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Light-Emitting Diodes: Progress in Plant Micropropagation

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Abstract

In commercial micropropagation laboratories, the light source is one of the most important factors controlling plant morphogenesis and metabolism of plant cells and tissue and organ cultures. Lamp manufacturers have begun to rate lamps specifically for plant needs. The traditional light source used for in vitro propagation is fluorescent lamps (FLs). However, power consumption in FL use is expensive and produces a wide range of wavelengths (350–750 nm) unnecessary for plant development. Light-emitting diodes (LEDs) have recently emerged as an alternative for commercial micropropagation. The flexibility of matching LED wavelengths to plant photoreceptors may provide more optimal production, influencing plant morphology and chlorophyll content. Although previous reports have confirmed physiological effects of LED light quality on morphogenesis and growth of several plantlets in vitro, these study results showed that LED light is more suitable for plant morphogenesis and growth than FLs. However, the responses vary according to plant species. This chapter describes the applications and benefits of LED lamps on chlorophyll in plant micropropagation. Two study cases are exposed, Anthurium (*Anthurium andreanum*) and moth orchids (*Phalaenopsis* sp.), both species with economic importance as ornamental plants, where LEDs have a positive effect on in vitro development and chlorophyll content.

Keywords: in vitro cloning, light quality, tissue culture, chlorophyll

1. Introduction

Micropropagation or in vitro plant cloning is being widely used for large-scale plant multiplication. This method enables the identical reproduction of the selected parents, following

the maintenance of genetic fidelity. In commercial micropropagation laboratories, the light source is one of the most important factors controlling plant morphogenesis and growth cells, tissue and organ cultures. Lamp manufacturers have begun to rate lamps specifically for plant needs. The traditional light source used for in vitro propagation is fluorescent lamps (FLs). Nevertheless, the power consumption in FLs is expensive and produces a wide range of wavelengths (350–750 nm) unnecessary for plant development. Light-emitting diodes (LEDs) have recently emerged as an alternative for commercial micropropagation. LEDs possess advantages such as less heat radiation, a monochromatic spectrum, greater durability, and low power consumption. The LED illumination system for in vitro culture provides light in the spectral region that is involved in photosynthesis and in the photomorphogenic responses in plants.

LED colors or combinations commonly used for in vitro culture are white, red, blue, and mixture rates of blue and red. It has been reported that red light is important for shoot and stem elongation, phytochrome responses and changes in plant anatomy [1]. In contrast, blue light is important in chlorophyll biosynthesis, stomatal opening, chloroplast maturation, and photosynthesis [2]. Blue and red combination LEDs have been used for studies in many areas of photobiological research such as photosynthesis [3] and chlorophyll synthesis [4].

In addition, several studies have shown positive effects of LED lamps on plant development during in vitro culture of different species such as *Fragaria × ananassa* [5, 6], *Musa* spp. [7], *Solanum tuberosum* [8], *Chrysanthemum* [9, 10], *Vitis riparia × V. vinifera* [11], *Brassica napus* [12], *Populus euroamericana* [13], and *Saccharum* spp. [14], among others. However, the response in LED systems depends on the wavelength to which the plants are exposed and varies according to the species [15].

This chapter describes the applications and benefits of LED lamps on chlorophyll in plant micropropagation. Two study cases are exposed, Anthurium (*Anthurium andreaeanum* Lind.) and moth orchids (*Phalaenopsis* sp.), both species with economic importance as ornamental plants, where LEDs have had a positive effect on in vitro development and chlorophyll content.

2. Plant micropropagation

Micropropagation is the asexual propagation of plants using the techniques of plant tissue culture (PTC). Plant tissue culture refers to growing and differentiation of cells, tissues, and organs isolated from the mother plant, on artificial solid or liquid media under aseptic and controlled conditions. The small organs or pieces of tissue plants used in PTC are called explants. Plant tissue culture medium provides inorganic nutrients and usually a carbohydrate to replace the carbon which the plant normally fixes from the atmosphere by photosynthesis. When carbon is supplied with sucrose and kept in low light conditions, micropropagated plantlets are not fully dependent on their own photosynthesis.

