Revista ÁRVORE
ISSN 1806 – 9088

Scientific Article

LIGHT PULSES IN THE MICROPROPAGATION OF *Corymbia torelliana* (F.Muell.) K.D.Hill & L.A.S.Johnson x *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson CLONE: in vitro ELONGATION AND ROOTING

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ABSTRACT

LED lamps emit light in specific spectrums, significantly enhancing the quantum efficiency of photosynthesis (QFP) and, consequently, plant growth and development. *Corymbia citriodora*, *Corymbia torelliana*, and some of their interspecific hybrids are being incorporated into clonal forestry programs. However, a major bottleneck in propagation is related to rooting. This study aimed to analyze the effects of light pulses with different spectral qualities on the in vitro elongation and rooting stages, using micropropagation techniques in a hybrid clone of *Corymbia torelliana* x *Corymbia citriodora*. Four light sources were utilized: (2F) fluorescent, (LED V+/A) red (99%) + blue (1%), (LED V/A+) red (1%) + blue (99%), and (LED V/A) red (72%) + blue (28%). The treatments compositions consisted of combining these light sources with different exposure durations of 7, 14, 21, and 28 days (without pulse), totaling 13 treatments. After 28 days of cultivation, the explants were evaluated based on the following parameters: survival (%), contamination (%), callogenesis (%), rooting (%), culture medium oxidation (%), shoot length (cm), and plant vigor (scores from 1 to 3). The results indicated that the lowest oxidation rate of the culture medium occurred with the V/A+ LED light source after 28 days. However, this result did not directly affect vigor or in vitro development. Additionally, under the tested conditions, using V+/A LEDs (a higher proportion of red) for 7 days stimulated shoot growth and root emission, leading to the best in vitro development.

Keywords: In vitro development; LEDs; Spectral quality.

How to cite:
Superbi, M. A., Brás, P. V., Castro, M. E. B. de, Xavier, A., & Otoni, W. C. (2024). Light pulses in the micropropagation of *Corymbia torelliana* (F.Muell.) K.D.Hill & L.A.S.Johnson x *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson clone: in vitro elongation and rooting. *Revista Árvore, 48*(1). https://doi.org/10.53661/1806-9088202448263750. Retrieved from https://www.revistaarvore.ufv.br/rarv/article/view/263750

Revista ÁRVORE
ISSN 1806 – 9088

PULSOS DE LUZ NA MICROPROPAGAÇÃO DE CLONE DE *Corymbia torelliana* (F.Muell.) K.D.Hill & L.A.S.Johnson x *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson: ALONGAMENTO E ENRAIZAMENTO in vitro

RESUMO – As lâmpadas LEDs emitem luz em espectros específicos, melhorando significativamente a eficiência quântica da fotossíntese (EQF) e, consequentemente, o crescimento e o desenvolvimento de plantas. As espécies *Corymbia citriodora*, *Corymbia torelliana* e alguns de seus híbridos interespecíficos, estão sendo incorporadas em programas de silvicultura clonal. Entretanto, o principal entrave da propagação é em relação ao enraizamento. O objetivo desse artigo foi analisar os efeitos do uso de pulsos de luz com diferentes qualidades espectrais nas etapas de alongamento e enraizamento in vitro, por meio da técnica de micropropagação, em um clone híbrido de *Corymbia torelliana* x *Corymbia citriodora*. Foram utilizadas 4 fontes de luz: (2F) fluorescente, (LED V+/A) vermelho (99%) + azul (1%), (LED V/A+) vermelho (1%) + azul (99%), LED V/A vermelho (72%) + azul (28%). A composição dos tratamentos consistiu na combinação dessas fontes de luz com diferentes tempos de permanência 7, 14, 21 e 28 dias (sem pulso), totalizando 13 tratamentos. Ao final de 28 dias de cultivo, os explantes foram avaliados nos seguintes parâmetros: sobrevivência (%), contaminação (%), calogênese (%), enraizamento (%), oxidação do meio de cultura (%), comprimento da parte aérea (cm) e vigor da planta (notas de 1 a 3). Os resultados indicaram que a menor taxa de oxidação do meio de cultura foi observada no tratamento com 28 dias na fonte de luz LED V/A+. Entretanto, esse resultado não afetou diretamente o vigor e nem o desenvolvimento in vitro. Além disso, nas condições testadas, o uso de LEDs V+/A (maior proporção de vermelho) por 7 dias estimulou o crescimento da parte aérea e a emissão de raízes proporcionando o melhor desenvolvimento in vitro.

Palavras-Chave: Desenvolvimento in vitro; LEDs; Qualidade espectral.

1. INTRODUCTION

The success of productivity in plantations of exotic forest species in Brazil is intrinsically linked to several factors, with clonal silviculture being a highly relevant component. A set of techniques has allowed the development of plantations, improving activities from the vegetative propagation of selected materials to the establishment and management of clonal forests (Xavier et al., 2021).

