

In vitro Studies on the Effect of Light Intensity on Plant Growth of *Phaius tankervilleae* (Banks ex L' Herit.) Bl. and *Vanda coerulea* Griff

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ABSTRACT The effect of light intensity on plant growth of *Phaius tankervilleae* and *Vanda coerulea* was investigated *in vitro*. Plantlets of *P. tankervilleae* and *V. coerulea* with leaves of 2 cm. long were cultured on suitable medium under different light intensities of 28, 37, 56, 74 and 93 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Plantlets of *P. tankervilleae* were cultured on modified Vacin & Went medium, supplemented with 100 g/l of banana without saccharose. Plantlets of *V. coerulea* were cultured on modified Vacin & Went medium, supplemented with 100 g/l of banana, 100 g/l potato extract and 20 g/l saccharose. The environment for all growth *in vitro* was maintained at $25^\circ \pm 2^\circ\text{C}$, with a 16-h photoperiod. Plant growth was observed two months after culturing. Dry matter accumulation, plant height, leaf number and leaf area development of these two species of plantlets were best at the light intensity of 74 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

KEYWORDS: *in vitro*, *Phaius tankervilleae*, *Vanda coerulea*, plant growth, light intensity.

INTRODUCTION

Thailand is one of the countries in Southeast Asia, which is blessed with an extraordinary abundance of natural wild orchids. Many species have beautiful flowers, which attract both local and foreign growers. Some orchids are not only ornamentally attractive with pleasant scents, but are also important medicinal plants, such as *Anoectochilus* spp., *Arundina graminifolia* (D. Don) Hochr, *Bletilla* spp. and *Habenaria* spp. (Zenghong et al, 1993). One of the significant impacts of orchid development during the last forty five years is a tremendous increase of orchid growers in the country. Road development and increasing land use demands and, especially, destruction of forests by intensive lumbering, are causing the number of orchid species to decrease rapidly. To prevent the extinction of some species and loss of genetic diversity, conservation of available orchid species is needed. The most powerful technique for these purposes is tissue culture propagation.

Several aspects such as wavelength (quality), intensity (quantity) and duration of light are important factors affecting plant growth (Arditti and Ernst, 1992). High light intensity substantially increased

the total number of expanded leaves, dry matter, sugar content and nitrogen absorbed in *Phalenopsis* (Kubota, 1993). But, excessive light intensity causes stunting of the stem and leaf of alpine plants (Datta, 1994). High light intensity stimulated growth, tillering and yield per tiller and increased the stem proportion of *Brachiria bizantha* and *Panicum maximum*. It greatly increased the number of sclerenchyma cells and their wall thickened in all organs (Deinum et al, 1996). Higher light intensity has more violet and ultra-violet radiation that cause the production of excess phenolic compounds in *Zosteria marina* (Vergeer et al, 1995). In cotton, leaf area was increased under low light intensity (Roussopoulos et al, 1998). Most lower plants like mosses and ferns, as well as several woodland wild flowers are retarded in their growth or killed by high-sunlight intensity (Datta, 1994).

P. tankervilleae is a sympodial terrestrial type and *V. coerulea* is a monopodial epiphytic type orchid (Seidenfaden and Smitinand, 1959). *V. coerulea* is a very important as a stud plant used in breeding programs for obtaining blue color. Both species have slow growth rates, hence their numbers in nature are decreasing.

Terrestrial orchids naturally need stronger light than epiphytic orchids. Although light is an important factor in micropropagation, reports on the effect of artificial light intensities on plant growth, particularly orchids, are rather scarce. Stronger light intensity costs more in terms of energy input. The suitable light intensity and duration will give the best product with economic cost. It was expected that *V. coerulea* needs lower light intensity than *P. tankervilleae* in *in vitro* culture. Hence, the aim of the present study is to determine the optimum light intensity for the best growth of *P. tankervilleae* and *V. coerulea*. The results of this study will provide information for managing light intensity economically in *in vitro* culture of these two orchids and others.

