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FORESTRY SCIENCE

Light-emitting diodes (LEDs) in development and response to water stress in *Eucalyptus benthamii* seedlings (Myrtaceae)

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ABSTRACT: Plants use light as a source of energy in the process of photosynthesis. Different levels of luminosity cause physiological and morphological changes in the plant, and its success depends on its adaptation to these different levels. Light emitting diodes (LED) have been proposed as a light source in controlled environments. The present research evaluated physiological and morphological aspects in *Eucalyptus benthamii* (Myrtaceae) seedlings kept under different colors of LED lamps and submitted to water stress. The experiment was carried out in a completely randomized design, in which the treatments were defined as: T1 (control, with white light); T2 (blue light); T3 (red light) and T4 (mixed blue and red light). Red light resulted in the best response to most morphological variables in plant growth. In response to water stress, blue light LEDs resulted in a better seedling response, with slower reduction of photosynthetic rate and other variables. This may indicate the possibility of reducing water deficit damage in seedlings acclimated to blue light prior to field planting.
Key words: seedling acclimatization, light supplementation, photosynthesis.

Uso de diodosemissores de luz (LEDs) no desenvolvimento e resposta a estresse hídrico em mudas de *Eucalyptus benthamii* (Myrtaceae)

RESUMO: As plantas utilizam a luz como fonte de energia no processo de fotossíntese. Diferentes níveis de luminosidade causam mudanças fisiológicas e morfológicas na planta, e o seu sucesso depende da sua adaptação a esses diferentes níveis. Diodos de emissão de luz (LED) têm sido propostos como fonte luminosa para ambientes controlados. O objetivo do presente trabalho foi avaliar aspectos fisiológicos e morfológicos em mudas de *Eucalyptus benthamii* (Myrtaceae) mantidas sob diferentes cores de lâmpadas LED e submetidas ao estresse hídrico. Foram realizados dois experimentos, sendo que um avaliou o efeito de diferentes cores de LEDs no crescimento das mudas e outro analisou o efeito das luzes na aclimatação das mudas e após exposição a deficit hídrico. O experiment foi conduzido em delineamento inteiramente casualizado, em que os tratamentos foram definidos como T1 (controle, com luz branca); T2 (luz azul); T3 (luz vermelha) e T4 (luz azul e vermelha mista). A luz vermelha resultou em melhor resposta para a maioria das variáveis morfológicas no crescimento das plantas. Emresposta ao estresse hídrico, os LEDs de luz azul resultaram em uma melhor resposta das mudas, reduzindo mais lentamente a taxa fotossintética e demais variáveis. Isso pode indicar a possibilidade de reduzir os danos de deficit hídrico nas mudas aclimatadas com luz azul anteriormente ao plantio a campo.
Palavras-chave: aclimatação de mudas, suplementação de luz, fotossíntese.

INTRODUCTION

Plants use light as a source of energy in the process of photosynthesis and respond to this light energy according to its intensity, wavelength, and direction in which it is being emitted. Plants perceive light through photoreceptors, such as phytochromes and cryptochromes, and respond to these receptors generating a series of specific physiological responses (MUNEER et al., 2014).

Light-emitting diode (LED) lamps are often used in place of fluorescent lamps, as LED light sources are more efficient, versatile, and economically viable for *in vitro* plant growth and regeneration, offering alternatives to obtain positive results in the field on a commercial scale (MONTEUUIS, 2015).

Different studies indicate better growth of plants under LED lighting conditions (HUNG et al., 2015; FERREIRA et al., 2017; LERIN et al., 2019). However, the effect of light sources on the morphogenesis of the species and clones of interest needs to be evaluated for protocol optimization. The red and blue wavelengths correspond to the absorption peak of chlorophyll a (660 nm) and chlorophyll b (460 nm) (TAIZ & ZEIGER, 2013).

According to DONG et al. (2014) and SAMUOLIENĖ et al. (2013) the great challenge of cultivation with artificial light is to provide controlled

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light intensities in sufficient quantity and quality for the development of plants. Light-emitting diodes (LED) have been proposed as a light source for controlled environments in agricultural facilities or in plant growth chambers. They have desirable characteristics, such as the ability to control the spectral composition, long durability, ability to emit specific wavelengths, relatively cold emission surfaces, in addition to having a reduced size, which facilitates handling and installation in growth chambers (LI et al., 2010; MUNEER et al., 2014).

