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Effect of Saline Stress and Calcium Nitrate on Lettuce Grown on Coconut Fiber

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Abstract

This study aimed to evaluate the use of saline solutions enriched with calcium nitrate in the production of lettuce grown in coconut fiber. The experiment was carried out from July to August 2017 in a greenhouse, at the Federal Rural University of the Semi-Arid Region (UFERSA), Mossoró-RN, Brazil. A randomized block design was used, in 2 × 5 factorial scheme, with three replicates. Treatments resulted from the combination of two lettuce cultivars [Elba (Curly) and Irene (Crisphead)] and five nutrient solutions (S1- standard nutrient solution; S2-S1 + NaCl (28.48 mmol L^{-1}); S3-S2 + $Ca(NO_3)_2$ (6.89 mmol L^{-1}); S4-S2 + $Ca(NO_3)_2$ (9.15 mmol L^{-1}); S5-S2 + $Ca(NO_3)_2$ (11.43 mmol L^{-1})]. Plants were harvest 30 days after transplantation and the following variables were analyzed: head diameter, stem diameter, number of leaves, fresh weight, dry weight, leaf area, specific leaf area and leaf succulence. The cv. Irene (Crisphead) is more tolerant to nutrient solution salinity compared with the cv. Elba (Curly). Nutrient solutions enriched with 50 and 100% of $Ca(NO_3)_2$ promoted better performance of the cultivars Elba and Irene, respectively, fertigated with saline nutrient solution.

Keywords: *Lactuca sativa*, hydroponic cultivation, salinity, soilless cultivation

1. Introduction

Lettuce (*Lactuca sativa* L.), belonging to the Asteraceae family, is the main leafy vegetable produced and consumed in the world, especially in the form of salads (Sala & Costa, 2012). In addition, it is the most cultivated and adapted vegetable to the protected system, especially hydroponics, standing out for its short cycle, which allows faster return of the invested capital.

Lettuce is a crop classified as sensitive to salinity, exhibiting a threshold of 1.3 dS m^{-1} in the saturation extract and 0.9 dS m^{-1} in the irrigation water (Maas & Hoffman, 1977). Nevertheless, tolerance to salinity is variable among varieties and, even within a varieties, between growth stages. In each growth stage, tolerance to salinity is controlled by more than one gene and highly influenced by environmental factors (T. J. Flowers & S. A. Flowers, 2005; Munns, 2005; Parida & Das, 2005).

The use of saline water to prepare nutrient solutions is a great challenge for researchers because, depending on the studied species, it may negatively affect the commercial yield of the crops. In lettuce in a floating system, Neocleous et al. (2014) observed reduction in fresh weight due to the lower absorption of water and nutrients, decrease in photosynthesis and in the restriction of CO_2 availability.

However, yield reduction can be compensated, at least partially, by the increase in the contents of anthocyanins in red lettuces, as well as by the improvement of freshness in green lettuces (Neocleous et al., 2014; Parida & Das, 2005).

The salinity of the root environment compromises plant development by reducing the osmotic potential of the solution, which is associated with the water stress caused by the difficulty to absorb water from the soil, accumulation of toxic ions in the tissues (Cl^- and Na^+) and ionic imbalance, leading to the reduction in the absorption of essential ions, such as NO_3^- and Ca^{2+} (Paulus et al., 2012; Soares et al., 2016; Cova et al., 2017).

One alternative to minimize the effects of salinity is the enrichment of the root zone with nutrients, which increases plant tolerance to salinity by relieving the damagescaused by Na^+ and Cl^- (Tzortzakis, 2009).

Calcium, in turn, is a constituent of the cell wall and acts in most processes of growth, development, maintenance and reproduction, being responsible for the mechanical resistance of vegetal structures, promotion of junction of cells and exoskeleton, besides controlling high turgor pressures and acting in the protection against physical and chemical injuries (Taiz & Zeiger, 2013).

In addition, increment of Ca content in leaf tissues can increase photosynthetic capacity and also chlorophyll synthesis (Fallovo et al., 2009). Hence, using Ca in the nutrient solution can be one method to reduce some physiological imbalances due to salinity, such as the absorption of micro- and macroelements (Tzortzakis, 2009; Borghesi et al., 2013).

In this context, the present study aimed to evaluate the effect of calcium nitrate enrichment in saline nutrient solution on two lettuce cultivars grown in coconut fiber.

