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Does light supplementation improve the initial growth of hops seedlings in a protected environment?

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ABSTRACT

The hops (*Humulus lupulus* L.) has significant importance for Brazil since its consumption in the manufacture of beer is primordial, although the country imports almost 100% of the hops consumed. Research in this area has increased recently, bringing new information and guidelines to support national production and provide a technical basis for improving planting and cultivation techniques. The present study aimed to evaluate the influence of different light supplementations (Control - environmental condition, 400 µmol m^{-2} s^{-1}, 600 $µmol^{-2}$ m s^{-1}, and 800 µmol m^{-2} s^{-1}) on the production of hops seedlings in a greenhouse. We found that the responses are distinct among the cultivars used, but light supplementation increases variables related to physiological processes and seedling growth. Thus, we conclude that light supplementation with photosynthetically active radiation is advantageous for the production of hops seedlings since positive effects were found on the physiological characteristics of gas exchange related to photosynthesis and plant development when 400, 600, and 800 µmol m^{-2} s^{-1} were applied. In addition, the intrinsic responses of each cultivar should be observed to adapt the production system.

Keywords: *Humulus lupulus* L., Indoor cultivation, Photosynthetic activity; Carboxylation.

Suplementação luminosa promove o crescimento inicial de mudas de lúpulo em ambiente protegido?

RESUMO

A cultura do lúpulo (*Humulus lupulus* L.) tem grande importância para o Brasil, visto que seu consumo na fabricação de cervejas é primordial, muito embora o País importe praticamente 100% do lúpulo consumido. Nos últimos anos as pesquisas nesse quesito têm aumentado, trazendo novas informações e diretrizes para embasar a produção nacional, a fim de fornecer embasamento técnico para aprimorar as técnicas de plantio e cultivo. Isto posto, o objetivo do presente trabalho foi avaliar distintas suplementações luminosas (Controle – condição ambiental; 400 µmol m^{-2} s^{-1}; 600 µmol m^{-2} s^{-1}; 800 µmol m^{-2} s^{-1}), na produção de mudas de lúpulo em casa de vegetação. Verificou-se que as respostas são distintas entre as cultivares utilizadas, porém, a suplementação luminosa resulta em incremento de variáveis ligadas aos processos fisiológicos e de crescimento das mudas. Desta maneira, concluímos que suplementação com radiação fotossintéticamente ativa é vantajosa à produção de mudas de lúpulo, devido aos efeitos positivos sobre as características fisiológicas de troca gasosa relacionadas à fotossíntese e ao desenvolvimento dos vegetais quando a intensidade utilizada é de 400, 600 e 800 µmol m^{-2} s^{-1}. Em complemento, as respostas intrínsecas de cada cultivar devem ser observadas a fim de adaptar-se o sistema de produção.

Palavras-chave: *Humulus lupulus* L., Cultivo indoor, Atividade fotossintética, Carboxilação.

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1. Introduction

Hops (*Humulus lupulus*) is a perennial, dioecious plant belonging to the Cannabaceae family and the Humulus genus. It is a vigorous climbing plant that can grow 7 to 8 meters high, with roots that can grow up to 100 meters long (Nguyen et al., 2020). Brazil is a major consumer of hops due to its tradition of beer consumption and brewing, although almost all hops consumed are still imported. According to data released by the Secretary of Foreign Trade of the Ministry of Industry, Foreign Trade and Services, 4,721 tons of hops were imported in 2021, with an expenditure of $82.078 million.

Although vigorous, hops are very susceptible to climate and solar radiation, making it difficult to grow outside their natural habitats in temperate climate regions. This susceptibility is even more evident regarding its flowering, which is controlled by plant maturity and daytime length (Tavares et al., 2017; Nguyen et al., 2020). According to Jastrombek et al. (2022), the major challenge for developing hops production in Brazil is the adaptation of cultivars to local geographic and climatic conditions.

