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Spectral quality influence on *in vitro* morphophysiological responses of *Eucalyptus dunnii* Maiden and *Eucalyptus grandis* W.Hill ex Maiden × *E. urophylla* S.T.Blake

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Abstract

Background: *In vitro* growth and development of plants in the micropropagation stages are influenced by several factors, including the light spectral quality, which has shown important effects on the photomorphogenesis. The work aimed to evaluate the photomorphogenic effect of spectral qualities on *in vitro* culture of *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla*.

Methods: Six light spectral qualities (i.e., red, white, blue, yellow, purple, and green) on *in vitro* multiplication, elongation, and adventitious rooting stages were evaluated through analysis of variance followed by a Tukey's test.

Results: White spectral quality was most adequate for *in vitro* multiplication of *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla*, as it resulted in less tissue oxidation, longer shoot length, and more buds per explant. Red, blue and yellow spectral qualities increased the chlorophyll *a*, chlorophyll *b*, and total chlorophyll (*a*+*b*) leaf contents of *Eucalyptus dunnii*. To promote *in vitro* elongation, white spectral quality was most suitable for *Eucalyptus dunnii*, and yellow for *Eucalyptus grandis* × *E. urophylla*, as these resulted in more shoot length and shoots per explant. Red, white, blue and purple spectral qualities increased the stomatal density of *Eucalyptus dunnii*; while the white and yellow were the better for *Eucalyptus grandis* × *E. urophylla.* To promote *in vitro* rooting, the white and yellow spectral qualities caused the best results for the *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla*, with longer root length and more roots per explant. *Eucalyptus dunnii* showed reduced adventitious rooting, regardless of spectral quality.

Conclusions: Light quality influence the morphophysiological responses of *Eucalyptus* in different stages of *in vitro* culture. Our results contribute to maximise the *in vitro* cloning of important eucalypts species.

Keywords: *in vitro* propagation; plant cloning; light spectrum; pigment content; photomorphogenesis.

Introduction

Species of *Eucalyptus* have a great economic importance due to their potential to provide feedstock, fuelwood and wood for industrial purposes, being grown in large-scale commercial forests (Carrillo et al. 2018). In Brazil, eucalypts forests are mainly made up of clonal varieties bred from superior genotypes that are better adapted to adverse environmental conditions and are resistant to biotic factors, thus ensuring greater productivity and fulfilment of the demand for more wood and other products (Abiri et al. 2020).

Among vegetative propagation techniques, micropropagation has been considered a tool in the production of clonal plants on a commercial scale,

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since it enables the mass multiplication of vegetative propagules in a short time and in a small area (Molinari et al. 2021; Silveira et al. 2020; Ashrafzadeh & Leung 2021). Nevertheless, *in vitro* growth and development of different species in micropropagation stages are influenced by several factors (Batista et al. 2018; Miranda et al. 2020; Faria et al. 2021; Faria et al. 2022).

One of the important factors for the morphogenesis of different plant tissues is the availability of light as an energy source for photoassimilation, carbon partitioning (Kölling et al. 2015), phenology (Nord & Linch 2009) and other important processes, therefore, variations in wavelength trigger different morphophysiological responses. Activation is dependent on genetic and epigenetic factors intrinsic to the species, as well as on their interaction for phenotypic expression (Gilmartin et al., 1990; He et al. 2011), however, this activation begins with the stimulation of photoreceptors that signal metabolic pathways causing direct effects on tissue cell division and differentiation (Gilmartin et al. 1990). Studies of different light spectra on the *in vitro* propagation of eucalypts species, provide important information to large-scale production of clones with high phytosanitary quality and high vigor (Souza et al. 2020a; Souza et al. 2020b).

Eucalypt micropropagation under different light qualities has been extensively studied showing positive results in plant health, inducing the increase of shoots and leaves (Souza et al. 2020a; Miranda et al. 2020), as well as seed germination (Rokich & Bell 1995). Besides, the use of cellophane plastic has shown to be a low-cost viable alternative as a filter for incident fluorescent light and providing the desired wavelength in micropropagation protocols (Souza et al. 2020b).

Consequently, we hypothesise that: (*i*) clones of *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla* would respond similarly to light spectra; and (*ii*) different light spectra would trigger large differences in morphophysiological features. In this context, the study evaluated the effect of spectral quality on *in vitro* multiplication, elongation and adventitious rooting stages for both clones.

Methods

Plant material and *in vitro* multiplication

Selected plants of *Eucalyptus dunnii* Maiden and *Eucalyptus grandis* W. Hill ex Maiden × *Eucalyptus urophylla* S. T. Blake (known as urograndis eucalypt) were used to obtain the explants. The plants were donated by the Institute of Forestry Research and Studies (IPEF, Brazil). Clusters of buds (i.e., explants), were previously *in vitro* established and maintained for 60 d in Wood Plant Medium (WPM) (Lloyd & McCown 1981), supplemented with 30 g L^{-1} of sucrose. Clusters with four buds (i.e., explant) were subcultured in glass flasks of 250 mL (6.0 × 7.0 cm, diameter × height) containing 50 mL of WPM supplemented with 0.5 mg L^{-1} of 6-benzylaminopurine (BAP), 0.05 mg L^{-1} of α-naphthaleneacetic acid (NAA), and 20 g L^{-1} of sucrose.

Explants were kept in a growth room at 24°C (± 1°C) with a 16-h photoperiod using two 0.60-m cool-white Philips T10 fluorescent light bulbs with 20 W power each and 6400-6500 K colour temperature. The irradiance of the two light bulbs combined (40 µmol m^{-2} s^{-1}) was measured with a photoradiometer model QSO-S Procheck + Sensor-PAR Photon Flux (Decagon Devices, Pullman, Washington, USA). Sixty days after inoculation, percentage of tissue oxidation (Souza et al. 2020b), shoot length per explant (cm), number of buds per explant, and photosynthetic pigment content [carotenoids, chlorophyll *a*, chlorophyll *b*, and total chlorophyll (*a*+*b*)] were evaluated.

The experiment was conducted as a completely randomised design with a factorial arrangement (2 × 6), testing two clones (i.e., *Eucalyptus dunnii* and *Eucalyptus grandis × E. urophylla*), and six spectral qualities (i.e., red, blue, green, purple, yellow, and white - control). Five explants were cultured per glass flask (experimental units), totaling twenty repetitions. Photosynthetic pigments analysis was performed randomly in three repetitions per treatment.

In vitro elongation

The *in vitro* elongation experiment followed the same protocols as the multiplication experiment, only differing on the number of explants per glass flask (experimental units), being three in the former and five in the latter. The *in vitro* elongation experiment had twelve repetitions per treatment.

Shoots measuring 1-cm in length (i.e., explant), from the multiplication stage, were inoculated in glass flasks of 250 mL (6.0 × 7.0 cm) containing 50 mL of WPM culture medium supplemented with 0.5 mg L^{-1} NAA, 0.05 mg L^{-1} BAP, and 20 g L^{-1} of sucrose. Thirty-five days after inoculation, tissue oxidation percentage, shoot length per explant (cm), number of shoots per explant, and leaf anatomical features [i.e., adaxial and abaxial epidermal thickness (µm), spongy parenchyma thickness (µm), palisade parenchyma thickness (µm), and stomatal density (mm^2)] were evaluated. Histological analysis was performed randomly in three repetitions per treatment.

In vitro adventitious rooting

Microcuttings (i.e., elongated shoots 2-cm-long) were inoculated in glass flasks of 250 mL (6.0 × 7.0 cm) containing 50 mL of WPM culture medium supplemented with 1.0 mg L^{-1} NAA, 0.5 mg L^{-1} of indole-3-butyric acid (IBA), 0.05 mg L^{-1} BAP, and 20 g L^{-1} of glucose and kept in a growth room. Thirty-five days after inoculation, rooting percentage, root length per explant (cm), and number of roots per explant were evaluated. Twenty explants considered elongated (> 3.0 cm) were selected and sectioned and kept per glass flask (experimental units), totaling twenty repetitions, and the experimental design was the same as in previous experiments (i.e., *in vitro* multiplication and elongation).

Culture medium preparation

The culture media used in all experiments were prepared with deionised water, adding 6 g L^{-1} of agar, and the

pH was adjusted to 5.80 ± 0.05 with NaOH (0.1 M) or HCl (0.1 M) before autoclaving at 121°C (1.0 kgf cm^{-2}) for 20 min.

Spectral quality

Spectral qualities red, blue, yellow, purple and green were provided, under all growth stages, by filtering the light output of the fluorescent light bulbs through double sheets of cellophane (Souza et al. 2020a) replaced every cycle. The light spectra were measured using a SPECTRA PEN Z850 portable spectrophotometer (Qubit Systems, Kingston, Ontario, USA). The spectra of each light source used in this experiment are shown in Figures 1A-F.

Analysis of photosynthetic pigments

Contents of photosynthetic pigments [carotenoids, chlorophyll *a,* chlorophyll *b*, and total chlorophyll (*a*+*b*)] were evaluated in the *in vitro* multiplication stage. The extraction was performed according to the method adapted from Lichtenthaler (1987). In a partially dark environment, 25 mg of leaf tissue was randomly collected in biological triplicate from explants from different flasks. A volume of 2.5 mL of dimethyl sulfoxide (DMSO) solution was pipetted into each sample and stored in the dark for 48 h. The absorbance (A) of the pigment extracted from the leaves was read at 480 nm, 649 nm, and 665 nm in a spectrophotometer (ThermoScientific,

