



Growth rate and morphology of *in vitro* cultivated *Dendrobium bigibbum* as affected by different photoperiod cycles

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ABSTRACT: The relationship between photoperiod cycles (8, 12, 16, and 20 hours) and the morphology (growth rate (stem and root), leaf characteristic and stomatal conductance) of *in vitro* cultivated *Dendrobium bigibbum* were evaluated. Longer photoperiod cycles promoted higher growth rates and stomatal density with reduction in stomatal aperture size but adversely affected leaf traits at 20 hours, resulting in reduced leaf number, size, and area. Meanwhile, photoperiod cycles did not affect the root growth rates.

KEYWORDS: *Dendrobium bigibbum*, *In vitro*, Morphology, MS Media, Photoperiod, Physiology

INTRODUCTION

Light is an essential factor that affects the growth and development of plants [1]. Consequently, photoperiod, the duration of light and darkness in a 24-hour cycle, has a significant influence on the morphology and physiology of plants [2]. In the case of *D. bigibbum*, there is limited research on how different photoperiods affect its growth and development during *in vitro* cultivation. Accordingly, interaction between the different environmental factors such as light intensity, photoperiodic cycles, temperature, humidity and plant growth regulators serve vital functions in a successful *in vitro* propagation [1, 3]. Photoperiodic cycles can dictate the optimum amount of plant growth regulators that could influence the plant development [2]. Hence, determination of the impacts of distinct photoperiod regimes on the morphology, physiology, and overall *in vitro* performance of *D. bigibbum* both in commercial production and conservation efforts related to this important ornamental plant. The study explored the effects of varying photoperiods on the morphology of the *in vitro* cultivated *D. bigibbum*.

MATERIALS AND METHODS

Plant tissue culture preparation and growing conditions

Lateral shoots of plantlets of *in vitro* grown *D. bigibbum*, measuring approximately five mm in length was carefully

selected from the mother plant and was transferred to another Murashige and Skoog (MS) culture bottle and it was incubated at 25°C inside the tissue culture cabinet. Standardized pre-treatment phase was implemented followed by the application of various photoperiod treatments. All explants underwent a two-week period of vegetative growth under a 16-hour photoperiod, with the vessels positioned under light-emitting diode (LED) lighting. This initial growth phase aims to establish a uniform starting point for the subsequent experimental treatments. The explants were carefully transferred to one of the four designated photoperiod treatments: 8, 12, 16, or 20 hours of photoperiod per day.

Based on most available studies on *in vitro* propagation of *Dendrobium* species, these selected durations are among the most preferred by the plant for maximum growth and development [3]. In the present study, plants were subjected to different photoperiodic cycles for additional sixty days, allowing sufficient time for the plants to respond and adapt to the different light regimes.

To maintain consistent environmental conditions and minimize external influences, all vessels was placed in a controlled environment with a constant temperature of 25°C ± 2°C. This controlled temperature provided an optimal growth environment, enabling the accurate assessment of the

effects of photoperiod on the morphology and physiology of *D. bigibbum*.

Morphological assessment

To evaluate the morphological changes, several key parameters was measured on the 1st day and the 60th day of the experimental period.

1. **Average growth rate:** The average growth rate was computed using the formula: Average Growth Rate = $\frac{S1-S2}{T}$,

Where: S1 = first measurement; S2= second measurement; and T= number of days between each measurement.

Additionally, any anomalies in morphology or growth were documented photographically throughout the experiment.

2. **Plant height:** The height of each plant was measured from the base of the stem to the tip of the highest leaf using a Vernier caliper. This measurement reflects the vertical growth and elongation of the plant.

3. **Leaf count and characteristics:** The number of leaves, longest leaf length and width, leaf size and area, and leaf color were recorded. These measurements provide insights into leaf development, expansion, and potential variations in leaf morphology. The leaf size and leaf area were computed using the following formula:

Leaf Size = Length × Width [4]

Leaf Area = $\frac{\pi}{4}$ length × width [5]

4. **Root Count and characteristics:** The number roots were counted and the longest root length was measured using a Vernier caliper. Roots that are white to beige in color are healthy roots while dark or brown roots was noted as sign of decay or stress.