The PTC techniques provide a new approach to plant propagation, being the best way to produce uniform plant germplasm and the regeneration of pathogen-free plants. To date,

commercial plant micropropagation has shown great productive potential; it is being used in hundreds of commercial laboratories for the propagation of species of agricultural and forestry importance. Commercial micropropagation of different species of economic importance is shown in **Figure 1**.

The commercial micropropagation process is carried out in the following stages:

Stage 0: Mother plant selection. Donor plants are selected and conditioned to be used to initiate *in vitro* cultures.

Stage I: *In vitro* establishing. The choice of the explant and its disinfection is carried out to initiate an aseptic culture.

Stage II: Multiplication. It is at this stage that mass propagation is performed, obtaining a large number of new individuals from minimal amounts of tissue.

Stage III: Elongation and rooting. The shoots must form their root system and at the same time increase their size to facilitate their manipulation and adaptation to the acclimatization conditions.

Stage IV: Acclimatization. It consists of a slow reduction of the relative humidity and gradual increases in the luminous intensity for a better adaptation to the external environment.

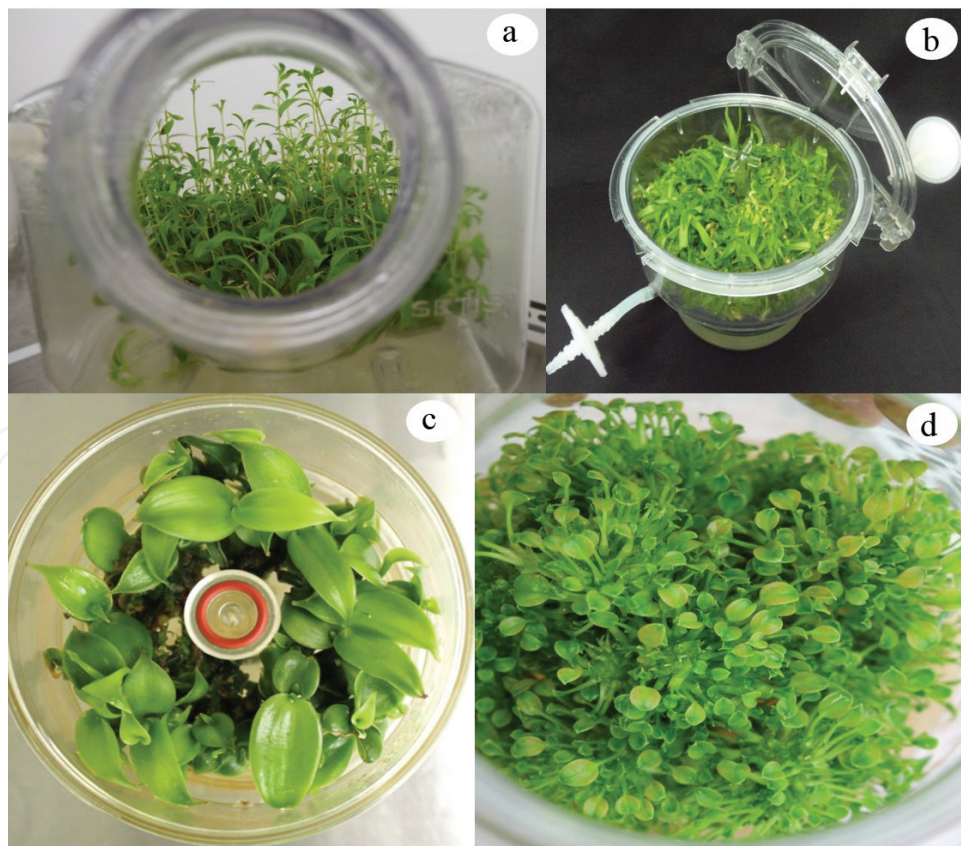


Figure 1. Commercial micropropagation of different species. (a) *Stevia rebaudiana*, (b) *Ananas comosus*, (c) *Vanilla planifolia* and (d) *Anthurium andreaenum*.

