Effect of Change in Light Quality on Physiological Transformation of *in vitro* Phalaenopsis ‘Fortune Saltzman’ Seedlings during the Growth Period

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Changes in light quality strongly affect several plant anatomical, physiological, morphological, and biochemical parameters of orchid tissue culture seedling growth. In this experiment, ways in which light quality influence plant photosynthesis, growth parameters, and carbon dioxide rhythms of different sizes (stage I, II, and III) of Phalaenopsis tissue culture seedlings were examined. Stage I (Seedlings of 1–2 cm in height with 1–2 leaves and 1–2 roots) tissue culture seedlings were grown under six different light qualities under a T5 fluorescent lamp: White, Red (610 nm), Red (658 nm), Blue (440 nm), Red (610 nm) + Blue (440 nm), and Red (658 nm) + Blue (440 nm). After 5 months, cultured seedlings exposed to the Blue (440 nm) treatment showed significantly higher responses in terms of leaf quantities and chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content. On the other hand, seedlings subjected to the Red (658 nm) treatment grew more stems and presented with higher fresh weight and leaf lengths compared to the results of other treatments. The number of roots increased under the Red (658 nm), Blue (440 nm) + Red (610 nm), and Blue (440 nm) + Red (658 nm) treatments. Moreover, seedlings subjected to Red light (658 nm) showed significantly higher levels of Rubisco enzyme activity than those subjected to the other treatments. Phosphoenolpyruvate carboxylase activities recorded during the nighttime in seedlings subjected to Red light (658 nm) were also significantly greater. The results showed that during stage I, the concentration of carbon dioxide rhythm ranged from 1500–1800 ppm and reflected a C3 photosynthesis system. As the seedlings matured, the carbon dioxide rhythm decreased to 400–800 ppm at night and reached stage III (CAM plant). After 5 months of culturing, the carbon dioxide rhythm of the Red (658 nm) treatment seedlings changed from C3 to CAM, while seedlings subjected to the other treatments were still in the intermediate stage (stage II). From these results, we conclude that to enhance seedling growth through commercial production, Red (658 nm) should be applied.

Key Words: CO₂ rhythm, micropropagation, physiological responses.

Introduction

*Phalaenopsis* is a monopodial and traditional horticultural epiphytic orchid species with high commercial value as a cut flower and potted plant. Several studies have been carried out on the growth and development of *Phalaenopsis* for large-scale production for commercial purposes. *In vitro* culture techniques have been successfully adapted for the mass propagation of orchid plantlets. Currently, plant tissue culture is mainly used for the rapid propagation of *Phalaenopsis* (Chen et al., 2012). The morphology and physiology of *in vitro* grown plants is regulated by various micro-environmental factors such as light, temperature, humidity, and carbon dioxide levels (Kozai and Smith, 1995). Light (spectral quality, photon flux, and photoperiod) is an important factor that influences the overall growth and development of plants *in vitro* (Huges, 1981). One study on the influence of light quality on the growth and development of *in vitro* grown Doritaenopsis showed that growth parameters are highest when plants are grown under red and blue light emitting diodes (LEDs) (Shin et al., 2008). Leaf lengths increase when plants are grown under red LED lights. Plant carbohydrate (starch, sucrose, glucose, and fructose) and leaf pigment (chlorophylls and carotenoids)
biosynthesis is significantly increased in plants grown under red combined with blue LED light relative to those grown under red or blue LED and fluorescent light treatments. Lin et al. (2011) investigated the effect of light quality on protocorm-like bodies (PLBs) of Dendrobium officinale. Chlorophyll and carotenoid concentrations were found to be significantly higher under blue LEDs and under different red to blue light ratios (1:2) relative to concentrations found under other light treatments (dark, fluorescent white light, and red LEDs). Although Phalaenopsis leaves perform CAM and take up CO₂ in dark conditions (Wang and Lee, 1994), the stomata of young orchid leaves do not exhibit CAM. It has been shown that orchids with succulent leaves are CAM plants, whereas those with thin leaves are mostly C3 plants (Chen and Lee, 2002). Huang (2006) found CO₂ circadian rhythm within in vitro Phalaenopsis seedlings was different from that of Chrysanthemum, a typical C3 plant. CO₂ rhythms of Chrysanthemum began at 3000 ppm under light periods, then gradually decreased to less than 500 ppm. However, once the dark period started, they increased to 3000 ppm, reflecting a typical C3 plant form. For the Phalaenopsis seedlings, CO₂ rhythms followed typical C3 physiological patterns 30 and 60 days after subculture. However, 90 days after subculture, the CO₂ rhythm under a light period showed an increase in CO₂ at the start of this period from 200 to 800 ppm and it declined to 200 ppm 6 h later. After seedlings were subjected to a dark period, the CO₂ concentration increased to 400 to 800 ppm, revealing a CAM physiological pattern. Apinya (2012) studied the effects of supplementary light treatments on the growth and development of Phalaenopsis Sogo Yukidian ‘V3’ tissue culture plantlets. Seedlings were clearly found to undergo three stages (C3, Intermediate, and CAM) based on respective CO₂ rhythms. Stage I involved a typical C3 pattern with the lowest CO₂ concentration (1200 ppm) occurring at night and concentrations peaking (1875 ppm) in the early morning. During stage II, concentrations were high (1600 ppm) in the early morning; however, CO₂ concentrations declined sharply to 680 ppm at night. The CO₂ concentration recorded during stage III peaked at noon (1400 ppm) and dropped to a low at night (200 ppm), more clearly reflecting a CAM type. Thus, the objective of this study was to examine how different light qualities influence photosynthesis, growth parameters, and CO₂ rhythm changes in Phalaenopsis ‘Fortune Saltzman’ tissue culture plantlets under different light quality conditions during growth periods.