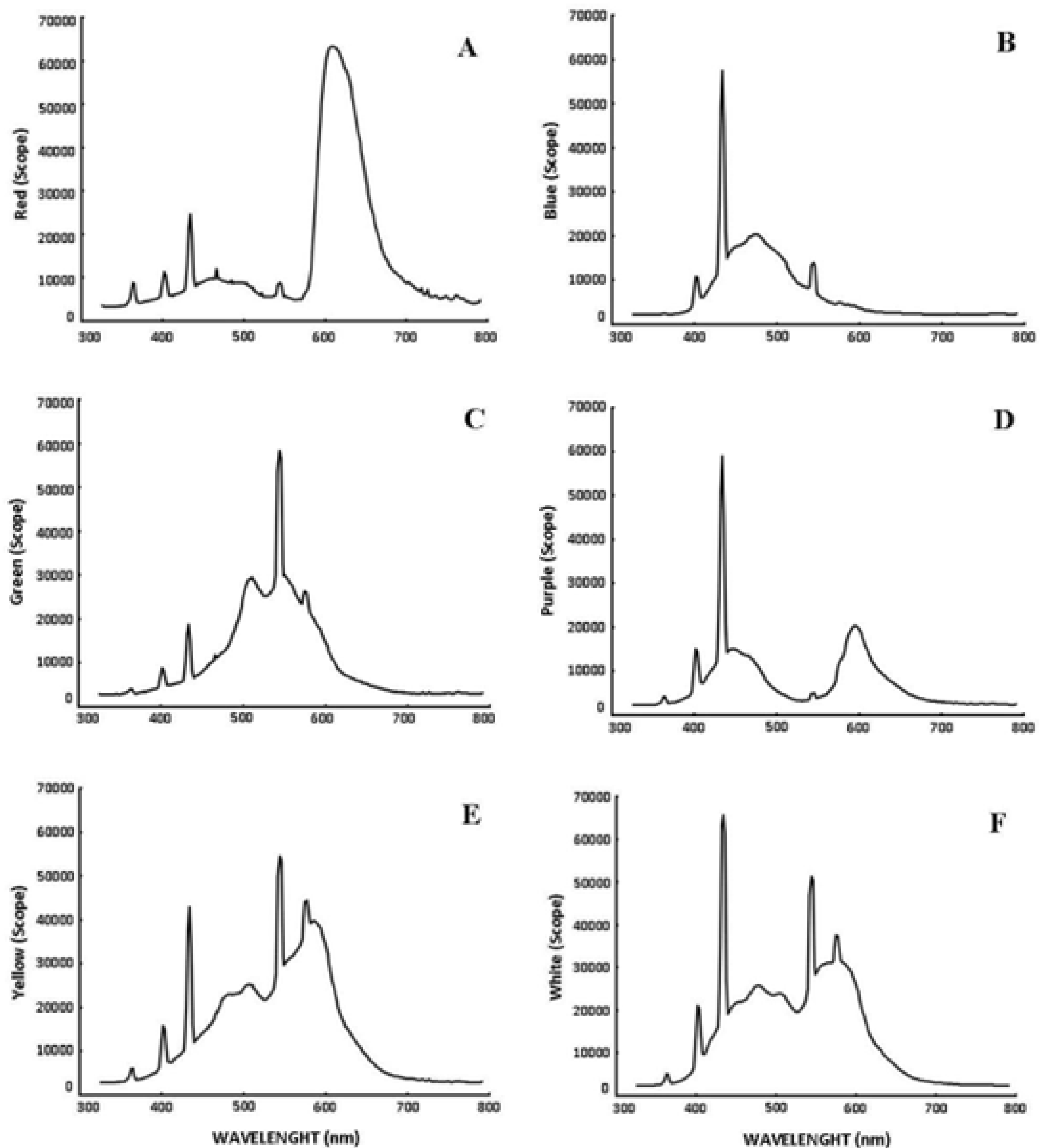

FIGURE 1: Variations in the wavelength (nm) emitted by the fluorescent light bulbs and filtered through double sheets of cellophane (μW cm^{-2} nm^{-1}). A = Red; B = Blue; C = Green; D = Purple; E = Yellow; F = White (no cellophane - control).

USA). Readings were performed in triplicate for each treatment. Chlorophyll and carotenoid contents were calculated following the equations described by Wellburn (1994): chlorophyll $a = [(12.19 \times A_{665}) - (3.45 \times A_{649})]$; chlorophyll $b = [(21.99 \times A_{649}) - (5.32 \times A_{665})]$; and carotenoids = $(1000 \times A_{480} - 2.14 \times Ca - 70.16 \times Cb)/220$. The results were expressed in µg of pigment per mg of leaf tissue fresh weight (µg mg^{-1}).

Histological analysis

Anatomical variables adaxial and abaxial epidermal thickness, palisade parenchyma thickness, spongy parenchyma thickness, and stomatal density were evaluated. Leaf samples, randomly collected from different plants in the same flask, in biological triplicate, were fixed in 70% acetic acid formaldehyde (AAF) solution for 48 h and then kept in 70% ethanol. The samples were dehydrated using an increasing ethyl alcohol series (80, 90, and 100%) for 30 min in each solution, and then stored in a 100% ethanol:historesin (1:1) (Leica®) solution in a hot oven overnight. After this, the samples were embedded in pure hydroxyethyl methacrylate resin (Leica®). Using a manual rotary microtome and a razor, the blocks containing the samples were cross-sectioned at 7-µm thickness. The sections were stained with toluidine blue solution, mounted on histological slides, and coated with stained-glass varnish. To obtain paradermal sections, the leaf samples were placed in a solution of sodium hypochlorite and distilled water (1:1) for 24 h so that tissue dissociation could occur. Then the tissues of the abaxial side were removed, stained with 1% safranin solution, and mounted on histological slides. The histological slides of the cross-sections and paradermal sections were observed under a Zeiss® light microscope and photomicrographed with an AxionCam ERc5s digital camera at micrometric scale using 20× and 40× objective lenses.

The thickness of the adaxial and abaxial epidermis, palisade parenchyma, spongy parenchyma, and total leaf blade tissues were measured at micrometric scale using the programs AxioVision version 4.8 and ImageJ at three different points of three photomicrographs from each treatment, the means of which were taken for analysis. The thickness of the midrib was also measured in three photomicrographs from each treatment to obtain a final mean. The thickness of the polar and equatorial diameter of stomata was measured in the five most homogeneous stomata in three photomicrographs from each treatment, from which the mean was calculated. The stomatal density was calculated as the ratio between the mean number of stomata and the mean area of the image present in three photomicrographs from each treatment.

Experimental design and data analysis

Statistical analyses were performed in R software, version 3.0.3 (R Core Team 2018), using the ExpDes package, version 1.1.2 (Ferreira et al. 2013). The variables that did not show a normal distribution according to the Shapiro-Wilk's test ($p > 0.05$) were arcsine-transformed. Heteroscedasticity was evaluated using Bartlett's test ($p > 0.05$). Response variables to the treatments were subjected to analysis of variance (ANOVA, $p < 0.05$) followed by Tukey's test ($p < 0.05$).

Results

Effect of spectral quality on *in vitro* multiplication

In the *in vitro* multiplication stage, there were significant effects of clones and spectral quality on morphophysiological features, 60 d after starting the *in vitro* culture ($p < 0.05$) (Figures 2A-E). The results indicated generally high percentages of phenolic oxidation of tissues across all treatments, although this did not prevent the development of explants of *Eucalyptus dunnii* (52.5% oxidation) or *Eucalyptus grandis* × *E. urophylla* (56.6% oxidation) (Figure 2A). The spectral qualities used had different effects on explant oxidation (there was not an interaction between clone and spectral quality), and the lowest tissue oxidation was observed with the white spectral quality (30.0%), which differed significantly from the other treatments (Figure 2B).

For shoot length, explants of *Eucalyptus grandis* × *E. urophylla* (1.1 cm) (Figure 2C) and explants from the white (1.2 cm) and blue (1.3 cm) spectral quality groups (Figure 2D) had the highest means, which differed significantly from the other groups. Explants of the *Eucalyptus grandis* × *E. urophylla* clone showed a higher number of buds than *Eucalyptus dunnii* under all the spectral qualities evaluated (Figure 2E). Regarding the best results obtained with the different spectral qualities, the white spectrum resulted in the most buds per explant (11.2 buds) for *Eucalyptus dunnii*, differing significantly from the green light; while the blue for *Eucalyptus grandis* × *E. urophylla* (25.7 buds), differing significantly from the red light (Figure 2E). The appearance of the explants of *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla* with regard to the morphological traits studied during their *in vitro* multiplication is shown in Figure 3.

Regarding the levels of the photosynthetic pigments studied, there was a difference in the response between *Eucalyptus dunnii* and the urograndis eucalypt with spectral qualities during the *in vitro* multiplication stage (Figures 4A-E). Only main effects were significant for carotenoid content, while for chlorophyll *a*, *b*, and total (*a*+*b*) there was clone and spectral quality interaction. The highest carotenoid content was observed in *Eucalyptus dunnii* (0.24 µg mg^{-1}), which differed significantly from *Eucalyptus grandis* × *E. urophylla* (0.11 µg mg^{-1}) (Figure 4A). The blue and yellow spectral qualities resulted in the highest carotenoid content (0.20 µg mg^{-1}), but there was no significant difference from the other treatments (Figure 4B). For the chlorophyll *a* (Figure 4C), chlorophyll *b* (Figure 4D), and total chlorophyll (*a*+*b*) (Figure 4E) contents, the *Eucalyptus dunnii* also showed higher levels than *Eucalyptus grandis* × *E. urophylla*. Regarding the spectral qualities analysed, red, blue, and yellow resulted in the highest chlorophyll (*a*+*b*) content for *Eucalyptus dunnii* (Figure 4E), while for *Eucalyptus grandis* × *E. urophylla*, no difference was observed between the light sources (Figures 4C, 4D, and 4E).

