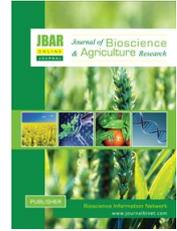


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## ***In vitro* regeneration of an *Oncidium* cultivar using different concentrations of BAP under different LEDs**

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### **ABSTRACT**

*Different developmental processes in plants are highly influenced by cytokinins. On the other hand, recently light emitting diode has garnered much reputation in plant tissue culture system due to its high energy efficiency and versatility. In the present study the effective concentration of 6-benzylaminopurine (BAP) on the organogenesis of *Oncidium Aloha 'Iwanaga'* was assessed and further evaluated under different LEDs. From the initial experiment, 0.1mg/l BAP was found to be the optimum concentration among others in terms of the average number of PLBs/explant and other parameters. The highest number of PLBs (28.1/explant) and shoots (2.0/explant) was obtained using 0.1mg/l BAP. Regarding root formation no response was observed under the treatment applied. In case of the LED treatments with 0.1mg/l BAP, the trial indicated that the number of PLBs increased under white (28.1/explant) and blue (26.2/explant) LED. Maximum shoot regeneration and fresh weight were observed under white LED compared to control. So, a combination of 0.1mg/l BAP with white LED could be proposed to be suitable for *in vitro* regeneration of *Oncidium Aloha 'Iwanaga'*.*

**Key Words:** *Orchid, Protocorm-like bodies, Shoot, Micropropagation and Organogenesis*

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### **I. Introduction**

Orchidaceae family is one of the largest and most diverse plant family producing beautiful flowers and exhibiting more than 25000 species and 700-800 genera (Samarfard et al., 1995). Among this huge number of species, *Oncidium* is one of the commercially important orchids. It produces brightly attractive flowers and highly adaptable to culture under a wide range of climatic conditions (Kalimuthu et al., 2007). Several factors work in conjunction which facilitates the reproduction of orchid in nature (Mondal et al., 2013). However, regulation of these factors is quite difficult which

leads to inadequate production of orchid propagules through conventional way. On the contrary, through tissue culture method, a large number of propagules of desired quality can be produced within least amount of time (Alam et al., 2020). Several studies reported the *in vitro* culture of *Oncidium* using multiple parts of plant as the explants such as root tips (Kerbaudy, 1984), leaf tissue (Chen et al., 1999), flower stalk internodes (Chen et al., 2000), leaf tip segments (Chen et al., 2003) and young leaves (Wu et al., 2004). Teixeira da Silva et al. (2005) stated protocorm-like bodies (PLBs) can be a good source of explant in the proliferation of orchids as these immature formations are meristematic and have strong potential for totipotency thus resulted in fast proliferation. Regarding growth promoters used in tissue culture system, cytokinin acts as the promoter of cell division and development of meristematic centers leading to the formation of organs, mainly shoots. Among different types of cytokinins, 6-benzylaminopurine (BAP) is a first generation synthetic cytokinin that elicits plant growth and development by stimulating cell division (Siddiqui et al., 2011). Several reports are available showing the impact of BAP on organogenesis of orchid genera like *Oncidium* (Rahman et al., 2005), *Cymbidium* (Shimasaki et al., 1990) and *Dendrobium* (Habiba et al., 2014). On the other hand, importance of light among the other environmental cues is undeniable as it is a fundamental necessity in each stage of a plant's life cycle (Sivakumar et al., 2006). Plants under natural illumination conduct multiple physiochemical activities in its leaf to store the energy in the form of carbohydrate, a process termed as photosynthesis. As such, the quality of the light source is of prime importance in regulating plant physiology and morphology. Over the year's fluorescent lamps (FLs) were used a common source of light but FLs have some disadvantages such as high electricity consumption with low output efficiency ultimately resulted in high production cost and it produces an unnecessarily wide range of wavelengths (350–750 nm). Recently light emitting diodes (LEDs) emerged as a new alternative light source with many advantages over the conventional one, such as longer life, wavelength specificity and narrow bandwidth, less heat radiation and low power consumption (Kaewjampa et al., 2012). The effects of BAP under different LEDs have been demonstrated in *Vanilla* and *Dendrobium* (Bello-Bello et al., 2016 and Habiba et al., 2014). However, till now no such research is done regarding the organogenesis of *Oncidium* Aloha 'Iwanaga'. In light of this, the present study was conducted to determine the appropriate concentration of BAP for the organogenesis of *Oncidium* and the impact of subsequent LED spectrum treatment on it.

