

Very Low and High Temperature Act as Stress Factor on Organogenesis in Protocorm-Like Bodies (PLBs) of *Dendrobium kingianum*

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Abstract: Temperature plays an important role in plant growth and is a natural regulator in the morphogenesis of *in vitro* plants. The effects of different temperatures on organogenesis of PLBs in *Dendrobium kingianum* were investigated in the present study. Protocorm-like bodies (PLBs) were cultured in modified Murashige and Skoog (MS) media and incubated in different temperatures for four weeks. The highest number of PLBs (5.1) was recorded in the media which incubated in 24°C (24hr). Increase in fresh weight showed higher values in 25°C (24hr). Whereas, the highest number of developing shoots (2.8) was recorded in 30°C (24hr). Very low and very high temperature showed inhibitory or died effects on PLBs and shoot formation. On the other hand, optimum temperature when combined with high and low temperature make stress condition and PLBs turn reddish color in *Dendrobium kingianum* after four weeks. This study clarifies that the preferable temperature for organogenesis of PLBs in *Dendrobium kingianum* is 22-28°C and low or high temperature act as stress factor on PLBs of this orchid.

Key words: *Dendrobium* • Protocorm-like body (PLB) • Temperature

INTRODUCTION

The Orchidaceae family, comprising about 25,000 species is considered as ornamentals plants and as such, they have been successfully and commercially grown globally as cut flowers and pot plants [1]. The genus *Dendrobium* (Orchidaceae) is a highly diversified group represented by more than 1,100 species in the world and distributed from Southeast Asia to New Guinea and Australia [2, 3]. Propagation of orchids is a complex process, which involves the environmental (structural and functional) and physiological changes and may get influenced by the internal and external signaling factors. Among various physical environmental factors applied to plants, the temperature stress is most relevant to plant growth and development in both the natural environment and *in vitro* culture. In general, the optimum temperature for plant growth *in vitro* is around 20-25°C.

Low temperatures are necessary for endogenous cytokinin and gibberellins accumulation, as well as photosynthetic enhancement, leading to sucrose gathering for flower bud initiation and stalk elongation [4-9]. High temperature environments strongly affect oxidative stress in *Phalaenopsis* orchids, resulting in inhibition of flower development [5, 10]. In *Cymbidium*, temperature $\geq 25^{\circ}\text{C}$ can cause flower bud blasting (abortion) in the early stages of development such as *Cymbidium pumilum* and *Cymbidium Sazanami* 'Haru-no-umi' [11, 12, 13]. A requirement for flower initiation of temperate *Cymbidium* hybrids was response to diurnal temperature fluctuation of 10-14°C. There are many reports on the effects of temperature in particularly of day/night temperature and temperature fluctuation used for *in vitro* cultivation of many orchids. However, to our knowledge, no data have been reported to support the requirement for a suitable of low and high temperature

for PLBs initiation in *Dendrobium kingianum*. The main objective of the present study was to investigate the effect of different temperature on the *in vitro* PLBs growth and development of a *Dendrobium kingianum*.

MATERIALS AND METHODS

Plant Materials and Culture Conditions: Protocorm-like bodies (PLBs) of *Dendrobium kingianum* were subcultured every two months on modified Murashige and Skoog [14] supplement with 412.5 mg/L ammonium nitrate, 950 mg/L potassium nitrate, 20 g/L sucrose and 2.2 g/L Phytigel (Sigma). 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 Kg cm⁻². 250 ml of UM culture bottles (AsOne, JAPAN) with plastic caps were used, each bottle receiving 30 ml of medium. Five PLBs explants were put in each culture vessel and three culture vessels were used for each treatment.

Temperature Treatments: PLBs of *Dendrobium kingianum* were incubated in sixteen conditions. Different temperatures (15, 20, 22, 24, 25, 28, 30, 35°C) were subjected in 24 hours and other culture vessels kept in 25°C (22hr) with additional (5, 10, 15, 20, 30, 35, 40, 45°C) for 2 hours for 4 weeks period. All treatments were also treated the same with continuous Toshiba fluorescent lamps (54 µmol m⁻² s⁻¹).

Data Collection and Statistical Analysis:

Visual observations were carried out weekly. The numbers of PLBs, the numbers of shoots, the percentage of PLBs, the percentage of shoot and fresh weight of PLBs were recorded after 4 weeks of culture. The experiment was a completely randomized design with 3 replications and each replicate contained 5 PLBs. The data were subjected to a one-way analysis of variance (ANOVA) and differences between means were tested using Tukey's honestly significant different test ($P \leq 0.05$).