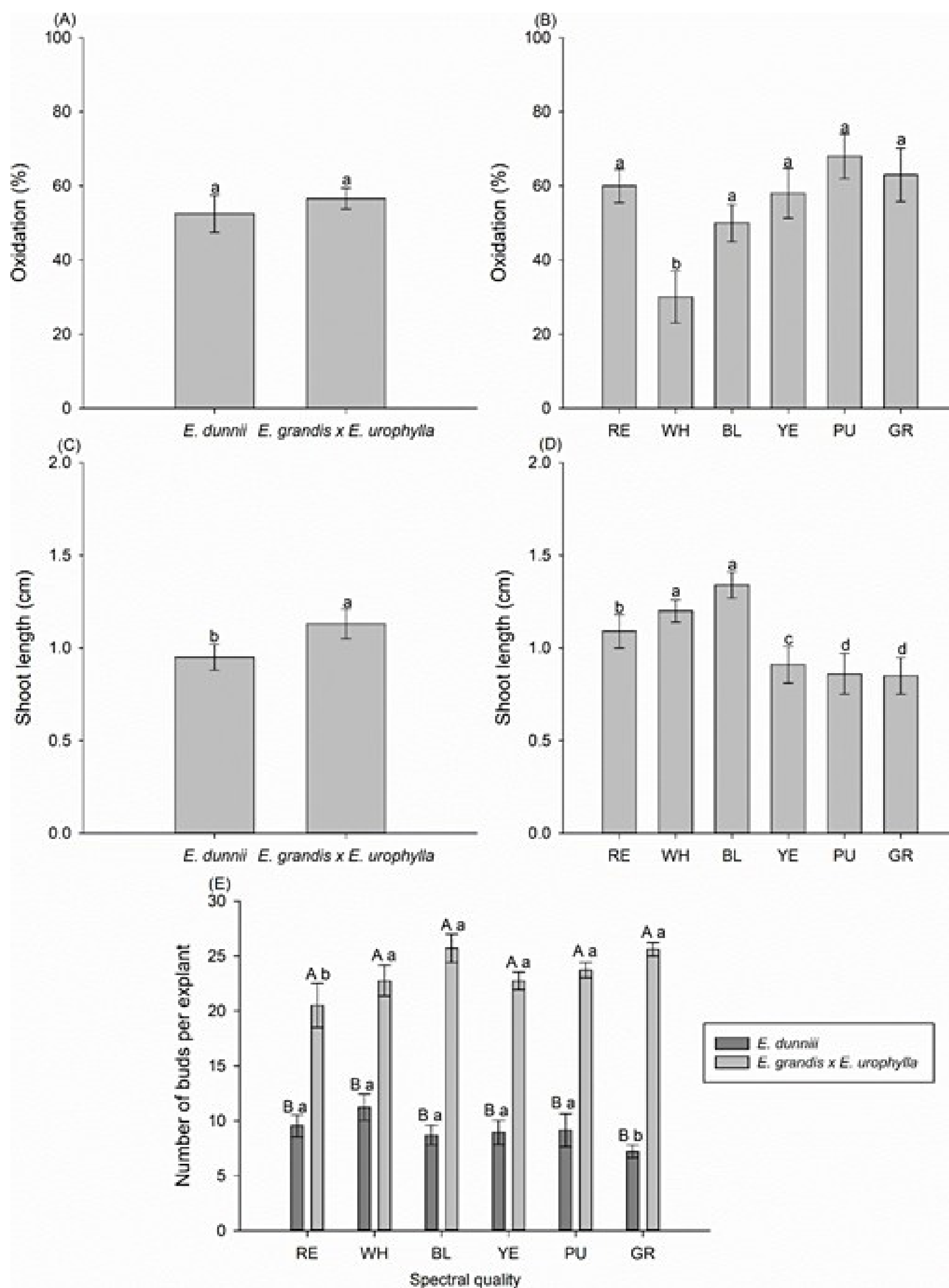

FIGURE 2: Morphological features measured during the *in vitro* multiplication stage of *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla* under different spectral qualities [i.e., red (RE), white (WH), blue (BL), yellow (YE), purple (PU), and green (GR)] at 60 d. (A) Oxidation percentage according to clone (n = 240, *p*-value = 0.512); (B) = Oxidation percentage according to spectral quality (n = 240, *p*-value = 0.014); (C) Shoot length per explant (cm) according to clone (n = 240, *p*-value = 0.001); (D) Shoot length (cm) according to spectral quality (n = 240, *p*-value = 0.001); and (E) Number of buds per explant (n = 240, *p*-value = 0.001). (A-D) Means followed by the same letters do not differ significantly at $p < 0.05$. (E) Means followed by the different uppercase letters represent significant differences when comparing different clones given the same spectral quality, and different lowercase letters represent significant differences when comparing spectral quality within the same clone at $p < 0.05$. Error bars represent the standard error of the mean.

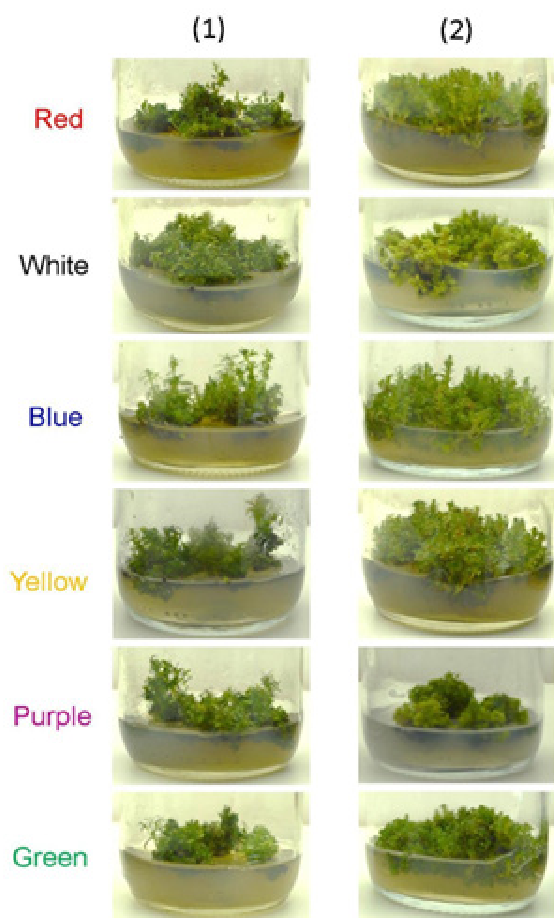

FIGURE 3: Visual characterisation of *Eucalyptus dunnii* (1) and *Eucalyptus grandis* × *E. urophylla* (2) explants on *in vitro* multiplication stage at 60 d according to spectral quality (i.e., red, white, blue, yellow, purple, and green).

Effect of spectral quality on *in vitro* elongation

Under the experimental conditions used during the *in vitro* elongation stage, only the main effect of clone (Figure 5A) but not light spectra (Figure 5B) or their interaction were significant on tissue oxidation. However, the factors had different effects on shoot length (Figure 5C) and number of shoots per explant (Figure 5D).

The oxidation percentage differed between *Eucalyptus dunnii* and the urograndis eucalypt, being the lowest values observed in *Eucalyptus dunnii* (38.0%) (Figure 5A). There was no significant difference in oxidation percentage between the different spectral qualities (Figure 5B). *Eucalyptus dunnii* had the greatest shoot length when grown under white light (3.1 cm), differing significantly from the yellow, purple, and green groups (Figure 5C). In the *Eucalyptus grandis* × *E. urophylla*, the yellow spectral quality resulted in the highest mean shoot length (3.2 cm), significantly longer than that under the white, blue, and green lights (Figure 5C).

The highest number of shoots per explant (9.4 shoots) was observed in the white light for *Eucalyptus dunnii*, only differing with the blue light (Figure 5D). In contrast, in the *Eucalyptus grandis* × *E. urophylla* clone, the best results were observed with the green (16.3 shoots), blue (13.6 shoots), and yellow (13.4 shoots) lights (Figure 5D). The appearance of the explants of *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla* with regard to morphological traits during *in vitro* elongation is shown in Figure 6.

The two clones showed statistical difference when analysing the anatomical features in the histological sections, in response to different spectral qualities during the elongation stage (Figures 7A-H). The highest means for the adaxial (Figure 7A) and abaxial (Figure 7C) epidermal thickness and spongy parenchyma thickness (Figure 7E) were observed for the *Eucalyptus dunnii* clone (11.98, 10.69, and 64.63 µm, respectively), which differed significantly from the means in *Eucalyptus grandis* × *E. urophylla* clone (11.19, 9.46, and 51.75 µm, respectively). There was no significant difference in the variables between the spectral qualities (Figures 7B, 7D, and 7F).

The palisade parenchyma thickness (Figure 7G) and stomatal density (Figure 7H) were affected by the interaction between the factors (clones and spectral quality). Yellow light resulted in the thickest palisade parenchyma in *Eucalyptus dunnii* (41.65 µm), differing significantly from the red and green qualities (Figure 7G). In contrast, there was no significant difference in the palisade parenchyma thickness of the *Eucalyptus grandis* × *E. urophylla* clone among light spectra (Figure 7G).

Exposure to red, white, blue, and purple light resulted in the highest stomatal densities in *Eucalyptus dunnii*, significantly differing from those grown under the yellow and green qualities (Figure 7H). However, the highest stomatal density in *Eucalyptus grandis* × *E. urophylla* was observed under white and yellow light, which differed significantly from red, blue, purple, and green (Figure 7H). The stomatal density on the abaxial surface of leaves of *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla* explants is shown in Figures 8A-L.

Effect of spectral quality on *in vitro* adventitious rooting

There was no interaction between clones and spectral quality on the *in vitro* adventitious rooting stage of the *Eucalyptus dunnii* or *Eucalyptus grandis* × *E. urophylla* clones after 35 d (Figures 9A-F). The rooting percentage (Figure 9A), root length (Figure 9C), and number of roots per explant (Figure 9E) were all influenced by clones. It was observed that 98.0% of *E. grandis x E. urophylla* clone exhibited roots (average of 2.5 roots per explant measuring 4.0 cm), while *E. dunnii* presented 3.0% of explant with roots (average of 0.03 roots per explant measuring 0.20 cm).

Regarding the effect of spectral quality on the adventitious rooting percentage (Figure 9B) and number of roots per explant (Figure 9F), the highest values were observed under the yellow light (53.0% of rooting and 1.5 roots per explant), but did not differ significantly from those under the other light sources. Regarding root length (Figure 9D), the use of white (2.4 cm) and yellow (2.7 cm) spectral qualities provided the highest

