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Influence of spectral quality on the rooting of *Corymbia* and *Eucalyptus* spp. minicuttings

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Abstract The pursuit of better adaptation in clonal plants seedling production processes based on the minicutting technique has expanded the use of species and hybrid combinations of genera *Corymbia* and *Eucalyptus* in the composition of commercial crops. The aim of the work was to evaluate the effect of spectral quality on the rooting of *Eucalyptus andrewsii*, *E. saligna*, *E. microcorys*, *E. cloeziana*, *E. pilularis*, *E. grandis*, *E. grandis* × *E. urophylla* and *Corymbia torelliana* minicuttings to help better understanding the production of clonal plants. *E. grandis* × *E. urophylla* and *C. torelliana* root anatomy was analyzed. The effects of spectral quality on the rooting of minicuttings were evaluated based on three sources (fluorescent, red and blue). Survival (SUR), callogenesis (CAL), oxidation (OXI) and rooting (RO) percentage; length (RL) and diameter of the largest root (ROD); mean number of roots per minicutting (NRM), root epidermis thickness (RET), root cortex diameter (RCD), diameter of the root vascular cylinder (DRVC) and root diameter (RD) were evaluated at 30 days. Based on the results, wavelength specificity was a useful technology to optimize the large-scale production of clonal plants of *Eucalyptus*. Fluorescent spectral quality was the most appropriate source in the rooting of *E. saligna* (68.7%), *E. microcorys* (43.7%), *E. pilularis* (75.0%) and *C. torelliana* (75.0%) minicuttings; blue spectral quality was the most appropriate for *E. andrewsii* (55.5%), *E. grandis* (75.0%) and *E. grandis* × *E. urophylla* (81.3%); and red spectral quality was the most appropriate for *E. cloeziana* (56.2%).

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Introduction

Crop expansion in non-traditional Brazilian zones and the pursuit of better adaptation in clonal plant processes have increased the species numbers and provided hybrid combinations of genera *Corymbia* D. Hill & L.A.S. Johnson and *Eucalyptus* L'Hér. Thus, the msean yield of forests is growing, mainly due to the combination of results deriving from genetic improvement, the use of advanced technologies, increasing vegetative propagation knowledge (Tambarussi et al. 2018, Yang et al. 2018, Díaz Sala 2020), as well as from advanced silvicultural practices (Xavier et al. 2013, Trueman et al. 2018).

Minicutting is the most applied propagation technique for producing clonal plants of species *Corymbia* and *Eucalyptus* species being adopted by most producers in the Brazilian forest sector (Kuppusamy et al. 2019, Estevez et al. 2020). This technique has led to considerable gains in plant production, mainly in rooting percentage, improved root systems, and shorter seedling formation time. It influences the quantity, quality, and performance, especially with homogeneous stands (Freitas et al. 2017, Lopes et al. 2019, Kuppusamy et al. 2019, Miranda et al. 2020).

Although this is a well-established technique, literature reports differences in minicutting rooting percentage between *Corymbia* and *Eucalyptus* species and between clones of the same species (Brondani et al. 2018, Luo et al. 2019, Estevez et al. 2020). Therefore, the success or failure of clonal production based on adventitious rooting depends on the knowledge about the influence of the endogenous and exogenous factors affecting rhizogenesis (Nakhooda & Watt 2017), such as growth regulators, genotype, gene expression, substrate composition, as well as environmental factors such as luminosity (spectral quality and luminous intensity), temperature and humidity (De Almeida et al. 2017, Díaz Sala 2020, Souza et al. 2020a, Zorz et al. 2020, Molinari et al. 2020).

Recent studies have reported that different wavelength levels influence plant metabolism (Souza et al. 2018, Faria et al. 2019, Abiri et al. 2020). According to Batista et al. (2018) blue (450 - 495 nm) and red (620 - 750 nm) lights influence plant morphogenesis, rhizogenesis, growth, and development. In addition to photosynthetic processes, light can act as an external regulation signal in several physiological processes that can influence plants' shoot and root development (Hsie et al. 2019, Faria et al. 2019). According to Miranda et al. (2020), Souza et al. (2020a), Souza et al. (2020b), and Souza et al. (2021) the use of systems that provide the appropriate spectral quality for vegetative propagation in *Corymbia* and *Eucalyptus* can promote greater development of the photosynthetic apparatus, resulting in high rates of multiplication, growth, rooting and subsequent acclimatization of plants to *ex vitro* conditions.

To better understand the limiting factors of the adventitious rooting phase in different eucalypts species with advanced ontogenetic age, the need for studies that promote the maximization of minicutting for the production of clonal plants on a commercial scale is evident. The work aimed to evaluate the influence of spectral quality for the optimization of rooting of minicuttings of *Eucalyptus andrewsii* Maiden, *E. saligna* Sm., *E. microcorys* F. Muell., *E. cloeziana* F. Muell., *E. pillularis.* Sm., *E. grandis* W. Hill, *E. grandis* × *E. urophylla* S.T. Blake and *Corymbia torelliana* (F. Muell.) K.D. Hill & L.A.S. Johnson.

Materials and Methods

Experimental material

Juvenile minicuttings were collected from *Eucalyptus andrewsii*, *E. saligna*, *E. microcorys*, *E. cloeziana*, *E. pilularis*, *E. grandis* and ministumps of *Corymbia torelliana's*, which were seminally produced from seeds of parent plants of test species from

Table 1 Composition of the nutritive solution for fertigation of *E. grandis* × *E. urophylla* ministumps.

