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Effect of 6-Benzylaminopurine (BA) and Hyaluronic Acid (HA) under White Light Emitting Diode (LED) on Organogenesis in Protocorm-Like Bodies (PLBs) of *Dendrobium kingianum*

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Abstract: Growth and development of PLBs cultured *in vitro* largely dependent on the presence of different plant growth regulators. In this study, potential effect of BAand hyaluronic acid (HA9; Shiseido, Japan) on organogenesis of protocorm-like bodies (PLBs) in *Dendrobium kingianum* under white light emitting diodes(LEDs) was investigated. PLBs of *D. kingianum*were explanted on modified MS medium supplemented with various concentration of BA and HA9. The results indicated that the application of BA at low concentration (0.1 g/L) increased average number of PLBs, shoots and their fresh weight. In case of HA9, the highest number of PLBs (17.5) was recorded at concentration of 0.1mg/L. While the highest number of shoots per explant (3.6) and the percentage of shoot formation (66.7) were observed at concentration of 1mg/L HA9. This study shows that low concentration of BA is a potential plant growth regulator and HA9 acts as a plant growth regulator like BA for organogenesis in PLBs of *Dendrobium kingianum.*

Key words: 6-Benzylaminopurine · Hyaluronic Acid · Protocorm-Like Body (PLB) · Light Emitting Diode (LED)

INTRODUCTION

An orchid is one of the unique plants group. Its attract almost every kind of individual including professional breeders, amateurs and normal collectors because of their naturally beautiful and uniquely shaped flowers that come in a wide spectrum of vibrant colors. Among orchids, *Dendrobium* occupies a foremost position with marvelous varieties. *Dendrobium kingianum*, commonly known as Pink Rock Orchid, Captain King's *Dendrobium*, is a plant of the genus *Dendrobium* and native to eastern Australia. Since the development of a method for non-symbiotic germination of orchid seeds by Knudson [1], tissue culture techniques have been used for large scale propagation of a number of orchid species and their hybrids [2, 3]. *Dendrobium* is micropropagated in tissue culture by protocorm-like bodies (PLBs) but the growth is slow. To stimulate more efficient microprapagated of PLB, has been directed to use plant growth regulators such as ; N-benzylaminopurine, 1-napthaleneacetic acid (NAA) and thidiazuron (TDZ), [4]. 6-benzylaminopurineis a first-generation synthetic cytokinin that elicits plant growth and development responses, settingblossomsand stimulating fruit richness by stimulatingcell division [5] and when added in appropriate concentrations in tissue culture media,it may regulate cell division, stimulate auxiliary and adventitious shoot proliferation, regulate differentiation, inhibit root formation, activate RNA synthesis and stimulate protein and enzyme activity [6].

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Hyaluronic acid, or HA, is a naturally occurring polymer which serves important biological functions in bacteria and higher animals including humans. It is composed of alternating units of N-acetyl-D-glucosamine and D-glucuronate. The functions of HA include cell adhesion and migration, dynamic processes that are mediated through interaction with extracellular matrix components, regulation of protein secretion, gene expression and cell proliferation and differentiation [7, 8]. Recently, LED as a new light source have drawn considerable interest as an alternative light source for *in vitro* propagation. LEDs have been considered as a novel radiation source for growth and development of plant species because of several characteristics in practical use [9, 10]. The most attractive features of LEDs are small mass, volume, solid state construction and long life [11, 12]. However, there is no available information about the effects of BA and HA9 as plant growth regulators for *Dendrobium kingianum* micropropagation under white LED. The objective of present study was to determine the optimum levels of BA and HA9 on organogenesis in *Dendrobium kingianum*.

MATERIALS AND METHODS

PLBs of *Dendrobium kingianum* were proliferated in the modified Murashige and Skoog [13] medium by transferring to a new medium. After excision of PLB into singles, further used as explants. MS medium supplemented with 412.5 mg/L ammonium nitrate, 950 mg/L potassium nitrate, 20g/L sucrose and 2g/L phytagel (Sigma) used as a culture medium. 6-benzylaminopurine (BA; Sigma, USA) at concentrations of 0, 0.1, 1, 10 mg/L and hyaluronic acid (HA9;Shiseido, Japan)at concentrations of 0, 0.01 0.1, 1, 10 mg/L added to culture media before sterilization. Five explants cultured in one vessel and three vessels were used for each treatment. Jars of 250ml (UM culture bottle, AAs one, Japan) with plastic caps containing 30mL of medium were used for culture vessels. The pH of the medium was adjusted to 5.5-5.8 using 0.1mM 2- (N- morpholino) ethanesulfonic acid sodium salt (MES-Na) before autoclaving at 121°C for 15 min. Five explants cultured in one vessel and three vessels were used for each treatment. Cultures were maintained at 25 ±1°C under white light emitting diodes during 16 h photoperiods for five weeks.