**Materials and Methods**

**Plant material and growth conditions**

Tissue seedlings of Phalaenopsis ‘Fortune Saltzman’, which are popular in the Taiwanese flower industry, were ordered from Chi Yueh Company, Taiwan. They were cultured on modified Hyponex medium (7N-6P₂O₅-19K₂O 1 g·L⁻¹ + 20N-20P₂O₅-20K₂O 1 g·L⁻¹). The medium was supplemented with 20 g·L⁻¹ sucrose, pH 5.7, and solidified with 8 g·L⁻¹ agar, 2 g·L⁻¹ peptone, 1 g·L⁻¹ activated charcoal, and 20 g·L⁻¹ potato (modified) 10 seedlings/flask with 10 replications for each treatment. Six different light quality treatments were used. Seedlings were cultured over a photoperiod of 12/12 h (day/night) in a 25 ± 2°C chamber (90 cm wide × 150 cm long × 39 cm height). Seedling stages were checked using a CARBOCAP Carbon dioxide module GMP222 connecting CO₂ probe (Vaisala, Finland) and an LI-1400 data logger mc (LI-COR, USA). The measurement range is 0 to 10000 ppm of CO₂. Accuracy at 25°C and 1013 hPa is ± (1.5% of range + 2% of reading). After sample flasks were checked for CO₂ concentrations, seedling stages were verified for CO₂ concentrations over a period of CO₂ fixation. The seedlings were separated into three different stages according to Apinya (2012):

- **Stage I**: Seedlings of 1–2 cm in height with 1–2 leaves and 1–2 roots
- **Stage II**: Seedlings of 3–4 cm in height with 2–3 leaves and 2–3 roots
- **Stage III**: Seedlings of 4–5 cm in height with 3–4 leaves and 3–4 roots

The experiment was initiated using the seedlings from stage I as the plant material.

**Light quality treatments**

Six T5 fluorescent lamp (Wellpower, Taiwan) light quality treatments were set up: White, Red (610 nm), Red (658 nm), Blue (440 nm), Red (610 nm) + Blue (440 nm), and Red (658 nm) + Blue (440 nm) (Fig. 1). The light treatments were set in different chambers that were covered by black cloth. The light intensity of each light treatment was controlled close to 32.14 μmol·m⁻²·s⁻¹ using an HR-350 spectrometer (HR-350; TAIWAN HIPOINT, Taiwan). The day/night light treatment was set to 12/12 h. The testing period ran from October 2013 to April 2014 at the National Pingtung University of Science and Technology Laboratory of Exploration & Conservation for Ornamental Germplasms.