5. **Stomatal size and density:** At the end of the experimental period, the abaxial surface of the leaf was coated with a clear nail polish, which was allowed to dry completely. Subsequently, the nail polish was removed using scotch tape and affixed to a glass slide. The specimen was then observed under a microscope equipped with high-power objective (HPO) lenses. ImageJ [6] was utilized to quantify the number of stomata in 20 randomly selected fields of view per sample. Image J was used based from the protocol prescribed by the software developed by the National Institutes of Health and Laboratory for Optical and Computational Instrumentation. The stomatal density and the stomatal aperture were computed using the following formula:

Density = $\frac{\text{number of stomata}}{0.0016 \text{ mm}^2}$

Stomatal Aperture = pore length × pore width

RESULTS AND DISCUSSION

Plant growth rate

Orchids exhibit species-specific adaptability to various light environments through morphological and physiological changes. The research categorized treatments based on hours of light exposure, with longer exposure times correlating with higher plant growth rates. Results as shown in Table 1 indicated that extended photoperiods allow for increased photosynthesis, leading to the production of energy-rich molecules essential for cell division and overall plant development. Among the four treatments, 8 hours of exposure to light resulted in the slowest plant growth rate. The treatment with 12 hours of exposure had a slightly higher growth rate. The treatment with 16 hours of exposure showed further improvement as compared to the later. Finally, the treatment with the longest exposure time of 20 hours had the fastest growth rate.

This suggests that longer exposure to the specific condition promotes better plant growth. Similarly, Gajdosik et al. [7] stated that extended periods of light exposure can positively impact the plant's ability to photosynthesize, leading to increased growth and potentially higher yields. Similarly, Hm et al. [8] found out that increasing the photoperiod during non-flowering times can effectively stimulate growth of *Chrysanthemum* sp. by increasing the plant height as compared to those under 15 hours and below.

Table 1. Plant growth rate of in-vitro grown *D.bigibbum* as affected by different photoperiod cycles

Photoperiod	Plant growth rate (mm/day)
8 hours	0.02 ^d ±0
12 hours	0.03 ^c ±0
16 hours	0.05 ^b ±0
20 hours	0.07 ^a ±0

*Values are presented as mean±sd of four treatments. Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance using Tukey's test.

Leaf count and characterization

The analysis of leaf count and characteristics in relation to varying photoperiod durations reveals that as the exposure to light increases, there is a decrease in the number of leaves, leaf size, and leaf area (Figure 1). As shown in Table 2, in terms of number of leaves, *D. bigibbum* subjected in 8-hr photoperiod had the most leaves of 11, largest average leaf size of 57.60 mm and largest leaf area of 46.18mm². In addition, increment in leaf morphological characters were also noted in *D. biggibum* subjected in 12 and 16-hrs photoperiod. Statistically, significant differences are noted between shorter and longer photoperiod treatments,

indicating shorter photoperiod cycles could promote leaf morphological characters. While longer photoperiods might be beneficial for certain aspects of plant development, it can also induce an adverse effect on leaf morphology and production.

Accordingly, plants adjust their leaf characteristics under prolonged photoperiods to minimize water loss through transpiration by reducing leaf size and number, optimizing water use efficiency. Despite these changes, studies suggest that the overall plant growth rate may not be negatively affected, as plants can adapt to extended photoperiods by enhancing photosynthetic efficiency and adjusting metabolic pathways [9], indicating an adaptive response to changing light conditions rather than a universal decline in growth. Some studies have shown that while leaf size and number might decrease under extended photoperiods, the plant's overall biomass or growth rate can remain unaffected or even increase due to enhanced photosynthetic efficiency or altered metabolic pathways [9, 10]. Therefore, the observed changes in leaf morphology and production could be part of a broader adaptive response to the changing photoperiod, rather than a direct indication of adverse effects on growth.

Table 2. Mean number of leaves, leaf size, and leaf area of in-vitro grown *D. bigibbum* as affected by different photoperiodic cycles

Photoperiod	Number of leaves	Leaf size (mm)	Leaf area (mm ²)
8 hours	11 ^a ±3.42	57.60 ^a ±16.07	46.18 ^a ±13.21
12 hours	10 ^a ±4.50	43.64 ^{ab} ±14.88	34.27 ^{ab} ±11.68
16 hours	10 ^a ±3.03	43.66 ^{ab} ±7.76	34.29 ^{ab} ±6.01
20 hours	8 ^a ±3.03	32.23 ^b ±7.85	25.31 ^b ±6.17

*Values are presented as mean±sd of four treatments. Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance using Tukey's test.