Statistical Analysis: Experimental data were collected by counting the number of PLBs, number of shoots and their fresh weight were measured. The data were statistically analyzed by calculating standard errors of the means (means ±SE) and significant differences assessed by Tukey HSD test (P?0.05) using Tukey software.

RESULTS

Effect of 6-Benzylaminopurine (BA) on Organogenesis in PLBs of *Dendrobium kingianum*: The effect of BA with different concentrations on organogenesis in PLB cultures of *Dendrobium kingianum*are shown in Table 1.

Table 1: Effect of BA under white LED on organogenesis in PLBs of *Dendrobium kingianum*

		PLB		Shoot		
Treatment	Concentration (mg/L)	Number	Rate (%)	Number	Rate %	Fresh weight (g)
BA	Control	12.1±1.9[b]	86.7	1.1±0.7[b]	20	0.212±0.05[b]
	0.1	19.5±1.9[a]	100	4.8±1.7[a]	53.3	0.493±0.11[a]
	1	16.9±2.9[a]	100	2.1±0.9[a]	46.7	0.196±0.04[b]
	10	9.1±1.5[b]	86.7	0.6±0.4[b]	13.3	0.106±0.02[b]

Values represent means ±SE followed by the different superscript letters show significant differences by Tukey HSD test (P□0.05)

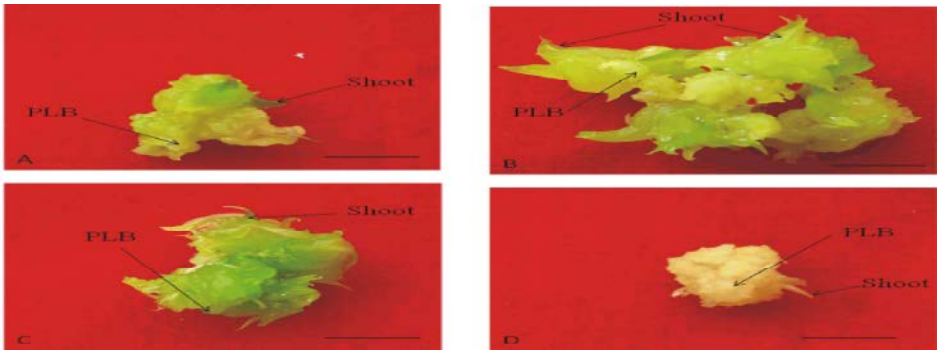

Fig. 1: Effect of BA under white LED on organogenesis in PLBs of *Dendrobium kingianum.* A: Control ; B: 0.1 mg/L BA; C: 1 mg/L BA ; D: 10 mg/L BA; Bars: 1cm

Table 2: Effect of HA9 under white LED on organogenesis in PLBs of *Dendrobium kingianum*

	PLB		Shoot		
HA9 (mg/L)	Number	Rate %	Average number	Rate %	Fresh weight (gm)
Control	12.1±1.8 [a]	86.7	1.1±0.7 [a]	20	0.212±0.05 [a]
0.01	16.5±3.4 [a]	100	2.1±1.2 [a]	40	0.228±0.08 [a]
0.1	17.5±2.1 [a]	100	0.7±0.4 [ab]	26.7	0.122±0.03 [a]
1	15.9±2.6 [a]	100	3.6±1.1 [a]	66.7	0.227±0.04 [a]
10	9.5±2.1 [b]	80.0	1.2±0.5 [a]	40	0.250±0.03 [a]

Values represent means ±SE followed by the different superscript letters show significant differences by Tukey HSD test (P□0.05)

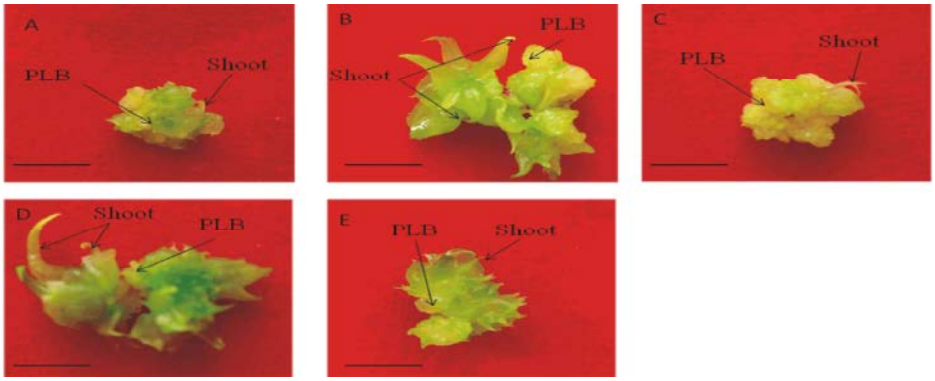

Fig. 2: Effect of HA9 under white LED on organogenesis in PLBs c of *D. kingianum*. A: Control ; B: 0.01 mg/L HA9; C: 0.11 mg/L HA9 ; D: 1 mg/L HA9; E: 10 mg/L HA9; Bars: 1cm

The highest number of PLBs per explant (19.5) was recorded MS medium supplemented with 0.1 mg/L BA and showed significantly different with control and high concentration of BA treatment.. The maximum percentage of shoot formation rate (53.3) and highest fresh weight (0.5g) after five weeks was observed with same concentration of BA treatment which significantly different with other treatment.

