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Effect of LED light quality on *in vitro* shoot proliferation and growth of vanilla (*Vanilla planifolia* Andrews)

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As an alternative to conventional lighting systems, light emitting diode (LED) has been demonstrated to be an artificial flexible lighting source for commercial micropropagation. The objective of this study was to determine the effects of different LED light quality on *in vitro* shoot proliferation and growth of *Vanilla planifolia*. To evaluate shoot proliferation, axillary bud cuttings (3 to 5 mm in diameter) of *V. planifolia* were used as explants and cultivated on Murashige and Skoog basal medium supplemented with 9.55 µM of 6-benzylaminopurine. To evaluate *in vitro* growth, unrooted shoots (2 cm in length) were used as explants cultivated on Murashige and Skoog basal medium without plant growth regulators. All explants were exposed to a 16 h photoperiod for 60 days under five different lights: fluorescent lamp, white LED, blue LED, red LED and blue plus red LED mixtures (Blue + Red = 1:1). The results indicated a clear increase in the number of shoots per explant under Fluorescent Lamp, White LED and Blue+Red LED light; these treatments produced more than 10 shoots. Shoot length was more than 3 cm in cultures under Blue, Red and Blue+Red (1:1) LEDs, and less than 3 cm in Fluorescent Lamp and White LEDs. Our results also showed that fresh weight, dry weight and dry matter were greatest in shoots under Blue+Red LED light. For shoot growth, plant height, number of leaves, number and length of roots, fresh weight, dry weight, dry matter and chlorophyll content were greater under Fluorescent Lamp and White LED. In conclusion, White or Blue+Red LED light may be used as an alternative light source for shoot proliferation, while White LED may be used for growth *in vitro*. These results demonstrate the effectiveness of light qualities using LEDs for micropropagation of *V. planifolia*.

Key words: Light quality, micropropagation, orchid, chlorophyll.

INTRODUCTION

In commercial micropropagation laboratories, 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

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on the morphogenesis and growth of plant cell, tissue and organ cultures (Reuveni and Evenor, 2007). Fluorescent lamps (FLs) are generally used to increase photosynthetic photon flux (PPF) level for *in vitro* culture. However, FLs contains unnecessary wavelengths (350 to 750 nm) that are of low quality for promoting growth and morphogenesis (Gupta and Jatothu, 2013). Recently, the use of light-emitting diodes (LEDs) as a radiation source for plants has attracted considerable interest for commercial micropropagation. The most attractive features of LEDs are their small mass, volume, wavelength specificity, long life, minimum heating and photon output that is linear with electrical input current (Brown et al., 1995; Massa et al., 2008; Gupta and Jatothu, 2013). The flexibility of matching wavelengths of LEDs to plant photoreceptors may provide more optimal production, influencing plant morphology and metabolism (Kim et al., 2004; Massa et al., 2008; Morrow, 2008). Although, previous reports have confirmed physiological and morphological effects of LED light quality on morphogenesis and growth of several plantlets *in vitro* (Hahn et al., 2000; Jao et al., 2005; Shin et al., 2008; Lin et al., 2011; Li et al., 2012; 2013), these study results showed that LED light is more suitable for plant morphogenesis and growth than FL (fluorescent lamp). However, the responses vary according to plant species.

Vanilla (*Vanilla planifolia*) is an economically important orchid that has been cultivated for its flavoring pods (Tan et al., 2011). In Mexico, *V. planifolia* is a species subject to special protection and its production is still incipient. It is propagated through clonal propagation techniques using stem cuttings. This conventional propagation method is slow, laborious, and economically unviable (Kalimuthu et al., 2006; Mengesha et al., 2012). Therefore, there is a need to search for an alternative way to mass produce this plant species. Recently, several studies have reported on the micropropagation of *V. planifolia* (Tan et al., 2011; 2013; Mengesha et al., 2012; Zuraida et al., 2013; De Oliveira et al., 2013). Prior to the present study, the effect of FL on *in vitro* propagation of vanilla has been reported but no research had been reported using LEDs. The objective of this study was to determine the effects of different LED light quality on *in vitro* shoot proliferation and growth of *V. planifoli*a.

