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LIGHT AND SUCROSE IN THE MICROPROPAGATION OF CLONES OF *Eucalyptus benthamii* AND *Eucalyptus dunnii*

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ABSTRACT

In photoautotrophic propagation systems, light quality and sucrose concentration are fundamental factors in the success of the technique, which aims to make the micropropagated plant more suitable for ex vitro acclimatization. The aim of this study was to evaluate the influence of different light conditions and sucrose concentrations on the in vitro multiplication and elongation of two clones of *Eucalyptus benthamii* and two clones of *Eucalyptus dunnii*. Four lighting conditions were tested: Light-emitting diode (LED) lamps emitting waves in the white spectrum (130 µmol m^{-2} s^{-1}), Red and Blue LED 3:1 (80 µmol m^{-2} s^{-1}) and fluorescent lamps at two intensities 100 and 64 µmol m^{-2} s^{-1}, under two sucrose concentrations, 0 and 30 g. L^{-1}, in the *E. benthamii* and *E. dunnii* culture medium. L^{-1}, in the JADS culture medium in two clones of *E. benthamii* and two clones of *E. dunnii*, kept in flasks (250 ml capacity) sealed with polypropylene lids and a gas exchange rate of 14 µL L^{-1} s^{-1}. At 30 days into the experiment, during the multiplication stage, the number of shoots, the size of the largest shoot, the quantification of total chlorophyll and carotenoids were assessed. All the light sources tested stimulated the development of the clones, except for fluorescent light with an intensity of 64 µmol m^{-2} s^{-1}, which affected the number and length of the shoots. Sucrose increased the multiplication and elongation of eucalyptus shoots, and the combination of white LED and 30 g. L^{-1} of sucrose resulted in higher levels of total chlorophyll and carotenoids.

Keywords: In vitro propagation; Photoautotrophy; Vegetative propagation; In vitro cultivation

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LUZ E SUCROSE NA MICROPROPAGAÇÃO DE CLONES DE *Eucalyptus benthamii* E *Eucalyptus dunnii*

RESUMO Nos sistemas de propagação fotoautotrófica a qualidade de luz e a concentração de sacarose são fatores fundamentais no êxito da técnica, que visa conferir à planta micropropagada maior adequação à aclimatização ex vitro. Assim, este estudo teve como objetivo avaliar a influência de diferentes condições de luminosidade e concentrações de sacarose na multiplicação e alongamento in vitro de dois clones de *Eucalyptus benthamii* e dois clones de *Eucalyptus dunnii*. Foram testadas quatro condições de luminosidade: Lâmpadas de diodo emissores de luz (LED) com emissão de ondas no espectro branco (130 µmol m^{-2} s^{-1}), LED Vermelho e Azul 3:1 (80 µmol m^{-2} s^{-1}) e lâmpadas fluorescentes em duas intensidades 100 e 64 µmol m^{-2} s^{-1}, sob duas concentrações de sacarose, 0 e 30 $g.L^{-1}$, no meio de cultura JADS em dois clones de *E. benthamii* e dois clones de *E. dunnii*, mantidos em frascos (250 ml de capacidade) selados com tampa de polipropeno e taxa de troca gasosa de 14 µL L^{-1} s^{-1}. Aos 30 dias de experimentação, na etapa de multiplicação, foi avaliado o número de brotações, tamanho da maior brotação, quantificação de clorofila total e de carotenoides. Todas as fontes de luz testadas estimularam o desenvolvimento dos clones, exceto a luz fluorescente com intensidade de 64 µmol m^{-2} s^{-1}, que afetou o número e o comprimento das brotações. A sacarose aumentou a multiplicação e o alongamento das brotações de eucalipto, e a combinação de LED branca com 30 g. L^{-1} de sacarose resultou em maiores níveis de clorofila total e carotenóides.