However, high concentration (10 mg/L) of BA reduced on every aspect of growth and development of PLBs. Effects of low concentration of BA has significant difference with high concentration treatment on organogenesis in PLBs of *Dendrobium kingianum* cultured *in vitro* under white LEDs.

Influence of Hyaluronic Acid (HA9) on Organogenesis in PLBs of *Dendrobium kingianum*: Application of HA9 promoted new explants. Effects of HA9 in modified MS medium on organogenesis in PLB of *Dendrobium kingianum* under white LED after five weeks of cultures are shown in Table 2. The highest number of PLBs (17.5) was recorded in the medium containing 0.1 mg/L HA9 and was significantly different with high concentration treatment. The maximum number of shoots (3.6) and percentage of shoot formation rate (66.7) and highest fresh weight (0.24gm) after five weeks was observed on the medium supplemented with 1 mg/L HA9.Higher concentration of HA9 (10 mg/L) had negative effect on PLBs and shoot formation. Meanwhile, PLB formation rate showed 100% at all treatments (BA and HA9) except for control (86.7%), 10 mg/L of BA(86.7%) and 10 mg/L of HA(80%).

Meanwhile, PLBs formation rate showed 100% at all treatments of BA and HA9 except for control (87%), 10mg/L BAP(86.7%) and 10 mg/L of HA9 (80%). In case of *Dendrobium* PLB formation at high concentration of BA and HA9 had negative impact

DISCUSSION

The purpose of the present study was to investigate the effects of BA and HA9 on organogenesis of PLB cultures in *Dendrobium kingianum*. Successful *in vitro* regeneration nearly always depends on the use of phytohormones. 6-benzylaminopurine is a wide spectrum plant growth regulator, it can accelerate growth of cell and stimulates basal shoot formation from explants.

6-benzylaminopurine is widely used for micropropagation of orchids because of its ability to induce organogenesis.This study showed that low concentrations of BA increased average no. of PLBs and their formation rate, number of shoots and fresh weight..BA especially at high concentrations usually inhibit the formation of roots and suppress growth,

besides reducing the influence of auxin in promoting root formation [14]. This study showed the same result that high concentration of BA inhibits growth and development of *Dendrobium* PLBs. Shimasaki and Fukomoto [15] declared that benzylaminopurine is effective for adventious shoot regeneration from hypocotyls segments of snapdragon. This study also confirmed the promotive effect of BA at low concentration.

Light is a fundamental environmental cue in the life of plants, playing crucial roles, directly and indirectly, in the regulation of plant development and growth [16] and plant growth regulators are generally used to efficiently proliferate PLBs and shoot and root formation in orchid plants *in vitro* [17]. The traditional light source used *in vitro* culture is fluorescent white light. This experiment was done under white LEDs and got good response of BA and HA9 on organogenesis of *Dendrobium*PLBs.

Application of HA9 resulted in significant promotion of PLBs and shoot formation, compared to the control phase and this new PLB formation occurred within very short time.Hyaluronic acid shortens the adaptation period of cells on the material surface and then cells enter the normal cell cycle quickly[18]. HA does not exhibit antigenicity and does not induce inflammatory or allergic reaction. Its degradation leads to formation of fragments that are not toxic to humans [19, 20]. Recently, in orchid tissue culture HA worked as a plant growth regulator in *Cymbidium dayanum* [21]*, Cymbidium insigne and Cymbidium finlaysonianum* [22] under white fluorescent light *in vitro* condition. Otherwise,hyaluronic acid shows high solubility in culture medium.It elicits systemic resistance in cucumber, tomato and pepper [23]. Present result clearly demonstrated that like BA, if hyaluronic acid added to modified MS media, acts as plant growth regulator to induce PLB and shoot formation. During the culture period there was no malformation observed in regenerated shoots. Therefore, much more work is still needed on hyaluronic acid for *Dendrobium* micropropagation.

CONCLUSION

Based on this research and discussion,it can be concluded that low concentrations of BA promotes organogenesis of PLBs in *D. kinianum* very speedily within short period of time. And results of this study also indicated that hyaluronic acid (HA9) can be used as plant growth regulator like BA because use of low concentration of HA9 on modified MS medium under white LED significantly affect growth of *D. kingianum* PLBs on the character of the average number of PLBs, average number of shoots and fresh weight.

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