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ORIGINAL ARTICLE

Use of light-emitting diodes on the morpho-anatomical characteristics of *Corymbia citriodora* seedlings

Uso de diodos emissores de luz sobre as características morfoanatômicas em mudas de *Corymbia citriodora*

Gabrieli Cristina Vitalli de Azevedo[1], Claiton Nardini[1*], Matheus Milani Pretto[2], João Antonio de Cristo[3], Erick dos Santos Silva[1], Elder Eloy[1], Braulio Otomar Caron[1], Denise Schmidt[1]

[1]Universidade Federal de Santa Maria – UFSM, Frederico Westphalen, RS, Brasil
[2]Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, Botucatu, SP, Brasil
[3]Meta Consultoria Agrícola, Canarana, MT, Brasil

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Abstract

With the increase in *Corymbia citriodora* cultivation in Brazil, it is crucial to evaluate its characteristics in different environments of seedling production, given the different acclimatization strategies under the prevailing environmental conditions in its cultivation. The objective of this study was to evaluate distinct spectral qualities of LEDs lamps in morpho-anatomical characteristics in *Corymbia citriodora* seedlings. The work was conducted from August 2018 to January 2019, in a growth room. The experimental design used was completely randomized, where the treatments were composed of four spectral qualities of light from white, red (660 nm), blue (450 nm) and blue + red (40% + 60%) LED lamps, with light intensity of 72 μmol m^{-2} s^{-1} each bench. For *Corymbia citriodora*, the different spectral qualities affected characteristics such as leaf blade thickness, leaf area, dry matter, stomatal density, decreased chlorophyll *b* and total chlorophyll content under red LED and caused lower epidermis thickness under blue LED cultivation. The use of white spectral quality is the most appropriate and favors the dispatch of seedlings at 102 days after emergence, both in the parameters evaluated for leaf area index and for dry matter.

Keywords: Lemon-scented gum*;* Light; LEDs; Leaf area index.

Resumo

Com o aumento do cultivo de *Corymbia citriodora* no Brasil, é importante avaliar suas características em distintos ambientes de produção de mudas, visto as diferentes estratégias de aclimatação sob as condições ambientais prevalecentes em seu cultivo. O objetivo deste estudo foi avaliar diferentes qualidades espectrais de lâmpadas LEDs nas características morfoanatômicas de mudas de *Corymbia citriodora*. O estudo foi conduzido no período de agosto de 2018 a janeiro de 2019, em sala de crescimento. O delineamento experimental utilizado foi o inteiramente casualizado, onde os tratamentos foram compostos por quatro qualidades espectrais de luz provenientes de lâmpadas LED branco, vermelho (660 nm), azul (450 nm) e azul + vermelho (40% + 60%), com intensidade luminosa de 72 μmol m^{-2} s^{-1} cada bancada. Para *Corymbia citriodora*, as diferentes qualidades espectrais afetaram características como, espessura do limbo foliar, área foliar, matéria seca, densidade estomática, reduziu o teor de clorofila *b* e clorofila total sob o LED vermelho e causou menor espessura da epiderme sob cultivo em LED azul. A utilização da qualidade espectral branca é a mais adequada e favorece a expedição das mudas aos 102 dias após a emergência, tanto nos parâmetros avaliados para índice de área foliar quanto para matéria seca.

Palavras-chave: Eucalipto cheiroso; Luz; LEDs; Índice de área foliar.

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1. INTRODUCTION

The lemon-scented gum, of the *Corymbia citriodora* species, is a large tree that grows abundantly in temperate and tropical regions of Australia, (Zheng et al., 2015), which has a high potential for acclimatization in different regions of the southern hemisphere. Its cultivation has increased in Brazil in recent years, due to its fast growing characteristic and edaphoclimatic adaptation, in addition to silvicultural quality characteristics and wood, widely used for building fences, charcoal and other multiple uses (Morais et al., 2010; Bernardi et al., 2012). Its leaves are used as raw material for essential oil extraction (Morais et al., 2010), having citronellal as the main chemical component that, due to its pleasant aroma, enters the composition of several flavoring products, soaps, detergents, perfumes, waxes, insecticides, among others (Andrade & Gomes, 2000).