One of the main factors that contribute to the decrease in seedling development, in addition to causing negative morphological, physiological, and nutritional changes, which influence the seedling's ability to resist adverse environmental conditions; and consequently, its quality is water stress (LISAR et al., 2012). The water stress to which a plant is subjected can be characterized as water deficit, more common, or as water saturation (flooding). Water deficiency stress induces a wide range of physiological and biochemical changes in plants; blocking cell growth and photosynthesis, in addition to changes in respiration, these being the first effects (VELÁZQUEZ & HERNÁNDEZ, 2013).

Taking these points into account, our hypothesis is that plants grown in different light lengths may show greater growth and/or tolerance to physiological and morphological aspects when subjected to water stress. In this way, supplementation with artificial light could be used as a form of pre-acclimatization to the field of seedlings aiming at greater hardening. Thus, the objective of the present research evaluated physiological and morphological aspects in *E. benthamii* seedlings kept under different colors of LED lamps and submitted to water stress.

MATERIALS AND METHODS

Clonal seedlings (a commercial clone) of *E. benthamii* developed and cultivated by a forestry company in the region were used. The clone was selected based on growth, shape and adaptation to the cold climate of the growing region. Clonal seedlings were chosen to avoid genetic variation between plants. The seedlings with approximately 120 days of age, produced at Forest Nursery in the municipality of Otacílio Costa (SC/Brazil) measuring an average of 25 cm in height and 2 mm stem diameter, were kept for 15 days in a shade house (50% shading), with temperature varying between 15 to 28 ºC and 70% of air humidity.

For the experiment testing the growth of plants subjected to different LED lights, the seedlings were transferred to 2L pots, which were filled with commercial substrate based on Pinus bark, with the addition of 6 g L^{-1} of Osmocote® (19-06-10) with 3-4 months of release. The pots were allocated in the growth room (25±2 ºC and 60±10 % relative humidity) being divided into 4 treatments, using 8 replicates of one seedling each.

The experiment was carried out in a completely randomized design, in which the treatments were defined as: T1 (control, with white light); T2 (blue light); T3 (red light) and T4 (mixed blue and red light). The specifications of each lamp were: (i) white lamps - 36", 1W, AC85 - 265 V and (ii) colored lamps - 36", 1W, AC85 - 265 V. Figure 1 shows the installation scheme of the experiments, showing the lights used. Each shelf was sealed with a blackout curtain to prevent light from interfering with another treatment. Three lamps were allocated per bench, three white in the control treatment, and two colored for one white in the colored light treatments.

Biometric evaluations of height and diameter were carried out in two stages, the first being on the day of implementation, and the second at the end of the experimental period, 33 days after implantation. The height of the seedlings was determined with the aid of a millimeter ruler, measuring from the base of the plant to the insertion of the highest leaf. The stem diameter was determined with the aid of a digital caliper. The measurements were carried out in the region of the neck of the seedlings. The data from these evaluations were used to obtain the increment in height and diameter of the neck, as well as the height-diameter ratio (h/d).

At the end of the experimental period, using the destructive method, the total biomass of the plants was manually collected. The samples were divided into root and shoot and placed in paper bags kept in an oven with forced air circulation (60 ± 3 °C) until reaching constant mass (grams) after 96 hours. Afterwards, the samples were weighted to determine the dry matter contents of the components on a precision scale (0.01 g). The Dickson quality index (DQI) was also determined. This was determined as a function of shoot height (H), collar diameter (DC), shoot dry matter weight (PMSPA) and root dry matter weight (PMSR), using the formula (DICKSON et al., 1960).

To evaluate the response of the seedlings to water stress, they were kept in 55 cm^3 tubes and placed in the growth room (25±2 ºC and 60±10 % relative humidity), under different lights. The experiment was carried out in a completely randomized design, in which the treatments were defined as: T1 (control, with white light); T2 (blue light); T3 (red light)