Nutrient	Nutritive solution(1) (mg L^{-1})	Molecular weight
N-NO^{3-}	60.00	14.00
N-NH^{4+}	30.00	14.00
P	12.00	30.97
Ca	30.00	40.08
K	80.00	39.10
S	18.92	32.06
Mg	12.00	24.31
Cu	0.10	63.54
Fe	2.00	55.85
Mo	0.02	95.94
Mn	1.60	54.94
Zn	1.96	65.37
B	1.08	10.81
Macro and micronutrient source	**QF / MW**	**(mg L^{-1})**
Potassium nitrate (Nuclear®)	KNO_3 / 101.10	206.8500
Monoammonium phosphate (Mallinckrodt®)	$NH_4H_2PO_4$ / 115.03	44.5700
Ammonium nitrate (Reagex®)	NH_4NO3 / 80.4	140.5000
Calcium sulfate (Vetec®)	$CaSO_4.2H_2O$ / 172.17	87.1817
Calcium nitrate (Labsynth®)	$Ca(NO_3)2.4H_2O$ / 236.15	57.1800
Magnesium sulfate (Mallinckrodt®)	$MgSO_4.7H_2O$ / 246.48	121.6680
Manganese sulfate (Ecibra®)	$MnSO_4.H_2O$ / 169.01	4.9223
Copper sulfate (Mallinckrodt®)	$CuSO4.5H_2O$ / 249.68	0.3929
Iron sulfate (Synth®)	$FeSO_4.7H_2O$ / 278.02	9.9520
Sodium - EDTA (Nuclear®)	Na_2-EDTA.2H2O / 372.24	13.3110
Sodium molybdate (Merck®)	$Na_2MoO_4.2H_2O$ / 241.95	0.0504
Zinc sulfate (Mallinckrodt®)	$ZnSO_4.7H_2O$ / 287.54	8.6000
Boric acid (Ecibra®)	H_3BO_3 / 61.83	6.2000

Notes: (1) pH was adjusted to 5.8 at 25°C with HCl and NaOH, both at 1 M. QF = chemical formula, MW = molecular weight.

the genera *Eucalyptus* and *Corymbia*; these genera were installed in 1974 (IPEF 1984). *Eucalyptus grandis* × *E. urophylla* (urograndis eucalypt) derived from ministumps, was propagated by cuttings and presented tissues with a higher ontogenetic age than the other investigated species.

The ministumps were established in a clonal mini garden (Figure 1A) in a semi-hydroponic gutter system in a medium sand bed (Table 1). The plants received nutrient solution by dripping, which was applied four times a day, totalling a daily flow rate of 4 L m^{-2}.

Rooting

Minicuttings were standardized in size (from 4 to 5 cm) and consisted of a pair of leaves - cut in half (Figure 1C) - from ministump shoots (Figure 1B) belonging to seven species and a hybrid. These minicuttings were staked in plastic trays (50 cm long × 26 cm wide) (Figure 1D) with 35-cm^3 cells filled with commercial substrate (Tropstrato Vida Verde®) and vermiculite, at the ratio of 1:1 (v/v). Next, the plastic trays were placed on shelves in the growth room at 24 ± 1°C for a 16-hour photoperiod. Two fluorescent lamps (Philips T10 cold white type of 0.60 m, power of 20 W (each) and color temperature of 6400-6500k) were used on each shelf. The irradiance of the two lamps (40 µmol m^{-2} s^{-1}) was measured in photoradiometer model QSO-S Procheck + Sensor-PAR Photon Flux (Decagon Devices, Pullman, Washington-USA). Plant humidity was maintained using a spray bottle twice a day, at a total daily volume of 0.5 L per tray.

Spectral quality

In order to compare the spectral quality, the experiment followed a randomized design, at 8 × 3 factorial arrangement - seven species and a hybrid (*E. andrewsii*, *E. saligna*, *E. microcorys*, urograndis eucalypt, *E. cloeziana*, *E. pilularis*, *E. grandis* and *C. torelliana*) and three spectral qualities (fluorescent, red and blue) - with thirty-two repetitions containing a minicutting