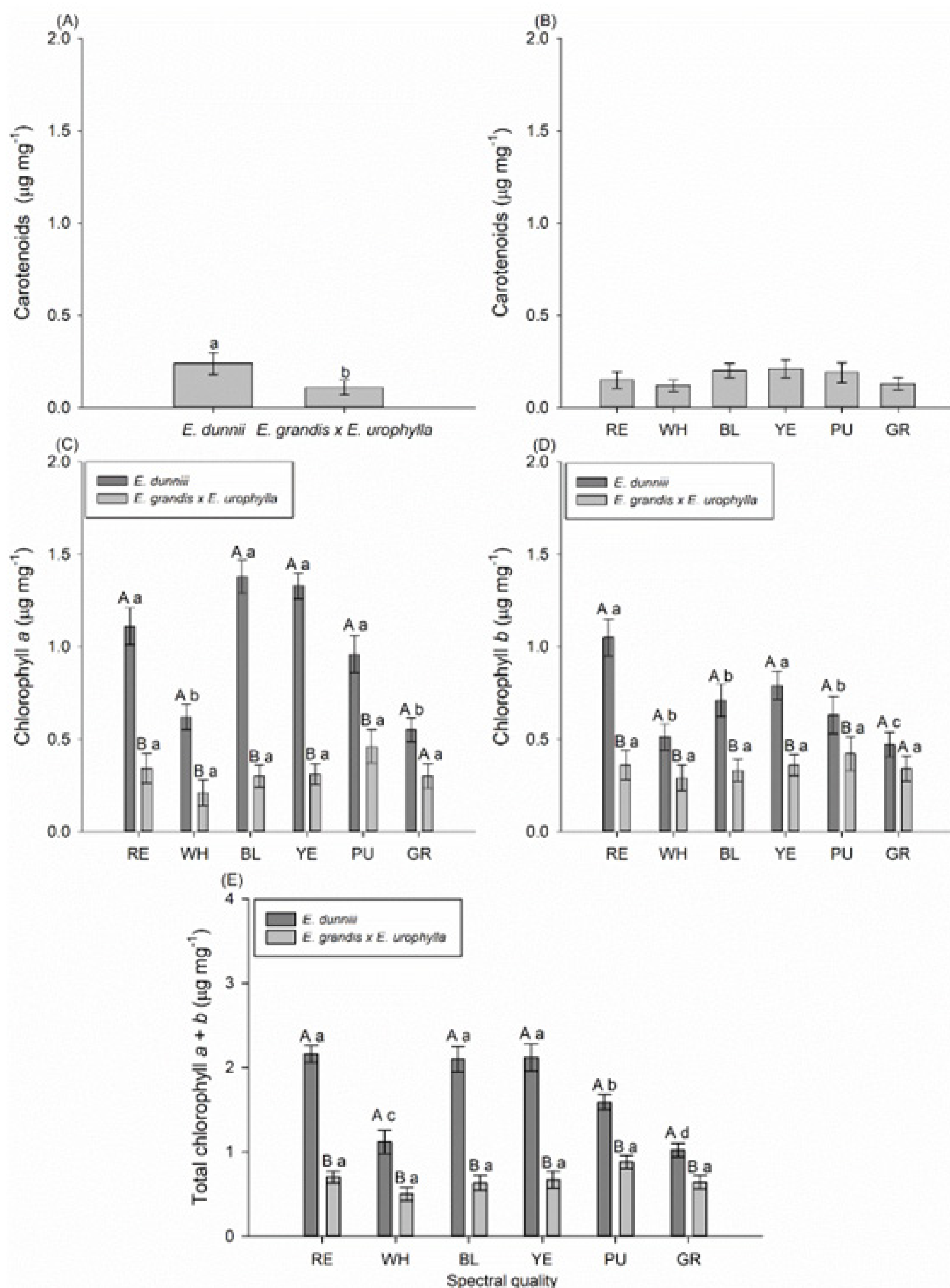

FIGURE 4: Photosynthetic pigment contents measured after 60 d during the *in vitro* multiplication stage of *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla* under different spectral qualities [i.e., red (RE), white (WH), blue (BL), yellow (YE), purple (PU), and green (GR)]. (A) Carotenoid content according to clone (n = 36, *p*-value = 0.001); (B) Carotenoid content according to spectral quality (n = 36, *p*-value = 0.222); (C) Chlorophyll *a* content (n = 36, *p*-value = 0.006); (D) Chlorophyll *b* content (n = 36, *p*-value = 0.007); and (E) Total chlorophyll (*a*+*b*) content (n = 36, *p*-value = 0.001). (A-B) Means followed by the same letters do not differ significantly by Tukey's test at $p < 0.05$. (C-E) Means followed by the different uppercase letters represent significant differences when comparing different clones given the same spectral quality, and different lowercase letters represent significant differences when comparing spectral quality within the same clone at $p < 0.05$. Error bars represent the standard error of the mean.

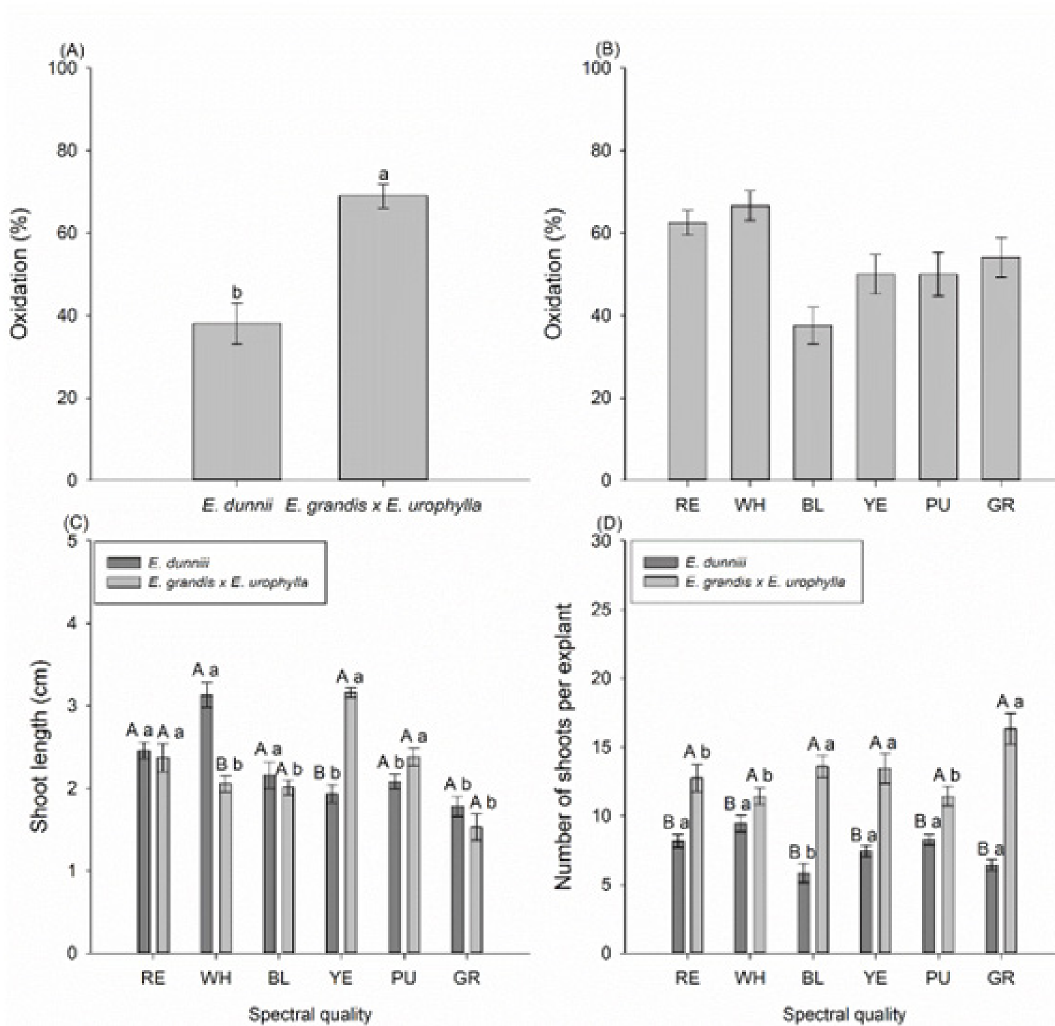

FIGURE 5: Morphological features measured after 35 d during the *in vitro* elongation stage of *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla* under different spectral qualities [i.e., red (RE), white (WH), blue (BL), yellow (YE), purple (PU), and green (GR)]. (A) Oxidation percentage according to clone (n = 144, *p*-value = 0.001); (B) Oxidation percentage according to spectral quality (n = 144, *p*-value = 0.329); (C) Shoot length per explant (cm) (n = 144, *p*-value = 0.003); and (D) Number of shoots per explant (n = 144, *p*-value = 0.001). (A-B) Means followed by the same letters do not differ significantly at $p < 0.05$. (C-D) Means followed by the different uppercase letters represent significant differences when comparing different clones given the same spectral quality, and different lowercase letters represent significant differences when comparing spectral quality within the same clone at $p < 0.05$. Error bars represent the standard error of the mean.

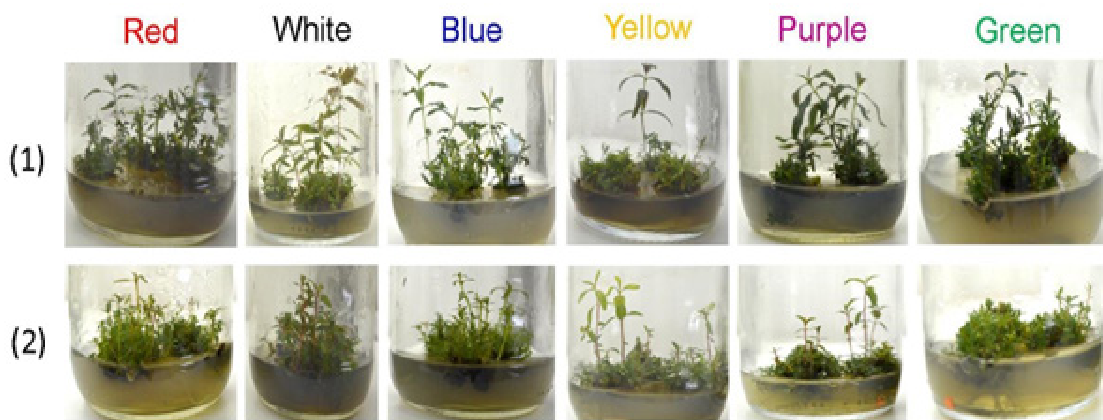

FIGURE 6: Visual characterization of *Eucalyptus dunnii* (1) and *Eucalyptus grandis* × *E. urophylla* (2) explants on *in vitro* elongation stage at 35 d according to spectral quality (i.e., red, white, blue, yellow, purple, and green).

