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Light quality affects growth and development of *in vitro* plantlet of *Vanilla planifolia* Jacks

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ABSTRACT

The use of diode lamps (LEDs) has proved to be an effective alternative for the *in vitro* culture of different plant species. LED light accelerates growth and physiological development of plantlets, besides lowering illumination costs. In this study, we evaluated the effect of different LEDs on the growth, development and physiology of *in vitro* plantlets of *Vanilla planifolia*. For that, five types of wavelengths were used: white light (W, 420 nm), red (R, 660 nm), blue (B, 460 nm), and a combination of blue and red LEDs (B:R, 1:1), also, a fluorescent light (Fl, 400–700 nm) control was included. Statistically significant differences were observed in growth, development and physiology of plants grown under LED and fluorescent lights. In the multiplication phase, LEDs B:R stimulated the elongation of shoots and chlorophyll synthesis. In the rooting phase, LEDs B stimulated shoot elongation, the number of roots formed and the number of leaves per shoot. Furthermore, treatments with different LEDs increased the synthesis of photosynthetic pigments. With regards to acclimatization, 100% survival was achieved in all regenerated plantlets, regardless of the type of LEDs used for illumination. This study demonstrated that the different LED lights produce a differential effect on growth and development during the *in vitro* culture of *V. planifolia*. These results can contribute to improve the micropropagation process of this species.

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1. Introduction

Vanilla (Vanilla planifolia Jacks.) is a tropical plant of the family *Orchidaceae* from which one of the world's most popular flavor additives is extracted (Soto-Arenas, 2003; Ramírez-Mosqueda and Iglesias-Andreu, 2015). The sexual reproduction of this species under cultivation is complex because of the presence of sterile seeds and a low germination rate, which does not exceed 1% (Soto-Arenas, 2003; Torres-González et al., 2011). For this reason, the plant is propagated asexually through cuttings. However, this method has limitations, such as being time-consuming and labor-intensive, and affecting the growth and development of the parent plant (Ramírez-Mosqueda and Iglesias-Andreu, 2016).

Plant tissue culture (PTC) resolves the issues mentioned above because it allows the rapid propagation of thousands of plants (Ramírez-Mosqueda and Iglesias-Andreu, 2016). In orchids, tissue culture techniques were used in conservation and micropropagation studies, evidencing the key role of the different types of light in regulating the growth and development of plants *in vitro* (Gupta and Jatothu, 2013).

Light (spectral quality, photon flux, and photoperiod) is one of the factors that influence the growth and development of different species

of plants *in vitro* (Hughes, 1981; Gupta and Jatothu, 2013). It is essential for various physiological processes in plants, such as photosynthesis, hence influencing growth and development, as well as morphogenesis (Gupta and Jatothu, 2013).

The light sources generally used for *in vitro* culture are tubular fluorescent lamps (FLs). FLs emit wide-spectrum light ranging from 350 to 750 nm, which contains low-quality wavelengths that are unnecessary for promoting growth (Kim et al., 2004). Light emitting diodes (LEDs) are a potential alternative light source for *in vitro* culturing, due to their wavelength specificity and narrow bandwidth, low amount of thermal emissions, low degradation, and long life (Bull et al., 1991; Gupta and Jatothu, 2013). The LED lights can eliminate wavelengths of light that are inactive for photosynthesis, this causes higher growth and development in plants (Gupta and Jatothu, 2013).

The LED lights most used in micropropagation of different plant species are: white light (W, 420 nm), red (R, 660 nm), blue (B, 460 nm), and a combination of blue and red LEDs (B: R, 1: 1). All (depending on the species study) produce different effects on morphology, physiology and plant morphogenesis. The assessment of the effect of different LED wavelengths on the growth and development of *in vitro* plantlets of various plant species has drawn particular attention (Gupta and Jatothu, 2013). However, despite the economic importance of *Vanilla planifolia*, the effect of different LED wavelengths on the morphogenesis and growth of this species under *in vitro* conditions has not been

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Table 1
Effect of light quality on the shoot proliferation of *Vanilla planifolia* after six weeks of culture.

Light quality	Number of shoots per explant	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
Fl	5.27 ± 0.50a	1.39 ± 0.13b	2.20 ± 0.05a	0.48 ± 0.15a
W	5.41 ± 0.41a	1.38 ± 0.11b	1.58 ± 0.07b	0.14 ± 0.01b
R	5.80 ± 0.48a	1.18 ± 0.04c	1.34 ± 0.02c	0.06 ± 0.01c
B	5.41 ± 0.46a	1.35 ± 0.07b	1.50 ± 0.01b	0.06 ± 0.01c
B:R	5.11 ± 0.40a	1.74 ± 0.08a	1.50 ± 0.05b	0.06 ± 0.11c

Figures represent the mean ± SE (standard error). Means with different letters are significantly different (Tukey, $p \leq 0.05$). Fl = fluorescent lamps; LEDs: W = white; R = red; B = blue; B:R = blue + red (1:1).

evaluated to date. Therefore, this study investigates the morphogenesis and growth of *V. planifolia* cultivated *in vitro* using LEDs of different wavelengths.