In this context, species of *Corymbia*, such as *Corymbia torelliana* (F.Muell.) K.D. Hill & L.A.S. Johnson and *Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson as well as their interspecific hybrids have been incorporated into clonal forestry cultivation initiatives. These species are significant in the forestry sector due to the quality and density of their wood and their resilience to harsh and adverse conditions (Assis, 2014). However, due to low rooting levels - less than 5% - companies in the forestry sector face difficulties in the vegetative propagation of the species. Therefore, rooting has been the main obstacle in the propagation of seedlings of the genus *Corymbia* (Reis et al., 2014).

Micropropagation is a widely used technique in agriculture that involves in vitro propagation from plant parts, enabling faithful reproduction of selected genotypes (Chen et al., 2002). The technique has been applied to multiply virus-free plants in less time and physical space (Abiri et al., 2020), and to enhance the efficiency of various processes within *Corymbia* seedling production (Brondani et al., 2018; Souza et al., 2019; Trueman et al., 2018). However, despite these advances, inducing rhizogenesis and managing environmental conditions remain significant challenges in the forestry sector.

The elongation process in micropropagation of stumps is essential to ensure that the shoots reach a desirable shoot length, given the need to maintain the plant architecture for the rooting stage (Xavier et al., 2021). Several factors impact the rooting of the stumps, including genetics, physiological conditions, mineral nutrition of the mother plant, storage and health of the stumps, environmental conditions, and carbohydrate levels in the vegetative propagules (Hartmann et al., 2011; Xavier et al., 2021). However, the development of in vitro environmental control systems has been proposed as a solution to overcome challenges

in the large-scale production of high-quality plants (Gago et al., 2014; Arencibia et al., 2017; Shukla et al., 2017).

Environmental control can be facilitated in commercial micropropagation since the light factor can be easily managed. LED (Light Emitting Diode) lamps can replace fluorescent lamps emitting a broad spectrum, including unnecessary and low-quality wavelengths for promoting plant growth (Ramírez-Mosqueda et al., 2017). The use of LED lamps has been a solution to overcome challenges associated with inefficient lighting, heat dissipation, high energy consumption and inadequate spectrum of traditional sources such as high-pressure sodium lamps, fluorescent lamps, metal halide lamps, and incandescent bulbs (Álvarez et al., 2014).

In in vitro environments, lamps can be managed to alter the photoperiod (light hours), light intensity (photon flux), and spectral quality (different wavelengths), influencing the morphology and growth of plant cells, tissues, and organs (Higuchi and Hisamatsu, 2016; Rehman et al., 2017; Zakurin et al., 2020). Therefore, LED lights have desirable characteristics such as spectral composition control, long durability, emission of specific wavelengths, and reduced heat emission and they are compact, easily manageable, and installable in growth chambers (Li et al., 2010; Muneer et al., 2014).

Adjustments to the wavelengths of LEDs create combinations of red and blue light that can generate specific physiological responses in plant photoreceptors, such as phytochromes (red light) and cryptochromes (blue light) (Muneer et al., 2014). Recent research has demonstrated how different light qualities affect plant metabolism (Batista et al., 2018), demonstrating that the absorption of blue and red light emitted by LED lamps exerts a substantial influence on plant development and physiology, representing about 90% of the emitted light (Lazzarini et al., 2018).

Considering the above, we hypothesize that pulses from different light sources enhance in vitro growth and development by stimulating stem elongation and rooting. Therefore, the objective of this study was to analyze the effects of using light pulses with different spectral qualities on the in vitro elongation and rooting stages through micropropagation techniques, in a hybrid clone of *Corymbia torelliana* × *Corymbia citriodora*.

2. MATERIAL AND METHODS

2.1 Study location and experimental material

The experiment was conducted at the Tissue Culture Laboratory II of the Institute of Applied Biotechnology for Agriculture (BIOAGRO) at the Federal University of Viçosa, in Viçosa, Minas Gerais, Brazil.

The plant material used for the explants was sourced from a clone of *Corymbia torelliana* (F.Muell.) K.D.Hill & L.A.S. Johnson × *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S. Johnson provided by the in vitro clonal germplasm bank of the Clonal Silviculture Research Group – DEF/UFV (CNPq, 2002), in partnership with the Celulose Riograndense Company - CMPC, located in Guaíba, Rio Grande do Sul, Brazil.

