



## Morphophysiology and postharvest quality of hydroponic lettuce cultivars grown under high temperature<sup>1</sup>

### Desempenho morfofisiológico e qualidade pós-colheita de cultivares de alface hidropônica em condições de temperatura elevada

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#### HIGHLIGHTS:

*Early bolting reduces the production, photosynthetic capacity, and postharvest quality of the lettuce cultivars.*

*The tolerance to early bolting under high temperature is a genetic trait that is not shown to be affected by pigment content.*

*Bolted cultivars show a reduction in photosynthetic capacity as a result of biochemical and/or stomatal limitations.*

**ABSTRACT:** Lettuce (*Lactuca sativa*) is the most economically important leafy vegetable in Brazil, with the crisp type being the most popular among producers. However, in regions with hot climates, lettuce has production limitations due to bolting, requiring knowledge on the behavior of cultivars available on the market. Thus, the objective of the present study was to characterize the performance of crisp lettuce cultivars in terms of their productive, physiological, and postharvest quality aspects when grown under hydroponic cultivation and high temperature conditions. The assay was performed in a greenhouse and the plants were grown in a hydroponic system of DFT type. The average temperature during the experiment was 30.33 °C. The experimental design used was completely randomized with nine treatments (Veneranda, Cristina, Olinda, Mônica, Cinderela, Grand Rapids, Itapuã Super, Crocantela, and Simpson Black Seed), with four replications. The cultivars were analyzed in terms of their productive, physiological, and postharvest quality performances. The Olinda and Cristina cultivars showed the best performance in growth, physiological, and postharvest variables. However, the cultivars had distinct sensory characteristics. Bolting negatively affected gas exchange, production, and postharvest quality of lettuce cultivars Veneranda, Mônica, Grand Rapids, and Cinderela. In addition, it was observed that the pigment content was not determinant for the expression of bolting. Therefore, this study recommends the use of cultivars Olinda and Cristina for the hydroponic cultivation under high temperature conditions.

**Key words:** *Lactuca sativa*, bolting, gas exchange, hydroponics

**RESUMO:** A alface (*Lactuca sativa*) é a hortaliça folhosa de maior importância econômica no Brasil, sendo o tipo crespa a mais popular entre os produtores. Entretanto, em regiões de clima quente, a alface apresenta limitações de produção devido ao pendoamento precoce, exigindo o conhecimento do comportamento das cultivares disponíveis no mercado. Assim, o objetivo do presente estudo foi caracterizar o desempenho de cultivares de alface crespa quanto aos aspectos produtivos, fisiológicos e de qualidade pós-colheita, cultivadas em condições de cultivo hidropônico e alta temperatura. O ensaio foi conduzido em condição de casa de vegetação, sendo as plantas cultivadas em sistema hidropônico tipo DFT. A temperatura média durante o experimento foi de 30,33 °C. O delineamento experimental utilizado foi o inteiramente casualizado com nove tratamentos (Veneranda, Cristina, Olinda, Mônica, Cinderela, Grand Rapids, Itapuã Super, Crocantela e Simpson Black Seed) e quatro repetições. As cultivares foram analisadas quanto ao seu desempenho produtivo, fisiológico e de qualidade pós-colheita. As cultivares Olinda e Cristina apresentaram melhor desempenho nos parâmetros de crescimento, fisiológicos e pós-colheita. Entretanto, estas cultivares apresentaram características sensoriais distintas. Verificou-se que o pendoamento precoce afetou negativamente as trocas gasosas, a produção e a qualidade pós-colheita das cultivares de alface Veneranda, Mônica, Grand Rapids e Cinderela. Além disso, observou-se que o teor de pigmentos não foi determinante para a expressão do pendoamento. Portanto, este estudo recomenda o uso das cultivares Olinda e Cristina para o cultivo hidropônico em condições de alta temperatura.

**Palavras-chave:** *Lactuca sativa*, pendoamento precoce, trocas gasosas, hidroponia



## INTRODUCTION

Global warming, along with the increase in world population, has posed a severe threat to food security on a global scale (FAO, 2015). The Intergovernmental Panel on Climate Change (IPCC) has estimated an increase of 1.5 to 4 °C in the average global temperature by the end of this century, which will lead to problems in the cultivation of plants (FAO, 2015), especially in hot climates, which are characterized by high temperatures and low annual thermal variability.

The effect of global warming ends up being aggravated in thermosensitive crops, such as lettuce (*Lactuca sativa* L. – Asteraceae), since the high temperature results in productive and qualitative limitations (Holmes et al., 2019; Fortes et al., 2020). In Brazil, lettuce has great economic importance, with the crisp type being the most commercialized (Sala & Costa, 2012; Santos et al., 2021). Most lettuce plantations in Brazil are soil-based; however, hydroponic systems are being increasingly adopted by Brazilian lettuce growers, mainly due to the reduction in risks as a result of the possibility of implementing environmental control, reducing production losses due to the climate (Sala & Costa, 2012; Velazquez-Gonzalez et al., 2022).

However, the cultivation of this species faces physiological and productive limitations related to the climate of some regions of the country, especially in the North and Northeast, since environments with temperature higher than 28 °C are considered stressful and stimulate the expression of bolting, inhibiting the expression of the productive potential of the crop and reducing its palatability due to the accumulation of latex in the leaves, which confers a bitter taste (Holmes et al., 2019; Liu et al., 2020).

In addition to the management of abiotic factors, the genetic material used for cultivation affects the adaptation of the crop to hot climates (Sala & Costa, 2012) and, consequently, the expression of early bolting. Thus, among the viable strategies for cultivation under hot climate conditions, the evaluation of cultivars with satisfactory production performance and environmental tolerance is indispensable (Holmes et al., 2019; Santos et al., 2021). Therefore, the objective of the present study was to characterize the performance of crisp lettuce cultivars in terms of their productive, physiological, and postharvest

quality aspects when grown under hydroponic cultivation and high temperature conditions.