Hops plants are most productive when adequate vegetative growth (twining stem) is achieved by the long day length before flowering is induced (Agehara, 2020). Maximizing vegetative growth before flowering is essential; for this to occur, the crop requires more than 15 hours of daylight during the early season and less than 15 hours at the end of the season (Jastrombek et al., 2022). For the production of high-quality seedlings, the correct management of light is necessary to favor the development of full-grown plants. Light is essential for the growth and development of plants. As an energy source, it drives photosynthesis in biomass accumulation, but specific wavelengths can also drive different processes (Christiaens et al., 2019).

Artificial light has proven an excellent solution in seedling production to mitigate the effects of poor radiation and low yield in hops cultivation. The use of supplemental light from LED lamps to control the flowering of plants in the field has received prominence since no Brazilian region provides the minimum photoperiod for the development of the hops crop, specifically the regions between latitudes 35° and 55° North or South of the equator (Jastrombek et al., 2022). Choosing an LED lamp with the optimal light spectrum composition for crop production is important. Red light is generally sufficient to inhibit flowering in short-day plants (as is the case with hops), and adding high-intensity far-red light is not desirable (Craig and Runkle, 2013).

The main advantage of LED technology compared to traditional light sources is the ability to control the spectral composition of light for specific applications (Morrow, 2008). Although the use of more modern lamps with appropriate wavelengths of light for each crop is beneficial to the production of hops strobiles, the arrangement of the lamps also influences harvest results since variations in wavelength and the amount of light received/emitted will have a direct influence on the flowering promotion. The present study aimed to analyze the effect of light supplementation on the initial growth and gas exchange of seedlings of two cultivars of hops.

2. Material and Methods

The present study was conducted in a climate-controlled greenhouse in the experimental sector of the Mato Grosso do Sul State University (Cassilândia- MS) from April to May 2022. According to Köppen's climate classification, the region has a rainy tropical climate (Aw-type) with rainy summers and dry winters. The greenhouse is covered on the side and surface ceiling with 150 microns of low-density polyethylene (LDPE) film, a light diffuser, and a Humil Cool (CELDEX®) climate control system, which is programmed to operate at 25°C.

The experimental design was entirely randomized (DIC) in a 2x4 factorial scheme and four replications. The treatments consisted of two hops cultivars (Cluster and Southern Cross) subjected to four light supplementations (Control; 400 µmol m^{-2} s^{-1}; 600 µmol m^{-2} s^{-1}; 800 µmol $m^{-2}s^{-1}$). Supplementation intensities with different photosynthetically active radiation were obtained by combining lamp power and lamp distance (400 µmol m^{-2} s^{-1} = 32,000 lumens at 43 cm distance from the plants; 600 µmol m^{-2} s^{-1} = 32.000 lumens at 35 cm distance from the plants; 800 µmol m^{-2} s^{-1} = 51,300 lumens at 30 cm distance from the plants). The lamps were installed to provide a uniform distribution of photosynthetically active radiation over the plants between 17:00 and 21:00 hours).

Cultivation was performed in commercial plastic tubes with a volume of 1 dm^3, containing soil classified as Neossolo Quartzarênico with the following particle-size characteristics: 125 g kg^{-1} of clay, 75 g kg^{-1} of silt and 800 g kg^{-1} of sand and chemical characteristics: pH ($CaCl_2$) = 4.2, organic matter =7.0 g dm^{-3}, P (resin) = 3 mg dm^{-3}, K^+ = 0.5 mmol dm^{-3}, Ca^{2+} = 7 $mmol_c$ dm^{-3}, Mg^{2+} = 4 $mmol_c$ dm^{-3}, H+Al = 30 mmol dm $^{-3}$, Al^{3+} = 12 mmol dm $^{-3}$, CEC = 42 mmol dm^{-3}, sum of bases= 12 mmol dm $^{-3}$, base saturation = 29%. The soil was previously corrected with calcined limestone, aiming to reach 80% base saturation, and fertilized with 150 g m^{-3} of P_2O_5, using 75 g m^{-3} simple superphosphate and 75 g m^{-3} phosphate fertilizer with micronutrients (Yoorin Master). One plant was grown in each tube, obtained by staking in a hydroponic system. Before the experiment, the plants were pruned to homogenize the size and emission of new shoots. The water supply was done according to need without specific quantities of irrigation.

