



LETTUCE GROWTH PROMOTED BY ARTIFICIAL LIGHTING USING LIGHT-EMITTING DIODES (LED)

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ABSTRACT

Understanding how the use of light-emitting diodes (LED) in artificial lighting affects plant growth is of considerable interest to Science because the application of specific light wavelengths to crops can enhance agricultural production. Therefore, the influence of LED lamps in the photosynthetically active spectrum (blue and red light) on the growth of lettuce (*Lactuca Sativa*) was evaluated. A factorial design was used to test 4 combinations of LED lighting (5:1; 3:1; 1:1 and 6:3) and 2 lettuce varieties (butterhead and crisphead). The plants were grown in pots in a controlled environment with a 12h-photoperiod, for 21 days. Leaf number (LN), leaf area (LA) and leaf fresh mass (LFM) were determined along with gas exchange variables (A: net photosynthesis; Ci: CO₂ internal concentration; and WUEi: instantaneous water use efficiency). Growth of lettuce plants was greatest in the source of variation of LED lighting with the combination 5:1, as well as the greatest photosynthetic efficiency. The crisphead lettuce was superior to the butterhead lettuce in all analyzed variables, which suggests a better adaptation to the treatments applied.

Palavras-Chave:

Fotoperíodo
Faixa espectral
Lactuca Sativa

CRESCIMENTO DE ALFACE PROMOVIDO POR ILUMINAÇÃO ARTIFICIAL FORNECIDA POR DIODOS EMISSORES DE LUZ (LED)

RESUMO

Compreender como a iluminação com diodos emissores de luz (LED) afeta o crescimento das plantas é de grande interesse para a Ciência, porque por meio da iluminação em comprimento de ondas específico para as culturas pode-se potencializar a produção agrícola. Nesse contexto, avaliou-se a influência de lâmpadas LED na faixa espectral fotossinteticamente ativa (luz azul e vermelha) no crescimento da alface (*Lactuca Sativa*). Utilizou-se um esquema fatorial com 4 combinações de luz de LED (5:1; 3:1; 1:1 e 6:3) e 2 variedades de alface (Lisa e Crespa). As plantas foram cultivadas em vasos, em ambiente fechado, com fotoperíodo controlado de 12 h durante 21 dias. Foram determinados o número de folhas (NF), área foliar (AF) e massa fresca das folhas (MFF), além das variáveis relacionadas às trocas gasosas (Pn: Fotossíntese líquida; Ci: concentração interna de CO₂ e EiUA: eficiência instantânea do uso da água). O maior crescimento das plantas de alface foi observado na fonte de variação de luz LED com combinação 5:1, bem como, uma maior eficiência na realização de fotossíntese. A variedade de alface crespa se apresentou superior à lisa em todas as variáveis analisadas, o que sugere uma melhor adaptação aos tratamentos aplicados.

INTRODUCTION

Artificial lighting provided by light-emitting diodes (LED) is a promising technology for the greenhouse industry. In addition, LED lamps have technical advantages over conventional artificial lighting sources such as HPS (High Pressure Sodium Lamp) lamps. A useful feature of LEDs is their inherent ability to provide spectral control with luminous flux customization, which allows plant photoreceptors to perceive light signals that can control morphology and improve product quality (CHINCHILLA *et al.*, 2018).

We worked with the hypothesis that the use of LED lighting at wavelengths between 400 and 720 nm, which comprises the spectral range of blue (400-520 nm) and red (610-720 nm) light, will improve the growth and quality of lettuce. The reason for this is that the absorption of light at these specific wavelengths are optimal for carrying out photosynthesis in plants, which will imply greater growth and biomass production (NAZNIN *et al.*, 2019, PALMER & VAN IERSEL, 2020).

However, questions such as which specific spectra and photosynthetic flux densities are required by different plant species and varieties at different stages of ontogenesis and which wavelength combinations should be selected for maximum productivity and optimal nutrition quality remain open (OLLE & VIRŠILE, 2013).

Lettuce is included in in this technological environment because it is a widely consumed vegetable worldwide (MEDEIROS *et al.*, 2019), and in Brazil, 4.8% of the amount (in grams) of vegetables purchased for home consumption is lettuce (CANELLA *et al.*, 2018). This fact prompts researchers to the application of technologies capable of improving production.

