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Effect of LED lights on the *in vitro* growth of *Pinus pseudostrobus* Lindl., plants

*Luis Alberto Marín-Martínez, Lourdes Georgina Iglesias-Andreu**

Instituto de Biotecnología y Ecología Aplicada (INBIOTECA), Universidad Veracruzana, Xalapa, Veracruz, Mexico

**Corresponding author: liglesias@uv.mx*

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Abstract: *Pinus pseudostrobus* Lindl. is a species endemic to Mexico and is widely used in reforestation programmes, as it is highly adapted to poor, shallow, limestone soils and has high commercial importance. However, it is necessary to preserve this genetic material since it is in trouble due to high rates of deforestation, land use change, and forest fires, so it is necessary to have effective strategies to obtain good quality seedlings. Due to the properties of LED (light emitting diode) lamps used for illumination in the production of *in vitro* plants, the effects of two different lighting systems (LED and fluorescent) on an *in vitro* culture were analysed for the morphological characteristics of the growth and photosynthetic pigment content in *P. pseudostrobus* seedlings. The length and root size of the seedlings were affected by the type of illumination, where a red LED light was the most effective at 30 days of evaluation. However, a blue LED light was equally effective as a red LED light at 60 days of seedling development. On the other hand, the fluorescent light was better in terms of the number of needles in the first stage, but we found the blue LED light to be better in the second stage. For the photosynthetic pigment content, the highest values were found with the blue LED light. The results showed that the LED lighting system favours the growth, development, and photosynthetic pigment content of the species under study.

Keywords: conifers; *in vitro* culture; light-emitting diodes (LEDs); plant tissue culture; seedlings

An *in vitro* plant culture is a technique that requires extremely demanding physical and chemical environmental control, so these factors must be controlled and optimised (Rizzo Zaldumbide 2020). Among the factors to be optimised are the composition of the culture medium, pH, temperature, humidity, light, and photoperiod (Castillo 2008), as they determine the growth and development of plant tissues (Loberant, Altman 2010).

Plant growth and development can be influenced by wavelengths between 300 and 900 nm, in addition to their intensity and duration, along with climatic factors (Casierra-Posada, Peña-Olmos 2015). Recent studies have shown how different portions of the visible spectrum affect plants metabolically (Rizzo Zaldumbide 2020). Blue light (B: 450–495 nm), red light (R: 620–750 nm), far-red light (FR: 750–850 nm), and even green light (G: 495–570 nm) all play important roles in the plant growth, morphogenesis, and phytochemical content (Golovatskaya, Karnachuk 2015; Cioć et al. 2018).

The traditional light source used in an *in vitro* culture is a tubular fluorescent lamp (LTF) (Lin et al. 2011) that emits a broad spectrum, so the physiological effects on plants are not very specific (Da Rocha et al. 2010). Therefore, alternative sources of energy

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and more efficient ways of illuminating crops must be sought (Loberant, Altman 2010). Light-emitting diodes (LEDs), an alternative to conventional light sources for *in vitro* plant growth, are available today. These, among others, have the advantage of having a small mass, volume, and power consumption, a longer lifetime, and high energy conversion efficiency, as well as adjustable light spectra (Río-Alvarez et al. 2014). Light-emitting diodes (LEDs) have a high potential for use as a light source in micropropagation (Loberant, Altman 2010).

Due to all of these advantages, LED lighting technology has been widely used in various crops having horticultural importance (Urrestarazu 2016; Moro-Peña 2020). However, there are few studies aimed at evaluating its effects in forestry and even fewer in the *in vitro* cultivation of coniferous species. In particular, LED luminaires, among others, allow the selection of specific wavelengths that will affect the growth and development of *in vitro* seedlings according to the species and growth stage. It has been found that a monochromatic red light can promote the germination of somatic embryos of *Pinus densiflora* (Kim, Moon 2014). Other authors have pointed out that R : B (1 : 1) can promote cell division of a *Populus euramericana in vitro* culture more than a monochromatic light or fluorescence (Kwon et al. 2015).