Fresh weight, dry weight, leaf length, root length, leaf quantity, root quantity, and stem height values were determined at each stage to confirm CO₂ rhythms and stages. The chlorophyll and carotenoid content analysis method used was modified from that presented by Arnon et al. (1954). Rubisco activity measurements were made according to Cheng and Fuchigami’s (2000) method with modifications. Phosphoenolpyruvate carboxylase (PEPC) measurements were recorded following Bradford (1976). Both measurements were also conducted twice at the beginning and end of the dark condition. Soluble sugar content measurements and starch content analyses were completed according to Yoshida et al. (1976). Finally, total nitrogen content was
analyzed according to Kjeldahl’s (1883) method.

Statistical analysis
The Windows v.9.0 SAS program (SAS Institute Inc., USA) was used to analyze the data. When a significant ($P < 0.05$) difference was found for a measured parameter, means were separated using Tukey’s HSD test (Zar, 1984).

Results
The effect of different light qualities on Phalaenopsis ‘Fortune Saltzman’ tissue culture seedling growth

After 1 month of culturing, no seedlings showed significant differences in terms of growth characteristics. However, after 5 months of culturing, seedlings subjected to the Red (658 nm) treatment showed significantly longer stems and leaves and higher fresh and dry weight values than those subjected to the other treatments (1.38 cm, 3.00 cm, 2.50 g, and 0.12 g, respectively). For the White light treatment, stem and leaf length and fresh and dry weight values reached 0.78 cm, 1.86 cm, 1.90 g, and 0.04 g respectively. The fresh and dry weight of the seedlings increased under Red (658 nm) and was significantly higher than other treatments. The number of roots increased under the Red (658 nm) and Blue (440 nm) + Red (610 nm) treatments as compared to White treatment. Leaf length increased under Red (610 and 658 nm) and Blue (440 nm) + Red (658 nm) treatments. Stem height increased under Red (610 and 658 nm) treatments. The number of leaves increased under the Blue (440 nm) treatments, averaging 5.2 leaves, while there was 3.8 leaves on average under the White light conditions. Blue light significantly promoted the formation of the most leaves compared to the other treatments (Table 1).

The effect of light qualities on chlorophyll, carotenoid, nitrogen, sugar, and starch content in Phalaenopsis ‘Fortune Saltzman’ tissue culture seedlings

The seedlings grown under Blue (440 nm) light conditions showed significantly higher levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content than those grown under other treatments. After 1 month of culturing, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content for the Blue (440 nm) treatment reached 0.237, 0.333, 0.569, and 0.12 g, respectively. After 5 months of culturing, the fresh weight, dry weight, number of roots, leaf length, and stem height increased under Red (658 nm) treatment and were significantly higher than other treatments. The number of leaves increased under the Blue (440 nm) and Red (658 nm) treatments, and the number of roots increased under the Blue (440 nm) treatment. The number of leaves increased under the Blue (440 nm) treatment and was significantly higher than other treatments. The number of roots increased under the Red (658 nm) and Blue (440 nm) + Red (610 nm) treatments as compared to White treatment. Leaf length increased under Red (610 and 658 nm) and Blue (440 nm) + Red (658 nm) treatments. Stem height increased under Red (610 and 658 nm) treatments. The number of leaves increased under the Blue (440 nm) treatments, averaging 5.2 leaves, while there was 3.8 leaves on average under the White light conditions. Blue light significantly promoted the formation of the most leaves compared to the other treatments (Table 1).

Table 1. The effect of different light qualities on growth in Phalaenopsis ‘Fortune Saltzman’ tissue culture seedlings after 5 months of culture.