## MATERIAL AND METHODS

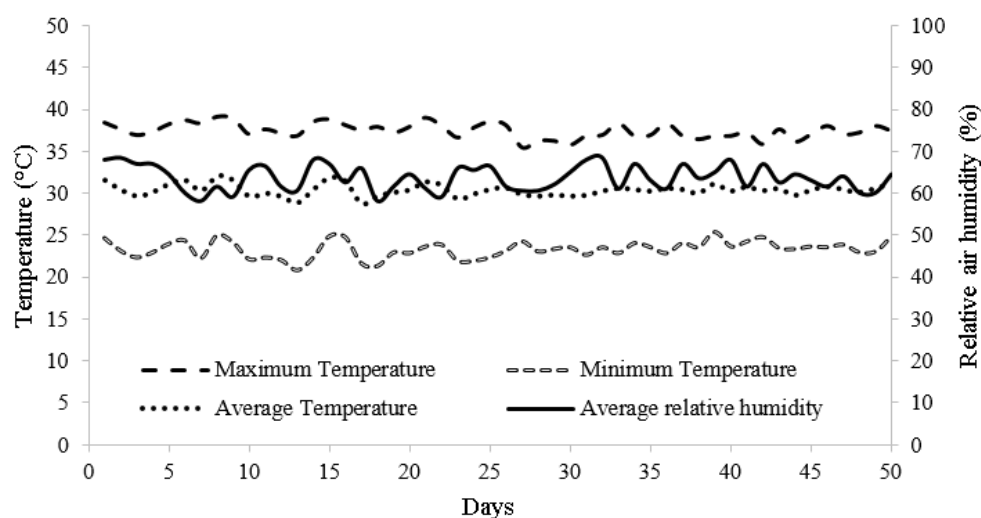
The experiment was performed from January 6 to February 24, 2021, under greenhouse conditions in the Soil Science Department of the Agrarian Sciences Institute of the Universidade Federal Rural da Amazônia - UFRA, Belém, Pará (1° 27' 11.08" S, 48° 26' 34.21" W, at an altitude of 9 m), Brazil. The climate of the experimental site is "Afi" according to Köppen's classification, with average temperature above 26 °C and annual rainfall of 3100 mm (Alvares et al., 2013).

At the center of the greenhouse, a thermo-hygrometer sensor was placed at 2 m above ground level to obtain the air temperature and relative humidity during the crop cycle (Figure 1). The maximum, minimum, and mean temperatures were  $37.40 \pm 1.01$ ,  $23.26 \pm 0.72$  °C, and  $30.33 \pm 1.81$  °C. The mean relative humidity of the air was 63.47%.

The experimental design used was completely randomized with nine treatments and four repetitions, for thirty-six experimental units. The experimental unit was composed of two lettuce plants each. The treatments were composed of different lettuce cultivars: Cinderella, Crocanta, Grand Rapids, Mônica, and Veneranda (Feltrin<sup>®</sup> Sementes, Farrroupilha, RS, Brazil), Cristina and Olinda (Hortival<sup>®</sup> Sementes, Vitória de Santo Antão, PE, Brazil), and Itapuã Super, and Simpson Black Seed (Isla<sup>®</sup> Sementes Ltda., Porto Alegre, RS, Brazil). The common characteristics of these cultivars, according to the producers, are tolerance to early bolting and tip-burn at elevated temperatures.

The seedlings were produced by sowing in polystyrene trays (128 cells) filled with coconut fiber substrate, at a density of three seeds per cell. The substrate has the following chemical characteristics according to Sampaio et al. (2021): 42 mg kg<sup>-1</sup> of N, 21 mg kg<sup>-1</sup> of P, 22 mg kg<sup>-1</sup> of K, 186 mg kg<sup>-1</sup> of Ca, 39 mg kg<sup>-1</sup> of Mg, 37 mg kg<sup>-1</sup> of S, 1 mg kg<sup>-1</sup> of B, 5 mg kg<sup>-1</sup> of Cu, 14 mg kg<sup>-1</sup> of Fe, 3 mg kg<sup>-1</sup> of Mn, 2 mg kg<sup>-1</sup> of Mo, and 4 mg kg<sup>-1</sup> of Zn.

Until seven days after sowing (DAS), the substrate had its moisture maintained using distilled water. Subsequently,



**Figure 1.** Temperature and average relative humidity of the air inside the greenhouse during the experimental period

thinning was performed (leaving only one seedling per cell) and the trays were transferred to nursery-type benches, where they received a nutrient solution of Hoagland & Arnon (1950) formulation 2, at 25% ionic strength.

The seedlings were transplanted to the hydroponic system with substrate at twenty DAS, when the plants had four leaves as described by Fortes et al. (2020). Sterilized ground silica was used as substrate in the assay. Pots with a capacity of 2 L were used to grow the lettuce during the trial. The substrate was sterilized by immersion in a 1% hydrochloric acid (HCl) solution for seven days and then washed with plenty of distilled water to remove acid residues.

The pots were drilled near their base, and a rubber tube mechanism was attached to control the drainage of the solution. In addition, the pots were covered with aluminized paper to avoid heating of the substrate and nutrient solution. Furthermore, the nutrient solution collectors were coated with aluminized metallic paint, and adapted using a support system in the neck of the bottles, to facilitate the drainage of the solution.

To cultivate the plants, the standard formulation of Hoagland & Arnon (1950) at 50% of the ionic concentration was used, and the pots were filled with 400 mL of this formulation. To prepare the solution, pure salts obtained from Sigma Aldrich™ were used. Plants received the following macronutrients from the nutritive solution: 3.00 mM L<sup>-1</sup> of KNO<sub>3</sub>, 2.00 mM L<sup>-1</sup> of Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, 0.50 mM L<sup>-1</sup> of NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, and 1.00 mM L<sup>-1</sup> of MgSO<sub>4</sub>·7H<sub>2</sub>O. Micronutrient stock solution was prepared using 2.86 g L<sup>-1</sup> of H<sub>3</sub>BO<sub>3</sub>, 1.81 g L<sup>-1</sup> of MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.22 g L<sup>-1</sup> of ZnSO<sub>4</sub>·5H<sub>2</sub>O, 0.08 g L<sup>-1</sup> of CuSO<sub>4</sub>·5H<sub>2</sub>O, and 0.02 g L<sup>-1</sup> of H<sub>2</sub>MoO<sub>4</sub>·H<sub>2</sub>O and Fe-EDDHA: 21.6 g L<sup>-1</sup> of EDDHA, 286 mL L<sup>-1</sup> of 1 M KOH and 24.9 g L<sup>-1</sup> of FeSO<sub>4</sub>·7H<sub>2</sub>O.

The replacement and drainage of the nutrient solution were made, respectively, in the early morning and late afternoon, manually daily. The water lost by evapotranspiration was supplied regularly based on the quantity exported, using distilled water.

The pH values were measured daily using a portable pH meter GroLine HI98118 (HANNA Instruments Inc., Woonsocket, Rhode Island, USA) and, when necessary, pH correction was performed using 1 M NaOH or 1 M citric acid solutions, keeping it within the recommended range of 5.5-6.5 for soilless cultivation.