Pinus pseudostrobus is one of the most important conifer species for the quality of its wood. At present, research on the *in vitro* culture of conifer species has used the traditional fluorescent lamp as a light source (Smirnakou et al. 2016), and there is no information on the effect of LED lights on the *in vitro* plant quality of this conifer species. Therefore, we proposed to develop the present work to evaluate the effect of the LED light quality on the growth and pigment content of *P. pseudostrobus* seedlings *in vitro*. The results of this study could provide baseline data to discuss the effect of LEDs on the micropropagation process of this species and help optimise the technical approach for the large-scale production of the *in vitro* culture of this valuable conifer species.

MATERIAL AND METHODS

The present study was carried out in the Biotechnology and Plant Tissue Culture Laboratory of the Universidad Veracruzana, located in the city of Xalapa, Veracruz, Mexico.

Plant material collection. A total of 20 "plus" trees were selected, defined as those being healthy, pest-free, with straight stems, a straight height, and a dominant diameter (Flores-Flores et al. 2014) in a natural stand of a natural *P. pseudostrobus* population, located in the municipality of Las Vigas de Ramírez, Veracruz, Mexico.

Plant material disinfection. Seeds were subjected to imbibition for 24 h in sterile distilled water (SDW); those seeds that were empty or with visible physical damage were discarded. In a laminar flow hood, the seeds were immersed for 3 min in a 96% ethanol solution and rinsed with SDW, then they were submerged again for 3 min in a 75% ethanol solution, rinsed with SDW, and then submerged again for 3 min in a 50% ethanol solution. After rinsing with SDW, they were again immersed for 3 min in a 25% ethanol solution and rinsed with SDW. Finally, they were incubated for 30 min in a 25% (v/v) solution of commercial sodium hypochlorite (NaClO) added with 0.5 mL of polyoxyethylene sorbitan monalaurate (Tween-20).

***In vitro* culture conditions.** The seeds were sown in 20 mL of a culture medium consisting of 1/2 WPM (Woody Plant Medium, Sigma®, Sigma Aldrich, USA) (McCown, Lloyd 1981), supplemented with 30 gL^{-1} of sucrose, 4 mgL^{-1} of gibberellic acid (GA_3) and 25 mgL^{-1} of hydrochloric cysteine. The pH of the culture medium was adjusted to 5.7, and 2.5 gL^{-1} of Phytagel (Sigma®, Sigma Aldrich, USA) was added as a gelling agent. The medium was poured into glass "G" type flasks with a 100 mL volume and finally it was sterilised at 1.5 $kg\cdot cm^{-2}$ at 120 °C for 15 min.

The evaluated seedlings were transferred 20 days after *in vitro* germination to a medium supplemented with 1/2 WPM (Woody Plant Medium, Sigma®, Sigma Aldrich, USA) (McCown, Lloyd 1981) supplemented with 60 gL^{-1} of sucrose, 2 mgL^{-1} of gibberellic acid (GA_3) and 25 mgL^{-1} of hydrochloric cysteine. The pH was adjusted to 5.7, and 2.5 gL^{-1} of Phytagel (Sigma®, Sigma Aldrich, USA) was added as a gelling agent. The medium was poured into glass "G" type flasks with a 100 mL volume and subsequently sterilized at 1.5 $kg\cdot cm^{-2}$ at 120 °C for 15 min.

P. pseudostrobus plants were grown *in vitro* at 25 °C, 80% relative humidity with a photoperiod of 16 h of light, with white LEDs (400–450 nm), red LEDs (700–800 nm), blue LEDs (400–500 nm), red + blue LEDs (1: 1) and a fluorescent light (400–450 nm). For the implementation of the LED light sources (SMD

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5050RBG, model IP65 strips), described by Ramírez-Mosqueda et al. (2017), the modules (Techno®, Zapopan, Mexico) supply 12 V and 1 W.

A completely randomised design was used, with four germinated *P. pseudostrobus* seedlings planted per flask and eight flasks for each of the five tested lighting treatments, for a total of 32 seedlings per tested lighting treatment, with three replicates per treatment. During a period of 60 days, evaluations were carried out every 30 days to determine the number of needles, root length, and total length.