Palavras-Chave: Propagação in vitro; Fotoautotrofia; Propagação vegetativa; Cultivo in vitro

1. INTRODUCTION

Eucalyptus cultivation has boosted the Brazilian economy and is an important factor in social transformation and the development of a green and more sustainable economy (Diniz, 2024; Ibá, 2024). However, this crop has commercially important genotypes with difficulties in their clonal propagation by forest nurseries (Souza, 2024). The micropropagation technique has been considered strategic in the forestry sector, especially for species with difficulties in producing clonal seedlings through minicuttings. Among its applications, we highlight the invigoration/rejuvenation of clones of commercial interest that are difficult to propagate by cuttings or minicuttings, with the aim of establishing them in clonal mini-gardens, in addition to the potential of this technique to promote clonal cleaning of the material, as well as mass propagation of superior genotypes with high phytosanitary quality (Xavier et al., 2021).

Subtropical species such as *Eucalyptus benthamii* and *E. dunnii* are among those considered difficult to propagate ex vitro. In in vitro plant propagation, several problems have been described, such as low multiplication rates, chlorosis, oxidation, hyperhydricity, callus formation and rooting difficulties (Murvanidze et al., 2022; Navroski et al., 2013; Souza et al., 2020), making it necessary to develop protocols that facilitate in vitro handling (Souza et al., 2023).

One of the aspects of *Eucalyptus* micropropagation that limits its operation on a large scale is directly related to the stress caused to the plant by the in vitro cultivation conditions. In plant tissue culture, the growing environment influences the plant's morphogenic responses (Fortini et al., 2021; Nguyen et al., 2020; Kozai, 2010; Khan et al., 2002; Louback, et al., 2021). Thus, the composition of the culture medium and the quality and quantity of light are essential for the proper development of the in vitro culture and its subsequent ex vitro acclimatization (Miranda et al., 2020; Souza et al., 2020; Souza, 2024).

The trend in the development of in vitro cultivation is the development of photoautotrophic conditions to replace the traditional heterotrophic and photomixotrophic conditions, where sucrose is excluded or reduced, respectively (Saldanha et al., 2014). The spectral quality

of light, as well as its intensity, interferes with chlorophyll synthesis, organogenesis, somatic embryogenesis, metabolite production, growth and development of plants in vitro (Batista et al., 2018; Murthy et al., 2024; Silva et al., 2020).

Changes in the wavelengths of the lamps are perceived by phytochromes and cryptochromes, triggering photomorphogenic responses in the plants (Taiz et al., 2017). In micropropagated plants of *Corymbia torelliana* x *C. citriodora*, the blue light band was responsible for increasing the number of elongated shoots (larger than 2 cm) and greater density of palisade parenchyma, while the red light band promoted the smallest gains in elongated shoots and greater formation of intercellular spaces in the mesophyll (Souza, 2024). Nonetheless, for *Eucalyptus urophylla* in vitro, fluorescent and LED lamps can be used for in vitro cultivation, without compromising clone development (Miranda et al., 2020).

Micropropagated seedlings need to adapt their structures developed under a heterotrophic system to the ex vitro autotrophic environment in order to reduce plant mortality. Therefore, changes in the amount of sucrose available in the culture medium and the photon flux density can help in this transition process, representing great value for the forestry industry in terms of successful massive clonal propagation.

Therefore, in order to stimulate photoautotrophic behavior, this study aimed to evaluate the influence of different light conditions and sucrose concentrations on the multiplication and elongation of two *Eucalyptus benthamii* clones and two *Eucalyptus dunnii* clones, grown in vitro by micropropagation.

2. MATERIAL AND METHODS

2.1 Plant material

The experiment was conducted at the Tissue Culture Laboratory II of the Institute of Biotechnology Applied to Agriculture and Livestock - BIOAGRO, at the Federal University of Viçosa, in Viçosa, Minas Gerais. Two clones of *Eucalyptus benthamii* (C1 and C2) and two clones of *Eucalyptus dunnii* (C3 and C4) kept in an in vitro clonal bank were used.