MATERIALS AND METHODS

Plant materials and culture media

For *in vitro* establishment and disinfection of axillary buds, the method described by Lee-Espinosa et al. (2008) was followed. Axillary buds of vanilla (*V. planifolia* Mansa morphotype), between 3 to 5 mm in diameter, were collected in Papantla, Veracruz, Mexico. To evaluate the effect of LEDs on *in vitro* shoot proliferation, twenty explants were used per treatment, distributed at a rate of three explants per culture vessel. The explants were placed in Magenta boxes (Sigma Chemical Company, MO, USA) containing 40 mL of MS (Murashige and Skoog, 1962) semisolid medium supplemented with 9.55 µM BAP (6-benzylaminopurine). The pH was adjusted to 5.8 before adding 0.22% (w/v) Gelrite® (Sigma Chemical Company, MO, USA) and autoclaving (20 min at 121°C). Number of shoots per explant, shoot length, fresh weight and dry weight were recorded after 60 days of culture. Moreover, dry matter content was calculated using dry weight/fresh weight x 100. To evaluate the effect of LED on *in vitro* grown plantlets, twenty unrooted shoots previously established *in vitro* that were 2 cm in length were used as explants per treatment. Shoots were distributed at a rate of five explants per culture vessel. The explants were placed in 1 L vessels containing 50 mL of hormone-free MS semisolid medium. Plant height, number of leaves, number and length of roots, fresh weight, dry weight, dry matter and chlorophyll content were evaluated after 60 days of culture.

Light treatments and culture conditions

The cultures of *in vitro* shoots and plantlets were illuminated using 100% fluorescent light (peak wavelengths from 545 to 610 nm), 100% white LED (460 and 560 nm), 100% red LED (660 nm), 100% blue LED (460 nm) and 50% red plus 50% blue LED (660 and 460 nm, respectively). The LED light source was aligned in a rectangle (40 cm × 120 cm). The LED system consisted of LED sticks (Model 5050-1M), a main controller (L63 × W35 × H22 mm) and a DC-12V adapter power supply (Shendk Model SDK-0605). All the cultures were incubated in a controlled environment at 24 ± 2°C, 50 ± 5% relative humidity and a 16 h photoperiod. In all treatments, the photosynthetic photon flux density (PPFD) was maintained to 25 µmol m^{-2} s^{-1}. PPFD was measured using a FieldScout Quantum Light Meter® (Field Scout Spectrum Technologies, Inc., IL, USA).

Chlorophyll content

Chlorophyll content in the third leaf of the plantlets, counting from the top downwards, was measured using a Field Scout CM 1000 Chlorophyll Meter® (FieldScout Spectrum Technologies, Inc., IL, USA).

Acclimatization

After 60 days of culture under *in vitro* conditions, rooted plantlets from different light treatments that were 5 to 10 cm high were transplanted into 50 × 30 × 7 cm trays with 1:1 mixture of peat-moss (Premier, Rivière-du-Loup, Canada) and Agrolita® (Agrolita, Tlalnepantla de Baz, Mexico) as substrate and then transferred to a greenhouse for acclimatization. Plantlets were watered three times a week and foliar fertilizer (Nitrofoska® foliar PS, COMPO, Zapopan, Mexico) was applied weekly. Once the plants had grown to a height greater than 20 cm, they were transplanted to their natural habitats (shady forest floor).

Experimental design and data analysis

A completely randomized design was used for all experiments and the experiments were replicated three times. Data were subjected to a one-way analysis of variance (ANOVA) using the SPSS program (Version 11.5 for Windows Inc., Chicago, IL, USA). Means were compared using Tukey's test ($p \leq 0.05$).

Table 1. Effect of light quality on shoot proliferation of Vanilla (*V. planifolia*) after 60 days of *in vitro* culture.