| Treatment | Fresh weight (g) | Dry weight (g) | Number of root | Leaf length (cm) | Height (cm) | Number of leaf |
|-----------|-----------------|---------------|----------------|-----------------|------------|---------------|
| White     | 1.90 ± 0.42 b   | 0.04 ± 0.005 b| 2.60 ± 0.52 b  | 1.86 ± 0.48 cd  | 0.78 ± 0.13 c| 3.8 ± 0.42 b  |
| B440      | 1.86 ± 0.37 b   | 0.03 ± 0.004 b| 2.40 ± 0.52 b  | 1.72 ± 0.26 d  | 1.00 ± 0.07 bc | 5.2 ± 0.42 a  |
| R610      | 1.68 ± 0.33 b   | 0.04 ± 0.005 b| 3.20 ± 0.79 ab | 2.66 ± 0.32 ab | 1.10 ± 0.29 ab | 3.8 ± 0.42 b  |
| R658      | 2.50 ± 0.32 a   | 0.12 ± 0.159 a| 3.60 ± 0.52 a  | 3.00 ± 0.31 a  | 1.38 ± 0.41 a | 4.0 ± 0.42 b  |
| B440+R610 | 1.80 ± 0.46 b   | 0.04 ± 0.004 b| 3.60 ± 0.84 a  | 2.36 ± 0.71 bc | 1.00 ± 0.15 d | 4.2 ± 0.79 b  |
| B440+R658 | 1.72 ± 0.43 b   | 0.04 ± 0.002 b| 3.20 ± 0.42 ab | 2.75 ± 0.57 ab | 1.02 ± 0.19 bc | 4.2 ± 0.67 b  |

* Means followed by different letters within same column showed significant differences among treatments based on ANOVA followed by Tukey’s test at $P<0.05$ (n=10).
3.484 mg·g⁻¹FW, respectively. The results for the White light treatment conditions were recorded as 0.221, 0.044, 0.265, and 2.126 mg·g⁻¹FW, respectively. After 5 months of culturing, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content under the Blue (440 nm) treatment reached 0.685, 0.338, 1.023, and 7.823 mg·g⁻¹FW, respectively. For the White light treatment, the results were recorded as 0.388, 0.201, 0.589, and 4.481 mg·g⁻¹FW, respectively (Table 2). This shows that Blue light (440 nm) significantly promotes the production of chlorophyll and carotenoid content. After 1 month of tissue culturing under Red (658 nm) treatment, seedlings showed the significantly highest nitrogen and sugar contents levels of all of the seedlings studied. Red (610 nm) treatment showed the significantly highest starch content. After 1 month of culturing, nitrogen and sugar content levels under Red (658 nm) treatment were 2.662%, 2.946 mg·g⁻¹FW. After 5 months of culturing, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content of the Red (658 nm) treatment were 3.100%, 2.909 mg·g⁻¹FW. After 1 month of culturing, the Red (658 nm) condition showed significantly highest starch content. After 5 months of culturing, Rubisco activity was highest under the Red (658 nm) treatment during the day. At night, Red (610 nm) and 658 nm) treatments generated higher levels of Rubisco activity during the day and night while the White and Blue (440 nm) + Red (610 nm) conditions generated higher nighttime levels. Only the Red (610 nm) condition generated more Rubisco activity during the day. After 5 months of culturing, Rubisco activity was highest under the Red (658 nm) treatment during the day. At night, Red (610 and 658 nm) treatments generated higher levels of Rubisco activity. The Red (658 nm) condition generated 115.97 μmol·mg⁻¹·min⁻¹ of Rubisco activity during the day and 91.521 μmol·mg⁻¹·min⁻¹ at night. The White treatment generated 75.430 μmol·mg⁻¹·min⁻¹ of Rubisco activity during the day and 41.665 μmol·mg⁻¹.

The effect of different light qualities on Rubisco and PEPC activity in Phalaenopsis 'Fortune Saltzman' tissue culture seedlings

In terms of photosynthetic enzyme activities, after 1 month of culturing, the Red (658 nm) condition showed significantly higher levels of Rubisco and PEPC activity during the day and night (1 hour after light and dark conditions). The Red (658 nm) condition generated Rubisco values of 36.353 μmol·mg⁻¹·min⁻¹ for the daytime and 32.597 μmol·mg⁻¹·min⁻¹ for the nighttime. Regarding PEPC activity, the Red (610 and 658 nm) treatment generated significantly higher levels during the day and night while the White and Blue (440 nm) + Red (610 nm) conditions generated higher nighttime levels. Only the Red (610 nm) condition generated more PEPC activity during the day. After 5 months of culturing, Rubisco activity was highest under the Red (658 nm) treatment during the day. At night, Red (610 and 658 nm) treatments generated higher levels of Rubisco activity. The Red (658 nm) condition generated 115.97 μmol·mg⁻¹·min⁻¹ of Rubisco activity during the day and 91.521 μmol·mg⁻¹·min⁻¹ at night. The White treatment generated 75.430 μmol·mg⁻¹·min⁻¹ of Rubisco activity during the day and 41.665 μmol·mg⁻¹.