RESULTS

Effect of Temperature on Organogenesis in PLBs:

The growth and development of PLBs in *Dendrobium kingianum* were significantly affected by different temperature treatments *in vitro*. As shown in Table 1 and Fig. 1, after 28 days of culture, the highest percentage of PLBs formation was 93.3%, under high temperature 25°C (24 hrs) and 25°C (22hrs) with interval to low temperature 20 °C (2 hrs). But the highest number of PLBs (5.1/explants) were found from the medium which were kept in 24°C (24hr) which was significantly different with very high and very low temperature.

The highest fresh weight of PLBs (0.134g) was found under temperature 25°C (24hr) after 4 weeks of culture. In contrast, the lowest percentage of PLBs formation and

Table 1: Effects of Temperature on organogenesis of PLBs in *Dendrobium kingianum*

Temperature(°C)	Avg. No. of PLB	Avg. No. of Shoot	Fresh Weigh (g)
15(24hr)	0.7±0.04 ^b	0.1±0.02 ^b	0.018±0.0
20(24hr)	1.2±0.09 ^b	0.2±0.03 ^b	0.043±0.0
22(24hr)	3.4±0.18 ^a	0.7±0.05 ^b	0.077±0.0
24(24hr)	5.1±0.25 ^a	1.3±0.11 ^a	0.129±0.0
25(24hr)	4.6±0.20 ^a	1.1±0.09 ^a	0.134±0.0
28(24hr)	3.9±0.19 ^a	1.9±0.12 ^a	0.133±0.0
30(24hr)	2.9±0.19 ^a	2.8±0.19 ^a	0.138±0.0
35(24hr)	0.2±0.04 ^b	0.4±0.06 ^b	0.020±0.0
25(22hr)+5(2hr)	2.5±0.15 ^a	0.7±0.08 ^b	0.097±0.0
25(22hr)+10(2hr)	3.2±0.16 ^a	0.5±0.07 ^b	0.072±0.0
25(22hr)+15(2hr)	4.4±0.28 ^a	1.3±0.10 ^a	0.124±0.0
25(22hr)+20(2hr)	3.2±0.10 ^a	1.1±0.08 ^a	0.109±0.0
25(22hr)+30(2hr)	3.5±0.14 ^a	1.7±0.13 ^a	0.114±0.0
25(22hr)+35(2hr)	3.9±0.20 ^a	1.7±0.12 ^a	0.104±0.0
25(22hr)+40(2hr)	2.1±0.10 ^b	1.0±0.10 ^a	0.077±0.0
25(22hr)+45(2hr)	0.0±0.00 ^b	.07±0.02 ^b	0.012±0.0

Values represent means ± SE followed by the different letters show significant differences by Turkey HSD test ($P \leq 0.05$).

Average number = Number of cultured explants with new PLBs or root or shoot / Total number of cultured explants.

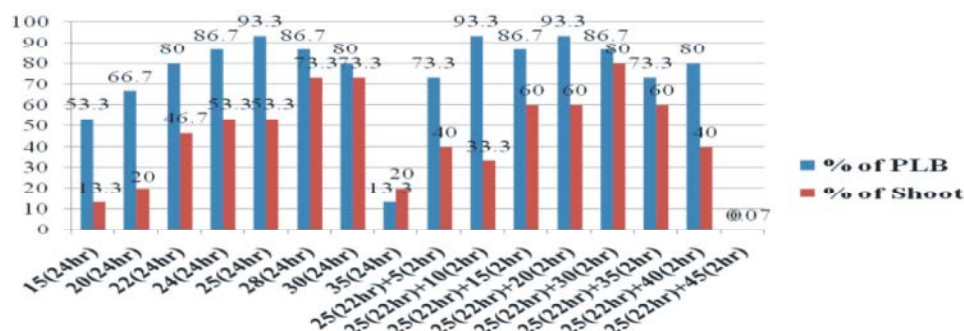


Fig. 1: Effect of different temperature on percentage of PLBs and Shoot formation of *Dendrobium kingianum*
 Percentage of PLBs/Shoot formation (%) = [(Number of cultured explants with new PLBs or shoot) / (Total number of cultured explants)] x 100.