The supplementation of photosynthetically active radiation in the climate-controlled greenhouse was provided by led grow lamps (Epistar, China) with red LEDs with a wavelength of 620-630 nm, blue LEDs with a wavelength of 440-445 nm, white LEDs of 5500-6500k and 2500-3300k, infrared LEDs with a wavelength of 730 nm, and ultraviolet LEDs with a wavelength of 380-410 nm, according to the following proportions 67%, 15%, 10%, 5%, and 3% of LEDs at each wavelength, respectively.

During the conduction of the experiments, meteorological variables in the growing environment were monitored and collected by measuring photosynthetically active radiation (µmol m^{-2} s^{-1}) with the Apogee device (MQ200) daily between 9 and 10 am. The data collection equipment was positioned over the growing tubes to intercept the radiation. In this sense, during the period of conduction of the experiment, it was verified a photosynthetically active radiation close to 400 µmol m^{-2} s^{-1}.

After 30 days of transplanting, the characteristics of net photosynthesis (A), stomatal conductance (gS), intracellular CO_2 concentration (Ci), and transpiration (E) were evaluated during the morning, when the plants are in full gas exchange activity, between 8 and 10 am, using a portable photosynthesis meter (LCi, ADC Bioscientific, Hertfordshire, UK) and instantaneous carboxylation efficiency (IICI) was calculated using the A/Ci ratio and water use efficiency (WUE) using the A/E ratio. The following characteristics were also evaluated: branch length, by measuring with a graduated ruler; number of shoots and number of leaves, by counting; relative chlorophyll content, using a digital chlorophyllometer (CCM-200, Opti-Sciences, Hudson, USA); leaf area, using the EasyLeafArea application (Easlon and Bloom, 2014); shoot dry mass, obtained after drying the material in an air-forced circulation oven at 65 °C until constant mass was obtained. The data were submitted to the analysis of variance (F test), and the means for the cultivars were compared by the t-test (LSD) and the means for the supplementation intensity by the Scott-Knott test, both 5% of probability.

3. Results and Discussion

For internal CO_2 concentration in the Cluster cultivar, the three light treatments did not differ but were superior to the control. In contrast, for Southern Cross, the control treatment was superior to the treatment with 800 µmol m^{-2} s^{-1}. This, in turn, was superior to the treatments with 600 µmol m^{-2} s^{-1} and 400 µmol m s^{-2-1} (Figure 1A). The transpiration was higher in the treatment with 400 µmol m^{-2} s^{-1}, and the other light levels, including the control, showed no statistical difference between them, demonstrating that the lowest dose of light tested increased the transpiration of hops seedlings (Figure 1B). For stomatal conductance, there was no difference between treatments for both cultivars assessed (Figure 1C).