To set up the experiments, clumps containing six to eight differentiated buds were grown in test tubes containing 10 mL of JADS culture medium (Correia et al., 1995), plus 0.8 $g.L^{-1}$ of PVP, 0.1 $g.L^{-1}$ of myo-inositol, 30 $g.L^{-1}$ of sucrose, 0.5 $mg.L^{-1}$ of benzylaminopurine (BAP) and 0.01 $mg.L^{-1}$ of naphthaleneacetic acid (ANA). The pH of the culture medium was adjusted to 5.8 before adding 6 $g \cdot L^{-1}$ of agar. The medium was then autoclaved at 1.5 atm and 121 °C for 20 minutes.

The cultures were kept in a growth room at 25 ± 2 °C, with a 16-hour photoperiod and light of 64 µmol m^{-2} s^{-1} (quantified by a LI-COR® radiometer, LI-250A Light Meter), provided by two tubular fluorescent lamps (Special Daylight, 40 W, Osram®, Brazil).

2.2 Sucrose and light quality

Two sucrose concentrations and four light conditions were tested in two stages of micropropagation development: multiplication and shoot elongation. In the multiplication phase, explants grown in vitro were transferred to glass flasks (250 mL capacity), sealed with polypropylene lids with a gas exchange rate of 14 µL L^{-1} s^{-1} (Batista et al., 2017), containing 40 mL of JADS culture medium, with the aforementioned modifications, in two concentrations of sucrose (0 and 30 g. L^{-1}), and kept in a growth room under four lighting conditions: four LED V/A red and blue 3:1 lamps (80 µmol m^{-2} s^{-1}, LabLumens®); 4F- 4 tubular fluorescent lamps (100 µmol m^{-2} s^{-1}, Osram®); 2F- 2 tubular fluorescent lamps (64 µmol m^{-2} s^{-1}, Osram®); LED B - two tubular white LED lamps (130 µmol m^{-2} s^{-1}, Tecnal®).

In the elongation phase, the conditions tested were similar to the multiplication phase, differing only in the growth regulators added to the culture medium. To elongate the shoots, 0.5 $mg.L^{-1}$ of BAP (benzylaminopurine) and 0.25 $mg.L^{-1}$ of AIB (indolbutyric acid) were used. The plants were cultivated in a growth room with a temperature of 25 ± 2 °C and a 16-hour photoperiod for 30 days.

2.3 Evaluations and data analysis

During the multiplication and elongation phase, the number of shoots (NS), the

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number of shoots larger than 2 cm (NS>2), the size of the largest shoot (SLS) and photosynthetic pigments were evaluated 30 days after the experiment was set up. To assess photosynthetic pigments, 0.24 mg samples of leaves were placed in 5 mL of DMSO solution (saturated with calcium carbonate) and kept in the dark for 48 h (Santos et al., 2008).

The absorbance of the samples was determined using 10 mm optical path quartz cuvettes in a Genesys 10UV spectrophotometer (ThermoScientific, USA). The wavelengths analyzed were 665, 645 and 480 nm and the equations for calculating the concentrations of chlorophyll a, b and carotenoids were followed as established by Wellburn (1994).

For the multiplication and elongation phases, a completely randomized design (DIC) was used, in a 2 x 4 factorial scheme (two sucrose concentrations and four light conditions), with 5 replicates and one observation per plot, obtained from the average of 4 explants. The statistical program R version 4.4.1 and the ExpDes.pt package (Ferreira, Cavalcanti & Nogueira, 2013) were used to analyze the data. For the characteristics evaluated, the data was transformed using $\sqrt{x+0,5}$ to meet the statistical assumptions, and the submitted to analysis of variance, with the means compared using the Tukey test at a 5% probability level.

3. RESULTS

The two sucrose concentrations evaluated and all the tested light sources (LED R/B 3:1 – four red/blue LED lamps at a 3:1 ratio; 4F – four fluorescent lamps; 2F – two fluorescent lamps; LED W – two white LED lamps) promoted shoot multiplication and elongation in the genotypes *Eucalyptus benthamii* and *Eucalyptus dunnii* evaluated in this study.