Photosynthetic pigment content. The methodology described by Porra et al. (1989) was used to determine the chlorophyll a, b, a + b, and carotene contents, for which the needles of the seedlings grown *in vitro* were taken as the plant material. In this case, 0.25 g of plant material from the shoot was weighed, then macerated in liquid nitrogen (N_2). 5 mL of acetone (80%) were added and centrifuged at 6 000 rpm for 12 min in 15 mL polypropylene centrifuge tubes.

The supernatant was transferred to new 15 mL polypropylene centrifuge tubes. Finally, readings were taken at 663.6 nm for chlorophyll a, 646.6 nm for chlorophyll b, and 440.5 nm for the carotenes using a spectrophotometer (JENWAY®, model 6715, Bibby Scientific Ltd, the United Kingdom). The following Equations (1–4) were used to calculate the photosynthetic pigment content:

$$\text{Chl}A = [(12.25 \times A663.6) - (2.55 \times B646.6)] \times \times V/100 \times W \quad (1)$$

$$\text{Chl}B = [(20.30 \times B646.6) - (4.91 \times A663.6)] \times \times V/100 \times W \quad (2)$$

$$\text{Chl}A + B = [(7.34 \times A663.6) + (17.76 \times B646.6)] \times \times V/100 \times W \quad (3)$$

$$\text{Car} = [(4.46 \times C440.5) - (\text{Chl}A + \text{Chl}B)] \times \times V/100 \times W \quad (4)$$

where:

V – volume (mL);
W – weight (g);
*A*663.6 – chlorophyll a reading: 663.6 nm;
*B*646.6 – chlorophyll b reading: 646.6 nm;
*C*440.5 – carotenoid reading: 440.5 nm.

The collected data were statistically analysed using the STATGRAPHICS software (Centurion XVI.I, 1982). The Shapiro-Wilk test was performed to examine the normality of the data, and then the differences between the treatments were identified by means of a one-factor analysis of variance (ANOVA), and the means were contrasted using Tukey's test ($P < 0.05$).

RESULTS AND DISCUSSION

Growth and development of seedlings. The results of the first measurement at 30 days showed the existence of significant differences in the growth of the seedlings under the various evaluated lighting treatments (white LED, red LED, blue LED, red + blue LED, and fluorescent light). It was found that with the red LED light, a greater plant length and root size were achieved compared to the other evaluated light treatments (Figure 1).

As in our study, several authors (Mengxi et al. 2011; Dutta Gupta, Jatothu 2013) detected significant differences in the seedling length, root size, and leaf number in seedlings grown *in vitro* under a red LED light spectrum (Table 1). The best results were obtained with a red LED light for the plant length and root size. However, for the number of needles, fluorescent and white LED lights were better as they presented a greater volume of needles.

The results of the second measurement, performed at 60 days, showed the existence of significant differences in the growth of the *in vitro* plants grown under the evaluated lighting treatments (white LED, red LED, blue LED, red + blue LED,

Figure 1. Effect of different lighting treatments on the *in vitro* growth of *P. pseudostrobus* seedlings at 30 days

F – fluorescent; W – white LED; R – red LED; B – blue LED; R + B – red + blue LED

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Table 1. Effect of different wavelengths of illumination on the *in vitro* growth of *P. pseudostrobus* seedlings at 30 days (mean ± SE)

Light sources	Plant length (cm)	Root size (cm)	No. of needles
Fluorescent	2.16 ± 0.20[d]	1.33 ± 0.15[d]	11.00 ± 0.57[a]
Red + blue LED	3.13 ± 0.15[c]	2.36 ± 0.25[c]	7.66 ± 0.57[d]
White LED	3.26 ± 0.05[c]	2.38 ± 0.15[c]	10.66 ± 0.57[ab]
Blue LED	4.80 ± 0.10[b]	3.65 ± 0.10[b]	9.33 ± 0.57[c]
Red LED	5.40 ± 0.10[a]	4.70 ± 0.10[a]	10.33 ± 0.57[b]

Means with different letters are significantly different (Tukey's test, $P < 0.05$)

Figure 2. Effect of different lighting treatments on the *in vitro* growth of *P. pseudostrobus* seedlings at 60 days

F – fluorescent; W – white LED; R – red LED; B – blue LED; R + B – red + blue LED

and fluorescent light). It was found that the greatest root length and size were obtained in the *in vitro* plants grown under both the red and blue LED light spectra (Figure 2).