From transplanting until the day of harvest, the plants were monitored in order to identify signs of early bolting, characterized by elongation of the stem, which grows in height and decreases in thickness (Fortes et al., 2020). The classification of the degree of plant bolting was performed according to a scale adapted from Holmes et al. (2019), where: 0 = no stem elongation 1 = visible stem elongation; 2 = stem elongation with initial formation of the pendulum structure; 3 = stem elongation and formation of inflorescence; 4 = formation of flowering.

At forty nine DAS, gas exchange was determined in the third pair of leaves, in the basipetal direction. Sampling was carried out between 08:00 a. m. and 12:00 a. m., using a portable infrared gas analyzer model LI6400XT (LICOR Biosciences, Inc.; Lincoln, Nebraska, USA) under external CO<sub>2</sub>

concentration of 400 μmol air<sup>-1</sup>, and artificial PAR of 1000 μmol photons m<sup>-2</sup> s<sup>-1</sup>. The variables evaluated were net photosynthesis rate (*A*, μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), stomatal conductance (*g<sub>s</sub>*, μmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), intercellular carbon (*C<sub>i</sub>*, μmol CO<sub>2</sub> mol air<sup>-1</sup>), transpiration (*E*, μmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), leaf temperature (LT, °C), and instantaneous water use efficiency (WUE, μmol CO<sub>2</sub> μmol<sup>-1</sup> H<sub>2</sub>O) - obtained by the ratio between *A* and *E*.

After the gas exchange analysis, the third pair of leaves was collected for the extraction and quantification of chlorophyll and carotenoid contents. For such, the methodology of Lichtenthaler (1987) was used, with the homogenization of 1 mg of fresh leaf tissue in a mortar containing 5 mL of acetone solution at 80%. The extract obtained was filtered and transferred to a 25 mL volumetric flask, and the volume was completed with 80% acetone solution. After that, absorbance was read in a spectrophotometer (Fento, model 700 S) at wavelengths of 470, 646.8, and 663.2 nm. The results were expressed in mg g<sup>-1</sup> of fresh matter.

At fifty DAS, the plants were harvested and compartmentalized into leaves, stem, and roots. The morphological variables determined were: (a) plant height (cm) - measured using a graduated ruler; (b) stem diameter (mm) - measured using a caliper; (c) leaf area (cm<sup>2</sup> plant<sup>-1</sup>) - determined using a leaf area integrator model LI-3100 (LICOR Biosciences, Inc.; Lincoln, Nebraska, USA); d) leaf fresh mass (g plant<sup>-1</sup>); e) stem fresh mass (g plant<sup>-1</sup>); and f) root fresh mass (g plant<sup>-1</sup>). Dry masses (in g plant<sup>-1</sup>) were obtained by drying fresh material at 60 °C in a forced air circulation oven until reaching constant weight. The masses were determined by weighing on a precision scale (0.001g).

In addition, leaf samples were collected to characterize the postharvest quality of lettuce. At this stage, the ascorbic acid content, total soluble solids (TSS), pH, titratable acidity (TA), and TSS/TA ratio were evaluated. Ascorbic acid content was quantified by Tillman's titrimetric method, using as titrant a solution of 2,6-dichloroindolifenol, at 0.02%, until it reached a light pink color (Strohecker & Henning, 1967; IAL, 2008). The extract was composed of 1 g of fresh lettuce leaves macerated, immersed in 50 mL of 0.5% oxalic acid solution. A 5-mL aliquot of the extract was taken and transferred to a 125-mL Erlenmeyer flask, and the volume was made up to 50 mL with distilled water and then titrated with chilled Tillman's solution until the turning point in duplicate per sample. For the blank tests, ascorbic acid standard solution containing 50 μg mL<sup>-1</sup> was used. The result was expressed as mg ascorbic acid per 100 g fresh mass.

The total soluble solids were determined using a digital refractometer with automatic temperature correction, transferring two drops of the extract from 1 g of macerated fresh leaf to the prism of the equipment (AOAC, 2012). The results were expressed in °Brix (%).

To determine the pH of the samples, 1 g of fresh macerated leaf was added to 30 mL of distilled water. After this, the sample was read using a benchtop pH meter (IAL, 2008) model PHOX P1000 (PHOX Suprimentos Científicos, Colombo, PR, Brazil). Titratable acidity - TA was determined according to the methodology of IAL (2008), through titration of the extract (1 g of fresh leaf homogenized and phenolphthalein indicator

at 1%, being completed to a volume of 50 mL using distilled water) with 0.1 M NaOH solution, with results expressed in % of citric acid. The TSS/TA ratio of the samples was calculated by the ratio between these two variables.

The data obtained were subjected to the Shapiro-Wilk normality test and Bartlett's homoscedasticity test. Upon meeting these assumptions, analysis of variance was carried out using the F-test ( $p \leq 0.05$ ) and, when significant, the means were tested using the Scott-Knott's test ( $p \leq 0.05$ ). The software environment RStudio (R Core Team, 2021) was used for these analyses.

## RESULTS AND DISCUSSION

The physiological performance was affected according to the cultivars evaluated (Table 1). As for net photosynthesis ( $A$ ), values ranging from 17.72 to 12.23  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  were observed, with Itapuã Super showing the best performance in this parameter, followed by Olinda and Crocantela, in different groups. In contrast, Grand Rapids and Veneranda formed the group that showed the lowest  $A$ , with an average of 12.46  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ .

For  $g_s$ , four groups were formed, the one with the highest conductance composed of Itapuã Super and Olinda, and the one with the lowest performance composed of Grand Rapids cultivar. In this context, values between 0.27 and 0.14  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$  were observed. In relation to  $C_i$ , values between 291.13 and 234.85  $\mu\text{mol CO}_2 \text{ mol}^{-1}$  were observed, with Veneranda and Grand Rapids standing out, with the highest and lowest  $C_i$ , respectively.

The formation of four groups of cultivars was observed for  $E$ , with mean values of 5.16 and 3.39  $\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ . Following the behavior observed for  $A$  and  $g_s$ , Itapuã Super showed higher  $E$  compared to the other cultivars, being included in a group with Simpson, which showed antagonistic behavior in relation to  $A$ . It was also observed that these cultivars showed higher LT and  $E$ .

For LT, values between 35.78 and 31.72 °C were observed, with higher temperature for Simpson and Grand Rapids, while the lowest temperature was observed for Olinda. Regarding WUE, values between 4.56 and 2.71  $\mu\text{mol CO}_2 \mu\text{mol H}_2\text{O}^{-1}$  were observed, highlighting the efficiency of Olinda, which showed higher WUE compared to the other cultivars. Veneranda and Simpson showed lower WUE.