The success of the productivity of lemon-scented gum plantations in Brazil has been due to the combination of several factors, including advances in its propagation (Souza et al., 2018). Thus, it is necessary to search for technologies that aim to increase the efficiency in the establishment of seedlings, as well as to reduce their permanence time in the nursery, resulting in the reduction of costs at this stage (Bernardi et al., 2012).

In the nursery or greenhouse stage, the more technified, environmental characteristics such as temperature and solar radiation can be manipulated (Beltrão et al., 2002; Veloso & Baptista, 2008) to optimize the cultivation, thus, it is increasingly frequent to adjust the ideal light, since this factor is related to photosynthesis and strongly influences the growth of plants (Gazolla-Neto et al., 2013). All plants have the ability to modify their anatomical structure in response to the light environment, aiming to increase the efficiency of the photosynthetic apparatus (Engel, 1989; Oliveira et al., 2016). However, still according to Oliveira et al. (2016), this response varies between the species and conditions to which they are subjected, such as their acclimatization capacity and the amount or quality of light.

Any variation in leaf structure, such as stomatal density, mesophyll size and shape, and vascular system differentiation, will affect plant physiological functions such as gas exchange, the distribution and content of pigments; characteristics that are directly related to photosynthesis and the process responsible for the accumulation of dry matter that will define the growth of the seedlings (Medeiros et al., 2015; Chen et al., 2016; Fernandez et al., 2017). For example, changes in leaf area can occur under stressful conditions, and then plants can reduce their leaf area by decreasing leaf division and expansion, changing their shapes and other mechanisms (Taiz et al., 2017), mitigating the effect of the stressful agent.

Artificial lighting, as a source of light, for plant growth has been increasingly used in various segments of agriculture (Torsian, 2020). Its use allows greater control over the growth of plants, since each species needs a specific amount and quality of light per day (Gupta & Jatothu, 2013). Currently it is possible to find some types of lamps, which act as a source of energy, aiming to improve the characteristics of plants. Light-Emitting Diodes (LEDs) are considered promising options, as they add several benefits such as low energy requirement, high efficiency in converting received energy into radiation, high durability, and can be used with one or more spectra for morphogenic responses and is considered a cold light, generating less heat than fluorescent lamps (Gupta & Jatothu, 2013). This allows their use in closed environments without harming the plants by burns and thus allows gains in light intensity (Garde, 2013). Chlorophyll, according to Kluge et al. (2015), efficiently absorbs light in the red and blue portions of the spectrum, so its use of monochromatic LEDs that emit light at specific wavelengths of the spectrum, can give plants and the production system an advantage as they boost photosynthetic metabolism, optimizing the process without using light that would not be used for photosynthesis (Yeh & Chung, 2009).

Considering all these aspects, this study wanted to identify the influence of different spectral qualities from LED lamps on the production of *Corymbia citriodora* seedlings and if there is any influence on the leaf area index, dry matter, chlorophyll, stomatal density and anatomy.

In this context, this study aimed to identify the influence of different spectral qualities from LED lamps on the morpho-anatomical characteristics of *Corymbia citriodora* seedlings.

2. MATERIAL AND METHODS

2.1. Study location and experimental design

The study was conducted in a growth room belonging to the Federal University of Santa Maria *campus* Frederico Westphalen, RS, Brazil, from August 2018 to January 2019 under a temperature of approximately 25 ºC, with a photoperiod of 8 hours of light. The experimental design used in the study was completely randomized, and the treatments were composed of four spectral qualities of light from white, red (660 nm), blue (450 nm) and blue + red (40% + 60%) LED lamps, with light intensity of 72 μmol m^{-2} s^{-1} each bench.

C. citriodora seeds used were acquired at the Forestry Science and Research Institute from in Piracicaba, São Paulo. Sowing was done on August 23, 2018, in 180 cm^3 conical tubes with six open-bottomed polypropylene grooves filled with organic substrate (Tecnomax®), totaling 56 tubes per tray. After sowing, the tubes were placed in the growth room and kept until the end of the evaluations and irrigation was performed daily, manually, and always while maintaining the substrate field capacity for all treatments. After eight days of sowing, the beginning of seedling emergence was verified.