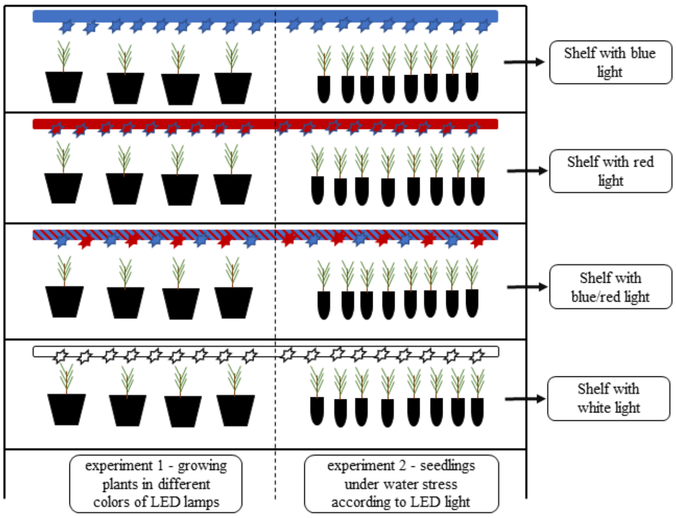

Figure 1 - Scheme of installation of the experiments, showing the LED (light-emitting diodes) lights used.

and T4 (mixed blue and red light), with the same characteristics of the lamps presented above. Fourteen replicates (one plant each) were used per treatment. The evaluation of the experiment was carried out after 14 days of acclimatization of the seedlings, and they were kept in the growth room.

After the initial measurement, irrigation of the plants was suspended for 24 hours to induce stress. During these 24 hours, measurements were taken at previously defined time intervals, initially every two hours, to define the effect of stress on the seedlings during this period. To measure photosynthetic variables, five seedlings were selected for each treatment, which were performed with the portable photosynthesis measurer IRGA (Infra-Red Gas Analyzer), model LI-6400 (LI-COR). Photosynthetic analyzes were performed 24, 30 and 48 hours after stress induction, respecting the randomness between treatments. The photosynthetically active radiation (PAR) used during the measurements was 700 µmol of photons m^{-2} s^{-1}, provided by an artificial light source (LI-6400-40), and the percentage of blue light was 10% of the PAR. The atmospheric concentration of CO_2 ranged from 390 to 400 µmol mol^{-1}.

With the IRGA, the variables of net photosynthetic rate (A - µmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (Gs - mol m^{-2} s^{-1}), transpiration (E - mmol H_2O m^{-2} s^{-1}), intercellular concentration of CO_2 in the mesophyll (Ci - µmol CO_2) and current external CO_2 concentration (Ca - µmol CO_2) were measured. These variables later made it possible to calculate the ratios of intercellular concentration of CO_2 in the mesophyll over the current external CO_2 concentration (Ci/Ca-µmol CO_2) and the plant water use efficiency (WUE – [µmol m^{-2} s^{-1}] $^{-1}$). WUE is a measure of the carbon gained by plants through photosynthesis relative to the water lost through transpiration, defined a A/E.

The normality of the data was ascertained with the Shapiro-Wilk test as was the homogeneity of variances with Bartlett's test. They were then submitted to an analysis of variance (ANOVA). And with significant F values ($P < 0.05$), Tukey's range test ($P < 0.05$) was used to find the differences of means. As a complementary analysis, the data were submitted to the elaboration of matrices of variance and covariance to proceed with the analysis of principal components (PCA).

RESULTS

In the evaluation of morphological variables (Table 1), diameter increment at root height (IDAC), height/diameter ratio (h/d) and root dry mass (MSR) showed no significant difference between treatments on the different LED lamps used in plant growth. The other variables showed a significant difference depending on the color of the LED used.

The seedlings under blue light showed a greater increase in height (HI), however, they did not statistically differ from the seedlings under red and

Table 1 - Morphologic variables obtained in the evaluation of *Eucalyptus benthamii* seedlings, according to the different LED lights (light-emitting diodes).

Treatment	---Variables---						
	HI(cm)	SDI(mm)	h/d	SDM(g)	RDM(g)	TDM(g)	DQI
Control Group (White Light)	5.48 b*	0.95	13.74	1.60 c	0.76	2.37 c	0.15 b
Blue Light	14.10 a	1.16	14.44	2.80 ab	0.95	3.76 ab	0.22 b
Red Light	10.38 ab	1.46	12.31	3.72 a	1.00	4.73 a	0.30 a
Mixed Light	8.22 ab	0.95	14.1	2.58 bc	0.80	3.38 bc	0.20 b

*Equal letters don't differ between treatments according to Tukey test at 5% probability. Where: HI: Height increment; SDI: Stem diameter increment; h/d: Height/diameter ratio; SDM: Shoot dry mass; RDM: Root dry mass; TDM: Total dry mass; DQI: Dickson quality index.

mixed light. The seedlings under red light showed heavier aerial dry mass (MSA) and total dry mass (MST), not statistically different from the seedlings under blue light in both variables. In Dickson's quality index (DQI), seedlings under red light also showed higher value, statistically different from all other treatments. The significant increase in the height increase of the seedlings under colored LEDs stands out immediately, in the comparison between blue and red light from the control.