The presence of 30 $g \cdot L^{-1}$ of sucrose increased the number of lateral shoots in all clones under the tested light conditions. In the absence of sucrose, the 4F light source was able to increase the number of shoots, showing overall values similar to the same light condition in the presence of sucrose (Table 1).

With regard to the length of the elongated shoots, both in terms of the number of shoots greater than 2 cm and the size of the largest shoot, the maximum estimated values were found at a concentration of 30 $g.L^{-1}$ and in the LED lights V/A 3:1, 4F and LED B (Table 2).

Table 1. Mean values of the number of shoots (NS) in relation to the different concentrations of sucrose (0 $g.L^{-1}$ and 30 $g.\ L^{-1}$) added to the culture medium, and in different light sources (LED V/A - two Red/Blue LED lamps; 4F- 4 fluorescent lamps; 2F- 2 fluorescent lamps; LED B- two white LEDS lamps) for different clones of *Eucalyptus benthamii* (C1 and C2) and *Eucalyptus dunnii* (C3 and C4), assessed at 30 days during the in vitro multiplication phase

Tabela 1. Valores médios do número de brotações (NB) em relação às diferentes concentrações de sacarose (0 $g.L^{-1}$ e 30 $g.L^{-1}$) adicionadas ao meio de cultura, e em diferentes fontes de luz (LED V/A - duas lâmpadas LED Vermelho/Azul; 4F- 4 lâmpadas fluorescentes; 2F- 2 lâmpadas fluorescentes; LED B- duas lâmpadas LEDS brancas) para diferentes clones de *Eucalyptus benthamii* (C1 e C2) e *Eucalyptus dunnii* (C3 e C4), avaliados aos 30 dias na fase de multiplicação in vitro

		NS Light conditions							
Sucrose ($g.L^{-1}$)	**Clone**	**LED V/A**		**4F**		**2F**		**LED B**	
0	C1	2,49	a AB	3,6	a A	2,4	a B	2,56	a AB
	C2	1,94	a A	2,65	a A	2,11	a A	1,78	a A
	C3	3,25	a A	2,99	a A	2,85	a A	2,56	a A
	C4	3,03	a A	3,45	a A	3,16	a A	2,4	a A
30	C1	5,8	a A	3,93	b B	4,25	a B	5,52	a A
	C2	2,49	c B	2,66	c B	3,02	c B	4,26	a A
	C3	5,25	ab A	5,05	a A	5,64	a A	5,69	a A
	C4	5,56	b A	3,93	b A	4,67	ab A	5,37	a A

Means followed by the same lowercase letter in the columns and uppercase letter in the rows, within each sucrose level, do not differ from each other by Tukey's test at 5% significance. Lowercase letters compare clones; uppercase letters, light conditions.
Médias seguidas pela mesma letra minúscula nas colunas e maiúscula nas linhas, dentro de cada nível de sacarose, não diferem entre si pelo teste de Tukey a 5% de significância. Letras minúsculas comparam clones; letras maiúsculas, as condições de luz.

Table 2. Mean values for the number of shoots larger than 2 cm (NS > 2) and the size of the largest shoot (SLS) in relation to the different concentrations of sucrose (0 gL^{-1} and 30 gL^{-1}) added to the culture medium, and in different light sources (LED V/A - two Red/Blue LED lamps; 4F- 4 fluorescent lamps; 2F- 2 fluorescent lamps; LED B- two white LEDS lamps), for different clones of *E. benthamii* (C1 and C2) and *E. dunnii* (C3 and C4), evaluated at 30 days of cultivation
Tabela 2. Valores médios do número de brotações maiores que 2 cm (NB > 2) e tamanho da maior brotação (TMB) em relação às diferentes concentrações de sacarose (0 gL^{-1} e 30 gL^{-1}) adicionadas ao meio de cultura, e em diferentes fontes de luz (LED V/A - duas lâmpadas LED Vermelho/Azul; 4F- 4 lâmpadas fluorescentes; 2F- 2 lâmpadas fluorescentes; LED B- duas lâmpadas LEDS brancas), para diferentes clones de *E. benthamii* (C1 e C2) e *E. dunnii* (C3 e C4), avaliados aos 30 dias de cultivo