Light treatment	No. of shoots/ explants	Shoot length (cm)	Fresh weight (mg)	Dry weight (mg)	Dry matter (%)
FL	10.2±0.52^{a}	2.6±0.14^{bc}	2418.7±135.59^{b}	221.8±13.15^{b}	9.1±0.10^{bc}
W	10.3±0.49^{a}	2.2±0.14^{c}	2236.2±189.99^{b}	211.2±19.24^{b}	9.4±0.10^{b}
B	5.6±0.37^{b}	3.5±0.11^{a}	1431.2±47.18^{c}	125.6±6.77^{c}	8.7±0.20^{c}
R	5.2±0.31^{b}	3.1±0.19^{ab}	1531.2±71.30^{c}	134.3±7.52^{c}	8.7±0.13^{c}
B+R (1:1)	10.8±0.59^{a}	3.4±0.09^{a}	2764.2±165.72^{a}	275.8±18.27^{a}	10.2±0.08^{a}

Abbreviations: FL = Fluorescent Lamp; W = White LED; B = Blue LED; R = Red LED and B+R = Blue and Red LEDs. Values followed by different letters denote statistically significant differences (Tukey, $p \leq 0.05$). Data represent mean ± SE.

RESULTS AND DISCUSSION

Effect of light quality on shoot proliferation

The shoot proliferation and growth of *V. planifolia* were significantly affected by different light treatments *in vitro*. The results indicated a significant increase in the number of shoots per explant under FL, W LED and B+R LED (1:1) light; these treatments produced more than 10 shoots. Explants under B and R LED conditions produced less than six shoots (Table 1). Shoot length was more than 3 cm in cultures under B, R and B+R (1:1) LEDs, and less than 3 cm in FL and W LEDs. Our results also showed that fresh weight, dry weight and dry matter were greatest in shoots under B+R (1:1) LED light, followed by FL, W, B and R LEDs (Figure 1).

Effect of light quality on the growth of plantlets

Different light qualities had variable effects on the growth of *V. planifolia* (Figure 2). Plant height, number of leaves, number and length of roots, fresh weight, dry weight, dry matter and chlorophyll content were greater under FL and W LED than under B LED, R LED and B+R LED (1:1) (Table 2). The growth variables were not significantly affected by FL and W LED light treatments. These treatments were beneficial for plantlet growth. Other studies appear to confirm that R LED retards development of plants *in* vitro (Lin et al., 2011; Edesi et al., 2014; Waman et al., 2015). Similarly, our results confirm that R LED affects the growth of *V. planifolia* plantlets *in vitro*. The present study showed that W LED light may be used as an alternative to FL for *in vitro* growth of *V. planifolia* in culture systems. After 60 days of *in vitro* culture, plantlets in all treatments were transferred to the greenhouse for acclimatization. Plant survival was 95% after 30 d growth under greenhouse conditions. The acclimatized *in vitro* plantlets were vigorous in aspect (Figure 3a) and developed normally when transplanted to soil (Figure 3b). Effect of LEDs on the micropropagation of orchid species has been studied. Fukai et al. (1997) reported in *Calanthe Satsuma* that the most vigorous protocorm development was obtained in the dark rather than by the irradiation of R, B, mixed light of R+B, and FL. In *Oncidium*, R LEDs significantly promoted the growth of PLBs, while B LEDs had the opposite effect (Xu et al., 2009). In *Dendrobium officinale,* R+B (1:2) LEDs are an effective light source for shoot growth (Lin et al., 2011). Godo et al. (2011) reported rhizoid formation in orange LED (590 nm) and R LED (625 nm) light in *Bletilla ochracea*. Our results do not coincide with those of Xu et al. (2009), Lin et al. (2011) and Godo et al. (2011). According to Kim et al. (2004), stem elongation could be promoted or inhibited by different synergistic interactions between blue or red light receptors and phytochrome depending on the species. Light quality also plays an important role in photosynthesis, influencing the way in which light is absorbed by chlorophyll (Tripathy and Brown, 1995; Topchiy et al., 2005). In our results, chlorophyll concentrations were greater in plantlets grown under FL and W LED light treatments, which is inconsistent with the idea that B light plays an important role in the synthesis of chlorophyll (Kurilcik et al., 2008; Li et al., 2010; 2012). According to Li et al. (2013), the chlorophyll content of *in vitro* plantlets grown under different light qualities may be correlated with the plant species or cultivar. In its natural habitat, *V. planifolia* grows on a shady forest floor, while other members of the Orquidacea family grow on open grassy or stony slopes.