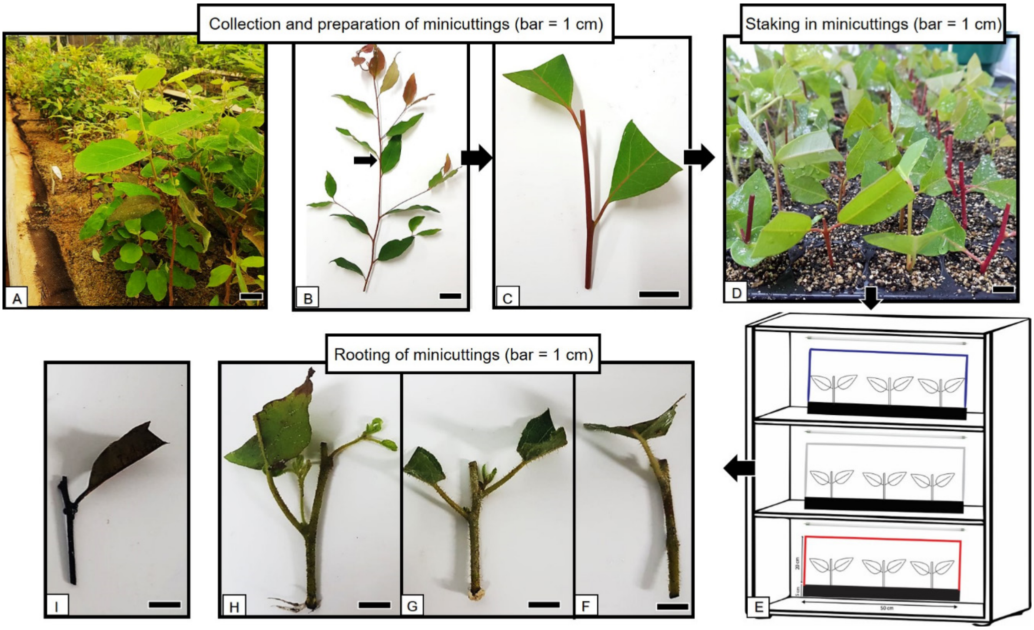

Figure 1 Ministumps' selection up to the rooting of *Corymbia* and *Eucalyptus* minicuttings: (A) Ministumps arranged in the clonal minigarden under semi-hydroponic system of the sand-bed channel type; (B) Selected sprouting, with emphasis on the median portion used to make the minicuttings; (C) Minicutting after preparation and standardization; (D) Minicuttings staked in plastic trays filled with commercial substrate and vermiculite; (E) Minicuttings arranged in the growth room based on different spectral qualities (fluorescent, red and blue) with the aid of a cellophane sheet; (F) Minicutting showing no response to rooting; (G) Minicutting presenting callus; (H) Rooted minicutting; (I) Oxidized minicutting. Bar = 1.0 cm Ministumps' selection up to the rooting of Corymbia and Eucalyptus minicuttings: (A) Ministumps arranged in the clonal minigarden under semi-hydroponic system of the sand-bed channel type; (B) Selected sprouting, with emphasis on the median portion used to make the minicuttings; (C) Minicutting after preparation and standardization; (D) Minicuttings staked in plastic trays filled with commercial substrate and vermiculite; (E) Minicuttings arranged in the growth room based on different spectral qualities (fluorescent, red and blue) with the aid of a cellophane sheet; (F) Minicutting showing no response to rooting; (G) Minicutting presenting callus; (H) Rooted minicutting; (I) Oxidized minicutting. Bar = 1.0 cm.

stake, each. Spectral qualities were enabled by filtering the light output of fluorescent lamps with cellophane foil, which was used to wrap the plastic trays (Figure 1E).

The light spectra were measured using a SPECTRA PEN Z850 portable spectroradiometer (Qubit Syculms-Kingston, Ontario-USA). The spectral distributions of each filter are shown in Figure 2.

Survival (SUR), callogenesis (CAL), oxidation (OXI), rooting (RO) percentage, length (RL) and diameter of the largest root (ROD), and the mean number of roots per minicutting (NRM) were evaluated at 30 days.

Histological analysis

The seven species and the hybrid provided the best rooting results (urograndis eucalypt and *C. torelliana*) based on the spectral qualities (fluorescent, red, and blue). They all were subjected to histological analysis.

Representative samples of leaves from each treatment were collected after thirty days and kept for 48 h in FAA solution (formaldehyde, acetic acid, 70% ethanol, 1:1:18), followed by transfer to ethanol 70%, and dehydrated in an alcoholic-ethyl series in increasing concentrations (80%, 90%, and 100%) for 30 min in each solution (Johansen 1940), and

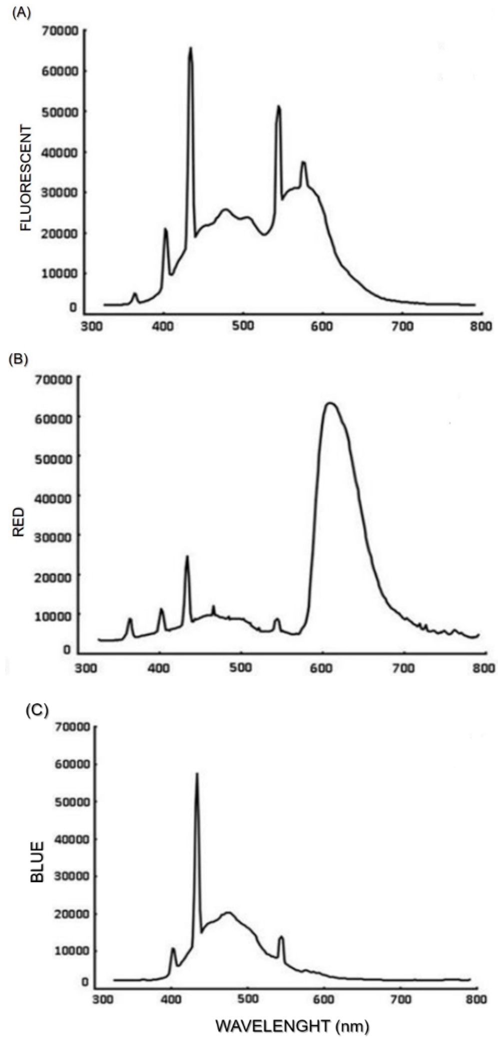

Figure 2 Variations in absolute irradiance (μW cm^{-2} nm^{-1}) and light wavelength applied to *E. andrewsii*, *E. saligna*, *E. microcorys*, *E. grandis* species × *E. urophylla*, *E. cloeziana*, *E. pilularis*, *E. grandis*) and *C. torelliana* under controlled growth room conditions. (A) Fluorescent lamp; (B) Red cellophane; (C) Blue cellophane.

subsequently soaked in historresin (Biosystems, Nussloch, Germany) in the proportion 1 :1 in a hot oven (overnight). The blockage was processed with pure hydroxyethyl methacrylate resin, and the cross-sections were obtained with a manual rotating microtome and a knife-thickness of 7 μm. Tissues were contrasted with toluidine blue (Vetec Química Fina Ltda, Rio de Janeiro, Brazil), mounted on Entellan histological slides (Merck KGaA, Darmstadt, Germany), and photomicrographed with an attached digital camera (AxionCam ERc5s) on micrometric and objective lens 20x and 40x. Root cortex diameter (RCD), the diameter of the root vascular cylinder (DRVC), and root diameter (RD), were randomly photographed at three different fields of view for the determination of root epidermis thickness (RET). Root area corresponded to 0.04 mm², and fifteen repetitions (5 anatomical sections × 3 fields of view) with one root. The calculation of 2 x RET + RCD + DRVC was used to measure root diameter (RD).