Requirements for the completion of each stage of micropropagation vary according to the method being utilized; it is not always necessary to follow each of the prescribed steps.

However, there are factors that affect the micropropagation process, including:

Factors that depend on the explant: Size, physiological age of the tissue, and explant position.

Factors that depend on the culture medium: Growth regulators, macro- and micronutrients, organic nitrogen, and carbon source.

Factors related to the incubation environment: Photoperiod, temperature, humidity, and light source.

Factors related to the incubation environment refer to incubators or growth rooms where temperature, humidity, and light can be controlled. In commercial micropropagation laboratories, the light source is one of the most important factors controlling plant development. Light quality (spectral quality), quantity, (photon flux) and photoperiod have a profound influence on the morphogenesis, growth and chlorophyll contents of a plant cell, and tissue and organ cultures.

The illumination systems allow wavelengths to be matched to plant photoreceptors to provide more optimal production and to influence plant morphology and metabolic composition [16]. Plants use energy between 400 and 700 nm and light in this region is called photosynthetically active radiation (PAR).

The growth and development of plants is dependent on light for:

Photosynthesis: The process whereby light energy is converted to chemical energy in the biosynthesis of chemicals from carbon dioxide and water.

Photomorphogenesis: The light-induced development of structure or form.

Phototropism: The growth response of plants which is induced by unilateral light.

In recent years, LEDs have emerged as an alternative for commercial micropropagation. LEDs possess various advantages such as less heat radiation, small mass, a monochromatic spectrum, greater durability, low power consumption, and specific wavelength. The flexibility of matching LED wavelengths to plant photoreceptors may provide more optimal production, influencing plant morphology and metabolism.

3. Spectral quality of LEDs

The traditional light source used for in vitro propagation is fluorescent lamps (FLs). However, power consumption in FL use is expensive and produces a wide range of wavelengths (350–750 nm) unnecessary for plant development, whereas monochromatic light-emitting diodes (LEDs) emit light at specific wavelengths. In this sense, LEDs can be fine-tuned to only produce the spectrums that plants need for morphogenic responses [17]. The response to LED light in micropropagation systems depends on light irradiance, photoperiod, and wavelength. The wavelength to which in vitro plants are exposed varies according to the species. Recent

studies compare the effect of FLs (545–610 nm) vs white LEDs (460 and 560 nm), red LEDs (660 nm), blue LEDs (460 nm), and the combination of blue and red LED (460 and 660 nm) treatments. LEDs affect in vitro rooting, number and length of new shoots, chlorophyll and carotenoid pigments, and other characteristics in plants. The spectral irradiance of LEDs is shown in Figure 2.