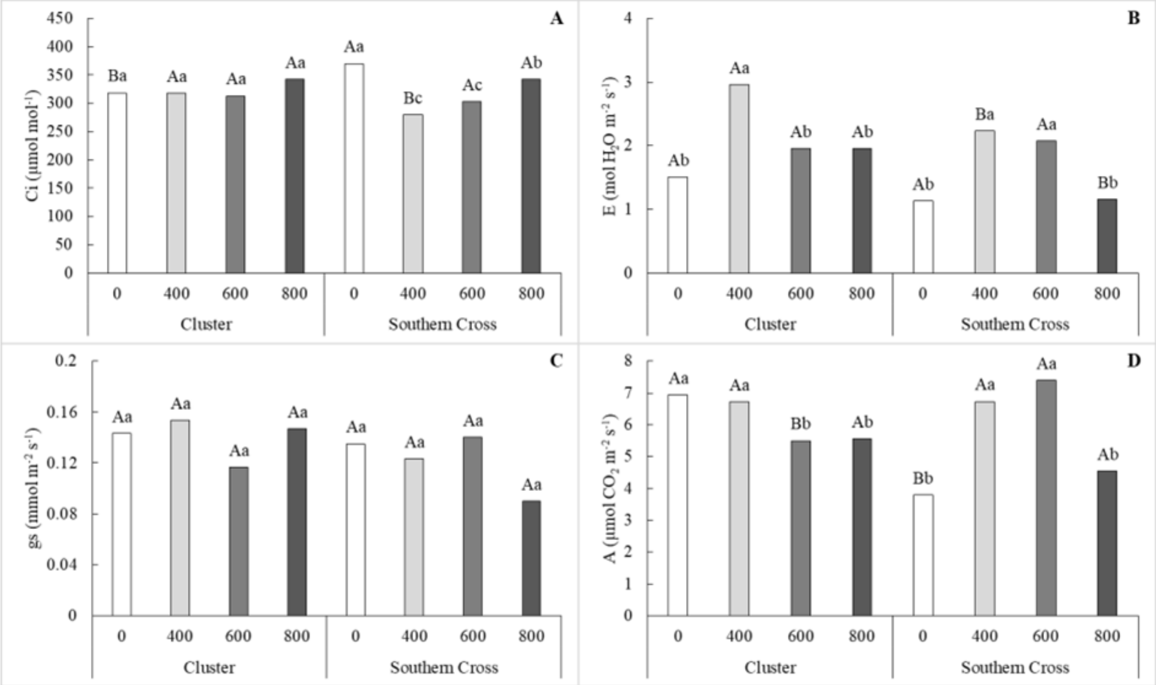

Figure 1. Internal CO_2 concentration (A), transpiration (B), stomatal conductance (C), and net photosynthesis (D) of *H. lupulus* seedlings submitted to light supplementation. Different letters on the bars indicate a significant difference between the means ($P < 0.05$). Bars represent mean values ($n = 4$). Lowercase letters compare treatments, and uppercase letters compare cultivars.

For Cluster, there were higher net photosynthesis rates in the control treatment and 400 µmol m^{-2} s^{-1}, while for the Southern Cross cultivar, higher values were obtained at light levels of 400 and 600 µmol m^{-2} s^{-1} (Figure 1D). The water use efficiency of the cultivars was divergent. While the Cluster cultivar obtained the highest water use efficiency in the control treatment, for the Southern Cross cultivar, there was no difference between the treatments. Also, among the cultivars, Cluster was superior only in the control treatment, while in the other treatments, Southern Cross stood out (Figure 2A).

For carboxylation efficiency, superiority was found for treatments with 0 and 400 µmol m^{-2} s^{-1} for Cluster and 400 and 600 µmol m^{-2} s^{-1} for Southern Cross. Among cultivars, Cluster was superior in the control treatment, while Southern Cross excelled when radiation of 600 µmol m^{-2} s^{-1} was applied (Figure 2B). For shoot length, it was found that supplementation with different light levels was more efficient when compared to the control treatment in the Cluster cultivar. For the Southern Cross cultivar, all treatments were similar. Among the cultivars, only Southern Cross was superior in the control treatment (Figure 3A).

For the number of branches, it was verified that the Cluster cultivar was superior in the 400 µmol m^{-2} s^{-1} and 600 µmol m^{-2} s^{-1}, evidencing that the treatment with the highest light intensity does not lead to a greater number of shoots. It was also found that for Southern Cross, there was a higher number of branches in the treatment with supplementation of 800 µmol m^{-2} s^{-1} of photosynthetically active radiation, in contrast to the other treatments, for which lower results were obtained, being the treatment with 400 µmol m^{-2} s^{-1} the one with the lowest efficiency (Figure 3B).