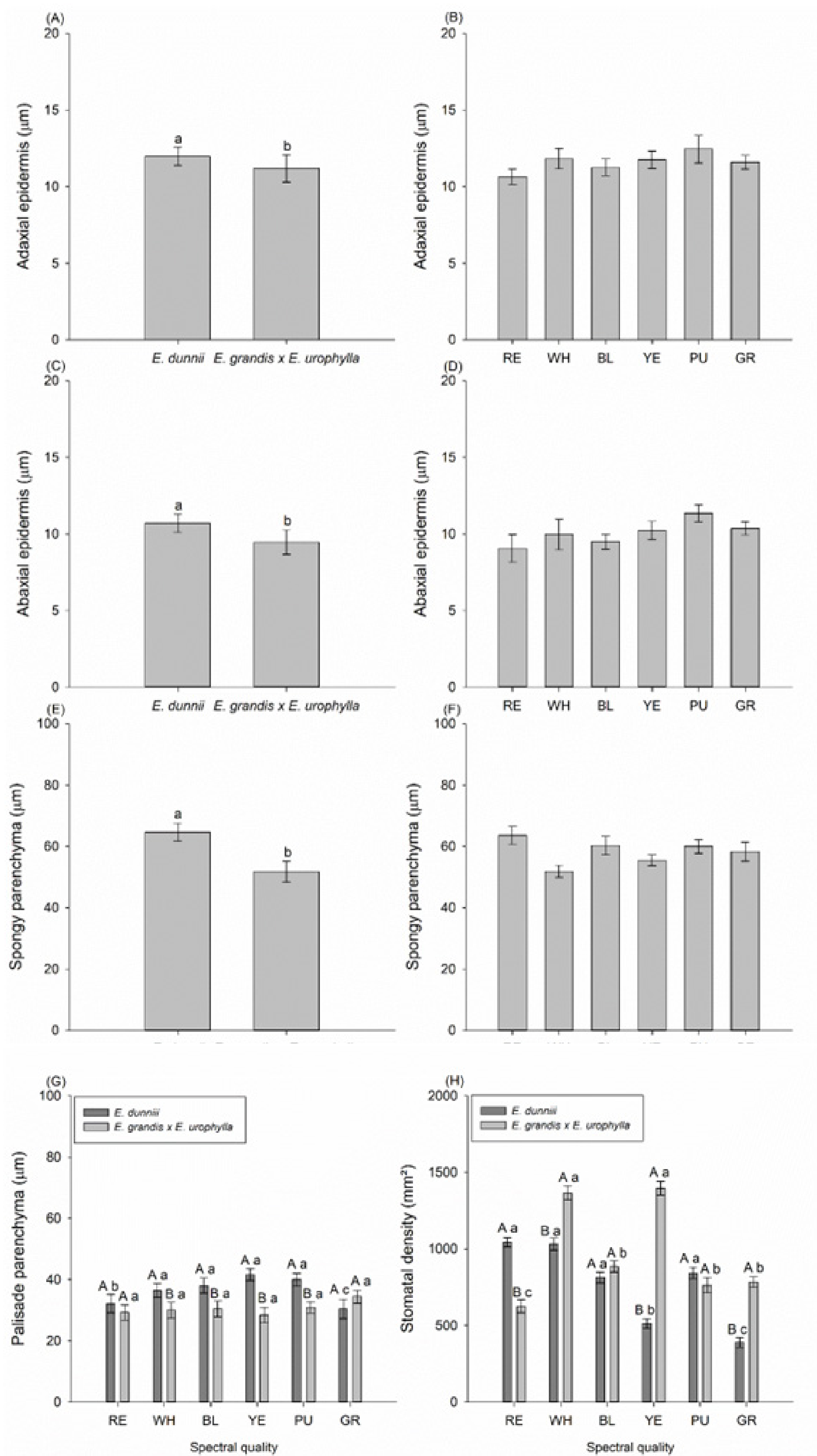

FIGURE 7: Anatomical features measured after 35 d during the *in vitro* elongation stage of *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla* under different spectral qualities [i.e., red (RE), white (WH), blue (BL), yellow (YE), purple (PU), and green (GR)]. (A) Adaxial epidermis thickness according to clone (n = 36, *p*-value = 0.001); (B) Adaxial epidermis thickness according to spectral quality (n = 36, *p*-value = 0.058); (C) Abaxial epidermis thickness according to species (n = 36, *p*-value = 0.001); (D) Abaxial epidermis thickness according to spectral quality (n = 36, *p*-value = 0.061); (E) Spongy parenchyma thickness according to species (n = 36, *p*-value = 0.001); (F) Spongy parenchyma thickness according to spectral quality (n = 36, *p*-value = 0.070); (G) Palisade parenchyma thickness according to clone and spectral quality (n = 36, *p*-value = 0.001). (H) Stomatal density according to species and spectral quality (n = 36, *p*-value = 0.001). (A-F) Means followed by the same letters do not differ significantly by Tukey's test at $p < 0.05$. (G-H) Means followed by the different uppercase letters represent significant differences when comparing different species given the same spectral quality, and different lowercase letters represent significant differences when comparing spectral quality within the same clone, by Tukey's test at $p < 0.05$. Error bars represent the standard error of the mean.

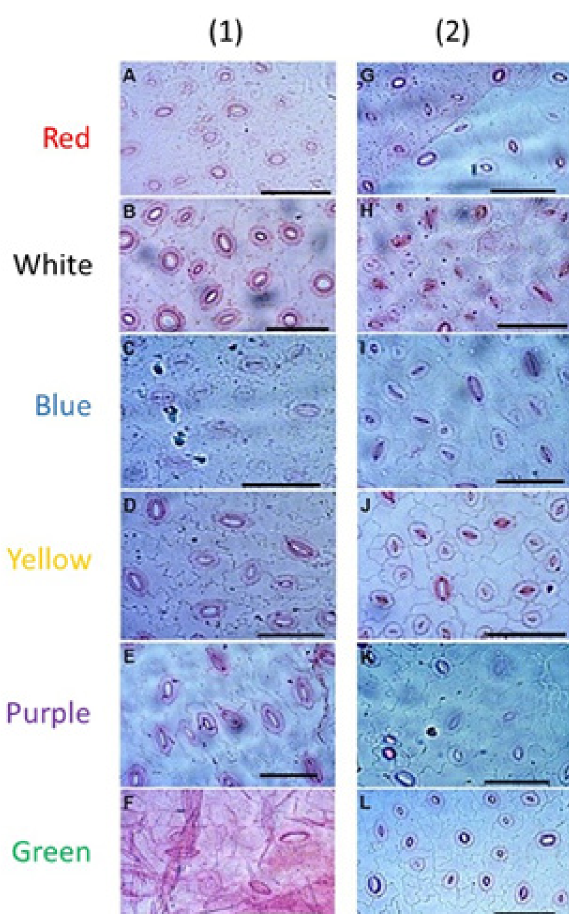

FIGURE 8: Abaxial leaf epidermis of *Eucalyptus dunnii* (1) and *Eucalyptus grandis* × *E. urophylla* (2) explants after 35 d during the *in vitro* elongation stage according to spectral quality (i.e., red, white, blue, yellow, purple, and green). Bar = 50.0 µm.

mean, differing significantly from the red and blue lights (Figure 9D). Thus, methods that aim to maximise rooting may become important strategies to be adopted in propagation systems. The appearance of rooted plants of *Eucalyptus grandis* × *E. urophylla* grown under the different light spectra and of *Eucalyptus dunnii* under the red spectral quality is shown in Figure 10.

Discussion

Effect of spectral quality on *in vitro* multiplication

Improving the micropropagation technique through the use for specific spectral qualities was studied in an attempt to establish efficient production systems of clonal plants for two eucalypts. Considering the established hypotheses, the results showed that clones generally responded differently to different wavelengths. The light source used in the *in vitro* multiplication stage influenced the oxidation percentage, shoot length and number of buds per explant of both *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla* clones, although with varying magnitudes (Figures 2A-E).

Lowest percentage of phenolic oxidation was observed under the white spectral quality for both clones (Figure 2B). In contrast, the use of white spectral quality resulted in greater oxidation in explants of *Corymbia torelliana* × *C. citriodora* (Souza et al. 2018). Tissue oxidation is a problem with the micropropagation of woody species, as reported in other studies (Oliveira et al. 2016; Souza et al. 2019; Souza et al. 2020b; Faria et al. 2021; Faria et al. 2022; Souza et al. 2022). These results may be linked to internal environmental factors that affect the vigor of explants, where smaller flasks tend to have reduced carbon dioxide concentrations and high ethylene concentrations, and may also be affected by light irradiation, air temperature, and relative humidity (Tisarum et al. 2018; Chen et al. 2019; Souza et al. 2021).

The best result observed in *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla* clones for shoot length was found under white and blue lights (Figure 2D). Due to the specificity of the wavelength, monochromatic light sources influence the photomorphogenic responses of explants grown in controlled environments, so they have emerged as a way to increase yield (Batista et al. 2018; Faria et al. 2019; Souza et al. 2022). Results described in the literature corroborate those found in this study, with spectral peaks of 450 nm in *Populus euramericana* (Kwon et al. 2015), *Acer saccharum* (Singh et al. 2017), *Corymbia citriodora* × *C. torelliana* (Souza et al. 2018), *Eucalyptus grandis* × *E. urophylla* (Souza et al. 2022) and *Zingiber officinale* (Gnasekaran et al. 2021) inducing more buds and greater shoot growth.

For the number of buds per explant, the white spectral quality provided the best results in the *Eucalyptus dunnii* clone, differing significantly from the green light (Figure 2E). Many studies have shown the superiority of white light sources for *in vitro* plant development (Batista et al. 2018; Abiri et al. 2020), although responses vary. Genotypic differences in the *in vitro* development have also been reported in different clones of *Eucalyptus*, such as *Eucalyptus grandis* × *E. urophylla* (Souza et al. 2020a; Miranda et al. 2020), *Eucalyptus benthamii* (Brondani et al. 2018), and *Eucalyptus globulus* (Oliveira et al. 2016). Thus, different results are observed for explants in the *in vitro* multiplication stage, the response varying according to the genotype and the culture conditions (e.g., spectral quality).