Plant physiological performance is highly influenced by environmental conditions, and plant adaptability is mainly

related to genetic characteristics (Taiz et al., 2017), which confers variable behaviors to cultivars or genotypes of the same species when subjected to similar climatic conditions (Lemos Neto et al., 2017; Fortes et al., 2020).

We observed that the limitation in  $A$  for Veneranda and Simpson may be related to biochemical factors, since they had high  $C_i$  and similar  $g_s$ , indicating that the  $\text{CO}_2$  reaching the cells of the leaf mesophyll is not being assimilated. Similar pattern was observed for Cinderela, but there was no limitation in  $\text{CO}_2$  delivery, despite the lower  $g_s$ . The accumulation of  $\text{CO}_2$  in the stomatal chamber indicates metabolic imbalances, such as the limitation of the regeneration of the RuBisCO (ribulose-1,5-biphosphate) enzyme, causing a reduction in net photosynthesis (Taiz et al., 2017; Pessaraki, 2021). The low WUE shown by these cultivars may be related to these factors.

It is known that photosynthesis is a highly thermosensitive process (Pessaraki, 2021), and in the case of Grand Rapids, the expressed limitation in gas exchange may be related to this factor, since this cultivar showed the highest LT. As a result, Grand Rapids had reduced  $A$  due to the limitation of the substrate for photosynthetic activity ( $\text{CO}_2$ ), which is evident in the low  $g_s$  and  $E$ . Mônica showed lower  $A$  than the other cultivars (except for Simpson), with no apparent limitations in relation to stomatal opening or  $\text{CO}_2$  assimilation, which reinforces the hypothesis of the limiting effect of the early bolting on the photosynthetic capacity of lettuce.

Once initiated the reproductive phase of plants, alterations in metabolic functioning occur, resulting in modification in processes of photoassimilate partition and remobilization of nutrients from roots and leaves aiming to support the growth, development and maturation of the floral structure, consequently affecting the efficiency of photosynthetic activity (Taiz et al., 2017; Pessaraki, 2021).

Regarding the high  $A$  of Itapuã Super, this can be attributed to the high  $g_s$  and the biochemical functioning of the photosynthetic apparatus, since no  $\text{CO}_2$  accumulation was observed, in contrast to what was observed for Veneranda. However, because of the large stomatal opening, this cultivar also showed high transpiration, and consequently, lower WUE.

As observed by Fortes et al. (2020) for the cultivars Isabela and Summer Crisp, despite showing the highest  $A$  among the evaluated cultivars, Itapuã Super appears to have a lower production efficiency than Olinda and Cristina, indicating that this cultivar may have a high consumption of carbohydrates in the respiration process, justifying the lower accumulation

**Table 1.** Mean gas exchange variables of lettuce cultivars grown in a hydroponic system under high temperature conditions

Cultivars	$A$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	$g_s$ ( $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )	$C_i$ ( $\mu\text{mol CO}_2 \text{ mol air}^{-1}$ )	$E$ ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )	LT (°C)	WUE ( $\mu\text{mol CO}_2 \mu\text{mol H}_2\text{O}^{-1}$ )
Veneranda	12.70 ± 1.13 f	0.24 ± 0.03 b	291.13 ± 1.45 a	4.68 ± 0.35 b	33.61 ± 0.81 c	2.71 ± 0.04 d
Cristina	15.24 ± 0.98 d	0.22 ± 0.01 b	267.53 ± 3.53 b	4.37 ± 0.08 b	33.60 ± 1.22 c	3.50 ± 0.29 c
Olinda	16.61 ± 0.15 b	0.25 ± 0.00 a	271.39 ± 1.29 b	3.64 ± 0.08 d	31.72 ± 0.05 d	4.56 ± 0.14 a
Mônica	14.96 ± 0.25 d	0.23 ± 0.01 b	272.15 ± 5.87 b	4.55 ± 0.66 b	34.34 ± 1.05 b	3.39 ± 0.52 c
Cinderela	14.13 ± 0.25 e	0.18 ± 0.00 c	252.67 ± 0.30 c	3.41 ± 0.14 d	33.83 ± 0.28 c	4.15 ± 0.25 b
Grand Rapids	12.23 ± 0.31 f	0.14 ± 0.01 d	234.85 ± 16.02 d	3.39 ± 0.00 d	35.81 ± 0.56 a	3.61 ± 0.09 c
Itapuã Super	17.72 ± 0.47 a	0.27 ± 0.01 a	267.61 ± 0.23 b	4.96 ± 0.50 a	34.37 ± 0.51 b	3.61 ± 0.28 c
Crocantela	16.06 ± 0.71 c	0.21 ± 0.02 c	250.01 ± 7.17 c	4.12 ± 0.52 c	34.50 ± 0.27 b	3.95 ± 0.34 b
Simpson B. S.	13.99 ± 0.21 e	0.22 ± 0.02 c	273.06 ± 8.06 b	5.16 ± 0.10 a	35.78 ± 0.66 a	2.71 ± 0.09 d

Means followed by the same letter within the column do not differ by the Scott-Knott's test ( $p \leq 0.05$ )



of mass. In this context, Cristina stands out, which had lower A than Itapuã Super, but with higher production.

The Olinda cultivar showed high A and gs, besides having lower E, LT, and consequently higher WUE. The performance of this cultivar in relation to gas exchange variables indicates that it has efficient water utilization mechanisms, especially in terms of maintaining leaf temperature, which leads to the full biochemical functioning and stability of the photosynthetic apparatus (Taiz et al., 2017).

LT maintenance is an important mechanism of tolerance to heat stress, since the enzymatic processes carried out in the leaves are modulated by temperature, so that with the elevation of temperature, some enzymes have their activity reduced or totally inhibited by thermal denaturation (Taiz et al., 2017; Pessaraki, 2021). Therefore, the performance of this cultivar indicates its suitability under high temperature conditions.

There were differences ( $p \leq 0.05$ ) in pigment content between lettuce cultivars (Figure 2). These pigments are essential for the photosynthetic process, especially in the photochemical stage, where chlorophyll a acts effectively, with the help of accessory pigments such as chlorophyll b and carotenoids, which act in the absorption and transfer of light energy to the reaction centers (Taiz et al., 2017).