2.2. Leaf area index and dry matter

The evaluations of leaf area and shoot dry matter were performed every 14 days and the plants were used to calculate the leaf area, later used for dry matter. Four plants were collected per treatment in each evaluation, totaling 36 collections in each treatment. The first collection was performed at 32 days after emergence (DAE), when the plants had two pairs of fully expanded leaves, and up to 144 DAE were performed, totaling nine collections. The evaluations ended when the plants reached ≥ 25 cm in height and stem diameter ≥ 2.5 mm, being this height (15 cm to 25 cm) recommended for transplanting in the field (Sturion et al., 2000).

For the evaluation of the leaf area, the methodology of Jadoski et al. (2012) was used. After collecting the four plants, all their leaves were removed, and these were digitized in a printer along with a reference scale. To determine the leaf area, the ImageJ® software (Ferreira & Rasb, 2012) was used, which captures the image of the leaves through a color contrast and compares this image with the reference scale, calculating the total area of the leaves.

The Leaf Area Index (LAI) was determined from the total leaf area and the soil area explored by each seedling, according to the following equation:

$$LAI=LA/SA$$

Where: LAI = leaf area index; LA = total leaf area of the plant, in m^2; and SA = the soil area used by the plant.

After evaluating the leaf area, the leaves were attached to the stems, then the samples were placed in paper bags and dried at 60°C until reaching constant mass. Afterwards, the weight of the dry matter, in grams, was determined with a precision balance. Both parts were considered for the evaluation of the total shoot Dry Matter (DM).

2.3. Chlorophyll content

To determine the content of total chlorophyll, chlorophyll *a* and chlorophyll *b*, the portable device ClorofiLOG (CFL1030 - FALKER) was used, an electronic chlorophyll content meter, which determines the results in Falker Chlorophyll Index (FCI). Chlorophyll ratio values were calculated by dividing chlorophyll *a* by chlorophyll *b* (*a*/*b*). The evaluations were carried out at the end of the seedling production cycle at 144 DAE in the laboratory, with four plants selected of each treatment and performing the reading on three leaves per plant from the third leaf from the top of the plant to the base.

2.4. Stomatal evaluation

The evaluation was performed at 144 DAE, using the third leaf from the apex to the base of the plant, fully expanded from three evaluated plants. The semi-permanent slides were made, adapting the methodology proposed by Weyers & Johansen (1985), through the epidermis impression technique. Universal instant adhesive (cyanoacrylate ester) was used by placing a drop on a glass slide. Both leaf faces, in the central region of the leaf, were pressed on the glass slide for 30 seconds, allowing the impression of the epidermis on the slide. The ready slides were submitted to image capture with the aid of an optical microscope with a camera attached, with a 20x magnification, with an area of 596 x 447 μm (0.2664 mm^2). To determine the number of stomata, the "Anati Quant 2" software (Aguiar et al., 2007) was used, based on the counting of stomatal cells.

2.5. Leaf anatomy

For the anatomical evaluation, the third leaf from the apex to the base of the plants, fully expanded, were used, being evaluated at 144 DAE. In order to carry out the anatomical evaluation of the internal tissues of the leaf blade, the methodology proposed by Carmello-Guerreiro (1995) was followed. The finished resin blocks were cut in the transverse direction with a thickness of approximately 5 μm with the aid of a cutting microtome. The histological sections were placed on slides and stained with astra blue for 120 minutes, and then images with an area of 596 x 447 μm were captured with the aid of an optical microscope with a camera attached, at a 20x magnification. With photomicrographed images and appropriate software highlighting the structures; the materials evaluated and characterized were: the thickness of the cuticle, epidermis, palisade parenchyma and spongy parenchyma.

2.6. Statistical analysis

The data were submitted to analysis of variance and the means were statistically analyzed using the SISVAR software (Ferreira, 2011). When there was significance, it was verified by the F tests ($p < 0.05$). Means were compared using the Scott-Knott test ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Leaf area index and dry matter

LAI values showed the same behavior throughout the study, for all treatments, initially showing a linear growth, followed by a maximum value and subsequent decrease. The highest values found were recorded between 60 and 102 DAE (Figure 1a). In the blue LED and blue + red LEDs, the LAI increased up to 60 DAE, from then on its value showed a decline until the end of the evaluations. For the white LED, there was an increase in the LAI up to 74 DAE, decreasing linearly from this date. The red LED showed a linear increase in LAI up to 102 DAE, decreasing after this period. In this treatment, the leaf area index kept increasing for practically the entire period of the experiment, indicating that there was an accumulation of fresh mass for a longer period, a fact that was not verified in the other treatments.