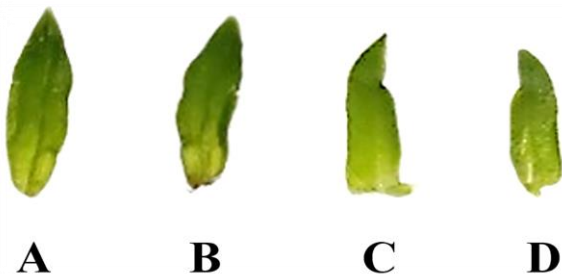


Figure 1. Leaves of in vitro grown *D. bigibbum* grown under (A) 8 hours, (B) 12 hours, (C) 16 hours, and (D) 20 hours of photoperiod

Leaf and root color

Leaf and root color analysis was done through discoloration of the plantlets. As observed, discoloration in plantlets also implicated slower growth rates. Meanwhile, *D. bigibbum* plantlets with green leaves and white to green roots, exhibited faster development and healthy growth. Leaf and root coloration serves as a vital indicator of plant health, wherein green leaves indicate healthy chlorophyll levels for photosynthesis and firm roots for active growth and proper nutrient uptake. The discoloration present in leaves and roots are common symptoms of plant stress caused by several factors including unfavorable environmental conditions, disease infestations caused by microbes, and nutrition deficiency [11]. Also, plant diseases can be identified through various signs such as lesions, color changes, and abnormal development in stems, leaves, buds, flowers, or roots, with symptoms like spots, dryness, and premature shedding indicating diseased conditions [12, 13].

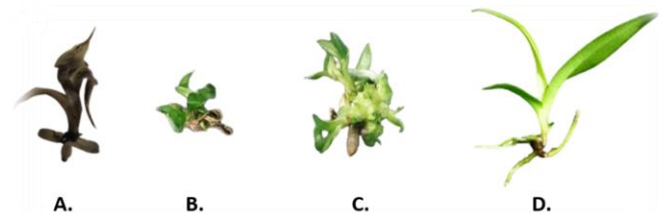


Figure 2. Tissue cultured *D. bigibbum* plantlets. (A) Discoloration present on roots and leaves; (B) Discoloration in some leaves; (C) Partial discoloration on the root; (D) Healthy Plant.

Root growth rate

The significance of root length in plants is closely tied to increased surface area, enabling efficient nutrient absorption, water uptake, and interactions with soil microbes for enhanced growth and resilience in diverse environments. Longer roots also provide better stability and anchorage, reducing the risk of plant damage in adverse weather conditions. As shown in Table 3, *D. bigibbum* subjected in a 8-hour photoperiod yielded the highest root growth rate of 0.085mm/day followed by 0.079mm/day in 20 hrs-photoperiod while the least of 0.045mm/day in 12 hrs-photoperiod cycles. Statistical analysis showed no significant differences among the treatments. Similarly, in a study of Miotto et al. [14], the overall growth of the root system is influenced not by photoperiod alone but by the interaction between light intensity, temperature and photoperiod. Alternatively, while most of the related studies focus on the above-the-ground morphological characteristics, Guo et al.

[15] specifically studied the effects of photoperiod on the formation of cherry radish root and found out that the cherry radish roots grow fastest and exhibit the most robust growth under a longer photoperiod.

Table 3. Root growth rate of in-vitro grown *D. bigibbum* as affected by different photoperiodic cycles

Photoperiod	Root growth rate (mm/day)
8 hours	0.085 ^a ±0.044
12 hours	0.045 ^a ±0.010
16 hours	0.048 ^a ±0.026
20 hours	0.079 ^a ±0.057

*Values are presented as mean±sd of four treatments. Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance using Tukey’s test.

Stomatal analysis

Stomata (Figure 3), the microscopic pores on plant leaves regulating gas exchange, play a crucial role in plant physiology. The results from Table 4 indicated no significant difference in stomatal characteristics across various photoperiod treatments, although a trend towards higher stomatal density and slightly reduced aperture was noted with longer light exposure. This suggests that while longer photoperiods lead to increased stomatal density, individual stomata tend to become smaller compared to those under shorter photoperiods, indicating an adaptive response to optimize gas exchange efficiency. Plants may enhance stomatal density in prolonged light to improve gas exchange capacity for carbon dioxide uptake and oxygen release, while reducing individual stomatal aperture helps regulate

transpirational water loss, maintaining physiological balance. This adjustment in stomatal characteristics allows plants to efficiently manage gas exchange and water conservation, ensuring optimal performance in extended light exposure. Studies by Franks et al. [16] highlight how plant species adjust stomatal development in response to environmental stimuli by modifying size and density, a phenomenon observed not only in living plants but also in herbarium samples and fossil records. Further research involving statistical analysis and gas exchange rate measurements would enhance understanding of how *in vitro D. bigibbum* plants adapt stomatal features to environmental changes [17].