Nonetheless, the relationship between blue and red light (R:B) needs to be better understood in lettuce production, and few studies have reported on the effects of the different combinations of colors on lettuce growth (WANG *et al.*, 2016). Thus, the objective of this study was to evaluate the effect of LED light bars available on the market in different combinations of the luminescence spectrum on the

growth and photosynthetic activity of two lettuce varieties.

MATERIAL AND METHODS

The experiment was carried out at the Laboratory of Storage and Processing of Agricultural Products (LAPPA) of the Academic Unit of Agricultural Engineering (UAEA) of the Center of Technology and Natural Resources (CTRN) of the Federal University of Campina Grande (UFCG), located between the geographic coordinates 7°12'58.67"S latitude and 35°54'35.71"W longitude.

The experiment was set up on four cement shelves in an environment protected from external influences. The treatments consisted of variations of horticultural RGB LED lights in different combinations of Red (R) and Blue (B) to compose four variations of alternating (R:B) LED bars (1:1, 3:1, 5:1 and 6:3) (Figure 1), commercially available, to grow two lettuce varieties: the butterhead (Regina) and the crisphead (Veneranda).

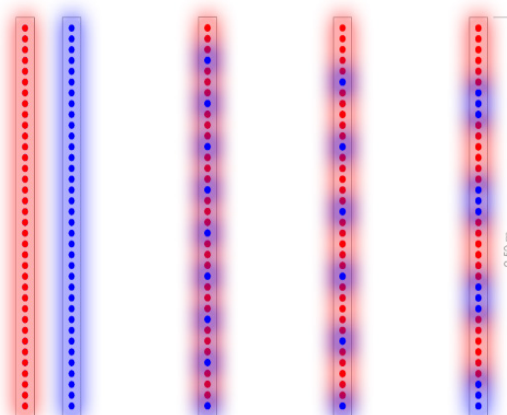


Figure 1. Detailed R/B light combinations (red/blue) to grow lettuce

The experiment was arranged in a 4x2 factorial, completely randomized design with six replications. The factors studied were 4 variations of LED light in the R:B ratios (5:1, 3:1, 6:3 and 1:1) and 2 lettuce varieties (Butterhead and Crisp), totaling 48 experimental units. Each experimental unit consisted of one plant per pot, spaced at 30 x 25 cm. All treatments maintained a photoperiod of 12 h. The plants were harvested at 21 days after transplanting (DAT).

RGB LEDs achieved peak outputs in the red

and blue regions with spectral energy at 445 nm in the blue region and 660 nm in the red region.

LED bars were 50 cm long, with 36 lamps and wattage output of 10 W. The bars were wired together and connected to a 12 v / 5 A power supply, for each shelf. They were attached to the top of the shelves, 30 cm above the plants, spaced at 15 cm, totaling 10 bars per shelf.

To regulate the photoperiod, the LED bars were connected to a programmable digital timer. The atmosphere of the experimental unit was not CO₂ enriched with during the experiment.

The seedlings of varieties Regina and Veneranda were supplied by a producer in the municipality of Lagoa Seca - PB. Seeds were sown in phenolic foam and 30 days after sowing (DAS) the seedlings were transplanted to 4.8 L-plastic pots. The pots were filled with about 5 kg of soil and had a layer of gravel and mesh placed at the bottom to facilitate drainage. The soil physical characteristics are described in Table 1.

Table 1. Physical characteristics of the soil used to grow lettuce

Physical Characteristics	Sand	Silt	Clay
Granulometry (%)	72.88	9.03	18.09
Texture Classification	Sandy Loam Soil		

Chemical fertilization (NPK) was applied according to the methodology proposed by Novais *et al.* (1991). Irrigation management was by 4 puncture tensiometers randomly installed at a depth of 15 cm, one on each shelf. A digital tensimeter measured the water tension in the soil daily. The critical moisture used as a parameter to determine when to irrigate was 15 kPa (MAROUELLI, 2008). To receive the lettuce plants, the soil was watered to its field capacity by applying a water depth of 33 mm at transplanting, and replenished when the established critical tension was achieved.