Our results clearly demonstrate that the spectral quality of light has an influence on the morphogenesis and growth responses of *V. planifolia.* According to Chung et al. (2010), light induces plant morphogenesis. Light is a signal that is received by a photoreceptor that then regulates the differentiation and growth of plants (Wang et al., 2001; Muleo and Morini, 2006). The light source generally used for *in vitro* culture is fluorescent lamps. However, LEDs have a long life and changing the light source is therefore less frequent, resulting in reduced labor costs. Also, LEDs generate very little heat, thereby minimizing the need for an extensive cooling system in plant growth facilities. This is the first report on measuring the effects of LED light on *V. planifolia in vitro*; LED light is a novel illumination system for micropropagation of this important species.

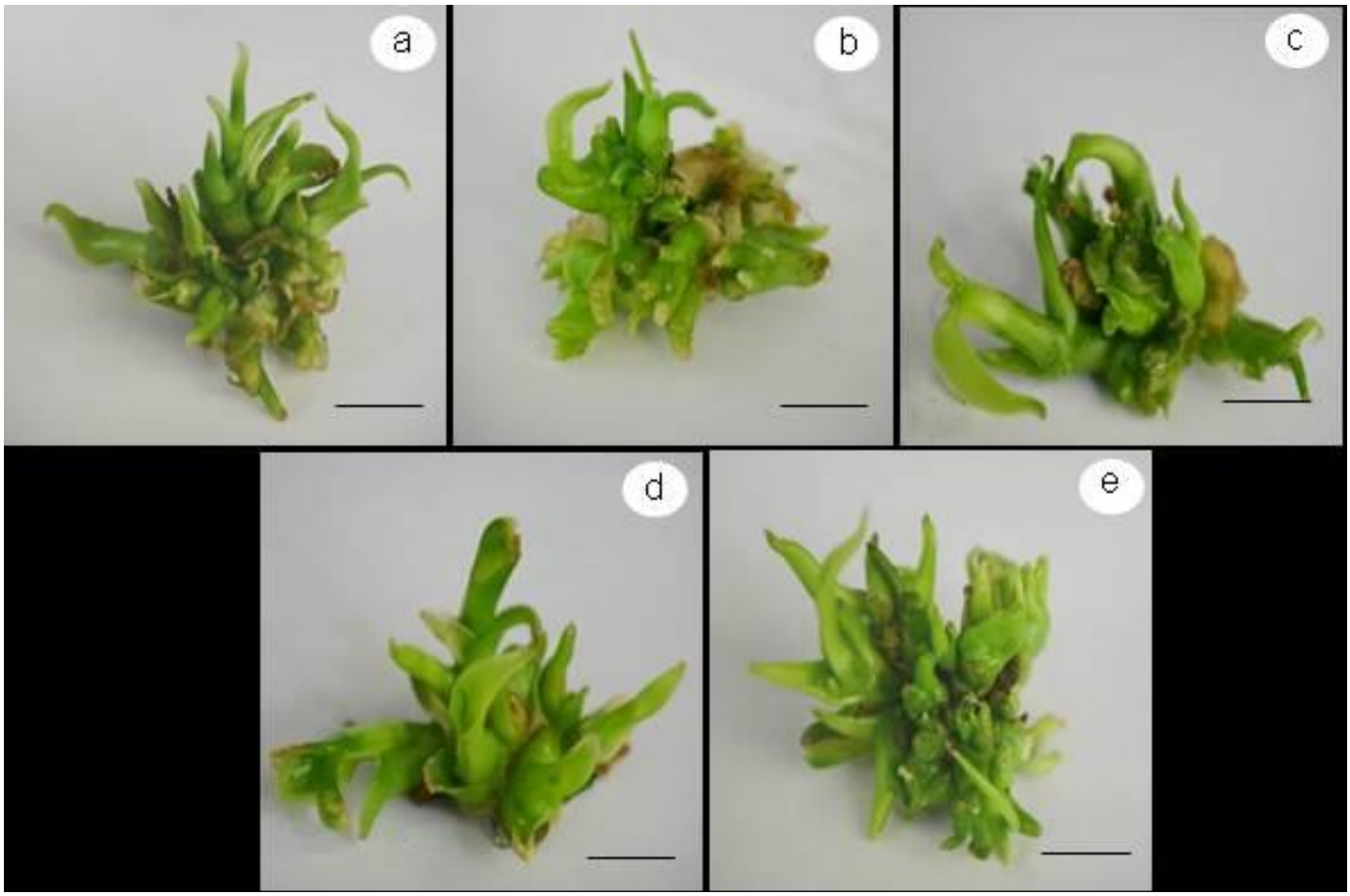

Figure 1. Effect of light quality on shoot proliferation of Vanilla (*V. planifolia*) after 60 days of *in vitro* culture. a) Fluorescent Lamp, b) White, c) Blue, d) Red and e) Blue+Red LEDs (*bar* 2.0 cm).

Figure 2. Effect of light quality on *in vitro* growth of plantlets of Vanilla (*V. planifolia*) after 60 days of *in vitro* culture. **a)** Fluorescent Lamp, **b)** White, **c)** Blue, **d)** Red and **e)** Blue+Red LEDs (*bar* = 2.0 cm).

In conclusion, the use of a LED light source was good at promoting the shoot proliferation and growth of vanilla plantlets. W or B+R LED (1:1) light may be used as an alternative light source for shoot proliferation, while W LED may be used for *in vitro* growth. These results demonstrated the effectiveness of a radiation system using