For the number of leaves, there was superiority only of the cultivar Southern Cross over Cluster in the control and 400 µmol m^{-2} s^{-1} treatments (Figure 4A), while for the relative chlorophyll content, there was a significant difference only between the cultivars in the 400 µmol m^{-2} s^{-1} and 800 µmol m^{-2} s^{-1} treatments, in which Cluster was superior (Figure 4B).

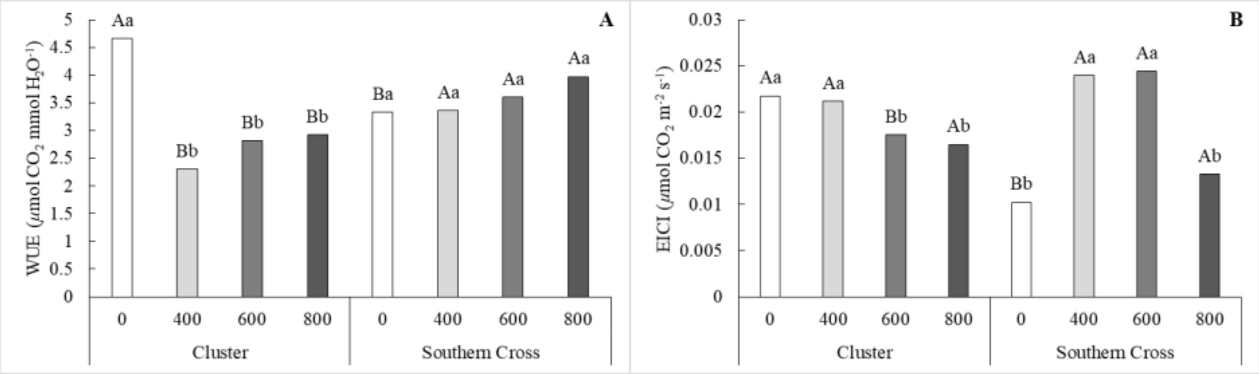

Figure 2. Water use efficiency (A) and intrinsic carboxylation efficiency (B) of *H. lupulus* seedlings submitted to light supplementation. Different letters on the bars indicate a significant difference between the means ($P < 0.05$). Bars represent mean values (n = 4). Lowercase letters compare treatments, and uppercase letters compare cultivars

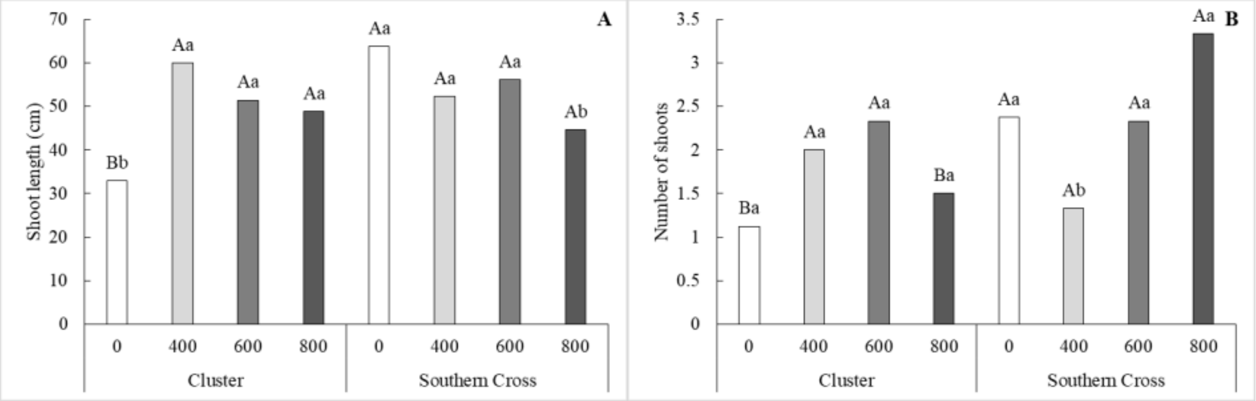

Figure 3. Shoot length (A) and number of shoots (B) of H. lupulus seedlings submitted to light supplementation. Different letters on the bars indicate a significant difference between the means ($P < 0.05$). Bars represent mean values (n = 4). Lowercase letters compare treatments, and uppercase letters compare cultivars.