Explants were multiplied in 55 mL test tubes containing 10 mL of JADS culture medium (Correia et al., 1995). The medium was composed of JADS salts with the addition of 30 g L^{-1} of sucrose, 100 mg L^{-1} of myo-inositol, 800 mg L^{-1} of Polyvinylpyrrolidone (PVP), and 5,5 g L^{-1} of agar for solidification, and the pH was adjusted to 5.8 ± 0.1. The culture medium was supplemented with 0,5 mg L^{-1} of 6-benzylamiporurine (BAP) and 0,01 mg L^{-1} of α-naphtaleneacetic acid (NAA) for the multiplication phase. The test tubes were capped with polypropylene lids, and the the culture medium was autoclaved at 1.5 atm and 121°C for 20 minutes.

The explants were subcultured on a culture medium containing the hormonal balance of the multiplication stage until the desired number of plants was obtained. After the multiplication stage, the explants were transferred to the JADS culture medium, but with 0,3 mg L^{-1} of BAP and 0,01 mg L^{-1} of NAA, for 15 days for the stabilization stage before the experiment.

For the experimental setup, the explants were standardized to 1,0 cm of aerial part and two leaf pairs (Figure 1), individualized and free of callogenesis at the base of the stump, and cultivated on JADS culture medium supplemented with 0,02 mg L^{-1} of BAP and 0,2 mg L^{-1} of IBA. The hormonal balance was adjusted to promote the elongation and rooting stages (Figure 1).

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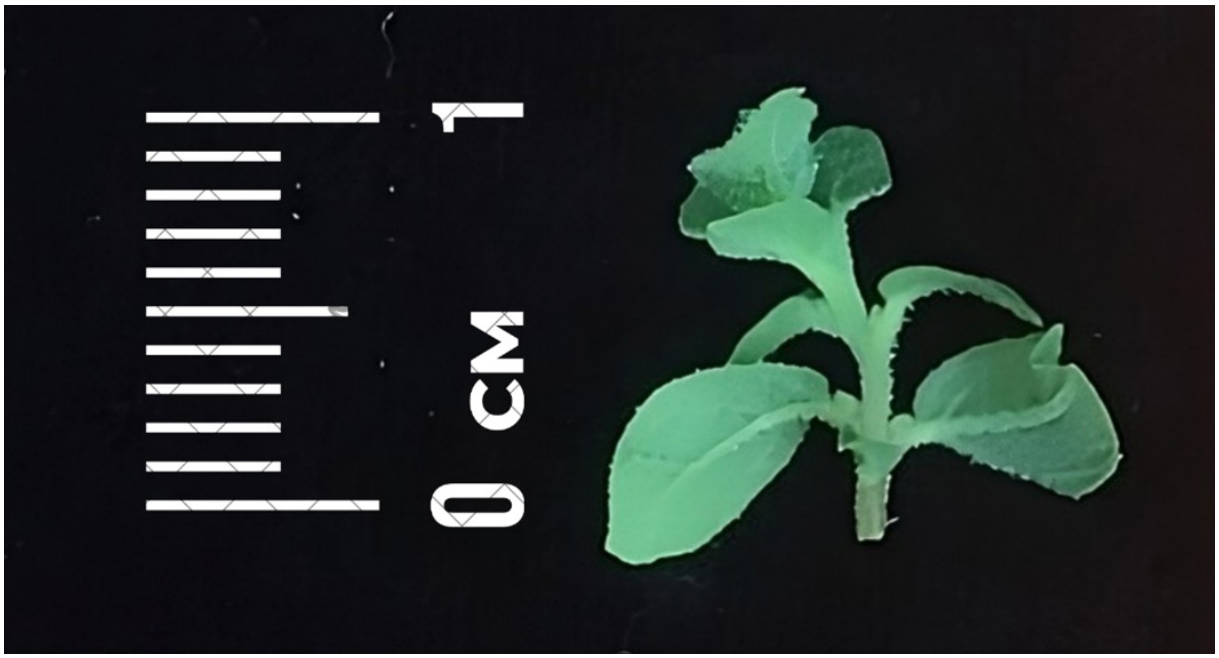

Figure 1. Standard explant, with approximately 1.0 cm of shoot and two pairs of leaves from the clone of *Corymbia torelliana* (F.Muell.) K.D. Hill & L.A.S. Johnson x *Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson

Figura 1. Explante padrão, com aproximadamente 1,0 cm de parte aérea e dois pares de folhas do clone de *Corymbia torelliana* (F.Muell.) K.D.Hill & L.A.S. Johnson x *Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson

2.2 Cultivation conditions and light source quality

After introducing the explants, the tubes were sealed with polypropylene caps and secured with micropore tape. The material was maintained in a growth room with a photoperiod of 16 hours, a constant temperature of 25 ± 2 ºC, a relative humidity of 60 ± 5%, and an irradiance of 60 µmol m^{-2} s^{-1} mas measured by a LI-COR® LI-250A Light Meter.