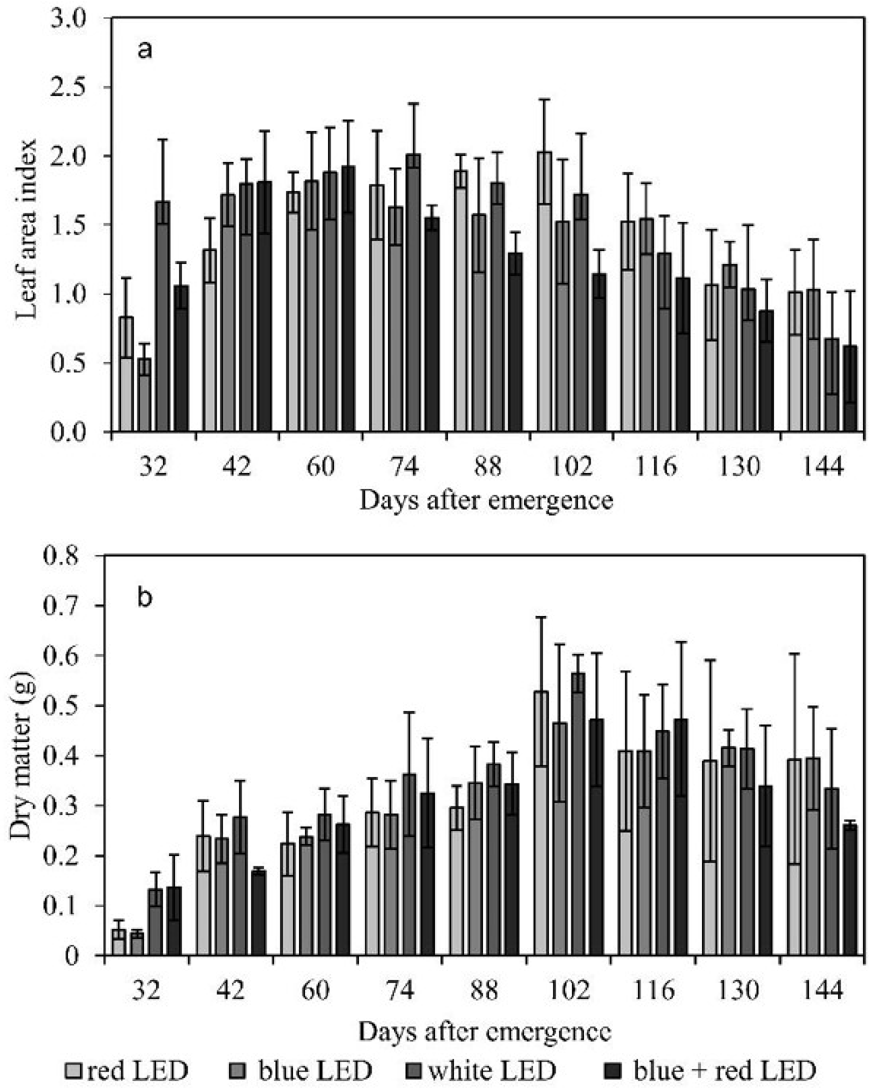

Figure 1: Dry matter and leaf area index of *Corymbia citriodora* cultivated under four LEDs over 144 days after emergence. Leaf area index (a); Dry matter (b).

The quantification of the LAI and its variation throughout the production cycle makes it possible to use it as a variable in growth models (Xavier et al., 2002). As the leaf area index increases, the accumulated values in dry matter increase, as these two components are strictly correlated. The LAI is an important variable to be evaluated, because through it the production of photo-assimilates is determined and the uptake of photosynthetically active radiation which is intercepted and transformed into dry matter (Caron et al., 2012).