Statistical analysis

Data analysis was performed with R Core Team software (2018) using the ExpDes package, version 1.1.2 (Ferreira et al. 2013). The data from the experiments were analyzed for homoscedasticity and regular distribution of residuals using the Hartley ($p > 0.05$) and Shapiro-Wilk tests ($p > 0.05$), respectively. According to the Hartley and Shapiro-Wilk tests, the data were transformed using the Box-Cox test. Then, they were subjected to analysis of variance (ANOVA, $p < 0.05$), and the means were compared using the Tukey test ($p < 0.05$). The bars represented in the graphs denote the standard deviation in relation to the mean value.

Results

Effect of spectral quality on minicutting rooting

Based on the analyzed features, there was a difference in the rooting of minicuttings among the species subjected to different spectral qualities at 30 cultivation days. SUR and CAL did not show an interaction between factors (species and spectral quality). Concerning the OXI of minicuttings, factors acted in a dependent manner.

The highest SUR values were recorded for *E. grandis*, which did not present minicutting mortality; this outcome was statistically different from that of *E. andrewsii*, which displayed 64.5% of minicutting mortality, on average (Figure 3A). The red spectral quality led to the highest SUR percentage (93.7%, on average) (Figure 3B); however, there was no statistical difference between red spectral quality and the other treatments. The high SUR percentage denote the potential of combining species' factors with specific spectral qualities.

Callogenesis is an undesirable aspect of the adventitious rooting process. Results have shown different CAL responses, depending on species and spectral qualities. *E. andrewsii* recorded the lowest mean CAL at the base of the minicuttings (2.0%, average); however, it did not statistically differ from the other treatments (Figure 3C). In addition, the red spectral quality has influenced CAL rate (21.0%, on average) and recorded higher values than the other treatments (Figure 3D).

The OXI of minicuttings was one of the variables influenced by species and spectral quality; it presented distinct responses. The lowest mean OXI percentage were recorded for species *E. pilularis* and *E. grandis*, subjected to red spectral quality (6.2%) (Figure 3E). On the other hand, *E. andrewsii* subjected to fluorescent spectral quality presented the highest OXI percentage (81.2%, on average) (Figure 3E).

Minicutting rooting (RO) was one of the variables influenced by spectral quality; it presented different

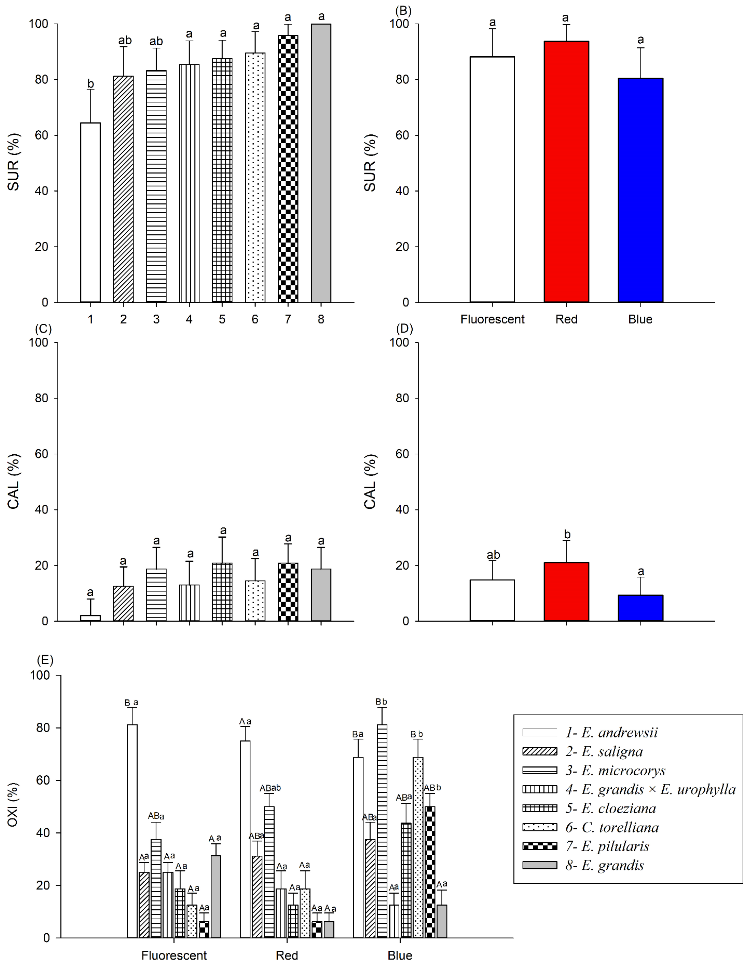

Figure 3 Features evaluated during the rooting of minicuttings based on different eucalypts species (*E. andrewsii*, *E. saligna*, *E. microcorys*, *E. grandis* × *E. urophylla*, *E. cloeziana*, *E. pilularis*, *E. grandis*, *C.torelliana*) and spectral qualities (fluorescent, red and blue). (A, B) Survival rate (SUR); (C, D) Callogenesis percentage (CAL); (E) Oxidation percentage (OXI). * (E) Uppercase letters represent statistical differences among different species in the same treatment (spectral quality), whereas lowercase letters represent statistical differences among different spectral qualities in the same treatment (species).

responses in the species. Fluorescent spectral quality recorded the highest RO means for *E. saligna* (68.7%), *E. microcorys* (43.7%), *E. pilularis* (75.0%), and *C. torelliana* (75.0 %) (Figure 4).