MATERIALS AND METHODS

Plantlets of *P. tankervilleae* (Banks ex L'Herit.) Bl. and *V. coerulea* Griff. with the leaves of 2 cm. long were each cultured *in vitro* on suitable medium (Sirijuntarut, 2000) under different light intensities of 28, 37, 56, 74 and 93 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (about 1,500, 2,000, 3,000, 4,000 and 5,000 lux; Arditti and Ernst, 1992) provided by day light fluorescent tubes. The plantlets of *P. tankervilleae* were cultured on a modified Vacin & Went (1949) medium in which ferric tartrate was replaced by the chelated iron of Murashige & Skoog (1962), supplemented with 100 g/l of raw banana and without saccharose. Whereas the plantlets of *V. coerulea* were cultured on the modified Vacin & Went medium including the minor elements, vitamins and iron of Murashige & Skoog, supplemented with 100 g/l of raw banana, 100 g/l potato extract and 20 g/l saccharose. The culture environment was maintained at $25 \pm 2^\circ\text{C}$, under continuous 16-h light and 8-h dark periods per day. Four plantlets were cultured per replication and ten replications were carried out for each treatment. Plant growth was observed two months after culturing. Leaf area development (leaf width, leaf length and leaf area) was measured by an area meter am 100 (Hall et al, 1993). In addition, leaf number, plant height and dry weight accumulation of whole plants were also determined. This experiment was carried out with completely randomized design and Duncan's New Multiple Range Test (DMRT) was used in statistical analysis (Chantalakana, 1991).

RESULTS

Plantlets of *P. tankervilleae* had slower growth than those of *V. coerulea* (Fig 1 and Fig 5). *P. tankervilleae*

plantlets exposed to light intensity of 74 $\mu\text{mol m}^{-2} \text{s}^{-1}$ seemed to be greener, higher and had more dry weight than the others. However, there was no statistical difference between every given light



Fig 1. The effect of light intensity at 28, 37, 56, 74 and 93 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on growth of *P. tankervilleae* during 2 months of culture. The best result was found at a light intensity of 74 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (second from right).

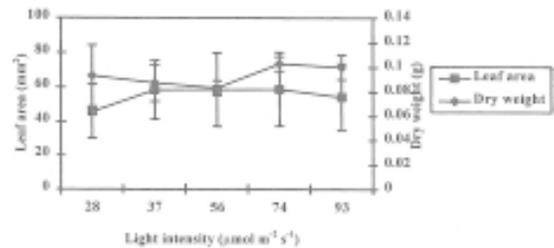


Fig 2. Effect of light intensity on leaf area and dry weight of *P. tankervilleae* after culturing for 2 months.

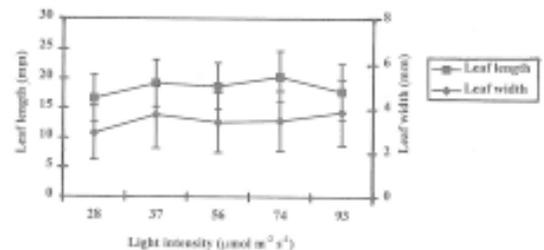


Fig 3. Effect of light intensity on plant height and leaf number of *P. tankervilleae* after culturing for 2 months.

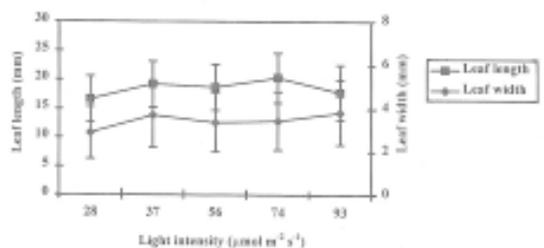


Fig 4. Effect of light intensity on leaf length and leaf width of *P. tankervilleae* after culturing for 2 months.



Fig 5. The effect of light intensity at 28, 37, 56, 74 and 93 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on growth of *V. coerulea* in 2 months of culture. The best result was found at a light intensity of 74 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (the second from right).

intensity for plant height, dry weight accumulation and leaf number (Figs 1, 2, and 4). Minimum leaf area and leaf width were found at a light intensity of 28 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figs 2, 4). Shorter leaf length was found at the light intensities of 28 and 93 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively ($P < 0.05$) (Fig 4). On the other hand, *V. coerulea* grew well at any given light intensity (Fig 5). Leaf area development, leaf width and dry weight accumulation were maximum at a light intensity of 74 $\mu\text{mol m}^{-2} \text{s}^{-1}$ ($P < 0.05$) (Figs 5, 6, and 8). The best dry weight was also found in plantlets exposed to light intensity of 74 $\mu\text{mol m}^{-2} \text{s}^{-1}$, followed by those exposed to 56, 93, 37 and 28 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively (Fig 6). Maximum leaf area development was observed in plantlets exposed to 74 $\mu\text{mol m}^{-2} \text{s}^{-1}$ followed by those exposed to 37, 56, 93 and 28 $\mu\text{mol m}^{-2} \text{s}^{-1}$ ($P < 0.05$) (Fig 6). However, the best plant height and leaf length were found in plantlets exposed to 37 $\mu\text{mol m}^{-2} \text{s}^{-1}$, followed by those exposed to 74, 56, 93 and 28 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figs 7, 8). There was no statistical difference of leaf number found in plantlets exposed to light intensity of 37 to 74 $\mu\text{mol m}^{-2} \text{s}^{-1}$. However, at 28 $\mu\text{mol m}^{-2} \text{s}^{-1}$, *V. coerulea* gave a leaf number less than the others ($P < 0.05$) (Fig 7). The plantlets at light intensity of 37 $\mu\text{mol m}^{-2} \text{s}^{-1}$ had the longest leaves, followed by those exposed to 74, 56, 28 and 93 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively (Fig 8). On the other hand, leaf width development is significantly better in plantlets exposed to light intensity of 74 $\mu\text{mol m}^{-2} \text{s}^{-1}$, followed by those exposed to 28, 37, 56 and 93 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively ($P < 0.05$) (Fig 8).