2. Method

The experiment was carried out from July to August 2017, in a greenhouse, in the experimental sector of the Department of Environmental and Technological Sciences of the Federal Rural University of the Semi-Arid Region (UFERSA), Mossoró-RN, Brazil (5°11′31″ S, 37º20′40″ W).

The greenhouse used in the study is 6.40 m wide and 22.5 m long, with an arched roof covered by 0.10-mm-thick, transparent low-densitypolyethylene, treated against the action of ultraviolet rays. Sides and front walls are equipped with anti-aphid net and the 0.30-m-high wall is made of reinforced concrete.

A completely randomized block design was used, in a 2 × 5 factorial scheme, with three replicates. Each replicate was formed by 4 plastic pots with capacity for 3.0 L, containing coconut fiber and one plant each.

Treatments resulted from the combination of two lettuce cultivars [‘Elba’ (curly) and ‘Irene’ (crisphead)] with five nutrient solutions, being the nutritional solution standard following the recommendation of Furlani et al. (1999) and the others salinized with sodium chloride. The amounts of salts used in the preparation of nutrient solutions are shown in Table 1.

Table 1. Quantities of fertilizers, sodium chloride, and electrical conductivity of nutritive solutions

Fertilizers	Nutritive solutions				
	S1	S2	S3	S4	S5
	---- mmol L^{-1} ----				
Monoammonium fosfate	1.30	1.30	1.30	1.30	1.30
Calcium nitrate	4.57	4.57	6.86	9.15	11.43
Potassium nitrate	4.95	4.95	4.95	4.95	4.95
Magnesium sulfate	3.32	3.32	3.32	3.32	3.32
NaCl	0	28.48	28.48	28.48	28.48
Rexolin® (mg L^{-1})	30	30	30	30	30
CE (dS m^{-1})*	1.5	3.82*	4.35	4.84	5.03

Note. * electrical conductivity after preparation of nutritive solutions. Rexolin®: micronutrients (11.6% of potassium oxide (K_2O), 1.28% of sulfur (S), 0.86% of magnesium (Mg), 2.1% of boron (B), 2.66% of iron (Fe), 0.36% of copper (Cu), 2.48% of manganese (Mn), 0.036% of molybdenum (Mo), and 3.38% of zinc (Zn).

Nutritive solutions were prepared using water from a deep water well at the university, with the following physicochemical characteristics: pH = 8.97; ECw = 0.62 dS m^{-1}; K^+ = 2.07 $mmol_c$ L^{-1}; Na^+ = 0.91 $mmol_c$ L^{-1}; Ca^{2+} = 2.87 $mmol_c$ L^{-1}; Mg^{2+} = 0.51 $mmol_c$ L^{-1}; Cl^- = 3.91 $mmol_c$ L^{-1}; CO_3^{2-} = 0.23 $mmol_c$ L^{-1}; HCO_3^- = 1.78 $mmol_c$ L^{-1}; SAR = 0.91 $mmol_c$ L^{-1}; SAR = 0.70 $(mmol_c\ L^{-1})^{0.5}$.

The pH of each solution was adjusted to 6.0-6.5 through the application of 0.1 mol L^{-1} KOH or HCl solution. After the solutions were prepared, electrical conductivity was measured using a benchtop conductivity meter (TECNOPON mCA150), are shown in Table 1.

Each nutrient solution was applied using a drip irrigation system composed of an electric pump, a plastic tank (60 L), lateral lines made of polyethylene hose (16 mm diameter) and 40 cm long microtube drippers with flow rate of 2.5 L h^{-1}.

Irrigation was controlled using digital timers programmed to perform different daily irrigation events, applying sufficient nutrient solution to meet crop water demand. The number of irrigation events and their duration varied along the cycle according to the crop requirement. In the first two weeks after transplantation, six irrigations were performed per day (7:00 a.m., 9:00 a.m.; 11:00 a.m., 01:00 p.m., 15:00 p.m., and 17:00 p.m.), lasting 1 minute each; in the last two weeks, eight irrigations were performed per day (7:00 a.m., 9:00 a.m.; 11:00 a.m., 12:00 a.m., 01:00 p.m., 02:00 p.m., 03:00 p.m. and 05:00 p.m.), lasting 1 minute and 30 seconds each.