At 21 DAT, leaf number (LN) was counted in each plant. To measure the LA, we used method of Benincasa (1986), where a sample with a known area of 9 cm² was taken from the leaf and dried in an oven to obtain its dry mass. Then, the total leaf area of each plant was calculated by dividing the product of the sample area and the total dry mass of leaves by the dry mass of the sample.

To obtain the fresh mass (g), leaves, stems and roots were separated, and then the fresh leaf mass (FLM) was determined by direct weighing on a precision balance.

The gas exchange measurements were performed on one leaf per plant, between 10 am and 12 pm, using the LCpro⁺ model gas exchange analyzer coupled to an IRGA (Infra Red Gas Analyzer). The equipment read the following variables: net photosynthesis (A) ($\mu\text{mol of CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), CO₂ internal concentration (C_i) ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), and instantaneous water use efficiency (WUE_i – A/E), which is calculated by the ratio between net photosynthesis and transpiration [$(\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}) / (\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1})$].

The data were analyzed by analysis of variance using the statistical software SISVAR (FERREIRA, 2011). The effects of different variations in lighting and variety combinations were compared by Tukey's test at 1 and 5% probability. In existing interaction between the factors, the effects of light treatments were unfolded as a function of the varieties.

RESULTS AND DISCUSSION

The summary of the analysis of variance for the Leaf Number (LN), Leaf Area (LA) and Fresh Leaf Mass (FLM) at 21 DAT of lettuce grown under LED lighting is presented in Table 2. A significant effect was found by the test of Tukey ($p < 0.05$) both for the isolated factors (Lighting and Variety), as for the interaction between them, in all the studied variables (Table 2).

The use of different combinations of light was determinant for leaf number of the lettuce varieties. Overall, the ratio 5:1 LED promoted the highest mean of leaf number per plant and the crisp variety presented the highest results, suggesting better growth patterns under these growing conditions (Figure 2). Concurrently, in the treatment 6:3 LED, there was a loss of all experimental plots and in the 1:1 LED there was the loss of the butterhead variety. The results may indicate that lighting with the proportions 6:3 and 1:1 could not be suitable for lettuce cultivation.

The mean leaf number of the crisp lettuce varied from 11.50 to 16.33 in the light treatment (LED 5:1),

Table 2. Summary of the analysis of variance for the data of Leaf Number (LN), Leaf Area (LA) and Fresh Leaf Mass (FLM) of lettuce at 21 DAT

Source of Variation	DF	LN	LA	FLM
		Mean squares		
Lighting (L)	3	643.25**	524848.45**	333.65**
Variety (C)	1	147.00**	331211.31**	6130.61**
LxC	3	88.05**	91235.32**	66.50**
Error	40	3.17	18934.29	5.47
CV (%)		10.15	24.83	15.20

DF: Degrees of Freedom; CV: Coefficient of Variation (transformation $(X+0.5)^{1/2}$; ** Significant at 1% and ns non-significant by Tukey's F test

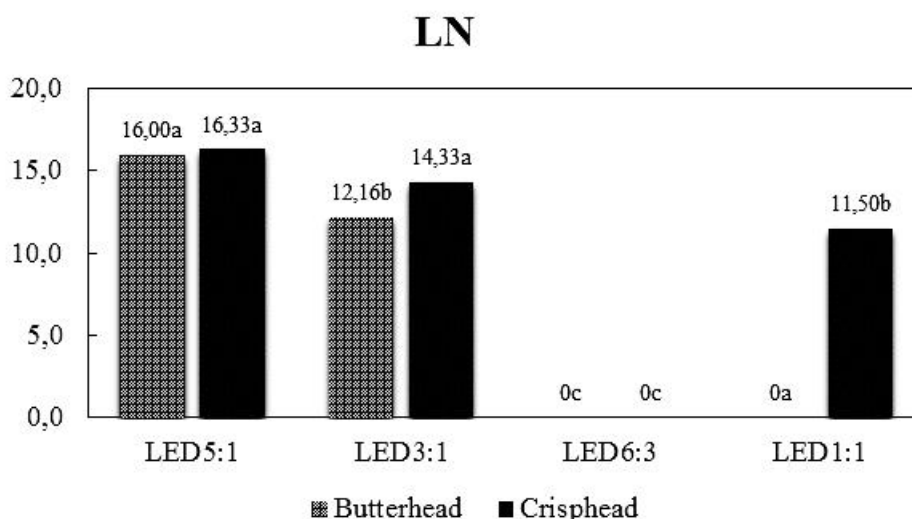


Figure 2. Leaf Number (LN) of lettuce grown under LED lighting at 21 days after transplanting (DAT)