DISCUSSION

In moderate light intensity, plants generally bear longer internodes, and are less tough and more

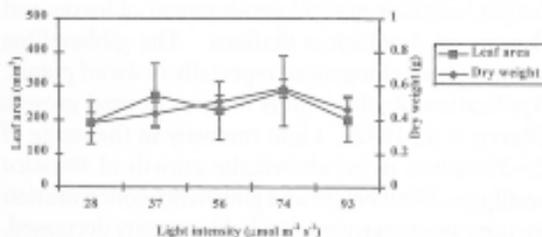


Fig 6. Effect of light intensity on leaf area and dry weight of *V. coerulea* after culturing for 2 months.

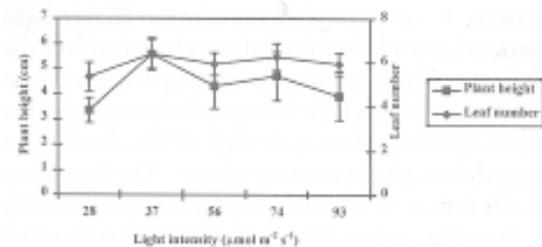


Fig 7. Effect of light intensity on plant height and leaf number of *V. coerulea* after culturing for 2 months.

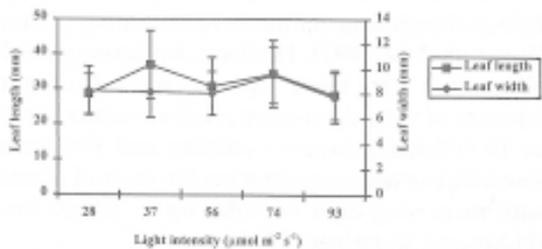


Fig 8. Effect of light intensity on leaf length and leaf width of *V. coerulea* after culturing for 2 months.

succulent with larger leaves than those grown in intense light (Barber and Anderson, 1992). Likewise, in papaya, the root formation of shoots or embryoids derived from callus or shoot tips occurred at a light intensities of 3,000 to 4,000 lux (Yie and Liaw, 1997). The results from this study revealed that plantlets of both *P. tankervilleae* and *V. coerulea* cultured on each suitable medium grew well at the light intensities of 37, 56 and 74 $\mu\text{mol m}^{-2} \text{s}^{-1}$. At the light intensities of 28 and 93 $\mu\text{mol m}^{-2} \text{s}^{-1}$, plantlets of both species studied generally grew less well. In this case, the light intensity of 28 $\mu\text{mol m}^{-2} \text{s}^{-1}$ seems to be insufficient for plant growth. On the other hand, the light intensity of 93 $\mu\text{mol m}^{-2} \text{s}^{-1}$ may be excessive for optimum growth regulation and photosynthetic activity. Plant growth is related to the function of growth hormones like auxin, which is sensitive to high light intensity. Cytokinins act in concert with auxin to cause cell division in plant tissue culture. Gibberellin deficient mutants have reduced plant

height but show normal development of leaves and flowers of *Arabidopsis thaliana*. The gibberellins control shoot elongation, especially in dwarf plants. Application of gibberellins restores normal growth (Raven et al, 1992). Light intensity in the range of 25-500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ altered the growth of *Brassica* seedlings. Plant height and gibberellin concentration increase progressively when light intensity decreased. In contrast, plant dry weight decreased with decreasing light intensity (Potter et al, 1999). Light is the ultimate substrate for photosynthetic energy conversion, it can also harm the plants. Higher light intensity causes photooxidation which involves the destruction of chlorophyll, resulting in less biomass production. High light intensity is damaging to the water-splitting photosystem II (PSII), leading to degradation of the reaction center. The frequency of this damage is relatively high when light intensity is increased, especially when combined with other environmental factors. There is an exception for lily plants in which no photoinhibition or damage to PSII was observed in the critical condition of strong light and high temperature in the culturing season (Sorrentino et al, 1997). Moreover, *Scindapsus* yielded plants that were more vigorous when the light intensity of the pretransplant stage was either 3,000 or 10,000 lux, whereas *Cordyline* and *Dracaena* showed progressive increases in the vigor of plants with increasing light intensity up to 10,000 lux (Miller and Murashige, 1976).