Four light sources, each with different spectral qualities, were used in the treatments: fluorescent light (2F); red+/blue LED with 99% red and 1% blue, with wavelengths of 650 nm and 450 nm, respectively; red/blue+ LED with 1% red and 99% blue, with wavelengths of 650 nm and 450 nm, respectively; and red/blue LED with 72% red and 28% blue, with wavelengths of 650 nm and 450 nm, respectively.

2.3 Experimental design and data analys

The experiment was conducted using a Completely Randomized Design (CRD), consisting of 13 treatments with 5 replications each, where each replication contained 8 tubes (each with one plant), resulting in a total of 40 plants per treatment and 520 plants overall for the experiment. Each treatment was composed of a combination of light source and duration in days (Figure 2).

Therefore, the treatments consisted of: T0 = 28 days 2F; T1= 7 days R/B+ and 21 days 2F; T2= 14 days R/B+ and 14 days 2F;T3 = 21 days R/B+ and 7 days 2F; T4 = 28 days R/B+; T5= 7 days R+/B and 21 days 2F; T6= days R/B+ and 14 days 2F; T7= 21days R+/B an 7 days 2R; T8 = 28 days 2F; T9 = 7 days R/B and 21 days 2F; T10= 14 days R/B and 14 days 2F; T11= 21 days R/B and 7 days 2F; T12 = 28 days 2F.

At the end of 28 days of culture, the explants were assessed for survival (%), contamination (%), rooting (%), callogenesis at the base of the stump (%), shoot length (cm), vigor (rated from 1 to 3), and medium oxidation (%). The experiment was replicated twice under identical conditions. To ensure standardization during the evaluation, the assessors were trained and participated in both experimental trials.

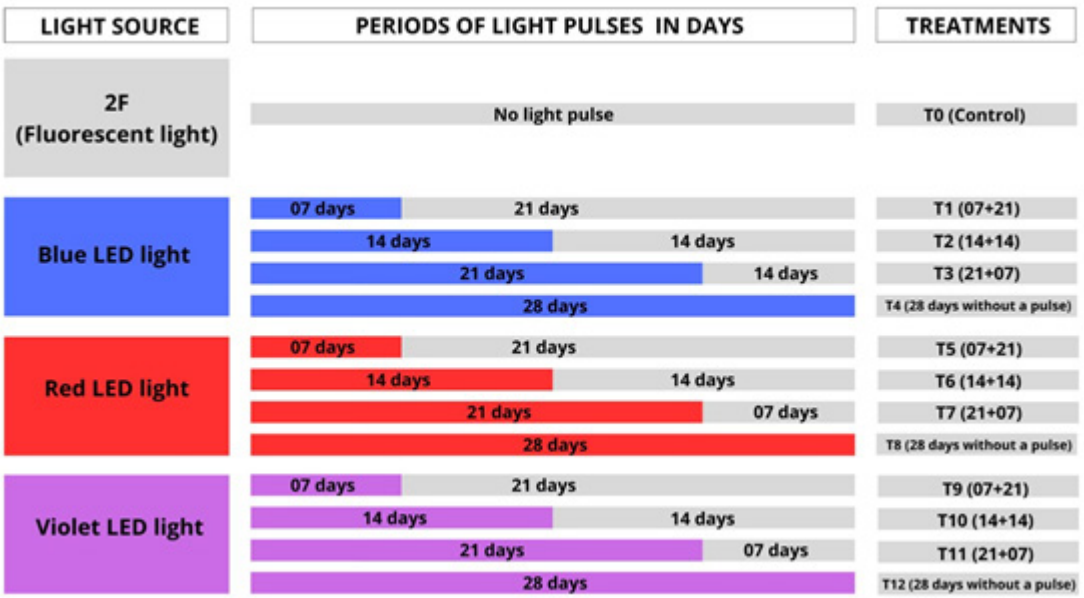

Figure 2. Schematic of the Light Pulse Application Used in the Experiment

Figura 2. Esquema de aplicação dos pulsos de luz usado no experimento

The vigor assessment methodology involved assigning scores ranging from 1 to 3, considering undesirable plant characteristics such as explant oxidation, leaf abscission, chlorosis, and hyperhydricity. Plants with a score of 1 exhibited at least two undesirable characteristics; plants with a score of 2 exhibited at least one undesirable characteristic; and plants with a score of 3 did not exhibit any undesirable characteristics. The final score for each treatment was calculated as the arithmetic mean of the scores assigned to the repetitions. Consequently, a score closer to 3 indicates higher vigor of the explant.

The percentage data that did not exhibit a normal distribution according to the Kolmogorov-Smirnov test were transformed using the equation: arc $\sin(\sqrt{(x/100)})$. The data were subjected to analysis of variance (ANOVA), and the means were compared using Tukey's test at a 5% significance level.