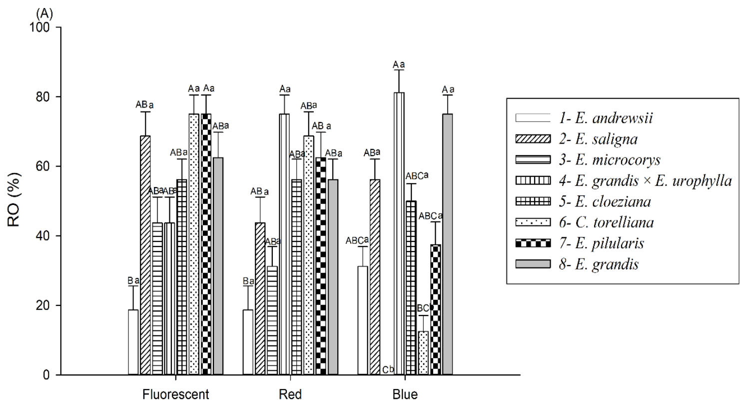

Figure 4 Rooting (RO) percentage recorded for minicuttings belonging to different eucalypts species (*E. andrewsii*, *E. saligna*, *E. microcorys*, *E. grandis* × *E. urophylla*, *E. cloeziana*, *E. pilularis*, *E. grandis*, *C. torelliana*) subjected to different spectral qualities (fluorescent, red and blue). *Uppercase letters represent statistical differences among different species in the same treatment (spectral quality), whereas lowercase letters represent statistical differences among different spectral qualities in the same treatment (species).

The *E. cloeziana* clone subjected to red spectral quality has shown the best response to RO (56.2%, average). *E. andrewsii* (55.5%), *E. grandis* (75.0%), and urograndis eucalypt (81.2%) subjected to blue spectral quality recorded the highest RO means (Figure 4). On the other hand, among all species subjected to fluorescent and red spectral qualities, *E. andrewsii* recorded the lowest RO performance, whereas *E. microcorys* recorded the lowest RO performance among species subjected to blue spectral quality (Figure 4).

RL and ROD did not show interaction between factors (species and spectral quality), whereas NRM presented interaction, depending on the tested ones.

RL and ROD has shown similar behavior: the highest values were recorded for urograndis eucalypt (0.92 cm and 0.41 mm, respectively); these values were significantly different ($p < 0.05$) from the values recorded for *E andrewsii* (0.32 cm and 0.16 mm, respectively, on average) and *E. microcorys* (0.30 cm and 0.15 mm, respectively), as shown in Figures 5A and 5C. Fluorescence spectral quality recorded the highest means (0.69 cm and 0.33 mm), but it did not statistically differ from the other (Figures 5B and 5D).

As for the NRM analyzed, species were influenced by different spectral qualities; this variable was crucial to induce minicutting rhizogenic processes. *E. saligna*, *E. microcorys*, *C. torelliana* and *E. piularis* subjected to fluorescent spectral quality recorded the highest mean number of roots per minicutting (1.57, 0.47, 1.93 and 0.94, respectively), as shown in Figure 5E. urograndis eucalypt and *E. cloeziana* subjected to red spectral quality recorded 1.36 and 0.80 roots per minicutting, respectively, on average; species *E. andrewsii* and *E. grandis* subjected to blue spectral quality recorded the highest mean number of roots per minicuttings (0.44 and 0.88, respectively), as shown in Figure 5E.

Similar to RO, the lowest NRM values (0.23 and 0.33, respectively, on average) were observed for species *E. andrewsii* subjected to fluorescent and red spectral qualities, whereas *E. microcorys* subjected to blue spectral quality did not show root induction (Figure 5E).

Effect of spectral quality on root anatomy

The investigated anatomical features showed different responses between species and spectral qualities (Figures 6 and 7). Urograndis eucalypt clone subjected to red spectral quality recorded the highest RET mean (13.8 μm); this value was significantly different from the blue spectral quality (10.8 µm), as shown in Figure 6A. *C. torelliana* subjected to red spectral quality presented a similar trend (14.09 µm); this value was statistically different from the one recorded for the treatment with fluorescent spectral quality (9.4 µm), as shown in Figure 6A.

The highest RCD mean was observed for urograndis eucalypt clone subjected to fluorescent spectral quality (870.7 µm); it was significantly different from values recorded for the other treatments, as shown in Figure 6B. The highest RCD means were recorded for *C. torelliana* subjected to red (555.8 µm) and blue (495.5 µm) spectral qualities (Figure 6B). These results have shown an inverse relationship between morphological NRM features and OXI and RO percentage, which recorded the best results for urograndis eucalypt subjected to red and blue spectral qualities and *C. torelliana* subjected to fluorescent spectral quality.