**Table 2.** The effect of different light qualities on chlorophyll and carotenoid contents in Phalaenopsis ‘Fortune Saltzman’ tissue culture seedlings after 1 month and 5 months of culture.

| Treatment       | Chl a (mg·g⁻¹FW) | Chl b (mg·g⁻¹FW) | Total Chl (mg·g⁻¹FW) | Carotenoid (mg·g⁻¹FW) | Chl a (mg·g⁻¹FW) | Chl b (mg·g⁻¹FW) | Total Chl (mg·g⁻¹FW) | Carotenoid (mg·g⁻¹FW) |
|-----------------|------------------|------------------|----------------------|-----------------------|------------------|------------------|----------------------|-----------------------|
|                 | After 1 month of culture | After 5 months of culture | After 1 month of culture | After 5 months of culture |
| White           | 0.221 ± 0.002  b | 0.044 ± 0.001  c | 0.265 ± 0.001  d | 2.126 ± 0.089  c | 0.388 ± 0.001  c | 0.201 ± 0.001  c | 0.589 ± 0.002  c | 4.481 ± 0.009  c |
| B440            | 0.237 ± 0.006  a | 0.333 ± 0.022  a | 0.569 ± 0.016  a | 3.484 ± 0.305  a | 0.685 ± 0.004  a | 0.338 ± 0.017  a | 1.023 ± 0.056  a | 7.823 ± 0.418  a |
| R610            | 0.195 ± 0.005  c | 0.095 ± 0.016  b | 0.295 ± 0.016  c | 2.785 ± 0.047  b | 0.370 ± 0.001  c | 0.150 ± 0.021  b | 0.540 ± 0.021  c | 3.390 ± 0.011  c |
| R658            | 0.195 ± 0.016  c | 0.055 ± 0.005  c | 0.250 ± 0.011  c | 2.815 ± 0.037  b | 0.398 ± 0.034  c | 0.199 ± 0.015  c | 0.597 ± 0.048  c | 4.670 ± 0.314  c |
| B440+R610       | 0.233 ± 0.001  c | 0.087 ± 0.003  b | 0.311 ± 0.005  b | 2.880 ± 0.132  b | 0.453 ± 0.005  b | 0.235 ± 0.001  b | 0.686 ± 0.005  b | 5.290 ± 0.008  b |
| B440+R658       | 0.196 ± 0.008  c | 0.050 ± 0.011  c | 0.246 ± 0.003  c | 2.500 ± 0.023  c | 0.390 ± 0.023  c | 0.202 ± 0.007  c | 0.592 ± 0.030  c | 4.544 ± 0.192  c |

* Means followed by different letters within same column showed significant differences among treatments, based on ANOVA followed by Tukey’s test at *P<0.05* (n=10).

**Table 3.** The effect of different light qualities on nitrogen, sugar, and starch contents in Phalaenopsis ‘Fortune Saltzman’ tissue culture seedlings after 1 month and 5 months of culture.

| Treatment | Nitrogen (%) | Sugar (mg·g⁻¹) | Starch (mg·g⁻¹) | Nitrogen (%) | Sugar (mg·g⁻¹) | Starch (mg·g⁻¹) |
|-----------|--------------|----------------|----------------|--------------|----------------|----------------|
|           | After 1 month of culture | After 5 months of culture | After 1 month of culture | After 5 months of culture |
| White     | 1.657 ± 0.050  b | 2.086 ± 0.018  c | 3.271 ± 0.009  d | 2.517 ± 0.014  e | 2.024 ± 0.028  d | 3.579 ± 0.008  e |
| B440      | 1.947 ± 0.029  d | 2.586 ± 0.027  e | 3.555 ± 0.034  f | 3.232 ± 0.010  f | 2.424 ± 0.044  e | 3.681 ± 0.023  d |
| R610      | 2.313 ± 0.026  c | 2.862 ± 0.048  b | 4.917 ± 0.019  a | 2.834 ± 0.021  c | 2.610 ± 0.031  b | 4.078 ± 0.003  b |
| R658      | 2.662 ± 0.479  a | 2.946 ± 0.040  a | 3.695 ± 0.018  b | 3.100 ± 0.011  a | 2.909 ± 0.009  a | 4.313 ± 0.003  a |
| B440+R610 | 1.890 ± 0.030  c | 2.641 ± 0.021  d | 2.844 ± 0.021  e | 2.942 ± 0.021  c | 2.409 ± 0.019  c | 4.083 ± 0.003  b |
| B440+R658 | 2.512 ± 0.023  b | 2.672 ± 0.012  c | 3.716 ± 0.025  b | 2.541 ± 0.022  d | 2.584 ± 0.031  b | 3.857 ± 0.022  c |