The dry matter values showed a similar behavior for all treatments, reaching a maximum value and then decreasing until the end of the evaluations, similar to what happened with the LAI (Figure 1b). The highest values were at 102 DAE for the red, blue and white LEDs, except for the blue + red LEDs, which were at 116 DAE. At 32 days, the red and blue LEDs showed low DM values compared to the white and blue + red LEDs. The DM in the red LED continued to grow with some small variations, up to 102 DAE and after that, it kept very close values until the end of the evaluations. The DM values for blue LED continued to increase until 102 DAE, with values below the other LEDs, until the last two evaluations, in which it presented results minimally superior to the white LED at 130 DAE and 144 DAE in relation to the red LED. The white and blue + red LEDs presented close and superior DM values compared to the red and blue LEDs at 32 DAE. The DM in the white LED continued to increase until 102 DAE, with results superior to the other LEDs, and then decreasing until the end at 144 DAE.

Plants with higher LAI and DM tend to have better chances of survival after field transplantation. For Freitas et al. (2013), all evaluated species, including *C. citriodora*, grown in 180 cm^3 tubes, showed a gain in shoot dry matter, which is considered an important factor for survival and initial development in the field. This factor will influence the success in the formation of forests, as they will have to resist adverse conditions found in the field in the first months after transplantation, that is, acclimatize to the environment (Gomes et al., 1991). It is also important to be careful with the time the seedlings stay in the nursery, as from a certain age in the nursery, the seedlings reduce their vegetative growth and can curl their root system, due to the reduction in the exploitable volume of substrate caused by the limited space in the tubes (Mafia et al., 2005).

3.2. Chlorophyll content

For the contents of photosynthetic pigments, it was possible to observe that the chlorophyll ratio (Figure 2a) and the chlorophyll *a* content (Figure 2b) were similar in all treatments. For chlorophyll *b* (Figure 2c) and total chlorophyll (Figure 2d), the seedlings grown in red LED showed lower FCI than the other treatments.

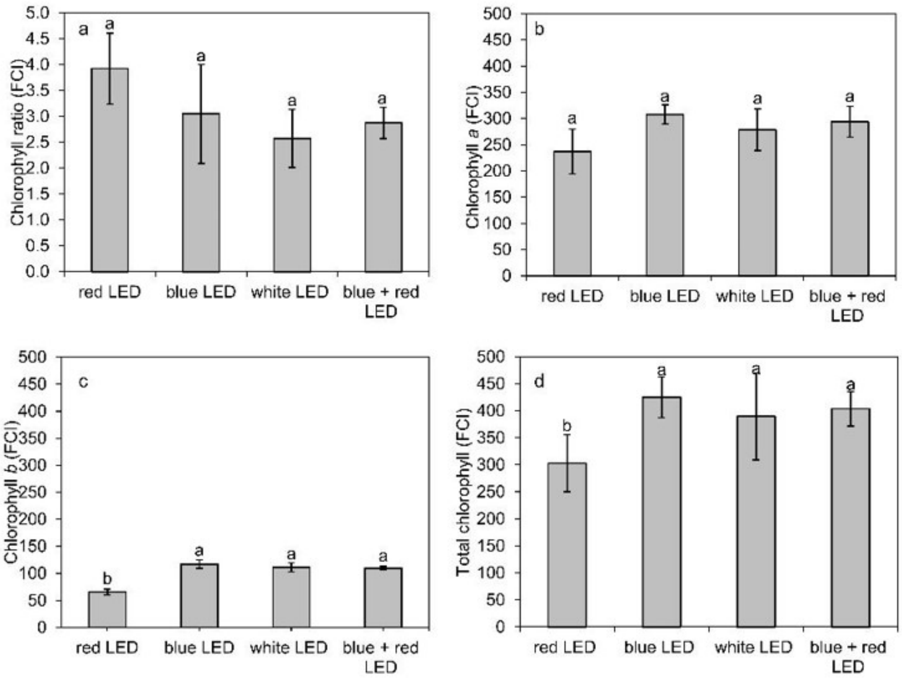

Figure 2: Chlorophyll Falker Content (CFL) of *Corymbia citriodora* cultivated under four LEDs at 144 days after emergence. Chlorophyll ratio (a); Chlorophyll *a* (b); Chlorophyll *b* (c); Total chlorophyll (d). Different letters in each column indicate significant differences ($P<0.05$) according to the Scott-Knott test.