at 21 DAT. Lee *et al.* (2015) found similar results for number of leaves of lettuce, ranging from 14.9 to 15.6 for different light treatments (90/30, 80/40, 70/50 and 60/60 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$). The authors reported that leaf growth in lettuce is more stimulated in red light and blue light than under fluorescent light at the same photon flux. The combination of blue and red light can be considered a growth regulator because the red light has higher quantum efficiency for photosynthesis (LI *et al.*, 2020), and blue light, which participates in numerous critical biological processes, including leaf expansion (WANG *et al.*, 2015). The results in Figure 3 show that the leaf area (LA) of the crisphead lettuce was greater than the leaf area of the butterhead lettuce (48% greater), suggesting that this variety is more resistant to the luminescence spectra. It also showed that the source of variation LED 5:1 produced better results.

The treatment with LED 1:1 resulted in a smaller leaf area for the crisphead lettuce. This treatment, in quantitative terms, has a higher incidence of

light in the spectral range from 400 to 520 nm, corresponding to blue light. Hernández and Kubota (2014) stated that excess blue light reduces leaf area and light interception in plants.

Contrasting with the results found in this study, Anuar *et al.* (2017) reported that the total leaf area of lettuce was not affected by the application of different red + blue LED light regimes (126, 170, 266, 201, and 183 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$) although the yield varied by 16%, which could be associated with differences in intensities of irradiance.

Additionally, the growth of lettuce leaves can be increased by high CO₂ exposure (MIYAGI *et al.*, 2016), in this way, the leaf area found in this study was affected by the low concentration of carbon dioxide, since the atmosphere of the greenhouse was not CO₂ enriched.

Plant yield results from the interaction of several environmental factors such as light, temperature, and carbon dioxide concentration. Because the treatment 5:1 LED produced the largest leaf area

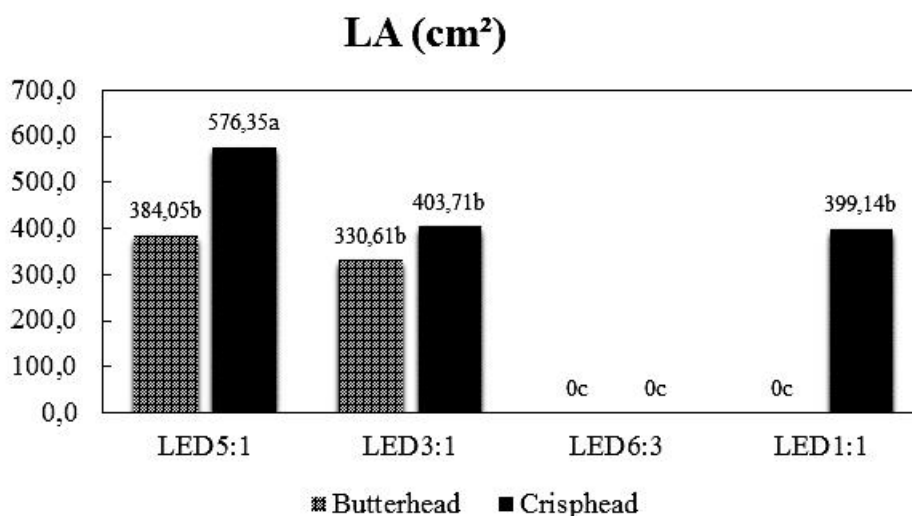


Figure 3. Leaf Area (LA) of lettuce grown under LED lighting at 21 days after transplanting (DAT)