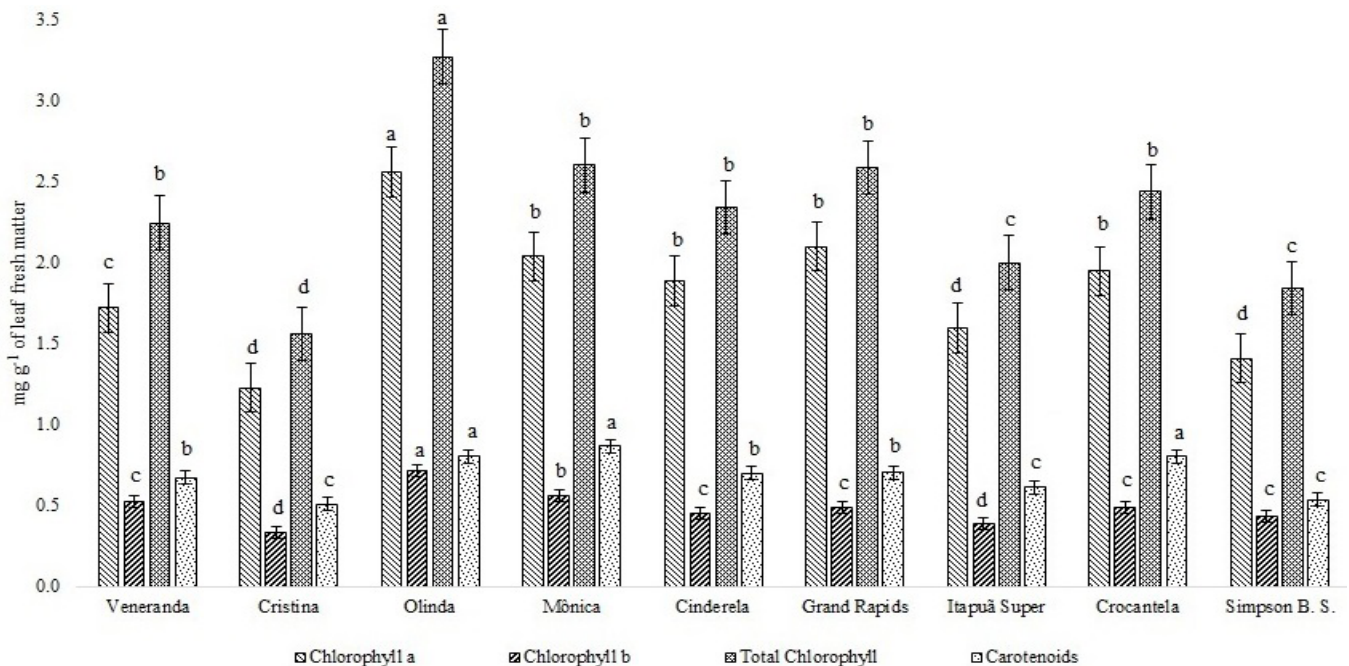
The Olinda cultivar distinguishes itself in terms of chlorophyll a and b contents (2.56 and 0.53 mg g<sup>-1</sup> of leaf fresh matter, respectively). However, an inverse behavior was observed for Cristina, which showed lower contents of these pigments (1.23 and 0.34 mg g<sup>-1</sup> of leaf fresh matter, respectively). Regarding the total chlorophyll content, the cultivars that showed bolting did not differ and also had higher levels than Itapuã Super, Simpson and Cristina. Regarding the carotenoid content, Olinda, Mônica and Crocantela had the highest value, while Cristina and Simpson had the lowest. Cinderela, Veneranda and Grand Rapids were included in the

same group according to the carotenoid content. Carotenoids have a protective function in chloroplasts against reactive oxygen species (Young, 1991; Pessaraki, 2021).

In this context, it was hypothesized that cultivars with lower contents of these pigments would show a greater tendency to express early bolting, since these pigments act as components of the non-enzymatic complexes of the plant's antioxidative defense system (Young, 1991; Pessaraki, 2021). However, the performance of Simpson, Itapuã Super, and Cristina indicates that the content of chlorophylls was not a determining factor for the expression of early bolting in our growing conditions. Thus, these results demonstrate that the contents of these pigments, despite indicating a greater adaptability of the plant to a stressful environment, do not affect the tolerance of expression of early bolting in lettuce under high temperature conditions, with this characteristic being related to genetic factors intrinsic to the cultivars.

Differences were observed between the cultivars ( $p \leq 0.05$ ) for the growth and production variables (Table 2). Regarding growth, specifically for plant height, five groups were established, with the highest value for the Mônica cultivar (23.48 cm), followed by the Veneranda and Cinderela cultivars (means of 20.75 and 18.93 cm, respectively). The group that showed the lowest average was composed of the Crocantela and Itapuã Super cultivars.

Regarding stem diameter, the opposite behavior was observed. Grand Rapids, Mônica, Veneranda, Cinderela, and Itapuã Super cultivars represented the group with the lowest diameter (average of 9.53 mm), while the Olinda cultivar showed the highest mean of 13.22 mm. As a consequence of these results, cultivars with greater height and smaller stem diameter showed signs of early bolting, with incidence score 1 for Cinderela and Grand Rapids and score 2 for Mônica and Veneranda. Crocantela, Itapuã Super, Cristina, Olinda, and



Means followed by the same lowercase letter do not differ by the Scott-Knott's test ( $p \leq 0.05$ ), indicating similarities between groups. Vertical bars indicate the standard error of the mean ( $n=4$ )

**Figure 2.** Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids contents in lettuce cultivars grown in a hydroponic system under high temperature conditions

**Table 2.** Plant height, stem diameter, number of leaves, leaf area, degree of bolting, and age at the beginning of bolting, and fresh and dry mass obtained from the evaluation of nine lettuce cultivars grown in a hydroponic system under high temperature conditions