Chlorophyll *a* and *b* are abundant in green plants (Taiz et al., 2017). Chlorophyll *a* is the pigment used to make photochemistry and accessory pigments (other pigments) including chlorophyll *b*; they aid in the absorption and transfer of light radiation to reaction centers (Streit et al., 2005). These pigments, therefore, are strictly linked to the photosynthetic efficiency of plants, consequently, to their growth and acclimatization to different environments (Santos et al., 2008).

The spectral quality influenced the levels of chlorophyll *b* in *C. citriodora* seedlings. According to Cardoso (2020), in crops under low light intensity, the leaves have higher levels of this pigment. Lower frequencies and therefore longer wavelengths correspond to red and orange light, while shorter wavelengths and higher frequencies correspond to blue and violet radiation. Thus, increasing the chlorophyll *b* content would be a way to compensate for the low light frequency. It is known that this type of chlorophyll makes it possible to capture photons of other wavelengths, so having a higher proportion of this pigment in these cases is an important characteristic (Vieira et al., 2010). Chlorophyll *b* captures energy from other wavelengths and then transfers it to chlorophyll *a*, which effectively acts in the photochemical reactions of photosynthesis and represents an adaptation mechanism to the condition of lower light intensity (Lima et al., 2011).

It can be inferred that *C. citriodora* seedlings, when cultivated under different spectral qualities, may present some alterations in their chlorophyll contents. According to Larcher (2004), ecologically the changes in chlorophyll biosynthesis by spectral variations can provide advantages in terms of growth and reproductive success of plant species. Thus, the results indicate an occurrence of chromatic adaptation of this species that may help in its photosynthetic performance.

3.3. Stomatal evaluation

Observing the variable stomatal density on the adaxial side, the blue + red LED was superior to the other treatments (Figure 3). On the abaxial side, no differences were observed between the evaluated LEDs. There was a change in stomatal density only on the adaxial side, due to the fact that this face is directly exposed to incident light. Studies show that a greater number of stomata can be found when the leaves are more exposed, for example, to solar radiation, indicating better control of stomatal conductance, which will reduce water losses through transpiration (Lleras, 1977).

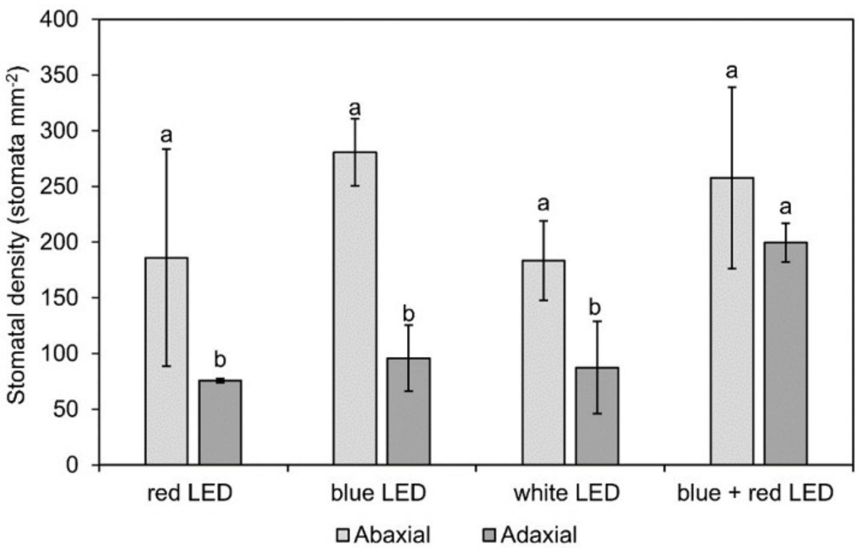

Figure 3: Number of stomata on the adaxial and abaxial leaf face of *Corymbia citriodora* cultivated under four LEDs at 144 days after emergence. Different letters in each column indicate significant differences (P<0.05) according to the Scott-Knott test.