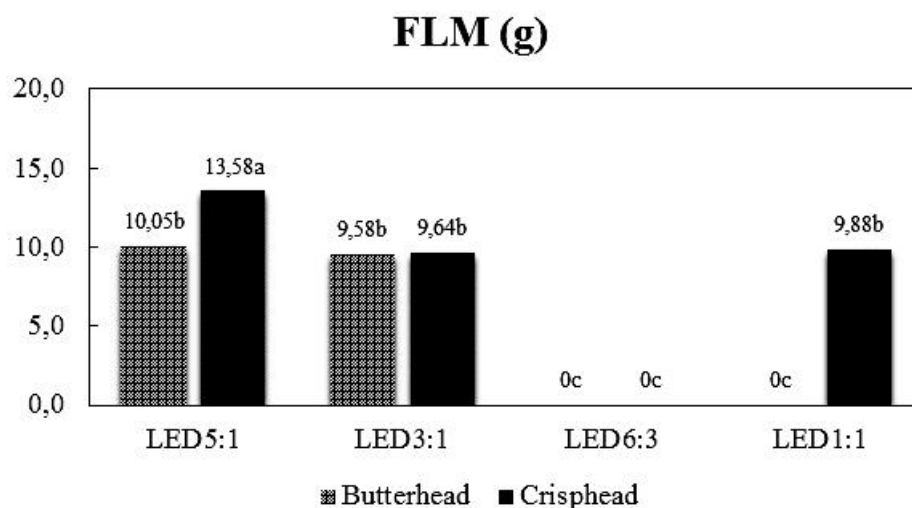


Figure 4. Fresh leaf mass (FLM) of lettuce varieties as a function of LED lighting at 21 DAT

(Figure 3), it is assumed that it had the greatest ability to perform photosynthesis, reflecting on crop productivity, which can be confirmed by the highest yield of plant biomass shown by Figure 4.

In the treatment with the highest production, the variety with the highest yield achieved fresh leaf mass of approximately 13.58 g.plant⁻¹. In a study on the effects of different light intensities on the yield of lettuce grown under similar environmental conditions, Weiguo *et al.* (2012) found that under LED light intensity of 600 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ the FLM was 162.89 g.plant⁻¹, which is in contrast to our findings. This indicates that in this experiment the plants produced are below the standards, hence

the reductions in the total biomass of lettuce suggest that the quality of light can modify growth, decrease the average weight of lettuce heads and, consequently, decrease market value. Lights not being bright enough for a favorable performance may have caused this result.

Lee *et al.* (2015) found that an optimal amount of red and blue LED irradiation may be 80/40 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$, which was the treatment with the highest leaf weight of 47.50 g.per plant⁻¹. Amoozgar *et al.* (2016) showed that use of blue + red LED lamps increased lettuce dry mass compared to blue alone.

Naznin *et al.* (2019) added 9.00% of blue light

to red light and obtained higher fresh and dry mass in lettuce (21.03% and 76.69%). These variable biomass responses may be due to the interaction with species, genotypes, duration and lighting methods, as well as other environmental factors (LI *et al.*, 2020).

Table 3 shows the summary of the analysis of variance related to the gas exchange variables. There is a significant effect of the electromagnetic radiation provided by the different combinations of light-emitting diodes (LED) on all variables. The variety of lettuce also had a significant effect on all variables. Considering the interaction between the factors (IxC), only A, Ci, and WUEi showed significant effects (Table 3).

The crisphead lettuce had the greatest potential for photosynthetic efficiency in all the physiological variables analyzed. Thus, it can be affirmed that this variety has better adaptability in carrying out photosynthesis in the growing conditions of this experiment.

The unfolding of the variable lighting as a function of lettuce varieties for the response variable A (Net Photosynthesis) is presented in Figure 5. It shows that the LED light treatment with the 5:1 combination produced the highest rates of net photosynthesis (A).

According to Wang *et al.* (2016), the combination of R (red) and B (blue) proved to be effective in managing photosynthesis, increasing photosynthesis rates with decreasing R:B ratio down to 1, i.e., equal proportion.

As mentioned, the optimal range of the blue and red spectra for photosynthesis are essential for its functioning. Plants under R (Red) light alone show reduced net photosynthetic rate compared to white or red (R) light supplemented with blue (B) (WANG *et al.*, 2015).