NS > 2 Light conditions									
Sucrose ($g.L^{-1}$)	**Clone**	**LED V/A**		**4F**		**2F**		**LED B**	
0	C1	0,71	a A	0,71	a A	0,71	a A	0,71	a A
	C2	0,71	a A	0,71	a A	0,71	a A	0,84	a A
	C3	0,71	a A	0,71	a A	0,71	a A	0,71	a A
	C4	0,71	a A	0,71	a A	0,71	a A	0,71	a A
30	C1	3,44	a A	3,53	a A	3,2	a A	3,5	a A
	C2	2,06	b AB	2,53	b A	0,71	d C	1,82	b B
	C3	1,64	b B	2,19	bc A	0,73	c AB	2,1	b AB
	C4	1,68	b B	1,86	c B	2,49	b A	2,05	b AB
SLS (cm)									
Sucrose ($g.L^{-1}$)	**Clone**	**LED V/A**		**4F**		**2F**		**LED B**	
0	C1	1,14	a A	1,21	a A	1,27	a A	1,23	a A
	C2	1,32	a A	1,19	a A	1,25	a A	1,4	a A
	C3	1,13	a A	1,21	a A	1,14	a A	1,14	a A
	C4	1,24	a A	1,22	a A	1,36	a A	1,27	a A
30	C1	2,26	b B	2,7	b AB	2,55	a B	3,05	a A
	C2	3,07	a A	3,09	a A	1,29	c B	2,79	a A
	C3	1,8	c A	1,97	c A	1,82	b A	1,82	c A
	C4	2	c AB	1,88	c B	2,27	a A	2,29	b A

Means followed by the same lowercase letter in the columns and uppercase letter in the rows, within each sucrose level, do not differ from each other by Tukey's test at 5% significance. Lowercase letters compare clones; uppercase letters, light conditions.
Médias seguidas pela mesma letra minúscula nas colunas e maiúscula nas linhas, dentro de cada nível de sacarose, não diferem entre si pelo teste de Tukey a 5% de significância. Letras minúsculas comparam clones; letras maiúsculas, as condições de luz.

The average values for the number of elongated shoots ranged from 0.71 cm to 3.53 cm and the size of the largest shoot from 1.13 cm to 3.07 cm. At a sucrose concentration of 0 $g.L^{-1}$ there was no change in the number of shoots and the size of the largest shoot in relation to the clones and lights. Both the lighting conditions and the presence or absence of sucrose in the culture medium caused different responses in the two phases of in vitro growth.

After 30 days of growing the shoots under different light and sucrose conditions, a significant difference was observed in the average values for chlorophyll and carotenoid content. In the absence of sucrose, the V/A 3:1 LED light stimulated the highest concentrations of chlorophyll, while when a concentration of 30 $g.L^{-1}$ was used, only the 4F light showed a lower average than the other light conditions. However, it is notable that the presence of sucrose increased the chlorophyll concentration in absolute terms for all the light conditions when compared to the absence of sucrose (Table 3).

4. DISCUSSION

Differences in in vitro plant growth are related to the supply of light, in its different spectra and intensities, as well as the availability of carbon to the plant, essential elements in the process of photosynthesis and

Table 3. Mean values of total chlorophyll (Total Cl) and carotenoid content in relation to the different concentrations of sucrose (0 g.L^{-1} and 30 g. L^{-1}) added to the culture medium, in different light sources (LED V/A = red/blue light, 4F = 4 fluorescent lamps, 2F = 2 fluorescent lamps, LED B = white light) for different clones of *Eucalyptus benthamii* (C1 and C2) and *Eucalyptus dunnii* (C3 and C4), assessed at 30 days of in vitro elongation