Cultivars	Height	Diameter	Number of leaves	Leaf area	Bolting start	Bolting
	(cm)	(mm)	(Leaves plant <sup>-1</sup> )	(cm <sup>2</sup> )	(days)	rate <sup>1</sup>
Veneranda	20.75 ± 4.52 b	9.43 ± 0.64 c	14.75 ± 0.96 d	922.45 ± 88.37 b	39	2
Cristina	6.48 ± 0.62 d	11.54 ± 0.90 b	16.25 ± 0.50 c	1025.82 ± 117.34 a	0	0
Olinda	7.55 ± 1.28 d	13.22 ± 0.91 a	22.00 ± 0.82 a	1165.35 ± 88.75 a	0	0
Mônica	23.48 ± 2.16 a	9.16 ± 0.52 c	17.00 ± 0.82 c	978.05 ± 37.06 a	36	2
Cinderela	18.93 ± 1.62 b	9.92 ± 0.69 c	13.75 ± 1.26 e	885.80 ± 59.12 b	37	1
Grand Rapids	14.70 ± 2.91 c	9.09 ± 0.96 c	13.50 ± 0.58 e	848.48 ± 89.13 b	38	1
Itapuã Super	4.96 ± 1.30 e	10.06 ± 1.18 c	15.50 ± 0.58 d	837.48 ± 199.52 b	0	0
Crocantela	2.56 ± 0.97 e	11.07 ± 1.44 b	14.25 ± 0.96 e	757.85 ± 241.49 b	0	0
Simpson B. S.	7.73 ± 1.51 d	11.69 ± 1.70 b	18.00 ± 0.82 b	925.98 ± 123.65 b	0	0

Cultivars	Fresh mass (g plant <sup>-1</sup> )			Dry mass (g plant <sup>-1</sup> )		
	Leaves	Stem	Roots	Leaves	Stem	Roots
Veneranda	28.91 ± 2.72 b	15.44 ± 0.91 a	5.51 ± 1.06 a	2.34 ± 0.15 b	1.68 ± 0.31 a	0.80 ± 0.06 a
Cristina	36.54 ± 6.03 a	6.83 ± 1.24 c	6.70 ± 1.36 a	3.15 ± 0.23 a	0.68 ± 0.14 c	0.63 ± 0.14 a
Olinda	39.64 ± 2.70 a	9.07 ± 2.46 b	7.99 ± 1.15 a	3.37 ± 0.11 a	0.84 ± 0.15 c	0.74 ± 0.11 a
Mônica	29.18 ± 2.01 b	14.59 ± 1.45 a	3.71 ± 0.44 b	2.06 ± 0.13 b	1.24 ± 0.19 b	0.64 ± 0.03 a
Cinderela	29.81 ± 2.00 b	15.82 ± 1.28 a	6.19 ± 2.36 a	2.42 ± 0.19 b	1.39 ± 0.11 b	0.79 ± 0.19 a
Grand Rapids	24.73 ± 1.92 b	10.23 ± 2.68 b	3.91 ± 2.15 b	2.09 ± 0.16 b	0.97 ± 0.25 c	0.53 ± 0.12 b
Itapuã Super	30.77 ± 7.66 b	4.53 ± 1.97 d	3.03 ± 1.96 b	2.32 ± 0.27 b	0.37 ± 0.15 d	0.41 ± 0.15 b
Crocantela	30.52 ± 9.34 b	1.74 ± 0.78 e	3.74 ± 1.55 b	2.02 ± 0.67 b	0.15 ± 0.06 d	0.40 ± 0.17 b
Simpson B. S.	29.22 ± 5.35 b	6.26 ± 2.29 c	3.10 ± 1.39 b	2.19 ± 0.44 b	0.64 ± 0.18 c	0.51 ± 0.10 b

Means followed by the same letter within the column do not differ by the Scott-Knott's test ( $p \leq 0.05$ ). <sup>1</sup> Classification of the degree of plant bolting adapted from Holmes et al. (2019)

Simpson Black Seed cultivars stood out, since they showed no signs of bolting.

These results indicate the tolerance of these cultivars to the growing conditions to which they were subjected. When the temperature of the growing environment is above 30 °C, lettuce has a tendency to accumulate gibberellin, promoting early bolting, (Liu et al., 2020), and both height and stem diameter are variables that indicate the resistance of the genetic material (Lemos Neto et al., 2017; Fortes et al., 2020).

Furthermore, the age until bolting of the evaluated cultivars should also be considered (Lemos Neto et al., 2017), along with the degree of bolting. In this context, the cultivar Mônica was the one that had the lowest tolerance, since it started the bolting earlier and in a more intense degree. Some limitations in the gas exchange process were observed for the bolted cultivars Mônica, Veneranda, Cinderela, and Grand Rapids, which may be related to the degree of expression of the bolting.

The Olinda cultivar had the largest number of leaves, while the Grand Rapids and Cinderela had on average 62.88% less. For leaf area, again, the cultivar Olinda distinguished itself, this time together with Cristina and Mônica cultivars, showing an average between 978.05 and 1165.35 cm<sup>2</sup>.

Based on this pattern, Olinda and Cristina showed the highest accumulation of fresh and dry leaf biomass, 23.81 and 32.21% higher, respectively, than the second group, which included the other cultivars. In relation to the accumulation of stem fresh mass, Cinderela, Veneranda, and Mônica had the highest values, with Crocantela obtaining the lowest mass. For stem dry mass, four groups were formed, with Veneranda showing the greatest accumulation (1.68 g plant<sup>-1</sup>), followed by another group, composed of Cinderela and Mônica (1.39 and 1.24 g plant<sup>-1</sup>, respectively).

The inferior performance in leaf biomass accumulation and the increase in stem mass can be attributed to the expression of early bolting, leading the plant to prioritize the formation

of reproductive structure over vegetative growth, causing a reduction in leaf biomass accumulation (Pessarakli, 2021). However, even though Crocantela, Simpson, and Itapuã Super did not show signs of bolting, they showed low performance in production and growth parameters, indicating poor adaptability of the genetic material to environmental and cultivation conditions (Negrão et al., 2020).

Souza et al. (2019), when evaluating the performance of Crocantela in southern Brazil in a region with maximum temperature during the experimental trial of 26.4 °C, verified higher values for biomass production compared to those observed in our study, indicating the effect of climate on the performance of this cultivar. Regarding the production of root fresh mass, two groups were formed, with Olinda, Cristina, Cinderela, and Veneranda cultivars belonging to the group with greater mass (between 6.70 and 5.51 g) and Itapuã Super, Simpson, Mônica, Crocantela, and Grand Rapids cultivars belonging to the group with lower mass (between 3.91 and 3.10 g). Similarly, for root dry mass two groups were formed, but with Mônica cultivar being among those that accumulated the most mass, resulting in an average 36.11% higher than that of the group with the lowest accumulation.

The functioning of the root system in hydroponic cultivation is a relevant aspect, since the roots are sensitive organs to external factors such as temperature, salinity and oxygenation. Moreover, the growth and final quality of the plants are affected by their functioning, due to their role in the absorption of water and ions, respiration and nutrient assimilation (Taiz et al., 2017; Velazquez-Gonzalez et al., 2022). These results demonstrate the greater tolerance and adaptability of the Olinda and Cristina cultivars to hot climates, since they had superior production and growth, and also did not show signs of bolting.