Eucalyptus dunnii had the highest levels of the photosynthetic pigments analysed [carotenoids, chlorophyll *a* and *b*, and total chlorophyll (*a*+*b*)] (Figures 4A-E). The biosynthesis of carotenoids and chlorophyll *a* and *b* is influenced by the clones and cultivation conditions, and certain conditions may result in greater photosynthetic efficiency (Gupta & Karmakar 2017; Oliveira et al. 2021). In addition, the effect of different wavelengths on the biosynthesis of photosynthetic pigments may depend on the tissues of the examined plants (Tisarum et al. 2018; Jung et al. 2021). The red, blue and yellow spectral qualities resulted in higher chlorophyll (*a*+*b*) content in *Eucalyptus dunnii*, and no variation was observed for urograndis eucalypt (Figure 4E). The primary photosynthetic pigments in higher plants, carotenoids and chlorophyll *a* and *b*, absorb

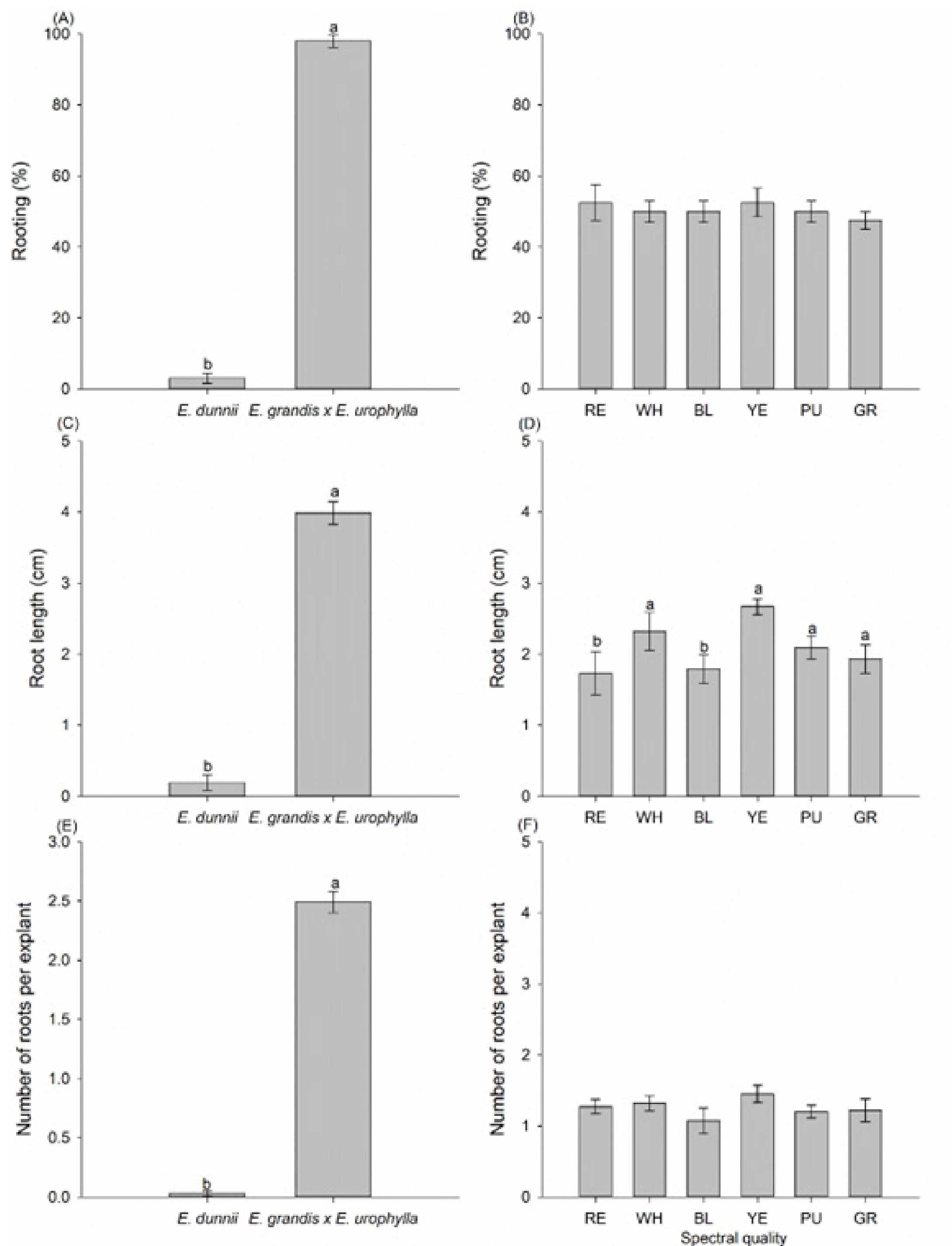

FIGURE 9: Morphological features measured on *in vitro* adventitious rooting of *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla* under different spectral qualities [i.e., red (RE), white (WH), blue (BL), yellow (YE), purple (PU), and green (GR)] at 35 d. (A) Rooting percentage according to species (n = 240, *p*-value = 0.001); (B) Rooting percentage according to spectral quality (n = 240, *p*-value = 0.067); (C) Root length (cm) according to species (n = 240, *p*-value = 0.001); (D) Root length (cm) according to spectral quality (n = 240, *p*-value = 0.001); (E) Number of roots per explant according to species (n = 240, *p*-value = 0.001); Number of roots per explant according to spectral quality (n = 240, *p*-value = 0.074). Means followed by the same letters do not differ significantly by Tukey's test at $p < 0.05$. Error bars represent the standard error of the mean.

FIGURE 10: *In vitro* rooted explants of *Eucalyptus grandis* × *E. urophylla* (left) under different spectral quality and *Eucalyptus dunnii* (right) under red light at 35 d. Bar = 1.0 cm.

certain wavelengths, especially blue and red, playing key roles in morphology control (Gupta & Karmakar 2017; Oliveira et al. 2021). The light absorption at specific wavelengths can promote a higher-energy state (Faria et al. 2019; Abiri et al. 2020). This excitation energy of the chlorophyll molecule can be used in the photochemical step, be lost in the form of heat, or cause damage to the photosynthetic apparatus, such as in the formation of blue free radicals (Abiri et al. 2020).

In *Gerbera jamesonii*, higher chlorophyll *a* and *b* contents have been observed under peak wavelengths of 450 and 600 nm (Pawłowska et al. 2018), and in *Stevia rebaudiana*, the amount of carotenoids in shoots exposed to wavelengths of 450 and 600 nm were reportedly higher than those in shoots exposed to 450 nm (blue LED) (Ramírez-Mosqueda et al. 2017). Therefore, knowledge of the relationship between wavelengths and plant growth patterns in micropropagation can lead to a better understanding of the clones studied.

Effect of spectral quality on *in vitro* elongation

The effects of specific wavelengths on each clone are reflected in the photomorphological responses of propagules grown in controlled environments. This technology is emerging as a useful way to optimise shoot development and yield. Wavelength was found to be an important factor on *in vitro* elongation, having direct implications on oxidation, shoot length, and the number of shoots per explant in *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla* (Figures 5A-D). The control of spectral quality is essential for plant cultivation, as it can optimise the photosynthetic rate and shoot development (Silva et al. 2017). Such optimisation was observed with the white light for *Eucalyptus dunnii* and the yellow light for *Eucalyptus grandis* × *E. urophylla* (Figure 5C).

Our findings are in line with the literature. Greater absorption of a broad light spectrum (450-600 nm) has induced a greater number of shoots and higher shoot development in *Eucalyptus urophylla* (Miranda et al. 2020) and *Populus euramericana* (Kwon et al. 2015). The use of white or yellow light in crops can increase plant growth and yield, as it allows light to better penetrate the leaves, maximising the photosynthetic rate when compared to the use of blue and red monochromatic lights, as observed in *Lippia rotundifolia* (Hsie et al. 2019). These studies show that plants need a broad light spectrum to optimise their photosynthetic processes, although such requirements vary by species.

On the analysed anatomical features, there was an influence of wavelength and clone after 35 d during the *in vitro* culture (Figures 7A-H). The highest values found for adaxial (Figure 7A) and abaxial (Figure 7C) epidermis thickness, spongy parenchyma thickness (Figure 7E), and palisade parenchyma thickness (Figure 7G) were observed in *Eucalyptus dunnii*. However, the highest stomatal density was observed in the *Eucalyptus grandis* × *E. urophylla* (Figure 7H). The morphological, anatomical, and physiological responses of plants vary by genotype, which are translated into changes in growth and development to adapt to changes in environmental conditions (Cioć et al. 2019; Miranda et al. 2020; Souza et al. 2020a; Nery et al. 2021).

Among the spectral qualities used, there was a significant interaction effect only on the palisade parenchyma thickness (Figure 7G) and stomatal density (Figure 7H). The use of yellow light resulted in the highest palisade parenchyma thickness in *Eucalyptus dunnii*, differing from the red and green lights (Figure 7G). Similar results, using a light source with a broad light spectrum, have been observed in *Ajuga genevensis* (Sahakyan et al. 2016), *Abies* × *borisii-regis* (Smirnakou et al. 2017) and *Polygala paniculata* (Nery et al. 2021). The adverse effect of red LEDs on plant development has been reported in *Curculigo orchioides* (Gupta & Sahoo 2015). Kwon et al. (2015) observed a greater number of dividing parenchyma cells in *Populus euramericana* when subjected to treatment with red/blue light, resulting in greater shoot and leaf development. In addition, Macedo

et al. (2011) reported that the blue wavelength (450 nm) led to an increase in epidermal, mesophyll, and palisade parenchymal cell thickness in the *in vitro* culture of *Alternanthera brasiliana*.

Regarding the stomatal density in the analysed clones (Figure 7H), it was found that the red, white, blue, and purple spectral qualities provided the best results for *Eucalyptus dunnii*; and the white and yellow for *Eucalyptus grandis* × *E. urophylla*. Li et al. (2018) observed that yellow and blue LEDs were more advantageous than green LEDs for the morphology and anatomy of *Solanum tuberosum* plants. In plants of *Capsicum chinense* grown in pots under red light, thicker and differentiated epidermal and parenchymal cells were found (Santana-Buzzy et al. 2005).

The wavelength of light influences the growth and development of plant cells, tissues, and organs, triggering different morphological and anatomical responses (Cioć et al. 2019; Miranda et al. 2020; Nery et al. 2021). Changes in the structural organisation of leaf cells can be observed at the anatomical level when plants are exposed to different environments, including different light spectra. These results are similar to those found by Souza et al. (2020b) in *Eucalyptus grandis* × *E. urophylla* explants under a broad light spectrum. Each spectrum can alter the expression levels of specific genes involved in the structural organisation of plant cells and in photosynthetic performance (Cioć et al. 2019; Silveira et al. 2020). In addition, studies show that exposure to red and green light leads to low stomatal conductance and that this is an ecological adaptation to avoid excessive leaf water loss (Aasamaa & Aphalo 2016).

Our morphological and anatomical results have important implications for the optimisation of the production of clonal plants through the micropropagation technique. The broad light spectrum, through the yellow and white light qualities, was found to be suitable for the *in vitro* elongation of explants in controlled environments.