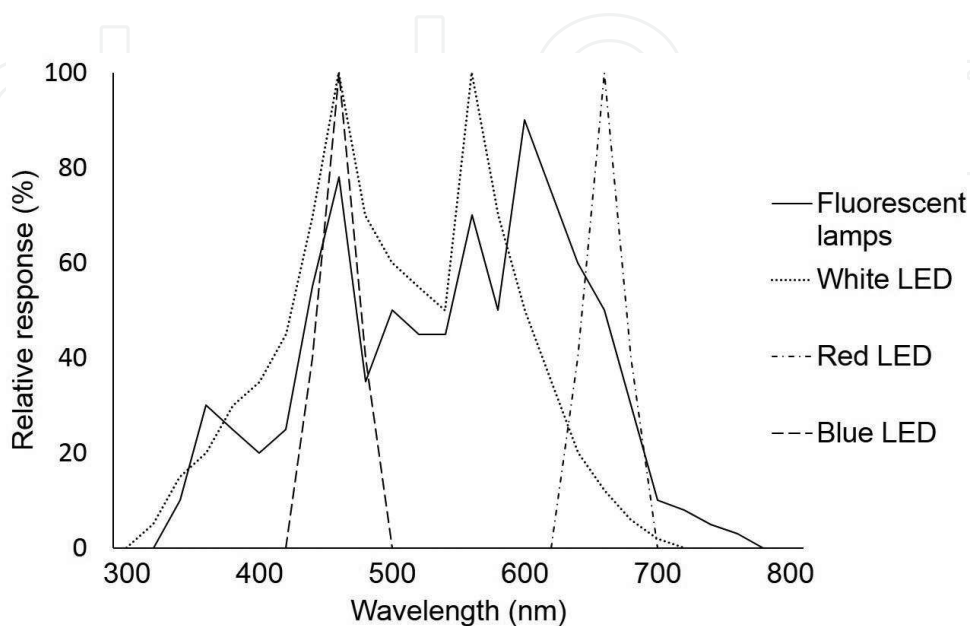


Figure 2. Spectral curves distribution in relative response of the LEDs and fluorescent lamps.

4. LEDs affect chlorophyll content

Several studies have shown important effects of LEDs on photosynthetic pigments during micropropagation of different species. Studies show that blue LEDs are a good light source for chlorophyll induction and that red LEDs decrease chlorophyll content. Dewir et al. [15] found that blue LEDs showed greater growth, vigor, and chlorophyll content in *Euphorbia milli*. Jao et al. [18] reported that blue LEDs promote growth and increase chlorophyll content in *Zantedeschia jucunda*. The same effect was observed by Li et al. [19, 20] during in vitro culture of *Gossypium hirsutum* and *Brassica campestris*, respectively. Kim et al. [9] and Moon et al. [21] emphasized the role of blue light on chlorophyll formation and chloroplast development in their work with *Chrysanthemum* and *Tripterospermum japonicum*, respectively. Monochromatic red LEDs with narrow peak emissions may cause an imbalance in the distribution of light energy between photosystems I and II, and thus be responsible for a reduction in net photosynthesis [3]. According to Li et al. [19], it has been observed that plantlets with lower chlorophyll content utilize the chlorophyll more efficiently than plantlets with higher chlorophyll content under red LEDs.

According to Soebo et al. [22], the possibility exists that red light may inhibit the translocation of photosynthetic products thereby increasing the accumulation of starch. Goins et al. [23]

observed higher photosynthetic rates and an increase in stomatal conductance in wheat leaves under mixed red and blue LEDs. Plant growth and development by increasing net photosynthetic rate was also observed in *Chrysanthemum* under mixed red/blue LED treatments and has been attributed to the similarities of the spectral energy distribution of red/blue to chlorophyll absorption [9].

The importance of blue light in stomatal opening has already been studied. It has been proposed that blue light received by phototropins activates a signaling cascade, resulting in fast stomata opening under a red light background [19]. The effect of light quality on stomatal characteristics has not yet been clearly determined, and differential stomatal behavior could be related to photosynthetic activity and plant growth.

According to Topchiy et al. [24], light quality also plays an important role in photosynthesis, influencing the way in which light is absorbed by chlorophyll. According to George [25], the level of chlorophyll so far obtained in tissue cultures is well below that found in mesophyll cells of whole plants of the same species, and the rate of chlorophyll formation on exposure of cultured cells to the light is extremely slow compared to the response of etiolated organized tissues. The greening of cultures also tends to be unpredictable, and even within individual cells, a range in the degree of chloroplast development is often found. In the carbon dioxide concentrations found in culture vessels, green callus tissue is normally photomixotrophic and growth is still partly dependent on the incorporation of sucrose into the medium [25]. However, green photoautotrophic callus cultures have been obtained from several different kinds of plants.