Plants were harvested 30 days after transplantation and the following variables were analyzed: head diameter (HD), measured using a graduated ruler, considering the average diameter of two perpendicular means, expressed in cm; stem diameter (SD), determined using a digital caliper in the region close to the substrate, expressed in mm; number of leaves (NL), determined by counting only leaves with commercial standard (not considering leaves less than three cm in length, dried and/or damaged); fresh weight aerial part (FW), obtained by weighing the plants on precision scale (0.01 g); dry weight (DW), determined after plants were dried in forced-air oven at temperature of 65 °C, until constant weight.

Besides these variables, leaf area (LA), specific leaf area (SLA) and leaf succulence (LS) were also determined. Leaf area was determined through the leaf disc method, using a volumetric ring with internal diameter of 2.5 cm (4.9 cm^2) to collect 20 leaf discs per plot. The leaf discs were placed in paper bags and dried in forced-air oven at temperature of 65 °C until constant weight. Leaf disc area and dry weight values were used to determine the leaf area of the plant, according to Equation 1.

$$LA = \frac{DA \times LDW}{DDW/N} \tag{1}$$

Where, LA: leaf area, cm^2; DA: leaf disc area, cm^2; LDW: leaf dry weight, g; DDW:leaf disc dry weight, g; N: number of discs used in the plot.

Specific leaf area was determined by the ratio between leaf area and leaf dry weight Equation 2.

$$SLA = \frac{LA}{LDW} \tag{2}$$

Where, SLA: specific leaf area, $cm^2\ g^{-1}$; LA:eaf area, cm^2; LDW: leaf dry weight, g.

Leaf succulence was determined through the ratio between leaf water content and leaf area, Equation 3.

$$LS = \frac{(LFW - LDW)}{LA} \tag{3}$$

Where, LS: leaf succulence, g H_2O cm^{-2}; LFW: leaf fresh weight, g; LDW: leaf dry weight, g; LA: leaf area, cm^2 $plant^{-1}$.

The obtained data were subjected to analysis of variance, and follow-up analyses were performed for variables that showed significant response to the interaction between factors. Means were compared by Tukey test at 0.05 probability level. The statistical analyses were performed using the program SISVAR (Ferreira, 2011).

3. Results and Discussion

The cultivars differed for head diameter (HD) only in the saline nutrient solution (S2), in which the cv. Irene surpassed the cv. Elba by 39.75% (Figure 1A). Additionally, nutrient solutions had no effect on this variable in the cv. Irene. On the other hand, the use of saline water to prepare the nutrient solution reduced HD by 38.83% in the cv. Elba, but nutrient solution enrichment with calcium nitrate inhibited the deleterious effect of salinity on this variable (Figure 1A).