Effect of spectral quality on *in vitro* adventitious rooting

Eucalyptus grandis × *E. urophylla* clone showed the best results of adventitious rooting (Figures 9A, 9C, and 9E). Adventitious rooting of *Eucalyptus dunnii* is a challenge in clonal propagation, varying from 3.0 to 46.5% (Brondani et al. 2011; Oberschelp et al. 2015; Souza et al. 2019). In contrast, the *Eucalyptus grandis* × *E. urophylla* has shown 80.0 to 100.0% (Gallo et al. 2017; Souza et al. 2020b; Miranda et al. 2020). Some species, such as *Hevea brasiliensis* are considered difficult-to-root because of the presence of an almost continuous cylinder of lignified tissue that, together with chemical barriers, hinder root emission (Almeida et al. 2017).

Under the different spectral qualities analysed, there was a significant difference between treatments only on the root length (Figure 9D), with the best results observed under the white and yellow lights. In *Abies* × *borisii-regis* explants, the best root development results were observed using a white light source (Smirnakou et al. 2017). In contrast, the rooting and survival percentages and the number of roots in *Corymbia citriodora* × *C. torelliana* and *Corymbia torelliana* × *C. citriodora* microcuttings were greatest when using the red/blue spectral quality (Souza et al. 2018).

Although light quality is an important factor for the *in vitro* culture, there are few studies on its effect in woody species. The absence of induction of adventitious roots is one of the main causes limiting cloning by the micropropagation technique (Almeida et al. 2017; Souza et al. 2022). Thus, because the efficiency of cultivation conditions in plant production varies between species, a broad light spectrum is often needed. Complementary studies are recommended to maximise the *in vitro* rooting of the *Eucalyptus dunnii* clone using other spectral qualities.

Conclusions

Light spectra triggered large and differential responses in morphophysiological features of *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla* clones. White spectral quality is most suitable for *in vitro* multiplication of *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla*. White spectral quality is also the most suitable for *in vitro* elongation of *Eucalyptus dunnii*, and the yellow for *Eucalyptus grandis* × *E. urophylla*. White and yellow spectral qualities are the most suitable for *in vitro* adventitious rooting of the *Eucalyptus dunnii* and *Eucalyptus grandis* × *E. urophylla* clones.

Competing interests

The authors declare that they have no competing interests.

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Abbreviations

WPM	Wood Plant Medium (Lloyd and McCown 1980)
BAP	6-benzylaminopurine
NAA	α-naphthaleneacetic acid
IBA	indole-3-butyric acid
DMSO	dimethyl sulfoxide
LED	light-emitting diode
AAF	acetic acid formaldehyde

Authors' contributions

SRPF was the primary author, conducted the experiments, analyses, review and discussion. DMSCS, SBF, MLMA, LVM GEB and DSG contributed to the analyses, review and discussion; GEB also provided supervision. TAM contributed to the review and discussion. All authors contributed to writing the manuscript.

References

Aasamaa, K., & Aphalo, P.J. (2016). Effect of vegetation shade and its components on stomatal responses to red, blue and green light in two deciduous tree species with different shade tolerance. *Environmental and Experimental Botany*, *121*, 94-101. https://doi.org/10.1016/j.envexpbot.2015.01.004

Abiri, R., Atabaki, N., Abdul-Hamid, H., Sanusi, R., Shukor, N.A.A., Shaharuddin, N.A., Ahmad, S.A., & Malik, S. (2020). The prospect of physiological events associated with the micropropagation of *Eucalyptus* sp. *Forests*, *11*(11), 1211. https://doi.org/10.3390/f11111211

Almeida, M.R., Aumond, Jr.M., Costa, C.T., Schwambach, J., Ruedell, C.M., Correa, L.R., & Fett-Neto, A.G. (2017). Environmental control of adventitious rooting in *Eucalyptus* and *Populus* cuttings. *Trees*, *31*, 1377-1390. https://doi.org/10.1007/s00468-017-1550-6

Ashrafzadeh, S., & Leung, D.W.M. (2021). Photoautotrophic micropropagation system (PAM): a novel approach deserving increased uptake for commercial plant production. *Vegetos*, *34*, 13-18. https://doi.org/10.1007/s42535-020-00182-x

Batista, D.S., Felipe, S.H.S., Silva, T.D., Castro, K.M., Mamedes-Rodrigues, T.C., Miranda, N.A., Ríos-Ríos, A.M., Faria, D.V., Fortini, E.A., Chagas, K., Torres-Silva, G., Xavier, A., Arencibia, A.D., & Otoni, W.C. (2018). Light quality in plant tissue culture: does it matter? *In Vitro Cellular & Developmental Biology – Plant*, *54*, 195-215. https://doi.org/10.1007/s11627-018-9902-5

Brondani, G.E., Dutra, L.F., Wendling, I., Grossi, F., Hansel, F.A., & Araujo, M.A. (2011). Micropropagation of an *Eucalyptus* hybrid (*Eucalyptus benthamii* × *Eucalyptus dunnii*). *Acta Scientiarum. Agronomy*, *33*(4), 655-663. https://doi.org/10.4025/actasciagron.v33i4.8317

Brondani, G.E., Oliveira, L.S., Konzen, E.R., Silva, A.L.L., & Costa, J.L. (2018). Mini-incubators improve the adventitious rooting performance of *Corymbia* and *Eucalyptus* microcuttings according to the environment in which they are conditioned. *Anais da Academia Brasileira de Ciências*, *90*(2; suppl. 1), 2409-2423. https://doi.org/10.1590/0001-3765201720170284

Carrillo, I., Mendonça, R.T., Ago, M., & Rojas, O.J. (2018). Comparative study of cellulosic components isolated from different *Eucalyptus* species. *Cellulose*, *25*, 1011-1029. https://doi.org/10.1007/s10570-018-1653-2

Chen, Y.-M., Huang, J.-Z., Hou, T.-W., & Pan, I.-C. (2019). Effects of light intensity and plant growth regulators on callus proliferation and shoot regeneration in the ornamental succulent *Haworthia*. *Botanical Studies*, *60*, 10. https://doi.org/10.1186/s40529-019-0257-y

Cioć, M., Kalisz, A., Żupnik, M., & Pawłowska, B. (2019). Different LED light intensities and 6-benzyladenine concentrations in relation to shoot development, leaf architecture, and photosynthetic pigments of *Gerbera jamesonii* Bolus *in vitro*. *Agronomy*, *9*(7), 358. https://doi.org/10.3390/agronomy9070358

Faria, D.V., Correia, L.N.F., Souza, M.V.C., Ríos-Ríos, A.M., Vital, C.E., Batista, D.S., Costa, M.G.C., & Otoni, W.C. (2019). Irradiance and light quality affect two annatto (*Bixa orellana* L.) cultivars with contrasting bixin production. *Journal of Photochemistry and Photobiology B: Biology*, *197*, 111549. https://doi.org/10.1016/j.jphotobiol.2019.111549

Faria, J.C.T., Ribeiro-Kumara, C., Costa, R.S.R., Nieri, E.M., Carvalho, D., Pinto, J.E.B.P., Sena Neto, A.R., & Brondani, G.E. (2022). Use of biodegradable polyester-based microvessels for micropropagation of mature *Eucalyptus microcorys*. *New Zealand Journal of Forestry Science*, *52*, 10. https://doi.org/10.33494/nzjfs522022x139x

Faria, J.C.T., Terra, J.A.P., Molinari, L.V., Delarmelina, W.M., Ribeiro-Kumara, C., Sena Neto, A.R., Carvalho, D., & Brondani, G.E. (2021). Use of polylactic acid microvessel to obtain microplantlets of *Eucalyptus microcorys* through indirect organogenesis. *3 Biotech*, *11*, 364. https://doi.org/10.1007/s13205-021-02822-8

Ferreira, E.B., Cavalcanti, P.P., & Nogueira, D.A. (2013). *ExpDes*: experimental designs package. R package version 1.1.2.

Gallo, R., Xavier, A., Oliveira, B.A., Moura, L.C., Miranda, N.A., Gatti, K.C., & Otoni, W.C. (2017). Micro-cutting propagation of *Eucalyptus grandis* × *Eucalyptus urophylla* through clumps of axillary buds using different containers and substrates. *Australian Journal of Crop Science*, *11*(10), 1327-1333. https://doi.org/10.21475/ajcs.17.11.10.pne607

Gilmartin, P.M., Sarokin, L., Memelink, J., & Chua, N.H. (1990). Molecular light switches for plant genes. *The Plant Cell*, *2*(5), 369-378. https://doi.org/10.1105/tpc.2.5.369

Gnasekaran, P., Rahman, Z.A., Chew, B.L., Appalasamy, S., Mariappan, V., & Subramaniam, S. (2021). Development of micropropagation system of

Zingiber officinale var. *rubrum* Theilade using different spectrum light-emitting diode (LED) irradiation. *Industrial Crops and Products*, *170*, 113748. https://doi.org/10.1016/j.indcrop.2021.113748

Gupta, S.D., & Karmakar, A. (2017). Machine vision based evaluation of impact of light emitting diodes (LEDs) on shoot regeneration and the effect of spectral quality on phenolic content and antioxidant capacity in *Swertia chirata*. *Journal of Photochemistry and Photobiology B: Biology*, *174*, 162-172. https://doi.org/10.1016/j.jphotochem.2017.09.033

Gupta, S.D., & Sahoo, T.K. (2015). Light emitting diode (LED)-induced alteration of oxidative events during *in vitro* shoot organogenesis of *Curculigo orchioides* Gaertn. *Acta Physiologiae Plantarum*, *37*, 233. https://doi.org/10.1007/s11738-015-1990-9

He, G., Elling, A.A., & Deng, X.W. (2011). The epigenome and plant development. *Annual Review of Plant Biology*, *62*, 411-435. https://doi.org/10.1146/annurev-arplant-042110-103806

Hsie, B.S., Bueno, A.I.S., Bertolucci, S.K.V., Carvalho, A.A., Cunha, S.H.B., Martins, E.R., & Pinto, J.E.B.P. (2019). Study of the influence of wavelengths and intensities of LEDs on the growth, photosynthetic pigment, and volatile compounds production of *Lippia rotundifolia* Cham *in vitro*. *Journal of Photochemistry and Photobiology B: Biology*, *198*, 111577. https://doi.org/10.1016/j.jphotobiol.2019.111577

Jung, W.-S., Chung, I.-M., Hwang, M.H., Kim, S.-H., Yu, C.Y., & Ghimire, B.K. (2021). Application of light-emitting diodes for improving the nutritional quality and bioactive compound levels of some crops and medicinal plants. *Molecules*, *26*(5), 1477. https://doi.org/10.3390/molecules26051477

Kölling, K., Thalmann, M., Müller, A., Jenny, C., Zeeman, S.C. (2015). Carbon partitioning in *Arabidopsis thaliana* is a dynamic process controlled by the plants metabolic status and its circadian clock. *Plant, Cell and Environment*, *38*, 1965-1979. https://doi.org/10.1111/pce.12512

Kwon, A.-R., Cui, H.-Y., Lee, H., Shin, H., Kang, K.-S., & Park, S.-Y. (2015). Light quality affects shoot regeneration, cell division, and wood formation in elite clones of *Populus euramericana*. *Acta Physiologiae Plantarum*, *37*, 65. https://doi.org/10.1007/s11738-015-1812-0

Li, R., Huang, W., Wang, X., Liu, X., & Xu, Z. (2018). Effects of yellow, green, and different blue spectra on growth of potato plantlets *in vitro*. *HortScience*, *53*(4), 541-546. https://doi.org/10.21273/HORTSCI12848-18

Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: Packer, L., & Douce, R. (eds). Methods in enzymology. vol 1. Academic, London, pp 350-381. https://doi.org/10.1016/0076-6879(87)48036-1

Lloyd, G., & McCown, B. (1980). Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. *Combined Proceedings of the International Plant Propagators Society*, *30*, 421-427.