5. Study cases

Anthurium (*A. andreaenum* Lind.) and moth orchids (*Phalaenopsis* sp.) are tropical species with worldwide economic importance as ornamental plants and cut flowers. These species are commonly propagated by suckers; however, this propagation method is relatively slow and can cause disease transmission. Micropropagation has emerged as an alternative for fast mass production of *A. andreaenum* and *Phalaenopsis* plants of high phytosanitary quality.

For *A. andreaenum*, nodal segments were excised from in vitro-derived adventitious shoots and were used as explants. For in vitro culture of *Phalaenopsis*, protocorms were used as explants. The explants were placed in a 500 ml jar containing 40 ml of MS [26] medium without growth regulators. The pH of the culture medium was adjusted to 5.8 with 0.1 N sodium hydroxide, 0.25% (w/v) Phytigel was added as a gelling agent and then it was autoclaved for 15 min at 120°C and 117.7 kPa. The nodal segments were exposed to white LEDs (460 and 560 nm), red LEDs (660 nm), blue LEDs (460 nm), the combination of blue and red LEDs (460 and 660 nm, respectively), and FLs (545–610 nm) as a control. The LED system (model: 5050-1M-RGB, 3M, MN, USA) consisted of strips remotely controlled with a 12 V DC power adapter (model: SDK-0605, 3M, MN, USA). The explants were incubated at $24 \pm 2^\circ\text{C}$ and for 16 h light photoperiod. In all treatments, the photosynthetic photon flux density (PPFD) was maintained to $25 \mu\text{mol m}^{-2} \text{s}^{-1}$. PPFD was measured using a FieldScout Quantum Light Meter®.

After 60 days of in vitro culture, shoot length (cm), number of leaves, rooted shoots, and chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents were evaluated. Chlorophyll content was determined according to the method of Harborne [27]. For experimental design and data analysis, a completely randomized experimental design was used for all experiments. For each treatment, ten culture vessels, containing three explants each, were used. An analysis of variance (ANOVA) and Tukey's comparison of means test ($p \leq 0.05$) were performed for each species using SPSS statistical software (version 22 for Windows).

For *A. andreanum*, treatments with white LEDs, blue LEDs, and the combination of blue and red LEDs showed the greatest plantlet length and number of leaves. The FL and red LED treatments showed similar responses in promoting the formation of plantlets and their leaves. All shoots were rooted and the highest root number was induced in cultures incubated in FLs and blue LEDs with 6.6 and 6.0 roots, respectively. The lowest root number (1.5) was recorded in cultures incubated in red LEDs (**Table 1**). Chlorophyll *a*, *b*, and total chlorophyll content was significantly higher in the blue LED treatment (0.692 mg g^{-1} fresh weight), while the lowest total chlorophyll content was found in the red LED and FL treatments with 0.327 and 0.375 mg g^{-1} fresh weight, respectively (**Figure 3a**).

In *Phalaenopsis*, treatments with FLs, white LED and the combination of blue and red LEDs showed the greatest plantlet length and number of leaves (**Table 1**) The white, red and blue LEDs showed similar responses in promoting the formation of plantlets and their leaves. All protocorms were rooted and had the same root number. Chlorophyll *a* content was significantly higher in the blue LED treatment (0.2813 mg g^{-1} fresh weight), while chlorophyll *b* content was higher in blue and the combination of blue and red LED treatments, with 0.1368 and 0.1468 mg g^{-1} fresh weight, respectively. Total chlorophyll ($0.421875 \text{ mg g}^{-1}$ fresh weight)