## II. Materials and Methods

Protocorm-like bodies (PLBs) of *Oncidium* Aloha 'Iwanaga' were proliferated to the modified Murashige and Skoog (MS) medium (Shimasaki et al., 1990) at the Lab of Vegetable and Floricultural Science, Faculty of Agriculture and Marine Science, Kochi University, Japan on December 2019. After excising PLBs into singles, each was used as explant. MS medium containing 412.5 mg/l ammonium nitrate, 950 mg/l potassium nitrate, 20 g/l sucrose and 2 g/l Phytigel (Sigma) was adjusted to pH 5.5–5.8 before autoclaving. BAP at various concentrations such as control (0), 0.01, 0.1, 1 and 10 mg/l were added to culture media. Jars (250 ml UM culture bottle; Asone, Japan) with plastic caps containing 30 ml of medium were used as culture vessels. Five explants were put in each culture vessel. Three culture vessels were used under each treatment. All cultures were maintained under white LED (NNLK41509, Panasonic, Japan) at  $25 \pm 1^{\circ}$  C for continuous photoperiod. Based on the results of the initial experiment culture media was prepared using 0.1mg/l BAP and each jar contained 30ml of medium. Five explants were put in each culture vessel and three culture vessels were used under each treatment. Jars were placed under different light sources e.g. green LED (Jefcom, LT20-G, peak wavelength: 517 nm), red LED (Jefcom, LT20-R, peak wavelength: 631 nm), blue LED (Jefcom, LT20-B, peak wavelength: 460 nm), white LED and white fluorescent lamp (FL20SS, Toshiba). Experimental data were collected after 6 weeks of culture by counting the number of PLBs, number of shoots and measuring the fresh weight (mg). The data were analyzed using one-way analysis variance (ANOVA) and differences between means were tested using Tukey's HSD test ( $p \leq 0.05$ ).