Macedo, A.F., Leal-Costa, M.V., Tavares, E.S., Lage, C.L.S., & Esquibel, M.A. (2011). The effect of light quality on leaf production and development of *in vitro*-cultured plants of *Alternanthera brasiliana* Kuntze. *Environmental and Experimental Botany*, *70*(1), 43-50. https://doi.org/10.1016/j.envexpbot.2010.05.012

Miranda, N.A., Xavier, A., Otoni, W.C., Gallo, R., Gatti, K.C., Moura, L.C., Souza, D.M.S.C., Maggione, J.H., & Santos, S.S.O. (2020). Quality and intensity of light in the *in vitro* development of microstumps of *Eucalyptus urophylla* in a photoautotrophic system. *Forest Science*, *66*(6), 754-760. https://doi.org/10.1093/forsci/fxaa027

Molinari, L.V., Souza, D.M.S.C., Avelar, M.L.M., Fernandes, S.B., Gonçalves, D.S., Faria, J.C.T., Carvalho, D.C., & Brondani, G.E. (2021). Effects of chemical sterilization of the culture media, porous membranes and luminosity on *in vitro* culture of *Eucalyptus grandis* × *Eucalyptus urophylla*. *Journal of Forestry Research*, *32*, 1587-1598. https://doi.org/10.1007/s11676-020-01240-5

Nery, L.A., Batista, D.S., Rocha, D.I., Felipe, S.H.S., Queiroz, M.C., Silva, P.O., Ventrella, M.C., & Otoni, W.C. (2021). Leaf development and anatomy of *in vitro*-grown *Polygala paniculata* L. are affected by light quality, gelling agents, and sucrose. *Vegetos*, *34*, 19-28. https://doi.org/10.1007/s42535-021-00192-3

Nord, E.A., Lynch, J.P. (2009). Plant phenology: a critical controller of soil resource acquisition, *Journal of Experimental Botany*, *60*(7), 1927-1937. https://doi.org/10.1093/jxb/erp018

Oberschelp, G.P.J., Gonçalves, A.N., Meneghetti, E.C., Graner, É., & Almeida, M. (2015). *Eucalyptus dunnii* Maiden plant regeneration via shoot organogenesis on a new basal medium based on the mineral composition of young stump shoots. *In Vitro Cellular & Developmental Biology – Plant*, *51*, 626-636. https://doi.org/10.1007/s11627-015-9715-8

Oliveira, L.S., Xavier, A., Lopes, A.P., Takahashi, E.K., & Otoni, W.C. (2016). *In vitro* multiplication and elongation of hybrid clones of *Eucalyptus globulus*. *Ciência Florestal*, *26*(1), 235-247. https://doi.org/10.5902/1980509821524

Oliveira, T., Balduino, M.C.M., Carvalho, A.A., Bertolucci, S.K.V., Cossa, M.C., Coelho, A.D., Leite, J.J.F., & Pinto, J.E.B.P. (2021). The effect of alternative membrane system, sucrose, and culture methods under photosynthetic photon flux on growth and volatile compounds of mint *in vitro*. *In Vitro Cellular & Developmental Biology – Plant*, *57*, 529-540. https://doi.org/10.1007/s11627-020-10147-z

Pawłowska, B., Żupnik, M., Szewczyk-Taranek, B., & Cioć, M. (2018). Impact of LED light sources on morphogenesis and levels of photosynthetic pigments in *Gerbera jamesonii* grown *in vitro*. *Horticulture, Environment, and Biotechnology*, *59*, 115-123. https://doi.org/10.1007/s13580-018-0012-4

R Core Team (2014). *R:* A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Ramírez-Mosqueda, M.A., Iglesias-Andreu, G., & Bautista-Aguilar, J.R. (2017). The effect of light quality on growth and development of *in vitro* plantlet of *Stevia rebaudiana* Bertoni. *Sugar Tech*, *19*, 331-336. https://doi.org/10.1007/s12355-016-0459-5

Rokich, D.P., & Bell, D.T. (1995). Light quality and intensity effects on the germination of species from the jarrah (*Eucalyptus marginata*) forest of Western Australia. *Australian Journal of Botany*, *43*(2), 169-179. https://doi.org/10.1071/BT9950169

Sahakyan, N., Petrosyan, M., & Trchounian, A. (2016). Comparative analysis of chemical composition and biological activities of *Ajuga genevensis* L. in *in vitro* culture and intact plants. *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*, *10*(7), 322-325.

Santana-Buzzy, N., Canto-Flick, A., Barahona-Pérez, F., Montalvo-Peniche, M.C., Zapata-Castillo, P.Y., Solís-Ruiz, A., Zaldívar-Collí, A., Gutiérrez-Alonso, O., & Miranda-Ham, M.L. (2005). Regeneration of Habanero pepper (*Capsicum chinense* Jacq.) via organogenesis. *HortScience*, *40*(6), 1829-1831. https://doi.org/10.21273/HORTSCI.40.6.1829

Silva, S.T., Bertolucci, S.K.V., Cunha, S.H.B., Lazzarini, L.E.S., Tavares, M.C., & Pinto, J.E.B.P. (2017). Effect of light and natural ventilation systems on the growth parameters and carvacrol content in the *in vitro* cultures of *Plectranthus amboinicus* (Lour.) Spreng. *Plant Cell, Tissue and Organ Culture*, *129*, 501-510. https://doi.org/10.1007/s11240-017-1195-6

Silveira, A.A.C., Lopes, F.J.F., & Sibov, S.T. (2020). Micropropagation of *Bambusa oldhamii* Munro in heterotrophic, mixotrophic and photomixotrophic systems. *Plant Cell, Tissue and Organ Culture*, *141*, 315-326. https://doi.org/10.1007/s11240-020-01788-4

Singh, A.S., Jones, A.M.P., Shukla, M.R., & Saxena, P.K. (2017). High light intensity stress as the limiting factor in micropropagation of sugar maple (*Acer saccharum* Marsh.). *Plant Cell, Tissue and Organ Culture*, *129*, 209-221. https://doi.org/10.1007/s11240-017-1170-2

Smirnakou, S., Ouzounis, T., & Radoglou, K. (2017). Effects of continuous spectrum LEDs used in indoor cultivation of two coniferous species *Pinus sylvestris* L. and *Abies borisii-regis* Mattf. *Scandinavian Journal of Forest Research*, *32*(2), 115-122. https://doi.org/10.1080/02827581.2016.1227470

Souza, D.M.S.C., Avelar, M.L.M., Fernandes, S.B., Silva, E.O., Duarte, V.P., Molinari, L.V., & Brondani, G.E. (2020a). Spectral quality and temporary immersion bioreactor for *in vitro* multiplication of *Eucalytpus grandis* × *Eucalyptus urophylla*. *3 Biotech*, *10*, 457. https://doi.org/10.1007/s13205-020-02447-3

Souza, D.M.S.C., Fernandes, S.B., Avelar, M.L.M., Frade, S.R.P., Molinari, L.V., Gonçalves, D.S., & Brondani, G.E. (2019). Mixotrophism effect on *in vitro* elongation and adventitious rooting of *Eucalyptus dunnii*. Cerne *25*(4), 394-401. https://doi.org/10.1590/01047760201925042638

Souza, D.M.S.C., Fernandes, S.B., Avelar, M.L.M., Frade, S.R.P., Molinari, L.V., Gonçalves, D.S., Pinto, J.E.B.P., & Brondani, G.E. (2020b). Light quality in micropropagation of *Eucalyptus grandis* × *Eucalyptus urophylla*. *Scientia Forestalis*, *48*(127), e3329. https://doi.org/10.18671/scifor.v48n127.03

Souza, D.M.S.C., Fernandes, S.B., Molinari, L.V., Avelar, M.L.M., Gonçalves, D.S., Faria, J.C.T., Teixeira, G.L., & Brondani, G.E. (2021). The influence of light quality in the *in vitro* cultivation of *Cattleya crispata* (Thunb.) Van den Berg. *Nativa*, *9*(5), 582-588. https://doi.org/10.31413/nativa.v9i5.12541

Souza, D.M.S.C., Fernandes, S.B., Silva, E.O., Duarte, V.P., Gonçalves, D.S., Carvalho, D., Teixeira, G.L., & Brondani, G.E. (2022). Effect of light intensity on *in vitro* introduction and multiplication of *Eucalyptus grandis* × *Eucalyptus urophylla*. *In Vitro Cellular & Developmental Biology – Plant*, *58*, 225-239. https://doi.org/10.1007/s11627-021-10237-6

Souza, D.M.S.C., Xavier, A., Otoni, W.C., Miranda, N.A., & Maggioni, J.H. (2018). Light quality in the *in vitro* introduction of *Corymbia* hybrid clones. *Revista Árvore*, *42*(6), e420604. https://doi.org/10.1590/1806-90882018000600004

Tisarum, R., Samphumphung, T., Theerawitaya, C., Prommee, W., & Cha-um, S. (2018). *In vitro* photoautotrophic acclimatization direct transplantation and *ex vitro* adaptation of rubber tree (*Hevea brasiliensis*). *Plant Cell, Tissue and Organ Culture*, *133*, 215-223. https://doi.org/10.1007/s11240-017-1374-5