Treatment	Shoot length (cm)	No. of leaves	Rooting (%)	No. of roots
<i>Anthurium andreanum</i>				
Fluorescent lamps	3.1 ± 0.1 b	4.9 ± 0.5 b	100.0 ± 0.0 a	6.6 ± 0.3 a
White LEDs	4.3 ± 0.2 a	5.7 ± 0.4 ab	100.0 ± 0.0 a	4.3 ± 0.6 b
Red LEDs	2.9 ± 0.2 b	5.0 ± 0.3 b	100.0 ± 0.0 a	5.0 ± 0.2 c
Blue LEDs	4.4 ± 0.4 a	6.8 ± 0.5 a	100.0 ± 0.0 a	6.0 ± 0.4 ab
Blue + red LEDs	4.0 ± 0.3 a	5.5 ± 0.3 ab	100.0 ± 0.0 a	2.5 ± 0.4 c
<i>Phalaenopsis</i> sp.				
Fluorescent lamps	17.0 ± 0.9 a	2.7 ± 0.2 a	100.0 ± 0.0 a	1.7 ± 0.2 a
White LEDs	14.5 ± 0.8 ab	2.3 ± 0.2 ab	100.0 ± 0.0 a	1.3 ± 0.2 a
Red LEDs	11.6 ± 0.7 b	1.7 ± 0.2 b	100.0 ± 0.0 a	1.5 ± 0.2 a
Blue LEDs	12.0 ± 0.4 b	1.7 ± 0.2 b	100.0 ± 0.0 a	1.3 ± 0.2 a
Blue + red LEDs	17.3 ± 0.6 a	2.7 ± 0.2 a	100.0 ± 0.0 a	1.7 ± 0.2 a

Table 1. Effect of LEDs on in vitro growth and rooting of *Anthurium andreanum* cv. Rosa and *Phalaenopsis* sp after 60 days of culture.

was higher in blue LED. The lowest total chlorophyll content was found in FL treatments and white LEDs with 0.1810 and 0.2500 mg g⁻¹ fresh weight, respectively (**Figure 3b**).

Our results indicate that FLs can be replaced by LEDs. The same effect was observed by Kurilčik et al. [10] and Lin et al. [28] during in vitro development of *Chrysanthemum* plantlets and *Dendrobium officinale* protocorms, respectively. In *Phalaenopsis*, LEDs had no effect on the number of roots, while in *A. andreanum* the highest number of roots was obtained in FLs and