As for the postharvest quality, differences were observed in all the variables evaluated ( $p \leq 0.05$ ) for the lettuce cultivars

**Table 3.** Postharvest variables obtained from the evaluation of lettuce leaves grown in a hydroponic system under high temperature condition

Cultivars	Ascorbic acid (mg 100g <sup>-1</sup> )	pH	TSS (%)	TA (% of citric acid)	TSS/TA ratio
Veneranda	19.565 ± 1.54 a	6.303 ± 0.06 a	4.113 ± 0.14 c	0.112 ± 0.00 b	36.771 ± 2.31 b
Cristina	18.844 ± 1.85 b	6.250 ± 0.19 a	4.750 ± 0.44 b	0.106 ± 0.01 b	45.774 ± 9.77 a
Olinda	21.014 ± 1.03 a	6.020 ± 0.09 b	5.300 ± 0.08 a	0.130 ± 0.01 a	40.975 ± 2.30 a
Mônica	18.478 ± 0.89 b	5.840 ± 0.08 c	4.425 ± 0.13 c	0.139 ± 0.01 a	32.058 ± 2.42 b
Cinderela	19.927 ± 0.51 a	6.100 ± 0.14 a	4.050 ± 0.19 c	0.093 ± 0.01 c	43.813 ± 3.05 a
Grand Rapids	17.754 ± 0.51 b	6.188 ± 0.18 a	4.350 ± 0.20 c	0.114 ± 0.02 b	39.476 ± 8.81 b
Itapuã Super	17.753 ± 0.51 b	6.100 ± 0.18 a	4.300 ± 0.35 c	0.098 ± 0.01 c	44.163 ± 2.42 a
Crocantela	18.841 ± 0.51 b	6.128 ± 0.05 a	3.350 ± 0.19 d	0.085 ± 0.01 c	39.563 ± 1.70 a
Simpson B. S.	18.840 ± 1.03 b	6.208 ± 0.05 a	3.100 ± 0.26 d	0.096 ± 0.02 c	33.065 ± 5.61 b

Means followed by the same letter within the column do not differ by the Scott-Knott's test ( $p \leq 0.05$ )

(Table 3). For the ascorbic acid, mean contents between 21.014 and 17.753 mg 100g<sup>-1</sup> were observed in the leaves. Two groups were formed in relation to the content, with emphasis on the group with the highest accumulation, composed of Olinda, Cinderela, and Veneranda cultivars.

Ascorbic acid in plant metabolism has its prominent role in mitigating the effects of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and its action along with glutathione is primordial for the efficiency of non-enzymatic antioxidant response complexes (Taiz et al., 2017; Paciolla et al., 2019), indicating a possible mechanism employed by Olinda and Cristina cultivars. However, for Veneranda cultivar, this mechanism proved to be inefficient, due to the stomatal limitation of physiological activity.

For the pH of the leaf extract, values between 5.84 and 6.30 were observed, indicating the influence of genetic variability in this variable. Average values above 6.0 were obtained for all the cultivars evaluated, which were in the same group, except for Mônica. This variable affects the digestibility of the leaves of some species; therefore, the higher the pH, the greater the digestion of the leaf material and free sugar content (Masoero et al., 2019). In contrast, under lower pH conditions, leaves tend to accumulate more water, lignin, cellulose, hemicellulose, and crude fibers (Masoero et al., 2019).

Regarding total soluble solids (TSS), the highest levels were observed in the leaves of Olinda cultivar. We observed that the cultivars which showed early bolting had a lower TSS content. TSS has been considered to be a factor that confers shelf life to vegetable products (Chitarra & Chitarra, 2005), hence being another interesting characteristic for the market. Therefore, the early bolting of lettuce can contribute to a product with a shorter shelf life.

For titratable acidity (TA), values between 0.085 and 0.139% were observed in the lettuce leaves, with higher levels in the Mônica and Olinda cultivars. The TA content interferes negatively in the palatability of vegetable products when in very high proportions relative to TSS; however, it contributes to making the vegetable material resistant to microbiological degradation, increasing the shelf life (Chitarra & Chitarra, 2005; Sampaio et al., 2021).

Regarding the taste of the leaves, the TSS/TA ratio evidenced a negative effect of bolting on the quality of the lettuce, since Mônica, Grand Rapids, and Veneranda cultivars showed the lowest performance in this parameter. Thus, two groups were formed: Cristina, Itapuã Super, Cinderela, Olinda, and Crocantela cultivars, with an average of 42.86% and

Mônica, Simpson, Veneranda, and Grand Rapids cultivars, with an average of 35.34%.

The TSS/TA ratio is indicative of the palatability of vegetables (Chitarra & Chitarra, 2005). Therefore, it was observed that the bolting influenced the palatability of the lettuce, resulting in lower ratio. This effect can be attributed to the fact that the bolting stage in lettuce confers bitterness in the taste of the leaves and consequently reduces their commercial quality (Holmes et al., 2019). Another factor to be considered is the adaptation of the cultivars to the growing system, which affects the postharvest quality of lettuce (Majid et al., 2021).

In this context, Olinda and Cristina distinguished themselves as cultivars of the crisp type suitable for cultivation in high temperature regions and can also meet the demands of different consumers, due to their different intensity of green color of the leaves.

The Olinda cultivar showed good productive performance, absence of inhibitions of biochemical and physiological nature, besides having characteristics of interest to the consumer market, such as the antioxidant potential, which contemplates a niche market that demands functional products with greater nutraceutical quality (Sala & Da Costa, 2012; Santos et al., 2021). The Cristina cultivar, on the other hand, represents a more traditional option for the consumer market in which demands for crisp lettuce with a lighter green color already exist (Sala & Costa, 2012).

## CONCLUSIONS

1. The lettuce cultivars Olinda and Cristina had the best productive performance and postharvest quality under high temperature conditions.
2. Early bolting limited the production, gas exchange capacity, and postharvest quality of lettuce.
3. Photosynthetic pigments content was not a determinant factor for the expression of bolting.