The DRVC has shown similar behavior to RCD in urograndis eucalypt; the largest root dimension (282.8 µm on average,) was recorded for the treatment with fluorescent spectral quality (Figure 6C). Concomitantly with the RCD results recorded for *C. torelliana*, blue and red spectral qualities recorded the highest DRVC values (194.7 µm and 189.7 µm, respectively, on average), as shown in Figure 6C.

Results recorded for RD were similar

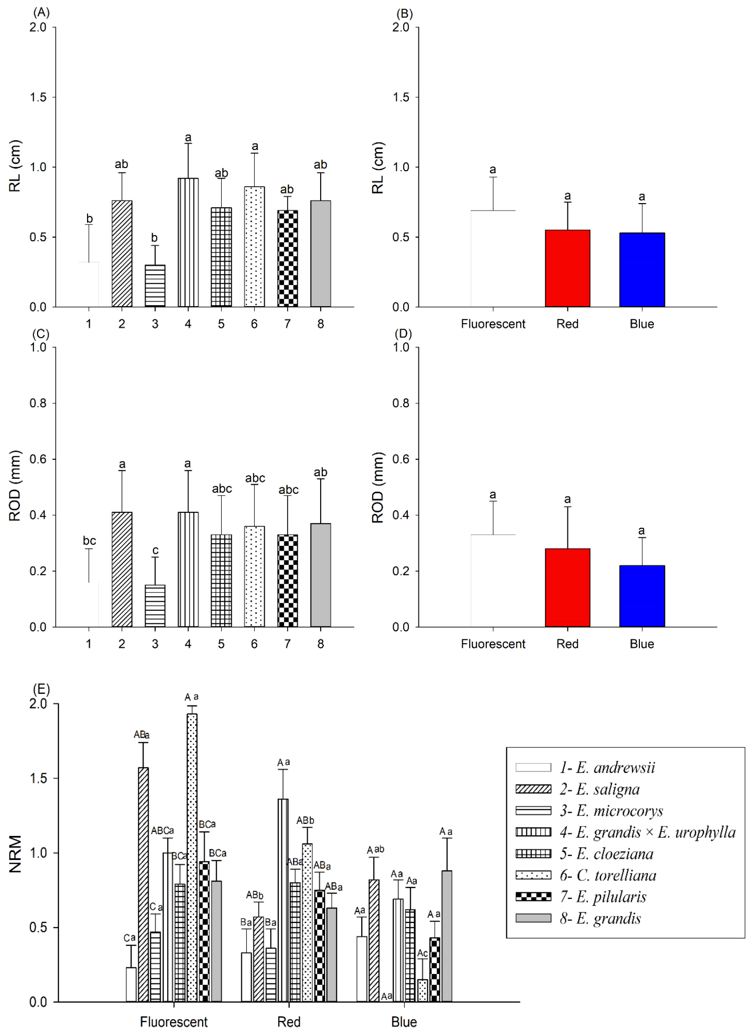

Figure 5 Features evaluated during the rooting of minicuttings from different eucalypts species (*E. andrewsii*, *E. saligna*, *E. microcorys*, *E. grandis* × *E. urophylla*, *E. cloeziana*, *E. pilularis*, *E. grandis* and *C.torelliana*) subjected to different spectral qualities (fluorescent, red and blue). (A, B) Root length (RL); (C, D) Root diameter (ROD); (E) Number of roots per minicutting (NRM). * (E) Uppercase letters represent statistical differences among different species in the same treatment (spectral quality), whereas lowercase letters represent statistical differences among different spectral qualities in the same treatment (species).

to the ones recorded for RCD and DRVC; the largest root dimensions were recorded for urograndis eucalypt clone subjected to fluorescent spectral quality (1180.2 μm, on average), as well as for *C. torelliana* subjected to red (773.5 μm, on average) and blue (715.0 μm, on average) spectral qualities (Figure 6D).

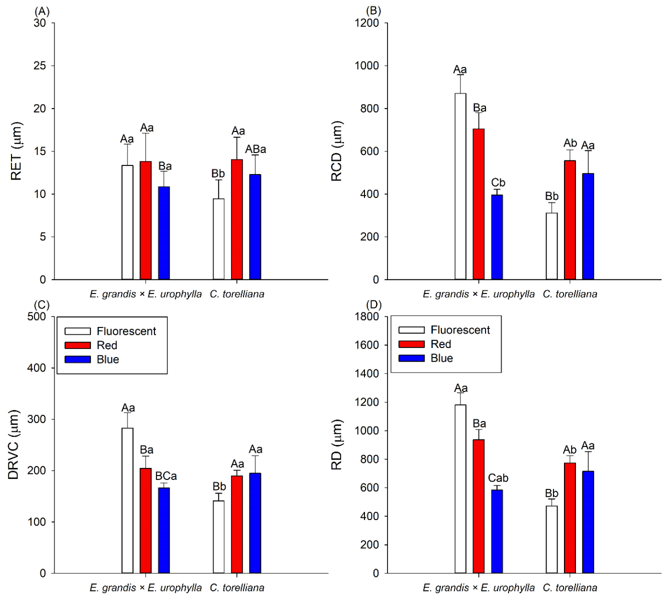

Figure 6 Anatomical features evaluated during the rooting of minicuttings from different eucalypts species (*E. grandis* × *E. urophylla*, *C. torelliana*) subjected to different spectral qualities (fluorescent, red and blue). (A) Root epidermis thickness (RET); (B) Root cortex diameter (RCD); (C) Diameter of the root vascular cylinder (DRVC); (D) Root diameter (RD). *Uppercase letters represent statistical differences among different spectral qualities in the same treatment (species), whereas lowercase letters represent statistical differences among different species in the same treatment (spectral quality).