The reduction in CO_2 concentrations becomes coherent due to the relationship of stomatal opening, entry of atmospheric CO_2, and, consequently, its fixation in forming new carbohydrates (Rocha et al., 2019). In complement, the concentration of CO_2 levels can present oscillation according to the carboxylation efficiency; that is, the fixation of CO_2 mediated by the Rubisco enzyme can be more or less efficient, depending on the conditions submitted to the plants, such as light, temperature, and mainly nutritional, due to macro elements such as nitrogen (Taiz and Zeiger, 2013).

It is important to define the radiation with the optimal light spectrum composition for cultivation since different genetic materials respond in a particular way to this aspect (Bauerle, 2021). Because they inhibit flowering in short-day plants, red light is usually sufficient, and it is not desirable to add a high intensity of far-red light (Craig and Runkle, 2013). However, Agehara and Gallardo (2021) warn that there is substantial variation in spectral sensitivity between crops, pointing out that these rules do not apply in all cases. Hence the need to know the hops production cycle and how each cultivar adapts to different light spectra, in addition to the type of lamp to be used and the light regime to be employed in cultivation.

As observed for the Southern Cross cultivar (Figure 1), light supplementation with LED lamps, with combinations of red and blue light intensity, was able to increase photosynthetic activity in aromatic species such as mint and parsley, even increasing the essential oil content of these species (Litvin et al., 2020). In plants of *Cannabis sativa*, belonging to the same botanical family as hops, recent studies indicate that light supplementation with red-blue wavelengths increases the concentration of THC (tetrahydrocannabinol) in plants (Hawley et al., 2018; Eichhorn et al., 2019).

This increase in the contents of aromatic compounds is also of interest for hops production since these compounds make up the main product of interest of the species (Korpelainen and Pietiläinen, 2021). The increase in gas exchange activity is accompanied by an increase in plant transpiration rate (Figure 1B). A similar effect was obtained for tomato, for which the simultaneous effect of increased net photosynthesis and transpiration rate of plants was observed as the intensities of light supplementation were increased, which also resulted in a significant increase in the dry mass of leaves, stem, and roots of the plants (Pan et al., 2020), corroborating the results obtained in the present study (Figure 4).