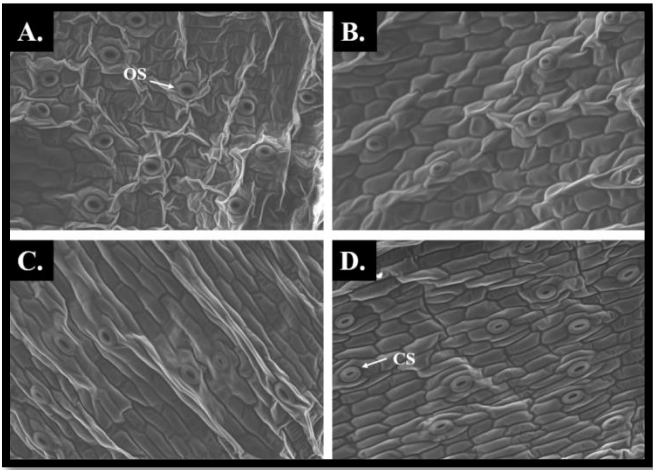


Figure 3. SEM imaging results of in vitro grown *D. bigibbum* plant leaves under varying photoperiod; (A) 8 hours, (B) 12 hours, (C) 16 hours, and (D) 20 hours. Open stoma (OS) and close stoma (CS)

Table 4. Analysis of stomatal characteristics of in-vitro grown *D. bigibbum* as affected by different photoperiod cycles

Photoperiod	Pore length (µm)	Pore width (µm)	Stomatal density (per mm ²)	Stomatal aperture (µm ²)
8 hours	4.26 ^a ±0.47	3.13 ^a ±0.52	7421.33 ^a ±1133.67	13.49 ^a ±3.77
12 hours	4.49 ^a ±1.14	2.93 ^a ±0.80	8343.67 ^a ±426.91	13.74 ^a ±7.42
16 hours	4.01 ^a ±0.87	2.83 ^a ±0.48	8407.00 ^a ±267.43	11.64 ^a ±4.46
20 hours	3.71 ^a ±0.17	2.42 ^a ±0.23	8501.00 ^a ±110.34	8.97 ^a ±0.80

*Values are presented as mean±sd of four treatments. Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance using Tukey’s test.

To sum up, based on the results, longer photoperiods of 16 and 20 hours promoted higher plant growth rates compared to shorter durations, but the 20-hour treatment adversely affected leaf morphology, resulting in reduced leaf number, size, and area. While root growth rates remained unaffected by photoperiod, stomatal density increased with longer light exposure, accompanied by a slight decrease in

stomatal aperture size, suggesting potential adaptations for water conservation.

The results of the present study are beneficial in the field of conservations of cultivation of *D. bigibbum* which is known in the field of pharmaceuticals. This is necessary to the optimized photoperiodic cycles that can interact with temperature, humidity, culture medium and plant growth

regulators conditions that could affect various developmental responses, physiological and morphological characteristics [18-20].

CONCLUSION

The study underscores the substantial influence of photoperiod duration on the growth, and development of in vitro cultivated *D. bigibbum* plants. Extended photoperiods were found to enhance plant growth rates, with the 16-hour duration being optimal for maximizing chlorophyll content, organic matter accumulation, and carbon assimilation, thereby improving photosynthetic efficiency and overall plant performance.

This study provides vital information on the optimum photoperiodic cycles necessary for in vitro culture of *D. bigibbum*.

DECLARATION

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Authorship Contributions

Concept and Writing: Dr. Eden S. David, Dr. Mary Jhane G. Valentino, Mr. Karl Joshua Antonio; Data Collection: Mr. Karl Joshua Antonio; Interpretation: Dr. Eden S. David, Dr. Mary Jhane G. Valentino, Mr. Karl Joshua Antonio

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Consent for publication

Not applicable.

Competing interests

The authors declared that there is no conflict of interest.

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