3. RESULTS

3.1 Survival, callogenesis, contamination, and explant vigor

The results indicated that the characteristics of contamination, survival, callogenesis at the base of the stump and vigor showed no significant differences in relation to the light source and duration of exposure, meaning they acted independently. All treatments achieved a high average survival rate of 99.4% ± 1.10% and high callogenesis at the base of the stump with 99.23% ± 1.20%. The maximum contamination observed was 10% ± 13.7% (Table 1).

However, after 28 days of the experiment, it is worth noting that the average scores assigned for vigor were above 2.6 (± 0.1949), demonstrating that few undesirable characteristics were observed in the explants. Therefore, none of the treatments hindered in vitro development.

3.2 Oxidation of the culture medium

Light pulses with different spectral qualities and the duration of exposure significantly (5%) influenced the oxidation of the culture medium after 28 days of in vitro cultivation. The lowest oxidation rate of the culture medium (2.5% ± 5.6%) was observed in treatment T4 (Table 2), which was kept without light pulses in the R/B+ LED light. Therefore, it was necessary to use a light source with a higher percentage of blue light (450 nm) for 28 days to inhibit the oxidation of the culture medium. The highest oxidation rates were observed in the following treatments: T0, T1, T2, T6, T7, T9, and T10,

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Table 1. Survival, Contamination, and Callus Formation Values at the Branch Base by Treatment, in Relation to Light Quality (Source) and Duration of Stay (in Days) in In Vitro Cultivation of *Corymbia torelliana* x *C. citriodora* Clone. T0= 28 days 2F; T1= 7 days R/B+ and 21 days 2F; T2= 14 days R/B+ and 14 days 2F; T3= 21 days R/B+ and 7 days 2F; T4= 28 days R/B+; T5= 7 days R+/B and 21 days 2F; T6= 14 days R/B+ and 14 days 2F; T7= 21 days R+/B and 7 days 2F; T8= 28 days 2F; T9= 7 days R/B and 21 days 2F; T10= 14 days R/B and 14 days 2F; T11= 21 days R/B and 7 days 2F; T12= 28 days 2F

Tabela 1. Valores de sobrevivência, contaminação e calogênese na base da estaca por tratamento, em função da fonte de luz (qualidade espectral) e tempo de permanência (em dias) no cultivo in vitro do clone de *Corymbia torelliana* x *C. citriodora*. T0= 28 dias 2F; T1= 7 dias V/A+ e 21 dias 2F; T2= 14 dias V/A+ e dias14 2F; T3= 21 dias V/A+ e 7 dias 2F; T4= 28 dias V/A+; T5= 7 dias V+/A e 21 dias 2F; T6= 14 dias V/A+ e 14 dias 2F; T7= 21 dias V+/A e 7 dias 2F; T8= 28 dias 2F; T9= 7 dias V/A e 21 dias 2F; T10= 14 dias V/A e 14 dias 2F; T11= 21 dias V/A e 7 dias 2F; T12= 28 dias 2F

Treatments	Survival (%)	Contamination (%)	Callogenesis (%)
T0	100	10	100
T1	100	7.5	100
T2	100	5	100
T3	100	2.5	100
T4	97.5	0	97.5
T5	100	0	100
T6	100	7.5	100
T7	97.5	5	97,5
T8	100	0	100
T9	100	0	100
T10	100	5	100
T11	100	2.5	97,5
T12	97.5	5	97.5

Table 2. Culture medium oxidation values by treatment, in relation to light source (spectral quality) and duration of stay (in Days) in vitro cultivation of *C. torelliana* x *C. citriodora* clone. T0= 28 days 2F; T1= 7 days R/B+ and 21 days 2F; T2= 14 days R/B+ and 14 days 2F; T3= 21 days R/B+ and 7 days 2F; T4= 28 days R/B+; T5= 7 days R+/B and 21 days 2F; T6= 14 days R/B+ and 14 days 2F; T7= 21 days R+/B and 7 days 2F; T8= 28 days 2F; T9= 7 days R/B and 21 days 2F; T10= 14 days R/B and 14 days 2F; T11= 21 days R/B and 7 days 2F; T12= 28 days 2F

Tabela 1. Valores de oxidação do meio de cultura por tratamento, em função da fonte de luz (qualidade espectral) e tempo de permanência (em dias) no cultivo in vitro do clone de *Corymbia torelliana* x *C. citriodora*. T0= 28 dias 2F; T1= 7 dias V/A+ e 21 dias 2F; T2= 14 dias V/A+ e dias14 2F; T3= 21 dias V/A+ e 7 dias 2F; T4= 28 dias V/A+; T5= 7 dias V+/A e 21 dias 2F; T6= 14 dias V/A+ e 14 dias 2F; T7= 21 dias V+/A e 7 dias 2F; T8= 28 dias 2F; T9= 7 dias V/A e 21 dias 2F; T10= 14 dias V/A e 14 dias 2F; T11= 21 dias V/A e 7 dias 2F; T12= 28 dias 2F

Treatments	Culture Medium Oxidation (%)
T0	65.0 d
T1	60.0 d
T2	57.5 d
T3	32.5 bcd

Cont...