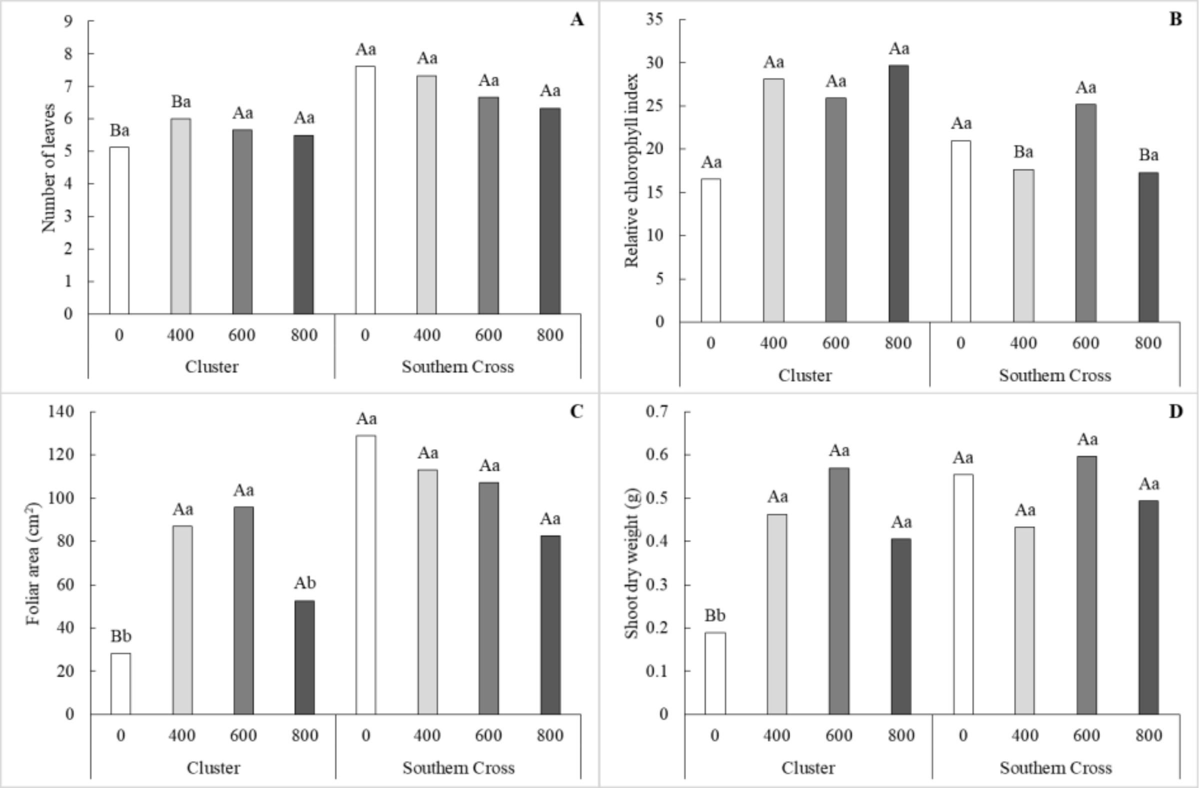

Figure 4. Number of leaves (A), relative chlorophyll index (B), foliar area (C), and shoot dry mass (D) of *H. lupulus* seedlings submitted to light supplementation. Different letters on the bars indicate a significant difference between the means ($P < 0.05$). Bars represent mean values ($n = 4$). Lowercase letters compare treatments, and uppercase letters compare cultivars.

In a study by Gloser et al. (2013), it was observed that hops belong to the anisohydric plants; that is, they are less conservative in their use of water, and this behavior was clear for the Cluster cultivar, showing that in the control treatment, there was the highest water use efficiency when compared to the treatments with artificial supplementation (Figure 2A), evidencing in this case that for this cultivar the use of LED light regime can influence the plant's ability to maintain adequate functionality of stomata.

The efficiency of atmospheric carbon uptake and use also differs among cultivars depending on the amount of photosynthetically active radiation supplied (Figure 2B). In this sense, the study developed by Bauerle (2021) demonstrated significant differences between four hops cultivars submitted to different CO_2 regimes and light supplementation. The author found that under CO_2 conditions close to those found in this study (415 μmol mol^{-1}), light supplementation can be more or less effective on gas exchange and plant development, depending on the responsiveness of the cultivar studied.

The present study reflects the importance of generating information about the adequacy of the environment for the production of seedlings, aiming to obtain a better quality of the plants. Also, the scarce information about the use of light supplementation aimed at the development of hops seedlings reflects the importance of this study as a source of information for the realization of future experiments with the culture, which has been gaining undeniable importance in the Brazilian economic scenario (Fagherazzi and Rufato, 2018).

4. Conclusions

Supplementation with photosynthetically active radiation is advantageous for hops seedling production because of the positive effects on the physiological characteristics of gas exchange related to photosynthesis and plant development when the intensity is 400, 600, and 800 μmol m^{-2} s^{-1}. In addition, the intrinsic responses of each cultivar must be observed to adapt the production system.

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