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## LITERATURE CITED

- Alvares, C. A.; Stape, J. L.; Sentelhas, P. C.; Gonçalves, J. L. M.; Spavorek, G.. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, v.22, p.711-728, 2013. <https://doi.org/10.1127/0941-2948/2013/0507>
- AOAC - Association of Official Analytical Chemistry. Official methods of analysis of the Association of Official Analytical Chemistry. Washington: AOAC, 2012.
- Chitarra, M. I. F.; Chitarra, A. B. Pós-colheita de frutos e hortaliças: fisiologia e manuseio. Lavras: UFLA, 2005. 783p.
- FAO – Food and Agriculture Organization of the United Nations. Climate change and food security: Risks and responses. Rome: FAO, 2015. 100p. Available on: <https://www.fao.org/3/i5188e/i5188E.pdf>. Accessed on: Feb. 2023.
- Fortes, J. F. M. de; Guimarães, M. A.; Lemos Neto, H. S.; Sampaio, I. M. G.; Silva, F. G. M. Productive and physiological performance of lettuce cultivars under hydroponic cultivation in the semi-arid region of the Northeast. *Revista Agro@mbiente On-line*, v.14, p.1-10, 2020. <https://doi.org/10.18227/1982-8470ragro.v14i0.5901>
- Hoagland, D. R.; Arnon, D. I. The water-culture method for growing plants without soil. *Circular California Agricultural Experiment Station*, v.347, p.1-32, 1950.
- Holmes, S. C.; Wells, D. E.; Pickens, J. M.; Kemble, J. M. Selection of heat tolerant lettuce (*Lactuca sativa* L.) cultivars grown in deep water culture and their marketability. *Horticulturae*, v.5, p.1-11, 2019. <https://doi.org/10.3390/horticulturae5030050>
- IAL - Instituto Adolfo Lutz. Métodos físico-químicos para análise de alimentos. São Paulo: IAL, 2008. 1020p.
- Lemos Neto, H. S.; Guimarães, M. A.; Tello, J. P. J.; Mesquita, R. O.; Do Vale, J. C.; Lima Neto, B. P. Productive and physiological performance of lettuce cultivars at different planting densities in the Brazilian Semi-arid region. *African Journal of Agricultural Research*, v.12, p.771-779, 2017. <https://doi.org/10.5897/AJAR2016.11961>
- Lichthenthaler, H. K. Chlorophyll and carotenoids-pigments of photosynthetic biomembranes. *Methods in Enzymology*, v.148, p.350-382. 1987. [https://doi.org/10.1016/0076-6879\(87\)48036-1](https://doi.org/10.1016/0076-6879(87)48036-1)
- Liu, R.; Su, Z.; Zhou, H.; Huang, Q.; Fan, S.; Liu, C.; Han, Y. LsHSP70 is induced by high temperature to interact with calmodulin, leading to higher bolting resistance in lettuce. *Scientific Reports*, v.10, p.1-9, 2020. <https://doi.org/10.1038/s41598-020-72443-3>
- Majid, M.; Khan, J. N.; Shah, Q. M. A.; Masoodi, K. Z.; Afroza, B.; Parvaze, S. Evaluation of hydroponic systems for the cultivation of lettuce (*Lactuca sativa* L., var. Longifolia) and comparison with protected soil-based cultivation. *Agricultural Water Management*, v.245, p.1-13, 2021. <https://doi.org/10.1016/j.agwat.2020.106572>
- Masoero, G.; Cugnetto, A.; Sarasso, G.; Giovannetti, G.; Nuti, M. Sunspots are correlated with foliar pH in grapevines. *Journal of Agronomy Research*, v.2, p.31-41, 2019. <https://doi.org/10.14302/issn.2639-3166.jar-19-3116>
- Negrão, L. D.; Sousa, P. V. D. L.; Barradas, A. M.; Brandão, A. D. C. A. S.; Araújo, M. A. D. M.; Moreira-Araújo, R. S. D. R. Bioactive compounds and antioxidant activity of crisphead lettuce (*Lactuca sativa* L.) of three different cultivation systems. *Food Science and Technology*, v.41, p.365-370, 2020. <https://doi.org/10.1590/fst.04120>
- Paciolla, C.; Fortunato, S.; Dipierro, N.; Paradiso, A.; De Leonardis, S.; Mastropasqua, L.; Pinto, M. C. Vitamin C in plants: From functions to biofortification. *Antioxidants*. v.8, p.1-26, 2019. <https://doi.org/10.3390/antiox8110519>
- Pessaraki, M. Handbook of plant and crop physiology. 4.ed. Boca Raton: CRC Press - Taylor & Francis Group, 2021. 1173p. <https://doi.org/10.1201/9781003093640>
- R Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2021. Available on: <<https://www.r-project.org/>>. Accessed on: Ago. 2022.
- Sala, F. C.; Costa, C. P. Retrospectiva e tendência da alfaceicultura Brasileira. *Horticultura Brasileira*, v.30, p.187-194, 2012. <https://doi.org/10.1590/S0102-05362012000200002>
- Sampaio, I. M. G.; Silva Júnior, M. L. Bittencourt, R. F. P. M.; Santos, G. A. M.; Lemos Neto, H. S. Production and postharvest quality of jambu in hydroponics under nitrogen application in nutrient solution. *Revista Ciência Agronômica*, v.52, p.1-8, 2021. <https://doi.org/10.5935/1806-6690.20210021>
- Santos, C. P.; Noboa, C. S.; Martinez, M.; Cardoso, J. C.; Sala, F. C. Morphological evaluation of lettuce genotypes grown under hydroponic system. *Horticultura Brasileira*, v.39, p.312-318, 2021. <https://doi.org/10.1590/s0102-0536-20210311>
- Souza, P. F.; Borghezán, M.; Zappelini, J.; Carvalho, L. R.; Ree, J.; Barcelos-Oliveira, J. L.; Pescador, R. Physiological differences of 'Crocantela' lettuce cultivated in conventional and hydroponic systems. *Horticultura Brasileira*, v.37, p.101-105, 2019. <https://doi.org/10.1590/S0102-053620190116>
- Strohecker, R.; Henning H. M. Análises de vitaminas: Métodos comprovados. Madrid: Paz Montalvo, 1967. 428p.
- Taiz, L.; Zeiger, E.; Møller, I. M.; Murphy, A. Fisiologia e desenvolvimento vegetal. 6.ed. Porto Alegre: ArtMed, 2017. 888p.
- Velazquez-Gonzalez, R. S.; Garcia-Garcia A. L.; Ventura-Zapata, E.; Barceinas-Sanchez, J. D. O.; Sosa-Savedra, J. C. A review on hydroponics and the technologies associated for medium- and small-scale operations. *Agriculture*. v.12, p.1-21, 2022. <https://doi.org/10.3390/agriculture12050646>
- Young, A. J. The photoprotective role of carotenoids in higher plants. *Physiologia Plantarum*, v.83, p.702-708, 1991. <https://doi.org/10.1111/j.1399-3054.1991.tb02490.x>