Cont...

Treatments	Culture Medium Oxidation (%)
T4	2.5 a
T5	60.0 bcd
T6	65.0 d
T7	52.5 d
T8	17.50 abc
T9	70.0 d
T10	62.5 d
T11	45.0 cd
T12	10.0 ab

demonstrating that both spectral quality and duration of exposure (in days) were crucial for highlighting the effect of the R/B+ LED light source.

3.3 Elongation and Rooting

After 28 days of cultivation, bud elongation was significantly (5%) influenced by light pulses with different spectral qualities and duration. The greatest shoot length (cm) (Figure 3) was achieved in treatment T5, which involved 7 days of pulsing with R+/B LED light followed by 21 days under 2F light,

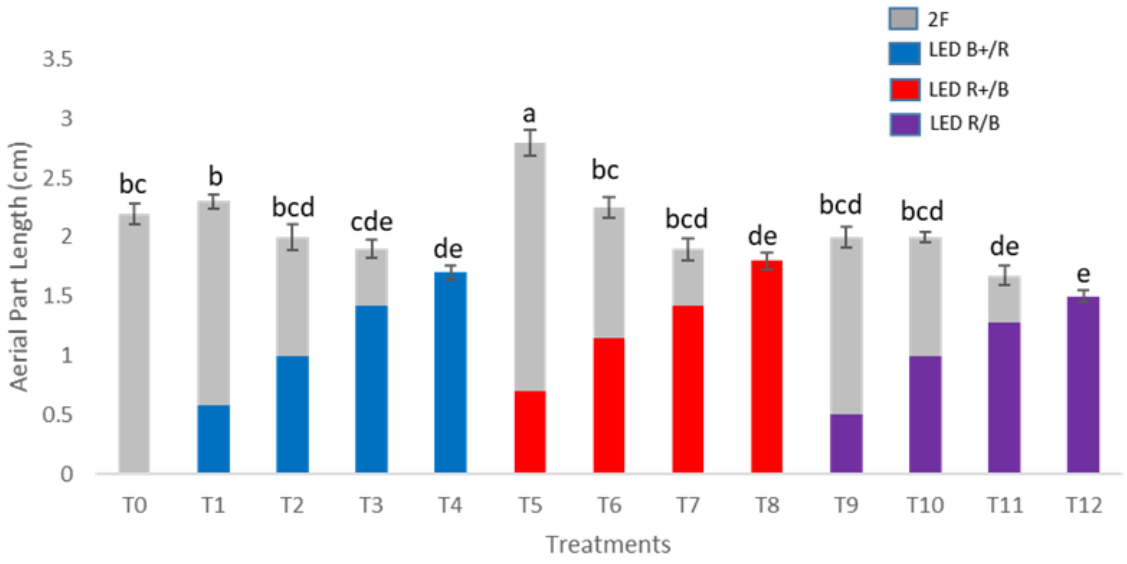

Figure 3. Aerial Part Length Obtained as a Function of Light Quality (Pulse) and Duration of Clone Stay (in Days) in In Vitro Cultivation of *Corymbia torelliana* x *C. citriodora* Clone. T0= 28 days 2F; T1= 7 days V/A+ and 21 days 2F; T2= 14 days R/B+ and 14 days 2F; T3= 21 days R/B+ and 7 days 2F; T4= 28 days R/B+; T5= 7 days R+/B and 21 days 2F; T6= 14 days R/B+ and 14 days 2F; T7= 21 days R+/B and 7 days 2F; T8= 28 days 2F; T9= 7 days R/B and 21 days 2F; T10= 14 days R/B and 14 days 2F; T11= 21 days R/B and 7 days 2F; T12= 28 days 2F

Figura 3. Comprimento da parte aérea obtida em função da qualidade de luz (pulso) e tempo de permanência do clone (em dias) no cultivo in vitro do clone de *Corymbia torelliana* x *C. citriodora*. T0= 28 dias 2F; T1= 7 dias V/A+ e 21 dias 2F; T2= 14 dias V/A+ e dias14 2F; T3= 21 dias V/A+ e 7 dias 2F; T4= 28 dias V/A+; T5= 7 dias V+/A e 21 dias 2F; T6= 14 dias V/A+ e 14 dias 2F; T7= 21 dias V+/A e 7 dias 2F; T8= 28 dias 2F; T9= 7 dias V/A e 21 dias 2F; T10= 14 dias V/A e 14 dias 2F; T11= 21 dias V/A e 7 dias 2F; T12= 28 dias 2F

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with an average length of 2.7 cm ± 0.241. This treatment promoted nearly threefold growth of the explant compared to its initial length, whereas treatment T12 resulted in a shoot length of less than 0.5 cm.