* Means followed by different letters within same column showed significant differences among treatments, based on ANOVA followed by Tukey’s test at *P<0.05* (n=10).
CO2 rhythm testing

The results showed that the CO2 rhythm of *Phalaenopsis* ‘Fortune Saltzman’ tissue culture seedlings can be clearly classified into 3 different stages. During stage I, CO2 concentrations reached a low (1050 ppm) at night and peaked (1800 ppm) in the morning through a C3 photosynthesis system. During stage II, CO2 concentrations decreased to 600–1000 ppm. As seedlings grew, the CO2 rhythm decreased to less than 100 ppm and peaked at noon (800 ppm) and then gradually decreased during the day. CO2 concentrations for some treatments (White, Blue (440 nm) + Red (610 nm), and Blue (440 nm) + Red (658 nm)) gradually decreased to roughly 800–1500 ppm. However, Red condition CO2 concentrations (610 and 658 nm) remained high at approximately 2000–3000 ppm. Blue condition CO2 levels (440 nm) were lowest at 600 ppm (Fig. 3). After 5 months of culturing, CO2 rhythms for all treatments except for the Red treatment (658 nm) occupied an intermediate stage with CO2 concentrations decreasing to 600–1000 ppm. Red treatment CO2 concentrations (658 nm) had already entered the CAM stage at this point. At this stage, the Red treatment CO2 concentrations (658 nm) increased during the light period (from 100 ppm to 800 ppm), and during the dark period they decreased to the initial levels (Fig. 4). These results show that Red (658 nm) light can promote the growth and development of *Phalaenopsis* ‘Fortune Saltzman’ tissue culture seedlings while moving from the C3 stage to the CAM stage sooner than those subjected to other light treatments. The CO2 rhythms of seedlings subjected to the Red (658 nm) treatment over stage I changed to CAM after 5 months of culturing.

Discussion

We showed that red light strongly affects shoot/stem elongation, phytochrome responses, and changes in plant anatomy (Schuerger et al., 1997). Previous studies have shown that plantlets grown under red light promote plant growth (Kim et al., 2004), showing that red light promotes fresh and dry leaf weight gain in the *Chrysanthemum*. Our results showed that Red treatments (658 nm) alone influenced seedling growth relative to Blue (440 nm) + Red (658 nm) conditions. We found that the quality of red light affects plant growth more than red light combined with blue light. Apinya

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**Table 4.** The effect of different light qualities on enzyme activity in *Phalaenopsis* ‘Fortune Saltzman’ tissue culture seedlings after 1 month and 5 months of culture.

| Treatment | Rubisco activity at the beginning of darkness (μmol·mg−1·min−1) | Rubisco activity at the end of darkness (μmol·mg−1·min−1) | PEPC activity at the beginning of darkness (μmol·mg−1·min−1) | PEPC activity at the end of darkness (μmol·mg−1·min−1) |
|-----------|-------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|
| White     | 18.05±0.54 c                                   | 16.59±5.66 bc                  | 4.75±0.05 b                     | 6.71±2.42 a                     |
| B440      | 15.17±1.68 d                                  | 17.63±0.55 bc                  | 5.59±0.05 ab                    | 4.44±0.03 b                     |
| R610      | 38.93±3.86 a                                  | 17.04±2.51 bc                  | 6.61±2.24 a                     | 6.72±2.35 a                     |
| R658      | 36.35±0.64 a                                  | 32.59±8.60 a                   | 6.78±2.40 a                     | 6.74±2.35 a                     |
| B440+R610 | 17.05±0.83 ed                                 | 13.66±1.00 e                   | 4.53±0.29 b                     | 4.25±0.04 a                     |
| B440+R658 | 21.31±2.73 b                                  | 20.90±5.78 b                   | 4.51±0.09 b                     | 4.44±0.02 b                     |