Although light is an important factor in micro-propagation, reports on the effect of artificial light intensities on plant growth, particularly of orchids, are rather scarce. This is chiefly because the higher light intensity necessary for some plants to mature are difficult to achieve and because of the space required by some plants at this stage. For relatively short time periods, plant performance probably reflects the photosynthetic process. This present study shows that light intensity plays a significant role not only on dry weight accumulation but also on plant height, leaf number, leaf shape and leaf area. The best results were found at 74 $\mu\text{mol m}^{-2} \text{s}^{-1}$. On the other hand, plantlets of *V. coerulea*, as well as *P. tankervilleae*, grown under the light intensity of 56 $\mu\text{mol m}^{-2} \text{s}^{-1}$ grew less than those grown under 37 and 74 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Generally, plant growth and development are affected by both internal factors including genotype and plant hormones and external factors such as light, temperature and moisture supply. This result may be due to the interaction between light intensity and internal factors which directly affect plant growth. The suitable light intensity

and duration will give the best result of product. Stronger light intensity costs more in terms of energy input. Consequently, the results suggest that light intensity of 37 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was sufficient to culture both *V. coerulea* and *P. tankervilleae* economically.

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REFERENCES

1. Arditti J and Ernst R (1992) Micropropagation of orchids. John Wiley & Sons Inc, USA.
2. Barber J and Anderson B (1992) Too much a good thing: light can be bad for photosynthesis. *TIBS* Feb 17, 61-6.
3. Chantalakana C (1991) Statistical analysis and research planning. 6th ed Thai Watana Panich Co, Ltd, Bangkok, Thailand.
4. Datta SC (1994) Plant Physiology. Wiley Eastern Inc, India.
5. Deinum B, Sulastri RD, Zeinab MHJ and Maassen A (1996) Effects of light intensity on growth, anatomy and forage quality of two tropical grasses (*Brachiaria brizantha* and *Panicum maximum* var *tricoglume*). *Netherlands J Agri Sci* 44(2), 111-24.
6. Hall DO, Scurlock JMO, Bolhar HR, Leegood RC and Long SP (1993) Photosynthesis and production in a changing environment. Chapman & Hall, UK.
7. Kubota S and Yoneda K (1993) Effect of light intensity preceding day-night temperatures on the sensitivity of *Phalaenopsis* to flower (Japanese). *J Jap Soc Hort Sci* 62(3), 595-600.
8. Miller LR and Murashige T (1976) Tissue culture propagation of tropical foliage plants. *In vitro* 12, 797-813.
9. Murashige T and Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15, 473-97.
10. Potter TI, Rood SB and Zanewich KP (1999) Light intensity, gibberellin content and the resolution of shoot growth in *Brassica*. *Planta* 207, 505-11.
11. Raven PH, Evert RF and Eichhorn SE (1992) Biology of plants. 5th ed, Worth publisher, USA.
12. Roussopoulos D, Liakatas A and Whittington WJ (1998) Cotton responses to different light-temperature regimes. *J Agri Sci* 131, 277-83.
13. Seidenfaden G and Smitinand T (1959) The orchids of Thailand. The Siam Society, Bangkok, Thailand.
14. Sirijuntarut M (2000) *In vitro* studies on seed germination and plant growth of *Vanda* and *Phaius*. MS Thesis in Environmental Biology, Faculty of Graduate Studies, Mahidol University, Bangkok, Thailand.
15. Sorrentino G, Cerio L and Alvino A (1997) Effect of shading and air temperature on leaf photosynthesis, fluorescence and growth in lily plants. *Scientia Horticulturae* 69, 259-73.
16. Vacin E and Went FW (1949) Some pH changes in nutrient solutions. *Bot Gaz* 110, 605-13.

17. Vergeer LHT, Aarts TL and Degroot JD (1995) The wasting disease and the effect of abiotic factors (light intensity, temperature, salinity) and infection with *Labyrinthula zosterae* on phenolic content of *Zostera marina* shoots. *Aquatic Bot* 52(1-2), 35-44.
18. Yie ST and Liaw SI (1997) Plant regeneration from shoot tips and callus of papaya. *In vitro* 9, 564-8.
19. Zenghong Y, Qitai Z, Zhizhou F, Kaiyong L and Heng L (1993) Orchids. The China Esperanto Press, Beijing, China.