The rooting of the stumps was also significantly influenced by light pulses with different spectral qualities and duration at a 5% significance level (Figure 4). The treatment with 7 days of pulsing with R+/B light followed by 21 days under 2F light (T5) achieved the highest rooting rate, reaching 55% (± 26%) of explants with root emission. This is a noteworthy average from the perspective of propagating hybrid clones of the genus *Corymbia*, given the recalcitrance of these materials to rooting.

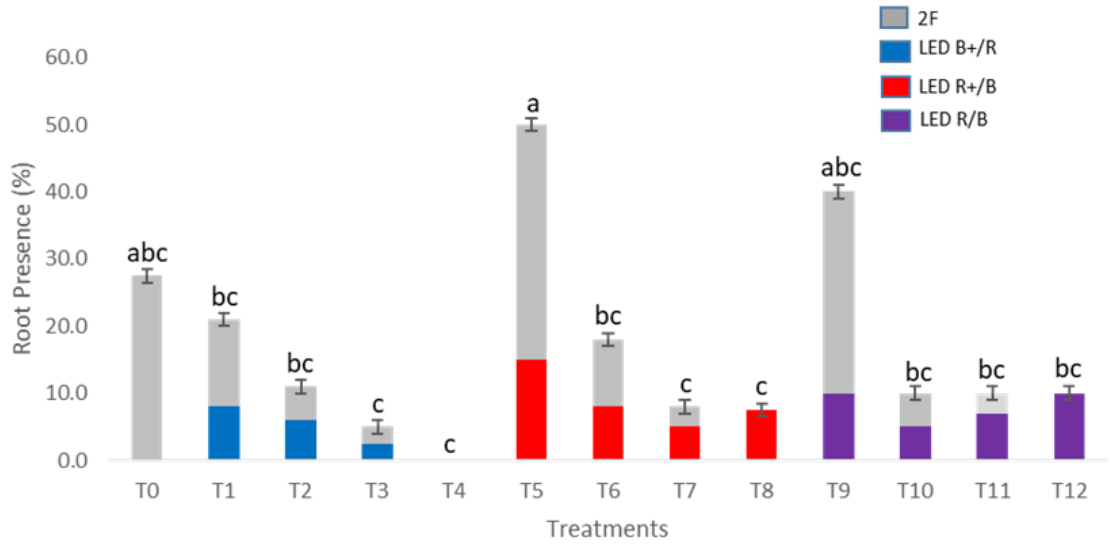

Figure 4. Root Emission as a Function of Light Quality (Source) and Duration of Stay (in Days) in In Vitro Cultivation of Corymbia torelliana x C. citriodora Clone. T0= 28 days 2F; T1= 7 days R/B+ and 21 days 2F; T2= 14 days R/B+ and 14 days 2F; T3= 21 days R/B+ and 7 days 2F; T4= 28 days R/B+; T5= 7 days R+/B and 21 days 2F; T6= 14 days R/B+ and 14 days 2F; T7= 21 days R+/B and 7 days 2F; T8= 28 days 2F; T9= 7 days R/B and 21 days 2F; T10= 14 days R/B and 14 days 2F; T11= 21 days R/B and 7 days 2F; T12= 28 days 2F.

Figura 4. Emissão de raiz em função da qualidade de luz (fonte) e tempo de permanência (em dias) no cultivo in vitro do clone de Corymbia torelliana x C. citriodora. T0= 28 dias 2F; T1= 7 dias V/A+ e 21 dias 2F; T2= 14 dias V/A+ e dias14 2F; T3= 21 dias V/A+ e 7 dias 2F; T4= 28 dias V/A+; T5= 7 dias V+/A e 21 dias 2F; T6= 14 dias V/A+ e 14 dias 2F; T7= 21 dias V+/A e 7 dias 2F; T8= 28 dias 2F; T9= 7 dias V/A e 21 dias 2F; T10= 14 dias V/A e 14 dias 2F; T11= 21 dias V/A e 7 dias 2F; T12= 28 dias 2F.

4. DISCUSSION

Our results confirmed the hypothesis that both aspects of the treatments (light spectrum quality and duration of exposure) promoted the in vitro development of the analyzed clone. It appears that the optimal time for inducing accelerated growth is 7 days under the R+/B LED light (with a higher red-to-blue light ratio), which provides the ideal combination of wavelengths in the red region at 650 nm (99%) and blue at 450 nm (1%). Therefore, the timely regulation of the light spectrum during the elongation and rooting phases highlighted the importance of spectral quality in the in vitro development of *Corymbia*, potentially yielding better results than fluorescent white light.