Principal component analysis (PCA) showed that the first two principal components account for 78.2% of the total variance. The first component and the second component account for 58.5% and 19.6% of the total variance, respectively. It can also be seen from the PCA that the blue light corresponds to and explains growth variables such as HI, SDM and TDM. Already red light on DQI and SDI (Figure 2).

In the response of seedlings to water stress, a significant difference was observed between treatments in all evaluations carried out during the 48 hours of follow-up after the induction of water stress (Figure 3).

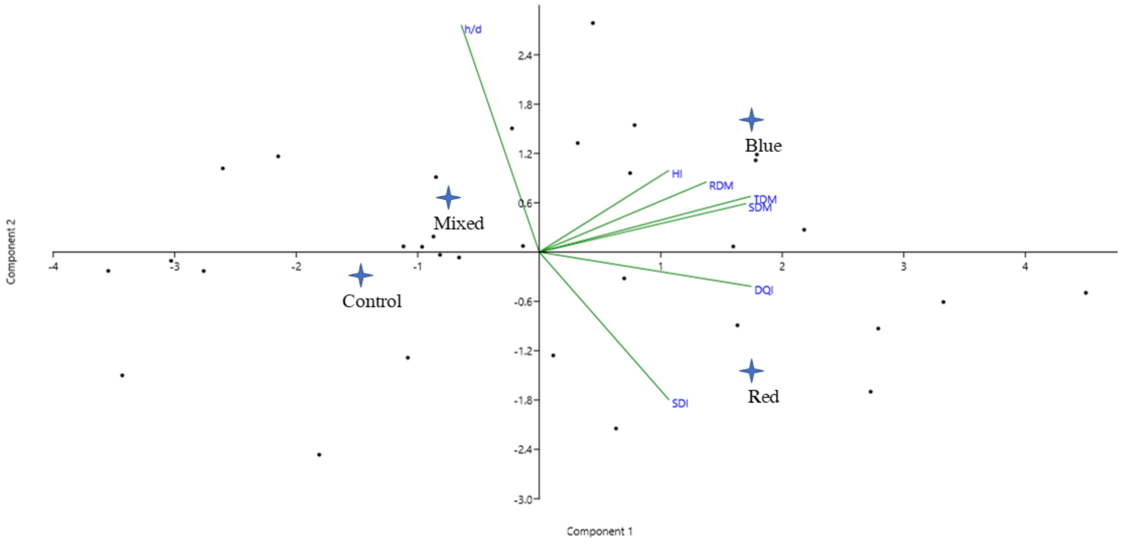

Figure 2 - Principal component analysis (PCA) of the variables analyzed in relation to LED (light-emitting diodes) light treatments on the growth of *Eucalyptus benthamii* seedlings. Where: HI: Height increment; SDI: Stem diameter increment; h/d: Height/diameter ratio; SDM: Shoot dry mass; RDM: Root dry mass; TDM: Total dry mass; DQI: Dickson quality index.