Tabela 3. Valores médios de teor de clorofila total (Total Cl) e carotenoides em relação às diferentes concentrações de sacarose (0 g.L^{-1} e 30 g.L^{-1}) adicionadas ao meio de cultura, em diferentes fontes de luz (LED V/A = luz vermelho/azul, 4F = 4 lâmpadas fluorescentes, 2F = 2 lâmpadas fluorescentes, LED B = luz branca) para diferentes clones de *Eucalyptus benthamii* (C1 e C2) e *Eucalyptus dunnii* (C3 e C4), avaliados aos 30 dias de alongamento in vitro

Total Cl (µg g^{-1}) Light conditions								
Sucrose (g.L^{-1})	**Clone**	**LED V/A**		**4F**		**2F**		**LED B**	
0	C1	13,21	b B	15,83	ab AB	24,37	a A	25,73	a A
	C2	36,89	a A	13,39	ab B	12,44	bc B	13,78	b B
	C3	32,22	a A	22,26	a AB	20,62	ab B	13,81	b B
	C4	13,51	b AB	9,71	b B	9,74	c B	21,54	ab A
30	C1	32,58	b B	18,72	b C	39,68	a AB	44,99	a A
	C2	20,97	c BC	33,92	a A	11,34	b C	24,27	b AB
	C3	27,92	bc B	28,22	ab B	40,89	a A	45,55	a A
	C4	49,34	a A	24,19	ab C	37,27	a B	43,79	a AB
Carotenoids (µg g^{-1}) Light conditions								
Sucrose (g.L^{-1})	**Clone**	**LED V/A**		**4F**		**2F**		**LED B**	
0	C1	2,05	b B	2,61	a AB	3,36	a AB	3,62	a A
	C2	5,03	a A	1,8	a B	1,35	c B	2,14	ab B
	C3	4,56	a A	3,15	a AB	2,97	ab AB	2,09	b B
	C4	2,37	b AB	1,67	a B	1,55	b B	3,47	ab A
30	C1	4,62	b B	2,88	b C	5,61	a AB	6,3	a A
	C2	3,37	b B	5,04	a A	1,79	b C	3,67	b AB
	C3	4,8	b AB	4,02	ab B	5,74	a A	6,1	a A
	C4	7,94	a A	4,18	ab C	5,78	a B	6,55	a AB

Means followed by the same lowercase letter in the columns and uppercase letter in the rows, within each sucrose level, do not differ from each other by Tukey's test at 5% significance. Lowercase letters compare clones; uppercase letters, light conditions.
Médias seguidas pela mesma letra minúscula nas colunas e maiúscula nas linhas, dentro de cada nível de sacarose, não diferem entre si pelo teste de Tukey a 5% de significância. Letras minúsculas comparam clones; letras maiúsculas, as condições de luz.

subsequent growth and development of a plant (Batista et al., 2018; Miranda et al., 2020; Murthy et al., 2024). Light essentially participates directly in photomorphogenesis, involving physiological regulation, growth and metabolic responses in plant organisms (Fan et al., 2022).

Higher multiplication and elongation rates are related to the use of sucrose as a source of energy and carbon for in vitro plants, which is an important component in culture media (Kozai, 1991; Nambiar et al., 2012). The *Eucalyptus* clones evaluated here proved to be responsive to the enrichment of the culture medium with sucrose and changes in light quality, increasing the production and length of the shoots.