The stomata of *C. citriodora* are present on both leaf faces, being classified as amphistomatic leaves (Moura & Franzener, 2014; Duarte, 2007). When five species of the Myrtaceae family were evaluated, the same characteristics were identified in relation to stomatal frequency. In addition, it has been shown that these species also have stomata often in greater numbers on the abaxial surface (Al-Edany & Al-Saadi, 2012). Stomatal development can be controlled, for example, by signals from the genome, environmental factors (Thiesen, 2019; Vráblová et al., 2018), by both light and water supply and can be located on the adaxial or abaxial side, and on both sides (Bucher et al., 2017). Also according to Bucher et al. (2017), species can change the size and density of stomata jointly on both sides, or they can occur independently.

Liu et al. (2014), when comparing the use of different spectral qualities with fluorescent lamps, found that there was an increase in stomatal density for all spectral qualities studied, with emphasis on the blue + red LED in the proportions of 50% blue LED and 50% red LED, which presented the highest number of stomata per mm^2 in relation to the other spectral qualities. In this study, similar results were found, which also suggests that the blue + red LEDs spectral quality, when analyzed on the adaxial leaf face, presented higher values when compared to the other spectral qualities. As for the abaxial face, regardless of the spectral qualities, the number of stomata was not influenced. For *Eucalyptus urophylla* cultivated *in vitro*, the stomatal density on the abaxial face of the leaves was also not influenced by the different spectral qualities (Miranda et al., 2020).

3.4. Leaf anatomy

In the present work, through the analysis of photomicrographs of the leaf, it was possible to observe the presence of adaxial and abaxial cuticle and epidermis, and palisade and spongy parenchyma under all studied LEDs, which were: red LED (Figure 4A), blue LED (Figure 4B), white LED (Figure 4C) and blue + red LEDs (Figure 4D). Two layers of palisade parenchyma next to the adaxial epidermis and next to the abaxial epidermis were found, also the presence of spongy parenchyma; therefore, the leaves are called dorsiventral leaves.

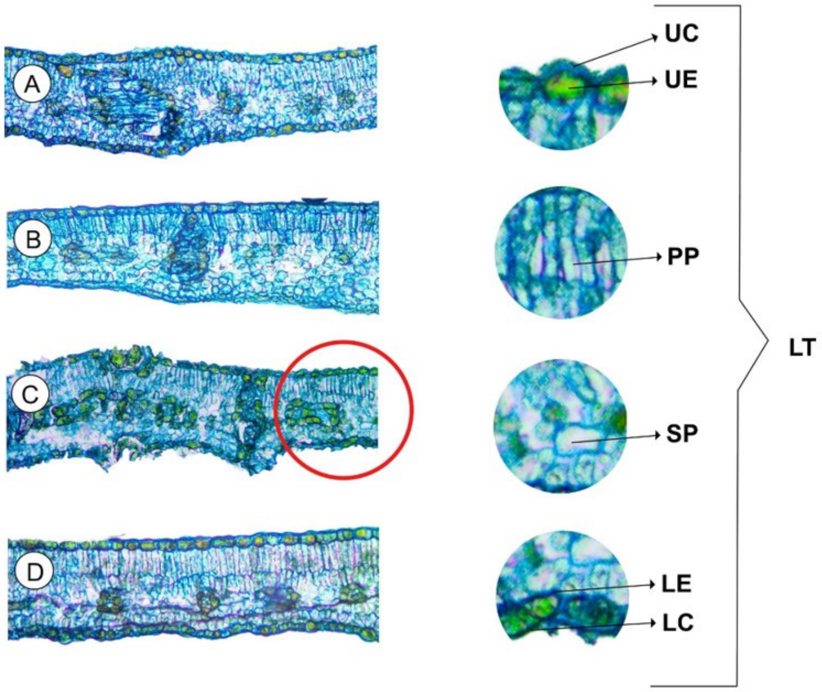

Figure 4: Cross-section of the leaf blade and anatomical structure of *Corymbia citriodora* cultivated under four LEDs at 144 days after emergence. (A) red LED, (B) blue LED, (C) white LED, (D) blue + red LEDs. UC: upper cuticle; UE: upper epidermis; PP: palisade parenchyma; SP: spongy parenchyma; LE: lower epidermis; LC: inferior cuticle; LT: leaf thickness.