2. Materials and methods

2.1. Plant material

This study used *V. planifolia* *in vitro* plantlets established in the Plant Tissue Culture laboratory of *Instituto de Biotecnología y Ecología Aplicada* (INBIOTECA) at the *Universidad Veracruzana*.

2.2. Light irradiation and growth conditions

Light quality experiments were performed in a culture room with $80 \pm 5\%$ relative humidity, photoperiod (16 h light/8 h dark), and 25 ± 2 °C. Light sources used in this experiment were:

- 1) FL (Control): fluorescent lamps emitting light at broad wavelengths of 400–700 nm.
- 2) W: White LEDs with a wavelength of 420 nm.
- 3) R: Red LEDs with a wavelength of 660 nm.
- 4) B: Blue LEDs with a wavelength of 460 nm.
- 5) B:R = 1:1: 50% blue light at a wavelength of 460 nm and 50% red light at a wavelength of 660 nm.

Explants in the shoot proliferation and rooting phases were incubated under the different LED treatments strips (IP65 model, SMD 5050 RGB supplying 12 V and 1 W per module, Techno Lite®, Zapopan, Jalisco). The distance between these and the light source was kept constant and equal in all treatments. Irradiation intensity of artificial light was set to $40 \mu\text{mol m}^{-2} \text{s}^{-1}$.

2.3. Effect of light quality on shoot proliferation

Nodal segments (0.5–1 cm in length) were exposed in semisolid MS (Murashige and Skoog, 1962) salts medium supplemented with 0.1 mg L^{-1} thiamine HCl, 0.5 mg L^{-1} nicotinic acid, 0.5 mg L^{-1} pyridoxin HCl, 2 mg L^{-1} glycine, 50 mg L^{-1} cysteine HCl, 100 mg L^{-1} myo-inositol, 2.1 mg L^{-1} benzyladenine (BA) as plant growth regulator (PGR) and 30 g L^{-1} sucrose. The pH of the medium was adjusted to 5.8 ± 0.2 ; afterwards, the medium was solidified with 2.5 g L^{-1} Gelrite™ (Sigma-Aldrich, St. Louis, MO) before autoclaving at 121 °C

and 124 kPa for 15 min. After six weeks of *in vitro* culture, the number of shoots, shoot size, number of leaves, fresh weight and dry weight were measured. Dry weight was obtained by oven drying at 60 °C for 48 h, and the content of photosynthetic pigments was determined.

2.4. Effect of light quality on rooting

Individual shoots (1–2 cm in length) were cultivated in semisolid MS medium at half its concentration containing also half the amounts of vitamins used in the propagation phase and 100 mg L^{-1} myo-inositol with no PGR supplementation. The pH of the medium was adjusted to 5.8 ± 0.2 ; afterwards, the medium was solidified with 2.5 g L^{-1} Gelrite™ (Sigma-Aldrich, St. Louis, MO) before autoclaving at 121 °C and 124 kPa for 15 min.

After six weeks of *in vitro* culture, the number of shoots, shoot size, number of leaves, fresh weight and dry weight of roots and stems were measured. Dry weight was obtained by oven drying at 60 °C for 48 h. Stomatal density and leaf area were determined from photomicrographs (100×) using the software Image-Pro. Total protein content and photosynthetic pigments of *in vitro* plants were also evaluated.

2.5. Chlorophyll content

Chlorophyll content was estimated from third leaves according to Porra et al. (1989). In brief, samples of 0.2 g of fresh leaves of each treatment were homogenized in a pre-chilled mortar using 20 mL of a 1:1 (v/v) mixture of 80% cold acetone and absolute ethanol. The homogenate was transferred to a 25 ml test tube, centrifuged at 6000 rpm for 12 min, and the supernatant collected. The absorbance was measured at 663 nm (Chl. a), 645 nm (Chl. b) and 441 nm for carotenes. The contents were determined using the following formulas:

$$\text{Chl a} = [(12.25 \times A_{663} - 2.25 \times A_{645})] \times V/100 \times W$$

$$\text{Chl b} = [(20.30 \times A_{645} - 4.91 \times A_{663})] \times V/100 \times W$$

$$\text{Chl a + b} = [(7.34 \times A_{663} + 17.76 \times A_{645})] \times V/100 \times W$$

$$\text{Car} = [(4.46 \times A_{441} - \text{Chl a} + \text{Chl b})] \times V/100 \times W$$

where V is the total volume of acetone extract (ml) and W is the fresh weight (g) of the sample.



Fig. 1. Effect of different LED wavelengths on the propagation of *Vanilla planifolia* *in vitro*. Fl = fluorescent lamps; LEDs: W = white, R = red, B = blue, and B:R = blue + red (1:1).

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