In our work, different results were presented among the different light sources and light spectra, with the best results for the plant and root length being observed with the red LED and blue LED light spectra. However, for the number of needles, we found the best results were achieved for the blue LED and fluorescent light (Table 2).

These results agree with that reported by Verma et al. (2018), who obtained similar results for an ornamental species (*Digitalis purpurea* L.) under the red and blue LED light spectra in terms of the seedling growth. However, Mendoza-Paredes et al. (2021) found that better growth of habanero bell pepper (*Capsicum chinense* Jacq.) seedlings was obtained under the blue LED light spectrum. According to Dutta Gupta and Agarwal (2017), the *in vitro* response of each species varies depending on the spectral light composition in which it grows.

As is known, the light-sensitive signal transduction pathways operating in the plant system are mainly regulated by red light-sensitive phytochromes, blue light-sensitive cryptochromes, and phototropins. Interactions between red and blue photoreceptors contribute to the response of plants to these light spectra. According to Berkovich et al. (2017), a red LED light tends to lengthen the plant height, while a blue light contributes to the formation of more compact and shorter plants.

Chlorophyll content. The results showed the existence of significant differences in the photosynthetic pigment content (chlorophyll a, b, a + b, and carotenoids) under the different evaluated lighting treatments (white LED, red LED, blue LED, red + blue LED, and fluorescent light). A higher chlorophyll content was found with the blue LED light (Figure 3).

Table 2. Effect of different illumination wavelengths on the *in vitro* growth of *P. pseudostrobus* seedlings at 60 days (mean ± SE)

Light sources	Plant length (cm)	Root size (cm)	No. of needles
Fluorescent	3.28 ± 0.20[c]	2.25 ± 0.15[c]	13.66 ± 1.6[a]
Red + blue LED	3.43 ± 0.15[c]	2.46 ± 0.25[b]	9.66 ± 0.57[c]
White LED	4.96 ± 0.05[b]	3.66 ± 0.15[b]	11.33 ± 0.57[b]
Blue LED	8.15 ± 0.10[a]	6.55 ± 0.10[a]	14.00 ± 0.57[a]
Red LED	8.33 ± 0.10[a]	6.70 ± 0.10[a]	11.66 ± 0.57[b]

Means with different letters are significantly different (Tukey test, $P < 0.05$).

Significant differences in the chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents were observed in the seedlings grown under the different light spectra (Figure 3). Higher of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents were observed in the seedlings grown under the blue LED light (Table 3). These results agree with those reported by different authors (Li, Kubota 2009; Novičkovas et al. 2012; Samuoliene et al. 2012; Hernández, Kubota 2016), who observed an increase in the photosynthetic pigments under a blue light spectrum in species such as the lettuce (*Lactuca sativa* L.), cucumber (*Cucumis sativus* L.), tomato (*Solanum lycopersicum* L.) and chili bell pepper (*Capsicum annuum* L.).