## III. Results and Discussion

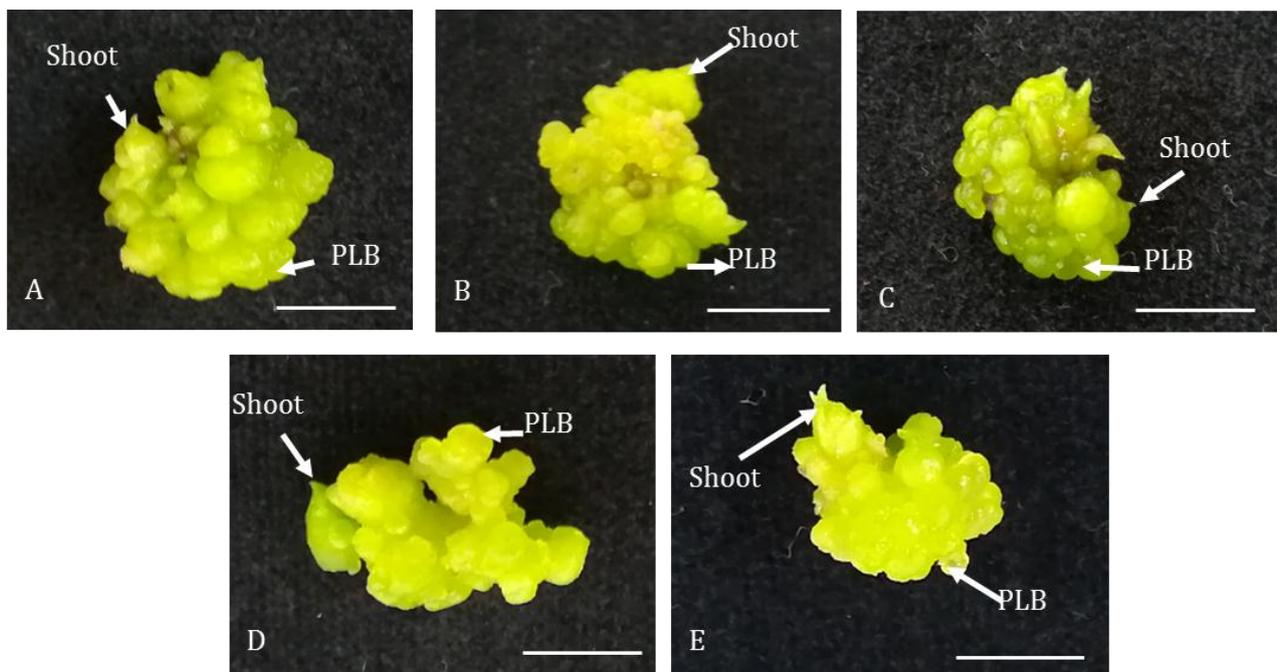
In the experiment, addition of BAP in media stimulated both PLB and shoot proliferation (Table 01). Maximum numbers of PLBs (28.1/explant) were found at 0.1mg/l and minimum (9.6/explant) was obtained with 0mg/l which was statistically similar to other concentrations. The highest formation rate of PLB (93.3%) was obtained with 0.1mg/l and the lowest (80.0%) was obtained with 0mg/l. No

significant variation was found with the number of shoots and fresh weight. The highest formation rate of shoot (86.6%) and the lowest (66.6%) was obtained with 0mg/l (Figure 01). The results in (Table 02) showed the effect of different light sources on the organogenesis of *Oncidium Aloha* 'Iwanaga' using 0.1mg/l BAP. Regarding light spectrum evaluation significant variation was found under different light conditions. The highest number of PLBs (28.1/explant) was obtained with white LED to which blue LED showed statistical similarity whereas the lowest was obtained in a white fluorescent lamp. The maximum formation rate of PLB (100%) was obtained with white and blue LED whereas the lowest (60.0%) was obtained with green LED. The highest number of shoots (2.0/explant) was obtained with white LED and the lowest (0.2/explant) was obtained with statistically similar red and blue LED. The highest fresh weight (260.0 mg) was obtained with white LED and the lowest was observed in green LED (Figure 01 and Figure 02).

**Table 01. Effect of BAP on the organogenesis of *Oncidium Aloha* 'Iwanaga'**

BAP (mg/l)	PLB		Shoot		Fresh Weight (mg)
	No./explant	Formation rate (%)	No./explant	Formation rate (%)	
0	9.6 ± 1.6 b	80.0	1.0 ± 0.2 a	66.6	160.3 ± 18.2 a
0.01	11.2 ± 2.0 b	86.6	1.0 ± 0.1 a	73.3	210.3 ± 22.4 a
0.1	28.1 ± 4.9 a	93.3	2.0 ± 0.3 a	86.6	260.0 ± 24.7 a
1	14.1 ± 2.3 b	86.6	1.0 ± 0.2 a	66.6	178.1 ± 23.1 a
10	13.7 ± 2.0 b	86.6	1.0 ± 0.2 a	66.6	173.9 ± 21.7 a

Values represent mean±SE followed by different letters which indicating significant differences by the Tukey HSD test ( $p \leq 0.05$ ).