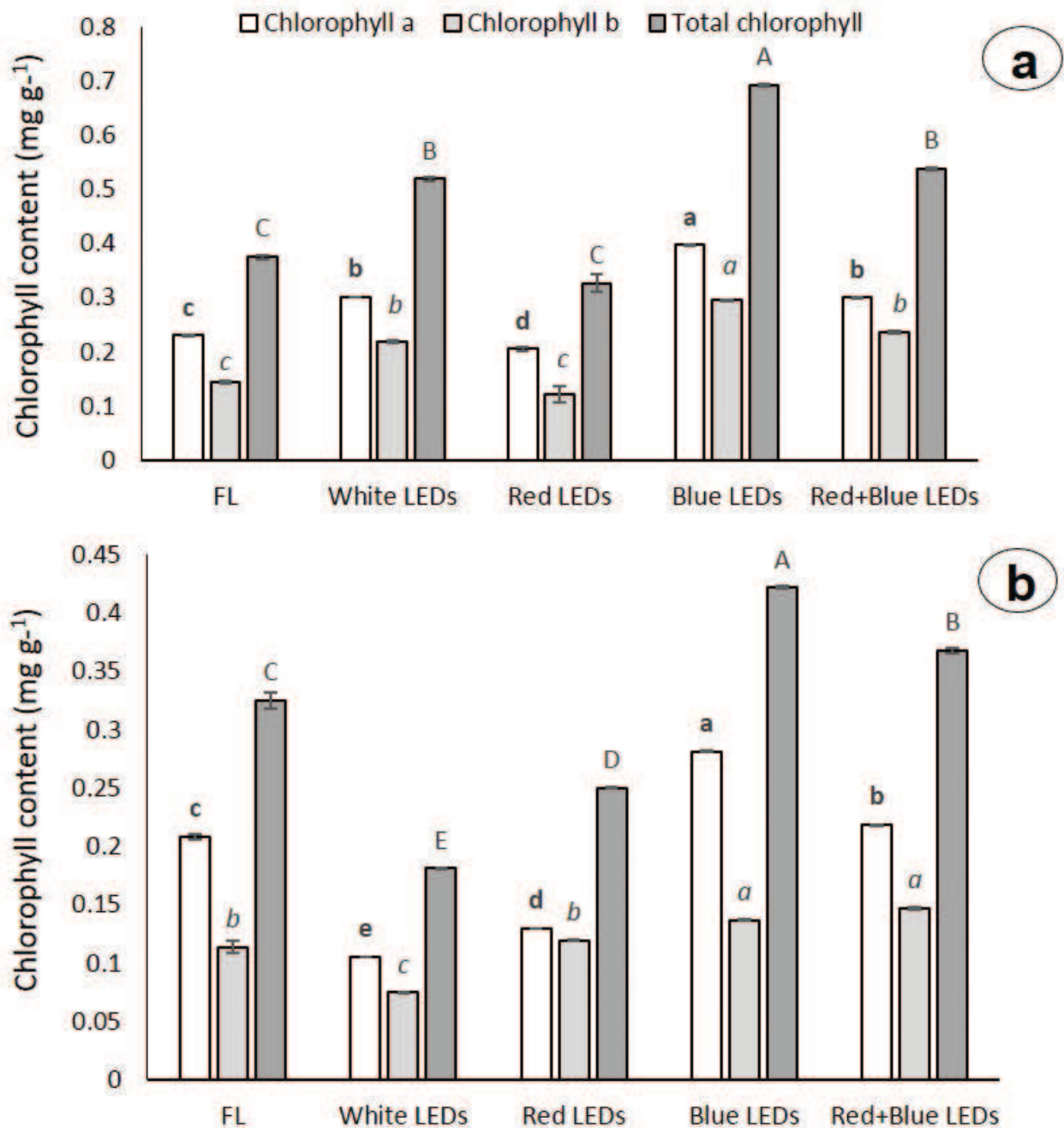


Figure 3. Effect of light quality on chlorophyll content in *Anthurium andreanum* (a) and *Phalaenopsis* sp. (b) after 60 days of culture in vitro. Different letters denote statistically significant differences according to Tukey's multiple range test at $p \leq 0.05$. Bars represent mean \pm SE.

blue LEDs. Similar results were reported by Cybularz-Urban et al. [29] and Waman et al. [7] in *Cattleya* and *Musa* spp., respectively.

According to Topchiy et al. [24], light quality also plays an important role in photosynthesis, influencing the way in which light is absorbed by chlorophyll. The present results demonstrated that the chlorophyll a, chlorophyll b, and total chlorophyll content appeared greater in plantlets growing under treatments containing blue light. Similar results were reported by Dewir et al. [15] where blue LEDs showed greater growth, vigor, and chlorophyll content in *E. milli*. Jao et al. [18] reported that blue LEDs promote growth and increase chlorophyll content in *Zantedeschia jucunda*. Our results are consistent with these studies in that the blue LEDs have an important role in the synthesis of photosynthetic pigments. This suggests that LEDs can also be used for improving the quality of *ex vitro* plantlets of *A. andreanum* and *Phalaenopsis* sp.

In conclusion, the use of light-emitting diodes (LEDs) as a radiation source for plants has attracted considerable interest for commercial micropropagation. The flexibility of matching LED wavelengths to plant photoreceptors may provide more optimal production, influencing plant morphology, and chlorophyll content. Although previous reports have confirmed physiological and morphological effects of LED light quality on metabolism and development of several plantlets in vitro, in our experience, LED light is more suitable for plant morphogenesis and growth than FLs. However, the responses vary according to plant species.

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