Table 2. Effect of light quality on the growth of plantlets of Vanilla (*V. planifolia*) after 60 days of *in vitro* culture.

Light treatment	Plant height(cm)	Number of Leaves	Number of roots	Root length(cm)	Fresh weight (mg)	Dry weight (mg)	Dry matter (%)	Chlorophyll content
FL	5.7±0.40^{a}	3.6±0.18^{a}	2.5±0.18^{a}	4.8±0.18^{a}	1197.5±54.30^{a}	74.2±5.65^{a}	6.6±0.50^{a}	107.6±0.37^{a}
W	5.9±0.27^{a}	3.5±0.18^{a}	2.6±0.18^{a}	4.5±0.23^{a}	1016.2±65.49^{ab}	70.5±4.96^{a}	6.2±0.44^{a}	108.7±0.64a
B	4.4±0.15^{b}	2.5±0.18^{b}	1.6±0.18^{bc}	3.5±0.13^{b}	868.7±30.43^{b}	50.1±1.85^{b}	4.4±0.16^{b}	90.0±1.18b
R	3.2±0.16^{c}	2.5±0.18^{b}	1.8±0.18^{b}	2.1±0.19^{c}	438.7±35.82^{cd}	32±2.09^{c}	2.8±0.18^{c}	82.7±0.70c
B+R (1:1)	4.1±0.23^{bc}	2.5±0.20^{b}	1.4±0.20^{c}	3.1±0.21^{b}	681.4±15.02^{c}	44.1±2.05^{bc}	3.9±0.18^{bc}	92.4±0.89b

Abbreviations: FL = Fluorescent Lamp; W = White LED; B = Blue LED; R = Red LED and B+R = Blue and Red LEDs. Values followed by different letters denote statistically significant differences (Tukey, $p \leq 0.05$). Data represent mean ± SE.

Figure 3. Plantlets of *V. planifolia* transferred from *in vitro* condition. **a)** plantlets in the process of acclimatization in the greenhouse after 30 days and **b)** plantlets growing in shady forest floor (natural habitat) after 60 days.

using LEDs for micropropagation of *V. planifolia* and subsequent acclimatization in *ex vitro* conditions.

Conflict of interests

The author(s) did not declare any conflict of interest.

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