In addition, R (red) alone or constant lighting with high amounts of B can adversely reduce net photosynthesis rate in many species due to

Table 3. Summary of analysis of variance for Net Photosynthesis (A), Internal CO₂ Concentration (Ci), and Instantaneous Water Use Efficiency (WUEi) of lettuce at 21 DAT

Source of Variation	DF	Mean squares		
		A	Ci	WUEi
Lighting (L)	3	1.036**	436182.20**	7.8666**
Variety (C)	1	0.392**	79327.52**	6.3147**
LxC	3	0.294**	92349.64**	1.4287*
Error	40	0.018	929.73	0.3890
CV (%)		20.06	6.32	32.14

DF: Degrees of Freedom; CV: Coefficient of Variation (transformation $(X+0.5)^{1/2}$; ** Significant at 1% and ns non-significant by Tukey's F test

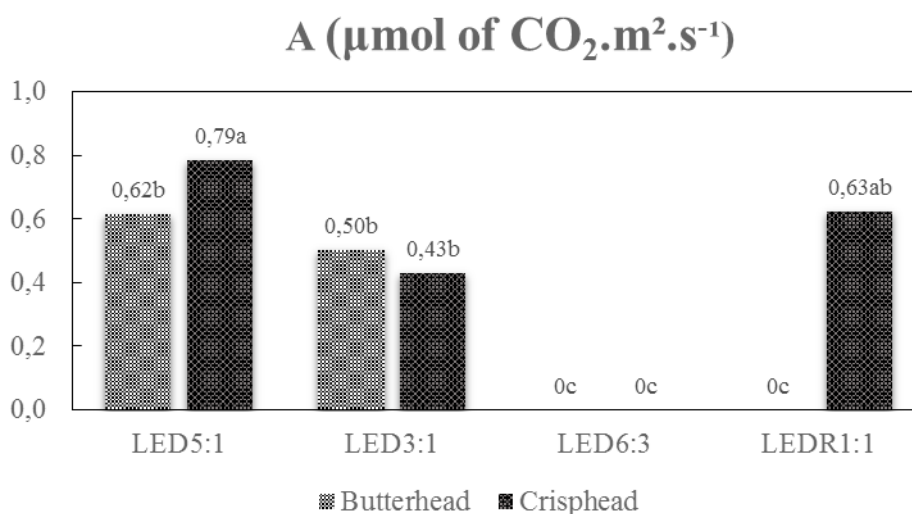


Figure 5. Net Photosynthesis (A) of lettuce grown under LED lighting at 21 DAT

chloroplast evasion responses (WADA *et al.*, 2003). Therefore, the variation in net photosynthesis rate is due to the different combination of red and blue light that compose the luminescence spectrum used in this experiment.

The lower rate of photosynthesis under LED lamps may also be attributed to the low nitrogen content in leaves, which occurs as a function of the chlorophyll and carotenoid content (KIM *et al.*, 2004).

The crisphead variety showed a higher rate of internal CO₂ concentration (Figure 6), being associated with stomatal conductance (Gs). For this reason, the stomatal limitation would be the main

factor in limiting the photosynthetic performance, because the larger the stomatal opening, the greater the CO₂ diffusion to the substomatic chamber (NASCIMENTO, 2009).

Jadoski *et al.* (2005) discussed that the increase in the CO₂ assimilation rate is related to its concentration inside the leaves, which may result from stomatal closure in response to abiotic stresses. The increase observed in the CO₂ assimilation rate can be directly related to the increase in transpiration.

Figure 7 show the instantaneous Water Use Efficiency (WUEi) of lettuce grown under different combinations of red and blue LED lighting.