* Means followed by different letters within same column showed significant differences among treatments, based on ANOVA followed by Tukey’s test at P<0.05 (n=10).
(2012) also found that seedlings under red light showed significantly longer stem and leaves compared to red light combined with blue light. In the present study, we found that Red (658 nm) treatments significantly promoted stem and leaf growth and fresh and dry weight gain. Red light was shown to increase in vitro Pelargonium plantlet stem elongation while blue light was found to inhibit shoot length (Appelgren, 1991). On the other hand, blue light significantly promoted the highest number of leaves. Poudel et al. (2008) also found that blue light was responsible for higher leaf quantities per explant in all genotypes of a Hybrid Franc grape. According to Massa et al. (2008), red wavelengths of between 600 and 700 nm can be absorbed by plant pigments. For Phalaenopsis, red light produces a high percentage of multiple inflorescences. These authors also found that hormones responsible for Phalaenopsis bud breakage and inflorescence elongation can be stimulated by red light (Tom et al., 2016).

In terms of chlorophyll and carotenoid contents, it can be concluded that blue light significantly affects pigment accumulation. Blue light is important for chlorophyll biosynthesis, stomata opening, enzyme synthesis, the maturation of chloroplasts, and photosynthesis (Tibbitts et al., 1983) and also affects the chlorophyll development control of Zantedeschia plantlets in vitro (Jao et al., 2005). Similar results were reported by Poudel et al. (2008), with chlorophyll content SPAD values and leaf numbers of stomata found to be highest among plants cultured under blue-light-emitting diodes.
in all genotypes of Hybrid Franc grapes. The results of the present experiment also show that Blue (440 nm) light treatments promoted the accumulation of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents more than other treatments examined.

Rubisco and PEPC are two key carboxylation enzymes used in photosynthesis. After 1 month of culturing, seedlings subjected to Red treatment (658 and 610 nm) showed significantly higher levels of Rubisco enzyme activity during the day, and the Red treatment (658 nm) generated the highest levels of Rubisco activity at night. Overall, Rubisco enzyme activity was higher during day and lower at night, serving as evidence of a C3 photosynthesis system. C3 plants convert CO$_2$ into a 3-carbon compound (PGA) with Rubisco during the day. On the other hand, CAM plants convert CO$_2$ into a 4-carbon intermediate (OAA) by using PEPC at night (Yamori et al., 2014). After 5 months of culturing, Red (658 nm) treatments showed greater PEPC enzyme activity at night (7.624 μmol·mg$^{-1}$·min$^{-1}$) than during the day (5.734 μmol·mg$^{-1}$·min$^{-1}$), serving as evidence of a CAM photosynthesis system. C3 plants convert CO$_2$ into a 3-carbon compound (PGA) with Rubisco during the day and the Red treatment (658 nm) showed the lowest growth due to CO$_2$ concentrations gradually decrease and exhibit C3, intermediate and CAM patterns similar to those found in our experiment. Decreasing CO$_2$ concentrations from C3 to CAM were also found in this experiment. Based on these experimental results, the system for detecting CO$_2$ concentrations within a culture flask has been proven stable and accurate.

**Conclusion**

After 5 months, cultured seedlings subjected to Blue (440 nm) treatment generated more leaves and presented higher levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content. On the other hand, seedlings subjected to the Red (658 nm) treatment had longer stems and leaves and higher fresh and dry weights than those subjected to other treatments. Root quantities increased under Red (658 nm), Blue (440 nm) + Red (610 nm), and Blue (440 nm) + Red (658 nm) treatments. The CO$_2$ rhythms of Red (658 nm) treatment seedlings changed from C3 to CAM. However, CO$_2$ rhythms of other treatments remained at an intermediate stage (stage II). This clearly shows that to enhance *in vitro* seedling growth in *Phalaenopsis*, Red (658 nm) treatments should be applied.

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