Elongation of the explants was essential for producing mini-stumps and facilitating the handling and architecture of the seedlings. Generally, higher photosynthetic quantum efficiency (PQE) results in increased dry matter, root system expansion, and stem elongation in many plant species (Bilodeau et al., 2019). The light source with a higher proportion of red light may have promoted

greater PQE when comparing the parameters (shoot length and rooting percentage) with treatment T0, which used only white light. It is important to highlight that even a small addition of blue light was sufficient to produce a superior effect on in vitro growth and development (Brown et al., 1995; Yorio et al., 1998).

Phytochromes play a fundamental role in acquiring information perceived by the aerial part of the plant. A low red ratio, for example, can influence root behavior, affecting both growth and morphology. The role of light quality in controlling root function may have been overlooked over the years, but some studies report results with woody species (Santos Junior et al., 2022), including *Eucalyptus* (Souza et al., 2019; Frade et al., 2023). Red light has been shown to stimulate root growth in *Enhalus acoroides* seedlings (Heemboo et al., 2023). Additionally, the addition of far-red light only during the initial growth phase of medicinal cannabis improved rooting without promoting stem elongation (Sae-Tang et al., 2024).

The lowest values for culture medium oxidation occurred under the R/B+ LED (with a higher proportion of blue light) with no pulsing. Although this was a significant result, this characteristic did not directly affect vigor or overall plant development. The influence of the light source with a higher proportion of blue light (R/B+ LED) may have been limited because not all absorbed blue photons are utilized in photosynthesis. Therefore, photosynthetic pigments did not fully realize the absorption of photons from the R/B+ LED. As a result, they did not contribute significantly to the growth and production of the explants. Of the total blue light absorbed, 20% might have been used by non-photosynthetic pigments or dissipated as heat or fluorescence (Bilodeau et al., 2019). In contrast, red LEDs emit a narrow light spectrum (620-750 nm), highly absorbed by chlorophyll and phytochromes (Muneer et al., 2014).

Light sources with specific wavelengths induce photomorphogenic responses in explants cultivated in controlled environments. This technology is beneficial for eucalyptus plants by optimizing in vitro development and productivity (Frade et al., 2023). In each stage of development, an optimal behavior will be desired for the explant. The wavelength was considered an essential factor in the in vitro multiplication of *E. grandis* × *E. urophylla*, as it reduced hyperhydricity, a higher number of shoots per explant, and greater shoot length (Souza et al., 2020a; Souza et al., 2020b).

Controlling plant morphology in vitro requires constant protocol updates due to the innovations emerging in the market. Artificial lighting technologies offer numerous possibilities for light manipulation and management. The light sources used in the experiments act in precise ways and do not always come with detailed descriptions of the spectral quality employed. However, the lack of reproducibility in protocols results in outcomes that are difficult to compare (Batista et al., 2018). The distinguishing feature of this work was achieving high rooting percentages for *Corymbia* compared to other studies with the genus (Reis et al., 2014), as well as specifying the proportions of each wavelength used, making the experiments comparable within the genus (Reis et al., 2014), as well as specifying the proportions of each wavelength used, allowing for experiment comparability.

The high maintenance cost of in vitro cultivation systems is justified by the expenses related to system setup, electricity, and the challenge of providing the irradiance necessary to reach the light saturation point (LSP) (Xavier et al., 2021). However, it is important to highlight the precedents that this work sets for testing growth acceleration strategies. This is especially relevant for regions with low solar intensity, where these methods could be explored as a supplementary light source. It is important to consider that in vitro cultivation conditions may differ from ex vitro conditions and should be adjusted according to the goals of seedling production.

5. CONCLUSION

Light pulses with different spectral qualities promoted both elongation and rooting. The R+/B LED light for 7 days, followed by a switch to 2F light for 21 days, was identified as the best combination of duration and spectral quality for the in vitro development of a *Corymbia torelliana* × *Corymbia citriodora* clone. This work contributed to advancing vegetative propagation techniques for the *Corymbia* genus. In addition, it opened new opportunities for the use of LEDs with specific spectral qualities, suggesting light pulses

Revista ÁRVORE
ISSN 1806 – 9088

as a method for accelerating growth during different phases of in vitro development.

6. ACKNOWLEDGEMENTS

We are grateful for the financial support of research from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Of the Laboratório de Cultura de Tecidos Vegetais II - BIOAGRO/UFV and Universidade Federal de Viçosa (UFV) for the suport. We acknowledge the support of the company Celulose Riograndense-CMPC in providing the genetic materials (clones).

AUTHOR CONTRIBUTIONS

Superbi, M. A.: Writing, reviewing, data analysis, conducting and executing experiments. Brás, P. V.: Writing, reviewing, detailed data analysis, conducting and executing laboratory experiments. Castro, M. E. B. de: Writing, reviewing, data analysis, conducting and executing experiments. Xavier, A.: Technical and scientific guidance, project supervision. Otoni, W. C.: Co-supervision, theoretical and methodological support.

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