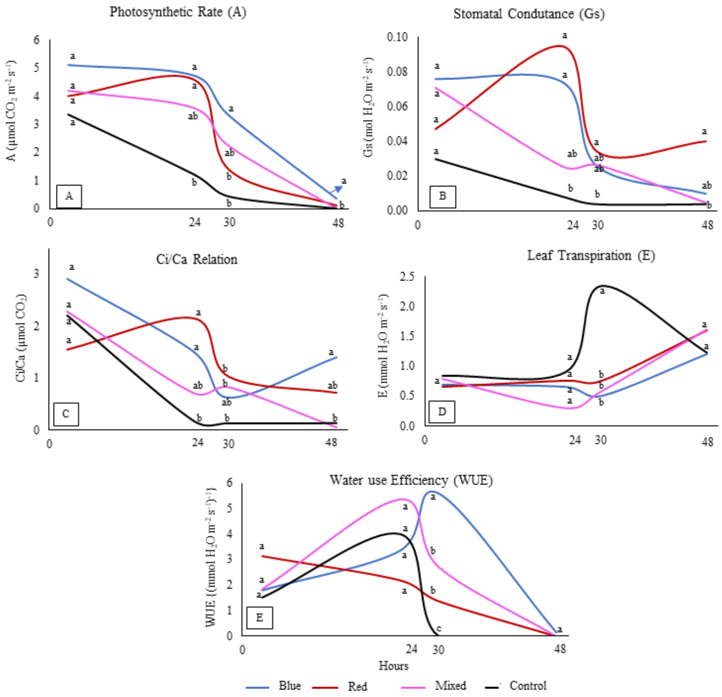

Figure 3 - A) Photosynthetic rate (µmol CO2 m-2 s-1), B) Stomatal conductance (H2O m-2 s-1), C) Ci/Ca ratio - Ci/Ca (Intercellular CO2 concentration/ external CO2 concentration ratio in µmol CO2), D) Leaf transpiration (H2O m-2 s-1), E) Water use efficiency – WUE (mmol H2O m-2 s-1) in *Eucalyptus benthamii* seedlings under water stress according to LED (light-emitting diodes) light during the experiment. Letters correspond to differences between treatment groups by Tukey test.

For photosynthesis (Figure 3a) there was no significant difference between treatments in the evaluation performed before induction. After 24 hours a significant drop in photosynthesis under white light (control), a moderate drop in seedlings under mixed light and a slight reduction in both blue and red light can be seen. After 30 hours, photosynthesis of seedlings under white light approaches zero, and finally hits zero after 48 hours, demonstrating the rapid effect of stress on seedling metabolism. The photosynthesis under mixed light drops after 30 hours, ending after 48 hours, also at zero. Until 30 hours, the drop in photosynthesis under blue light is slight, remaining almost three times higher than the photosynthetic rate of seedlings under red light, which feel greater effect from the same point onwards, both end the experiment with photosynthesis close to zero, but not completely zeroed.

For stomatal conductance (Figure 3b), the drop in seedlings under blue light is lighter, with these being noticeable after 30 hours of drought, while the others show effects after 24 hours for white and mixed light, and 30 hours for red light. The seedlings under white and mixed light ended the experiment with zero conductance.

For external and internal carbon ratio (Ci/Ca) (Figure 3c), in the seedlings under blue light, the drop was slight, being noticeable after 30 hours, and showing recovering slightly after 48 hours of drought. The ratio in seedlings under red light had a slight increase after 24 hours, and a strong drop after 30, also recovering slightly, but falling again after 48 hours, the same behavior was observed in seedlings under mixed light. The ratio in seedlings under white light dropped sharply after 24 hours and remained stable after that.

Transpiration (Figure 3d) remained stable in all treatments up to 24 hours. After 30 hours, the transpiration of seedlings under white light increased sharply and recovered slightly after 48 hours. The transpiration of the other seedlings remained stable until 48 hours of drought, when a significant effect of stress on this variable was observed.

The water use efficiency (WUE) (Figure 3e) of all seedlings remained stable until 24 hours of drought. After 30 hours, only the seedlings under blue light did not show a drop in this variable, and the efficiency of the seedlings under white light had already reached zero at that point. After 48 hours, only the seedlings under blue light had not zeroed in terms of WUE.

Principal component analysis (PCA) showed that the first two principal components account for 87.8% of the total variance (Figure 4). The first component and the second component account for 65.3% and 22.5% of the total variance, respectively. Blue and red light explain most of the variables such as photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), stomatal conductance (H_2O m^{-2} s^{-1}) and leaf transpiration (H_2O m^{-2} s^{-1}). Blue and red lights (mixed) have a greater influence on the *WUE* variable.

DISCUSSION

We observed that in the growth of *E. benthamii* seedlings, supplementation with blue and red light promoted an improvement in morphological characteristics such as increase in height, dry mass and DQI. Studies indicate that red light promotes leaf and stem elongation via stimulation of gibberellin biosynthesis, through the control of mitosis and cell proliferation (MANIVANNAN et al., 2015).

In the production of raspberry seedlings cultivars Batum and Dorman Red, ROCHA et al. (2013) observed that the fresh mass of the plant grown under the red LEDs presented superior results than the plants grown under the blue and green LEDs. BELLO-BELLO et al. (2016), evaluating the quality of light (fluorescent lamp, white LEDs, red LEDs, blue LEDs, and blue plus red LEDs) in the growth of shoots of *Vanilla planifolia* Andrews, reported that the highest average fresh mass per plant was obtained with the treatment consisting of blue LEDs plus red LEDs.