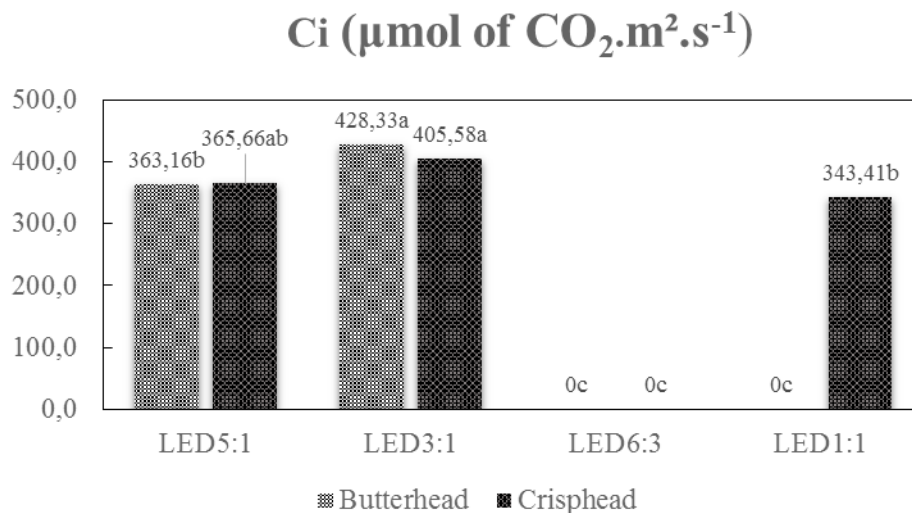


Figure 6. Internal CO₂ (Ci) concentration of lettuce grown under LED lighting, at 21 DAT

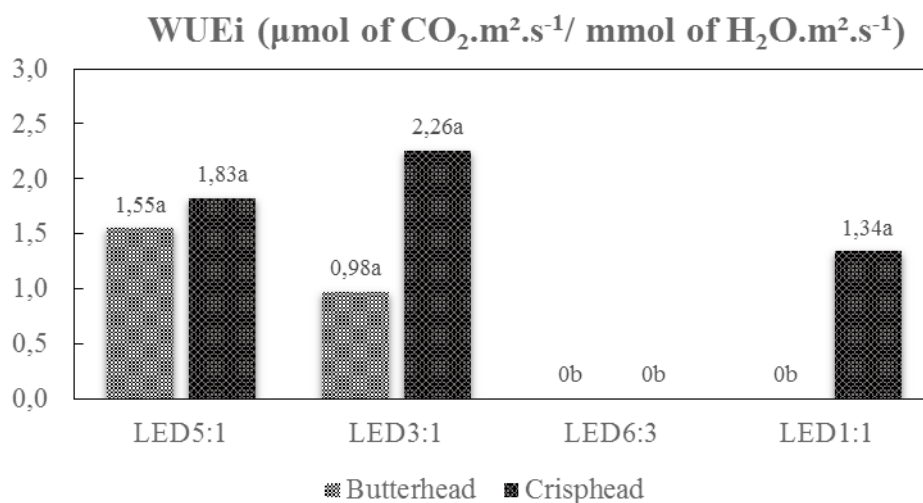


Figure 7. Instantaneous Water Use Efficiency (WUEi) of lettuce grown under LED lighting at 21 DAT

The LED light source variation with the combination 5:1 was more efficient in terms of instantaneous water use efficiency.

The crisphead lettuce was more efficient in using water for photosynthesis, which shows that this type of variety can be more adapted to the cultivation of indoor crops (production chambers).

Pennisi *et al.* (2019) report that water use efficiency differs by light treatment applied and are to be associated with changes in the stomatal behavior.

For Kerbauy *et al.* (2008), the instantaneous water use efficiency (WUEi) was significant under different irrigation depths, in a manner that the light levels the lettuce plants were subjected to limited the efficiency of the physiological process. It is of note that the lower the water availability, the lower the degree of stomatal opening in order to reduce water loss; as a consequence, WUEi is higher, maintaining the minimum water balance, and it is still reported that C3-type plants such as lettuce have limited CO₂ uptake under water deficit.

Over the 21 days of the experiment, the water tension did not reach its critical state, that is, irrigation was not necessary, establishing a minimum water use in the cultivation environment.

CONCLUSIONS

- The highest growth and production of lettuce was recorded in the source variation of LED lighting with the 5:1 combination.
- The 5:1 LED lighting treatment was more efficient for plants carrying out photosynthesis.
- The butterhead lettuce cultivar presented greater sensitivity to LED lighting, while the crisphead lettuce presented greater resistance.

Limitations and Recommendations

- The optimal R:B ratio still requires further study, as well as the length of the photoperiod and the environmental conditions for this type of production.

AUTHORSHIP CONTRIBUTION STATEMENT

LIMA, S.C.: Conceptualization, Data curation, Investigation, Writing – original, Writing – review & editing; **PEDROZA, J.P.:** Project administration, Supervision, Validation, Visualization; **SABOYA, L.M.F.:** Formal Analysis, Methodology, Supervision; **MORAES NETO, J.M.:** Supervision, Visualization; **MELO, D.F.:** Data curation, Methodology; **GUIMARÃES, R.F.B.:** Data curation, Methodology.

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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