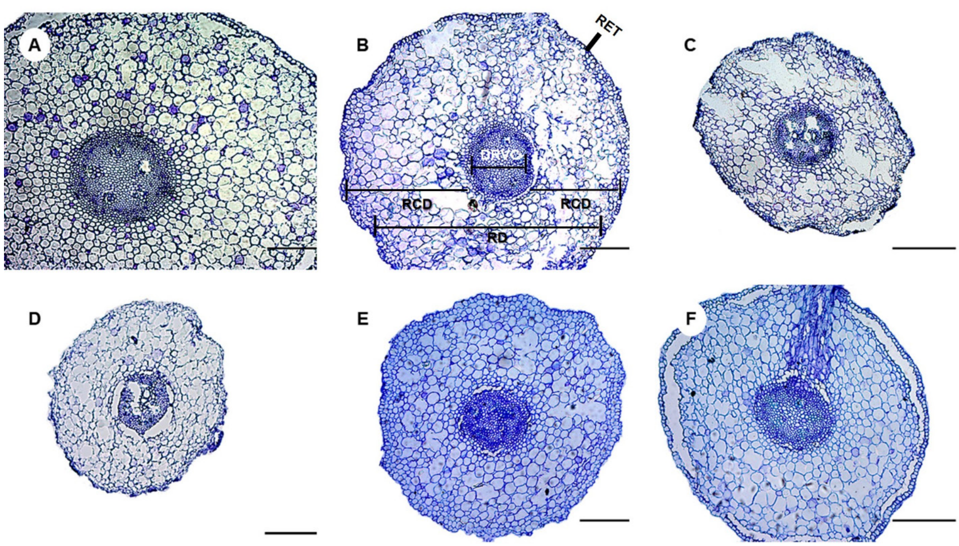

Figure 7 Anatomical features evaluated during the rooting of minicuttings. (A) *E. grandis* × *E. urophylla* subjected to fluorescent spectral quality; (B) *E. grandis* × *E. urophylla* subjected to red spectral quality; (C) *E. grandis* × *E. urophylla* subjected to blue spectral quality; (D) *C. torelliana* subjected to fluorescent spectral quality; (E) *C. torelliana* subjected to red spectral quality; (F) *C. torelliana* subjected to blue spectral quality. RET: Root epidermis thisckness; RCD: Root cortex diameter; DRVC: diameter of the root vascular cylinder; RD: Root diameter. Bar = 200 μm.

Discussion

Effect of spectral quality on minicutting rooting

The improvement of vegetative propagation protocols capable of influencing plant development was investigated to help establish efficient production systems to produce clonal plants on a large scale. The current study's survival rate results were better than those reported in other studies based on minicutting technique application to genus *Eucalyptus* and *Corymbia*. Overall, minicuttings of species belonging to genera *Eucalyptus* and *Corymbia* present survival percentage ranging from 60% to 85% (Trueman et al. 2018, Estevez et al. 2020).

Results similar to the current study were observed for *Microlaelia lundii* (Rchb.f. & Warm.) subjected to red spectral quality, which recorded increased rooting and survival percentage (Favetta et al. 2017). Braga et al. (2009) recorded a higher mean number of roots and longer length of the most significant root for *Dendranthema grandiflorum* microcuttings grown in a greenhouse under red shading. According to Hung et al. (2015), strawberry cultivars subjected to treatments with red photons recorded higher survival and rooting percentage than those treated with fluorescent lamps (90-94% vs. 76%).

However, studies about the effect of culture exposure *in vitro* to red and blue light spectra, either alone or in combination, have found significantly improved plant morphogenesis (Abiri et al. 2020, Rodrigues et al. 2020). Thus, minicuttings' survival may depend on the adopted plant material (genotype) and culture condition, such as spectral quality specificity.

If one considers rhizogenesis maximization, results in the current study have shown the development of adventitious roots presenting low external callus percentage - i.e., without indirect organogenesis - in minicuttings of eucalypts species subjected to blue spectral quality (9.3%). Brondani et al. (2018) observed similar results for callogenesis at the base of minicuttings in *Corymbia citriodora* (Hook.) KDHill & LAS Johnson (14.6%), *Eucalyptus urophylla* ST Blake (13.4%) and *Eucalytpus benthamii* Maiden et Cambage (1.6%).

According to Díaz - Sala (2020), the presence of calluses in rooting propagules impairs root functionality and compromises the quality of plants; thus, it has an undesirable effect on rhizogenesis. Different culture environments can change the metabolism of propagules; this process leads to enzyme denaturation and reduces nutrient absorption, which affects rhizogenic capacity (De Almeida et al. 2017, Batista et al. 2018, Díaz Sala 2020).

The use of spectral quality was an essential factor for the OXI percentage of the tested species, since it helps reducing the mortality and increasing the rooting of minicuttings. Phenolic oxidation is one of the main limiting factors for tissue regeneration (Abiri et al. 2020); thus, methodologies aimed at overcoming or reducing tissue oxidation are important strategies to be adopted in propagation systems.

Therefore, specific spectral quality application based on the minicutting species was suitable to clone highly productive genotypes in large-scale production systems. Fluorescent spectral quality was the most suitable treatment for the rooting of *E. saligna*, *E. microcorys*, *E. pilularis* and *C. torelliana* minicuttings. According to Oliveira et al. (2021), the absorption of a broad light spectrum enables a greater energetic state of chlorophyll molecules, making it possible to maximize the photosynthetic percentage.