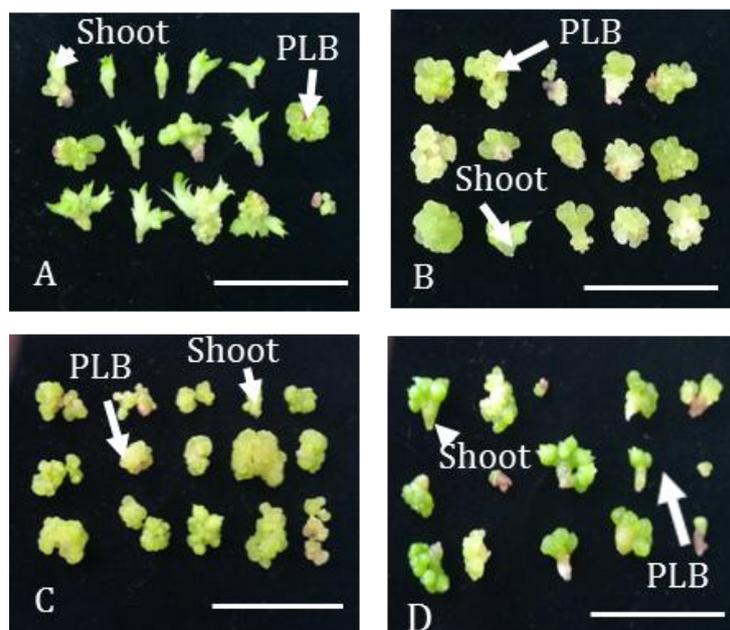


**Figure 01. Effect of BAP on the organogenesis of *Oncidium Aloha* 'Iwanaga'; A: 0mg/l; B: 0.01mg/l; C: 0.1mg/l; D: 1.0mg/l and E: 10mg/l BAP; Bar=1cm.**

**Table 02. Effect of different light sources on the organogenesis of *Oncidium Aloha* 'Iwanaga' using 0.1mg/l BAP**

Light sources	PLB		Shoot		Fresh weight (mg)
	No./explant	Formation rate (%)	No./explant	Formation rate(%)	
green LED	10.5 ± 3.7 b	60	1.7 ± 0.3 ab	80	95.5 ± 18.4 b
red LED	19.4 ± 2.9 ab	93.3	0.2 ± 0.2 b	13.3	125.0 ± 14.0 b
blue LED	26.2 ± 3.2 a	100	0.2 ± 0.1 b	86.6	166.3 ± 26.5 ab
white LED	28.1 ± 4.9 a	100	2.0 ± 0.3 a	86.6	260.0 ± 24.6 a
white fluorescent lamp	8.7 ± 2.3 b	86.6	0.8 ± 0.4 ab	66.6	97.9 ± 16.8 b

Values represent mean±SE followed by different letters which indicating significant differences by the Tukey HSD test ( $p \leq 0.05$ ).



**Figure 02.** Effect of different light sources on the organogenesis of *Oncidium Aloha* 'Iwanaga' using 0.1mg/l BAP; A: green LED, B: red LED, C: blue LED and D: white fluorescent lamp, Bar=1cm

Cytokinin constitutes a major class of plant growth regulator that is involved in a wide range of physiological processes. BAP is the most commonly used cytokinin in tissue culture (Nasiruddin et al., 2003). From the initial part we found the best results regarding PLB and shoot proliferation at 0.1mg/l compared with other concentrations (Table 01). Similar to our data (Habiba et al., 2014) confirmed the promotive effect of BAP at low concentrations. Habiba et al. (2014) also got a good response of BAP on the organogenesis of *Dendrobium* under white LED. Solarte et al. (2010) stated that the promotive effect of white LED is because of its spectral composition which includes blue (430-480 nm), green (495-570 nm) and red (590-670 nm) wavelengths that absorbed by photosystems II ( $\leq 680$  nm) and I ( $\leq 700$  nm) as maximum efficiency required in both photosystems. Current results found white LED very effective in PLB regeneration.

#### IV. Conclusion

Determining the effective concentration plant hormone increased the generation of orchids. From the data of our experiment we found 0.1mg/l BAP effective compared to other concentrations and white LED accelerate PLB formation using that certain concentration.

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#### Conflict of Interest

The author declared that there is no conflict of interest

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