Other authors have reported the use of 30 g.L^{-1} of sucrose in the micropropagation process as an agent to promote multiplication or elongation in *Eucalyptus* (Brondani et al., 2009; Borges et al., 2011; Navroski et al., 2013). In *Corymbia* hybrids, different sucrose concentrations and light quality also influenced the productivity of propagules, photosynthesis, production of metabolic compounds, enzyme activity, accumulation of fresh and dry biomass, as well as plant survival when acclimatized ex vitro (Souza, 2024). In *Vernonia condensata* plants, a reduction in sucrose also negatively affected in vitro growth parameters (Fortini et al., 2021). Carbon sources such as sucrose are

necessary in the culture medium due to light restriction for photosynthetic activity and low CO_2 concentrations under in vitro conditions, especially when the plant is in the initial stage of culture establishment and micropropagation, where it lacks photoassimilates in sufficient concentrations to adequately perform its metabolic functions (Kozai, 1991; Kozai & Kubota, 2001; Nambiar et al., 2012; Taiz et al., 2017).

All the light sources tested stimulated the multiplication and elongation of the buds. Thus, with the expectation of minimizing electricity consumption, reducing the maintenance costs of light sources and improving the production efficiency and quality of micropropagated *Eucalyptus* plants, the use of LED lamps for in vitro cultivation of *E. benthamii* and *E. dunnii* proved to be a viable alternative. In *Eucalyptus urophylla*, the use of Blue LED, Far Red LED, Red/Blue LED and Red LED light did not affect the productivity of micro-cuttings when compared to fluorescent light (Miranda et al., 2020).

The reduction or elimination of sucrose and the use of light systems with different spectra can increase the ability of in vitro plants to carry out photosynthesis. Such conditions, which stimulate photoautotrophic behavior, will facilitate the plant's ex vitro acclimatization, reducing seedling mortality (Jeong & Sivanesan, 2018; Kozai, 1991; Souza, 2024). However, in some plants, an increase in sucrose results in a lower rate of photosynthesis, associated with inhibition of chlorophyll biosynthesis (McCarthy et al., 2016).

According to Yuan et al. (2015), the biosynthesis and accumulation of carotenoids is also regulated by the presence of sucrose in the cell metabolism. The chlorophyll and carotenoid content showed higher values at a sucrose concentration of 30 $g.L^{-1}$ in all the lights, where only the 4F light reduced the chlorophyll and carotenoid concentrations. At 0 $g.L^{-1}$, only the V/A LED increased the accumulation of chlorophylls and carotenoids. In *Fagopyrum tataricum*, higher concentrations of carotenoids were found in shoots exposed to white LED light compared to shoots exposed to red LED light (Tuan et al., 2013). In sugar cane, white LED also increased the levels of photosynthetic pigments in the plants, regardless of sucrose concentration (Ferreira et al., 2016). For *Eucalyptus urophylla*, white LED also increased chlorophyll and carotenoid levels (Miranda et al., 2020).

This research opens up new perspectives for implementing an efficient photoautotrophic system for *Eucalyptus* in large-scale propagation. It is suggested that further research be carried out evaluating higher photon flux densities, changes in the composition of the culture medium, such as other sources of sugars (maltose, galactose, glucose) and an increase in CO_2 in a photoautotrophic propagation system, with the aim of improving the micropropagation process of the *Eucalyptus* genus, allowing more vigorous micropropagated plants to be achieved, with greater ease in acclimatization, reducing losses in this critical stage of the process.

5. CONCLUSION

The use of sucrose added to the culture medium stimulates the multiplication and elongation of *Eucalyptus benthamii* and *Eucalyptus dunnii* buds. Fluorescent lamps, red/blue LED and white LED, can be used in in vitro culture for the multiplication and elongation of buds. However, the fluorescent lamp with an intensity of 64 $\mu mol\ m^{-2}\ s^{-1}$ reduced the size of the largest bud and the number of buds larger than 2 cm.

The white LED and 30 $g.L^{-1}$ of sucrose promoted higher overall averages of total chlorophyll and carotenoids for the clones tested. The use of LED lamps favored the gain of photosynthetic pigments compared to fluorescent lamps, as well as showing greater homogeneity of responses in different clones.

6. ACKNOWLEDGEMENTS

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AUTHOR CONTRIBUTIONS

Gatti, KC: Conceptualization; Data curation; Formal analysis; Writing-original draft; Writing-review & editing. Souza, MDH: Formal analysis; Writing-original

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