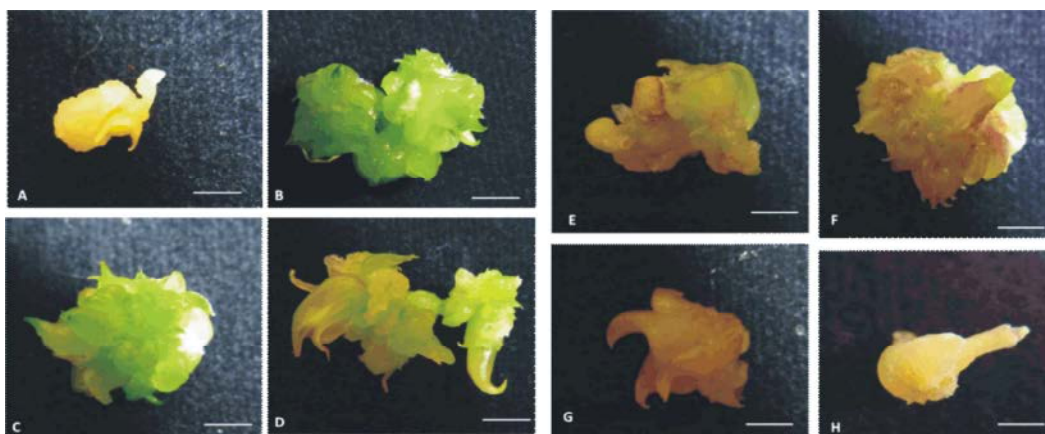


Fig. 2: Effect of different temperatures on organogenesis of PLBs in *Dendrobium kingianum*; A. 15°(24hr); B. 24°(24hr); C. 25°(24hr); D. 30°(24hr); E. 25°(22hr)+5°C(2hr); F. 25°(22hr)+15°C(2hr); G. 25°(22hr)+40°C(2hr); H. 25°(22hr)+45°C(2hr)

fresh weight of PLBs were found under temperature at 25°C (22hr) interval with high temperature 45°C (2hr) which failed to show any response and gradually turned brown and died later after 2 weeks of culture.

However, all high temperatures above 35°C inhibited of both PLBs formation and fresh weight of PLBs. Among the sixteen different temperature treatments, high temperature 30°C (24hr) showed the greatest effect on the number of shoots per explant and percentage of shoot induction was 73.3% with average 2.8 shoot per explant after 28 days of culture.

At 24 and 25°C temperature produced good quality PLBs (Fig. 2) and At temperature 25°C(22hr) with time interval (2 hours) of low temperature at 5 and 15°C and interval to high temperature 40°C and 45°C(2 hrs) PLBs turn reddish color and survived producing stress condition

DISCUSSION

Temperature is one of the most important factors regulating plant development through photosynthesis in term of rate of carbon assimilation. For instance, high temperatures cause increased respiration and photosynthesis while, low temperature can result in poor growth. In orchid, high temperature produce a growth of protocorms of *D. purpurella* was obtained at 23°C [15]. It was response to flower initiation of *Phalaenopsis* and *Zygopetalum* Redvale 'Fire Kiss' under 20-25°C and 25.4-28.6°C, respectively [5-9, 13]. In our present study we got best performance on protocorm growth at 24°C. In *P. pusilla*, an epiphytic orchid, found the best temperature for shoot elongation and leaf development was obtained when the temperature was at 27°C. But at 22°C and 32°C were inhibited in *P. pusilla* [16]. Flowering of *Phalaenopsis* was inhibited in plants grown at 28°C or greater [17]. The promotion of flowering in several orchid

genera by exposure to low temperature can be considered a vernalization process, in which the flowering initiation stage [11]. The results of present study showed that comparatively high temperature induce shoot formation.

Odontioda grown at the coolest constant temperatures of 14 or 17 °C had the greatest increase in pseudobulb diameter and thus the greatest final pseudobulb diameter [7]. In contrast, pseudobulb development and flower formation in *Cymbidium ensifolium* var. *misericors* were accelerated at warm temperature of 25-30 °C [18]. However, plants acclimatized under extreme temperature conditions showed symptoms of wilting, chlorophyll degradation and growth reduction [19]. For example, *Phalaenopsis* seedlings grown under extreme temperatures (11°C or 37°C) was decrease F/F_m and high temperatures remained produce oxidative damage, resulting in biochemical, physiological and morphological changes [4,5, 10,11, 20, 21]. Similar response to extreme temperature has been reported in our study which =15 °C or ≥35 °C, thereby inhibiting growth and development of PLBs. Therefore, at optimum temperature of *Dendrobium kingianum* PLBs growth was between 22-28°C. At optimum temperatures the protocorms of *Dactylorhiza majalis* become larger before the bud differentiated, apparently because the chronological age was more important for the onset of bud development than seedling size [22]. Robiah *et al.* [23] reported that PLBs of *Dendrobium Sonia-28* at 25°C the highest percentage of shoot growth and survival rate which reduces as the temperature was lowered to 5°C. PLBs cultured at low temperature (5°C) causes stress to the cells due to the osmotic flow of water out from the cells. Consequently, PLBs secrete a higher amount of peroxidases under stress. This could be due to the need for lignification process of the plant cells in order to avoid unnecessary out flow of water [24]. In our study we found that less than 15°C causes stress condition on PLBs of *Dendrobium kingianum*.

CONCLUSION

In conclusion, *Dendrobium kingianum* should be grown at temperature between 22-28 °C to avoid cold and heat stress which produced below 15°C and above 35°C. At 22-28°C temperature ranges, should be further applied to large scale orchid production *in vitro*. Present study results demonstrated that very low and very high temperature act as stress factor on organogenesis of PLBs in *Dendrobium kingianum*.

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