Red spectral quality was the most suitable treatment for urograndis eucalypt and *E. cloeziana*, whereas species *E. andrewsii* and *E. grandis* presented the best results under treatment with blue spectral quality. Wavelength specificity of monochromatic spectral qualities has influenced photomorphogenic responses of propagules grown in controlled environments, which made it a valuable technology to optimize rooting and yield rates (Batista et al. 2018, Faria et al. 2019).

Response variations between genotypes have also been observed at the rooting stage of *Corymbia* and *Eucalyptus* minicuttings (Douglas et al. 2016, De Almeida et al. 2017); this outcome indicates considerable influence. Some species, such as urograndis eucalypt (Souza et al. 2020a) and *Hevea brasiliensis* (Willd. Ex A. Juss) Müll. Arg., (Medrado et al. 1995) are hard to root due to the presence of an almost continuous cylinder of lignified tissues that, together with chemical barriers, hinders root emission. However, the association among wavelengths, growth patterns, and plants' minicutting rooting enables a better understanding of vegetative propagation protocols to be applied to the species investigated in the present study.

If one considers root development in length and diameter, the herein adopted spectral qualities had similar effects on the tested species. There was variation in morphophysiological responses of different species to wavelengths ranging from 400 to 500 nm to optimize plant adaptations to changes in environmental conditions (Lazzarini et al. 2017). However, even non-woody plant species, such as *Cucumis sativus* L., *Capsicum annuum* L. (Snowden et al. 2016) and *Lavandula angustifolia* Miller (Rodrigues et al. 2020), developed at wavelengths ranging from 400 to 500 nm, presented low development and rooting percentages. *Abies* × *borisii-regis* Mattf explants subjected to fluorescent light sources presented the best development and rooting results (Smirnakou et al. 2016).

The NRM recorded for the species investigated in the current study was influenced by different spectral qualities. *E. saligna*, *E. microcorys*, *C. torelliana* and *E. piularis* subjected to fluorescent spectral quality have shown the highest NRM values. urograndis eucalypt and *E. cloeziana* subjected to red spectral quality recorded the best NRM results, as well as *E. andrewsii* and *E. grandis* subjected to blue spectral quality. Assumingly, the effects of light on adventitious root formation are mostly indirect; besides, they may influence the hormonal balance between auxin and cytokinin and carbohydrate availability and distribution in plants (De Almeida et al. 2017, Pinto et al. 2020).

Therefore, the lack of adventitious root induction is one of the main factors limiting cloning processes based on the minicutting and micropropagation techniques (De Almeida et al. 2017). Thus, the effectiveness of monochromatic light in cultivated plant production can change from species to species, as well as requires the combination of light spectra.

Effect of spectral quality on root anatomy

The use of spectral quality directly impacted urograndis eucalypt and *C. torelliana* root anatomy; species subjected to red spectral quality recorded the highest RET values (Figures 7B and 7E). Similar results were recorded for *Capsicum chinense* Jacq. plants grown in pots, which presented thicker and differentiated epidermal cells (Santana-Buzzy et al. 2005). Culture *in vitro* led to thicker epidermis formation in *Lavandula angustifolia* grown in a growth room under red shading and thinner epidermis formation in plants grown under blue shading (Rodrigues et al. 2020). Different light spectra can influence the growth and development of plant cells, tissues, and organs and trigger different morphophysiological and morphoanatomical responses (Abiri et al. 2020, Díaz Sala 2020).

Variables RCD, DRVC and RD presented similar behavior; urograndis eucalypt clone subjected to fluorescent spectral quality recorded the largest dimensions (Figure 7A). Broad light spectrum can increase auxin transport through the vascular system, which can lead to a larger number of cells and, consequently, to leaf expansion, stem elongation and rooting (Lee et al. 2016). *C. torelliana* subjected to red and blue spectral qualities recorded the highest values (Figures 7E and 7F). The herein observed increased cortex diameter development, mainly in comparison to the red spectral quality, may be associated with higher stomata density, which increased photosynthetic yield (carbohydrate production and translocation s in leaves), and superior storage in the parenchyma of reserve organs such as roots. The red spectrum enabled more significant root development in *E. grandis* and *E. globulus* (Ruedell et al. 2013).

Morphogenesis and rhizogenesis can be influenced by the intensity and quality of light treatments applied during plant production (De Almeida et al. 2017, Abiri et al. 2020). Spectral quality enabled optimizing clone production protocols to be used to several forest species, although the responses appeared to vary considerably depending on the species (Batista et al. 2018, Zhao et al. 2020). Results in the current study have important implications in optimizing production systems applied to eucalypts clonal plant based on the minicutting technique. Wavelength specificity - through spectral quality - has proved viable and essential for the survival and rooting of minicuttings in controlled environments. However, complementary studies must be conducted to investigate the inverse relationship of root growth between morphological and anatomical features.

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Conclusions

Our study provided evidence that the wavelength specificity influences the morphological and anatomical characteristics studied for the rooting of minicuttings, making it possible to optimize the production of clonal plants on a large scale.

Fluorescent spectral quality showed the best results for the rooting of *E. saligna*, *E. microcorys*, *E. pilularis* and *C. torelliana* minicuttings.

For the blue spectral quality, the best results were observed in the rooting of *E. andrewsii*, *E. grandis* and urograndis eucalypt minicuttings.

Red spectral quality was more suitable to be used for *E. cloeziana* minicutting rooting.

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