Plant growth, leaf water potential and crop production are critically limited or even suppressed by water restriction (HOSSAIN et al. 2016). It was reported that the treatment of two *Melissa officinalis* genotypes with LED lights significantly improved tolerance to water stress (AHMADI et al. 2019) probably by maintaining leaf turgor pressure at satisfactory levels and attenuating the effects of stress. Furthermore, promising results indicated that drought tolerance in two lemon balm genotypes was increased by LED pretreatment, since the relative water content in LED-treated plants remained like that of seedlings kept in a greenhouse (AHMADI et al. 2019).

The smaller decrease in photosynthesis of plants conducted under blue light during the water stress process may be related to the physiological process that occurs. Blue light plays a crucial role in

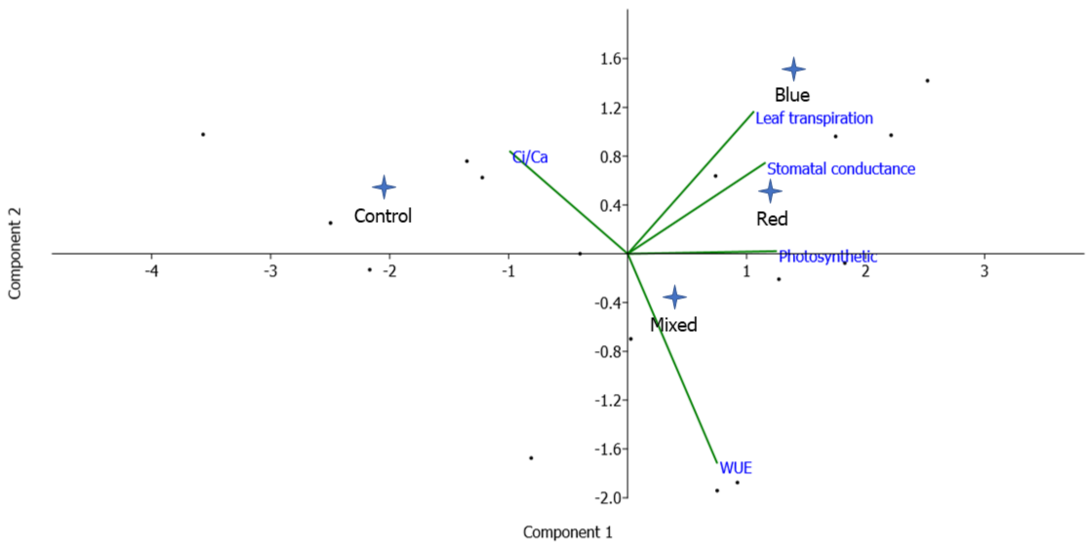

Figure 4 - Principal component analysis (PCA) of the variables analyzed in relation to LED (light-emitting diodes) light treatments under water stress in function to LED light of *Eucalyptus benthamii* seedlings. Where: Photosynthetic rate (µmol CO2 m-2 s-1), Stomatal conductance (H2O m-2 s-1), Ci/Ca ratio - Ci/Ca (Intercellular CO2 concentration/external CO2 concentration ratio in µmol CO2), Leaf transpiration (H2O m-2 s-1), and Water use efficiency – WUE (mmol H2O m-2 s-1).

water relations and gas exchange; and consequently, in growth and tolerance to stress factors. Furthermore, it exerts a positive and coordinated influence on the plant genome and plastid, which influences the development of chloroplasts in plant cells and the synthesis of chlorophyll. The absorption of blue light by chlorophylls promotes a higher energy state than that of red light due to the higher energy existing in blue light (TAIZ & ZEIGER, 2013).

Among the various narrow spectrum lights, blue and red LEDs are mainly used for plant growth because their wavelengths of approximately 460 and 660 nm respectively are highly effective for chlorophyll absorption, which results in optimal photosynthetic efficiency (JOHKAN et al. 2010; GUPTA & JATOTHU, 2013).

The experiment indicated advantages in using colored LEDs regarding the response of seedlings to water stress, and seedlings under white LEDs showed more severe and faster physiological effects. However, the use of blue LEDs can be recommended, and the seedlings of this treatment resisted for longer, and when there were drops, these were lighter.

CONCLUSION

The red light resulted in greater development in most of the evaluated traits, and in the traits in which it did not obtain better results, it did not statistically differ from the seedlings under blue light.

In response to water stress, blue light LEDs resulted in a better response of seedlings, slower reducing photosynthetic rates such as photosynthesis. This may indicate the possibility of treating the seedlings before planting in the field, to reduce possible effects of water deficit.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS’ CONTRIBUTIONS

The authors contributed equally to the manuscript.

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