Results found by Duarte (2007), for *C. citriodora*, corroborate the results found in this study; they identified two to three layers of palisade parenchyma on the adaxial surface and, facing the abaxial surface of the leaf, spongy parenchyma with intracellular spaces. On the other hand, Moura & Franzener (2014), found the presence of three layers of palisade parenchyma on the adaxial face of the leaf of *C. citriodora* and on the abaxial face; they identified one to two layers of this same parenchyma.

A dense cluster could be observed (Figure 4D), with few intracellular spaces of the spongy parenchyma when cultivated under the spectral quality blue + red LEDs, while for the other spectral qualities only spongy parenchyma with regular distribution was identified (Table 1). The leaves of plants with denser spongy parenchyma may not have required an increase in their intercellular spaces for better use of available light. The spongy parenchyma in dorsiventral leaves of dicotyledons promotes an increase in light dissipation, by reflection at the gas-liquid interfaces of these cells (Lambers et al., 2008), and this higher proportion of spongy parenchyma in the shade leaves increases the absorption of light luminosity, due to its greater internal dispersion (Vogelman et al., 1996). This study could justify the densification, as the plants received light from two spectra in which the maximum absorption by chlorophyll occurs, as previously mentioned; therefore, there was no need to maximize the absorption of light that reached the leaves.

By analyzing the cross-sections, it was possible to identify that the cuticle thickness of the adaxial and abaxial faces did not show any difference between the LEDs used (Table 1). The leaf blade thickness showed higher values when exposed to the spectral qualities white LED (112.67 μm) and blue + red LEDs (107.47 μm). For the thickness of the evaluated parenchyma, it was found that there was no significant difference between the treatments, but this small difference resulted in a greater thickness of leaf blade, according to Nascimento et al. (2014), which indicates that there was, in this case, an adaptive effect of the species to the quality of light. Still, according to Taiz et al. (2017), increasing leaf thickness is considered an acclimation strategy to improve light energy capture and, consequently, favor greater photosynthetic efficiency, and this ability to modify leaf anatomy prevents or reduces abiotic effects.

Table 1: Anatomical leaf structure parameters of *Corymbia citriodora* cultivated under four LEDs at 144 days after emergence.

Parameter (μm)	red LED	blue LED	White LED	blue + red LEDs	CV (%)
Upper cuticle	4.18 a*	3.16 a	4.63 a	4.41 a	20.27
Upper epidermis	7.80 a	6.89 a	7.35 a	9.27 a	18.13
Palisade parenchyma	38.42 a	34.02 a	40.68 a	41.81 a	8.95
Spongy parenchyma	34.13 a	41.47 a	48.14 a	41.02 a	11.81
Lower epidermis	8.14 a	4.63 b	8.36 a	7.35 a	17.98
Lower cuticle	4.07 a	2.94 a	3.50 a	3.62 a	24.94
Leaf thickness	96.73 b	93.12 b	112.67 a	107.47 a	8.17

*Different letters in each row indicate significant differences ($P<0.05$) according to Scott-Knott test.

When analyzing the covering of *C. citriodora* leaves, it was found that they have a single layer of epidermal cells, covered by a cuticle layer (Figure 4). As for the thickness, it was observed that in the adaxial epidermis there was a similarity in all LEDs. On the abaxial surface, it was found that for the blue LED, there was less thickening when compared to the other treatments (4.63 μm). According to Taiz et al. (2017), the epidermis presents itself as the outermost layer of plant cells and usually consists of a single layer of cells.

The use of LED lamps with different spectral qualities resulted in some distinct morpho-anatomical responses. These studies have implications and consequences about the use of LED lamps on the leaf anatomy of plants in order to improve the production of forest seedlings, as in clonal mini-gardens for obtaining cuttings, possibly improving seedling quality.

4. CONCLUSION

The different spectral qualities affected some of the growth characteristics, such as leaf blade thickness, leaf area, dry matter, stomatal density, decreased chlorophyll *b* and total chlorophyll content under red LED and caused thinner epidermis under cultivation under blue LED. For the cultivation of *Corymbia citriodora* seedlings using LED lamps, the use of white spectral quality is the most appropriate and favors the dispatch of seedlings at 102 days after emergence, both in the parameters evaluated for leaf area index and for dry matter.

5. ACKNOWLEDGEMENTS

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