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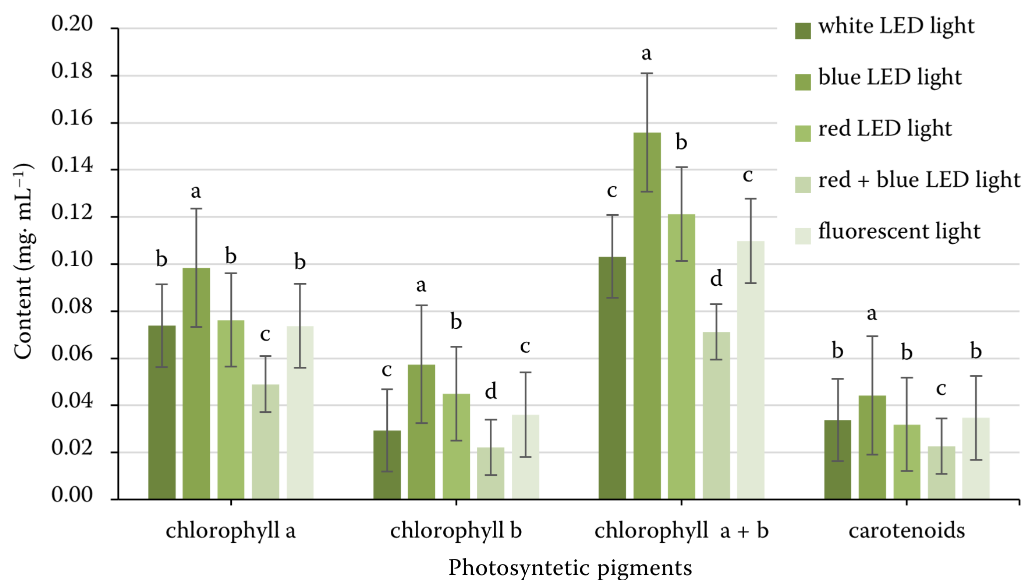

Figure 3. Effect of different sources of light on the chlorophyll and carotenoid content in *P. pseudostrobus* seedlings obtained in *in vitro* culture (mean ± SE)

F – fluorescent; W – white LED; R – red LED; B – blue LED; R + B – red + blue LED; means with different letters were significantly different (Tukey test, $P < 0.05$)

Table 3. Effect of different light sources on chlorophyll and carotenoid content of *P. pseudostrobus* seedlings grown *in vitro*; mean ± SE

Light sources	Photosynthetic pigment content (mg·mL⁻¹)			
	chlorophyll a	chlorophyll b	chlorophyll a + b	carotenoids
Red + blue LED	0.048 ± 0.004[c]	0.021 ± 0.013[d]	0.071 ± 0.010[d]	0.022 ± 0.0009[c]
Fluorescent	0.073 ± 0.001[b]	0.035 ± 0.003[c]	0.109 ± 0.004[c]	0.034 ± 0.0005[b]
White LED	0.073 ± 0.002[b]	0.029 ± 0.003[c]	0.103 ± 0.005[c]	0.033 ± 0.0008[b]
Red LED	0.076 ± 0.008[b]	0.044 ± 0.012[b]	0.121 ± 0.021[b]	0.031 ± 0.002[b]
Blue LED	0.098 ± 0.013[a]	0.057 ± 0.025[a]	0.155 ± 0.038[a]	0.044 ± 0.002[a]

Means with different letters were significantly different (Tukey test, $P < 0.05$)

These results agree with the expectations considering that the plants respond differently to certain wavelengths, activating the cryptochrome system and matching the absorption spectra of the chlorophyll and carotenoids. As it is known, both chlorophylls a and b and the carotenoids initiate photosynthesis by capturing light energy and converting it into chemical energy (Caffarri et al. 2014).

Therefore, chlorophyll is an indicator of the crop vigour and productivity; moreover, the chlorophyll content has been used as an index of the plant photosynthetic capacity (Bowyer, Leegood 1997), so a reduction in its content can be considered a plant's response to stress (Tenga et al. 1989). However, it is considered that photomorphogenesis is the physiological process that allows the development and growth of plants under the control of light (Kendrick, Kronenberg 2012). Therefore, improving the quality of the light using LED lights can improve photosynthetic performance in plants (Batista et al. 2018), as presented in our case study where the performance was improved under LED light spectra.

CONCLUSION

The results of this study showed that *P. pseudostrobus* responds differentially to different wave-

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lengths of light-emitting diodes (LED). A red LED light was beneficial during germination and the first growth stage, while red and blue LED lights were beneficial for improving the quality of the *P. pseudostrobus* seedlings in terms of the plant height and root length, which constitutes a viable alternative to contribute to reforestation programmes, as well as for use in forestry and agroforestry.

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