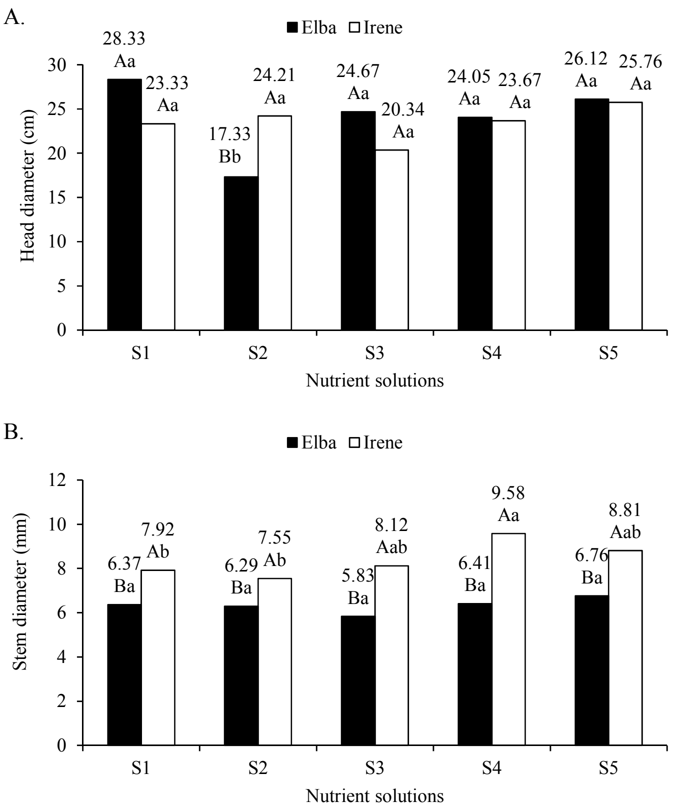


Figure 1. Head diameter (A) and stem diameter (B) of lettuce cultivars produced in coconut fiber and fertigated with saline nutrient solutions enriched with calcium nitrate (Means followed by the same letters, uppercase for cultivars and lowercase for nutrient solutions, do not differ by Tukey test at 0.05 probability level). [S1: standard nutrient solution; S2: S1 + NaCl (28.48 mmol L^{-1}); S3: S2 + $Ca(NO_3)_2$ (6.89 mmol L^{-1}); S4: S2 + $Ca(NO_3)_2$ (9.15 mmol L^{-1}); S5: S2 + $Ca(NO_3)_2$ (11.43 mmol L^{-1})]

Negative effect of salinity on lettuce head diameter has also been found by other authors, such as Santos et al. (2010). Guimarães et al. (2016), working with seven lettuce cultivars, observed that the effect of salinity on this variable varied according to the cultivar.

Stem diameter (SD) was significantly different between the cultivars. The cv. Irene was superior to the cv. Elba in all nutrient solutions, with greatest differences in the solutions S4 (49.45%) and S3 (39.28%), whereas the lowest difference occurred in the solution S2 (20.03%). The nutrient solutions caused no significant response on SD in the cv. Elba, leading to mean value of 6.33 mm. On the other hand, the cv. Irene showed significant response to the treatments, with higher values in the solutions S3, S4 and S5, which indicates that it may have higher Ca demand compared with the cv. Elba (Figure 1B).

According to Mota et al. (2001), stem diameter is a characteristic of great importance for the fast food industry, because the stem is manually removed for subsequent slicing of the lettuce head. Therefore, the thicker the stem, the faster it is removed, increasing industrial yield.

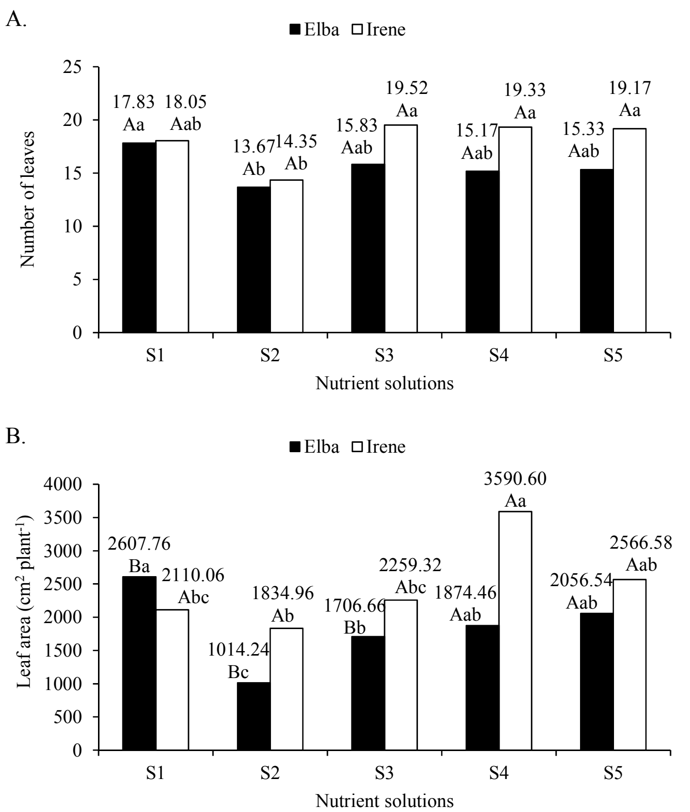


Figure 2. Number of leaves (A) and leaf area (B) of lettuce cultivars produced in coconut fiber and fertigated with saline nutrient solutions enriched with calcium nitrate (Means followed by the same letters, uppercase for cultivars and lowercase for nutrient solutions, do not differ by Tukey test at 0.05 probability level). [S1: standard nutrient solution; S2: S1 + NaCl (28.48 mmol L^{-1}); S3: S2 + $Ca(NO_3)_2$ (6.89 mmol L^{-1}); S4: S2 + $Ca(NO_3)_2$ (9.15 mmol L^{-1}); S5: S2 + $Ca(NO_3)_2$ (11.43 mmol L^{-1})]

Regarding the number of leaves, except for the solutions S1 and S2, in which there was no difference between the cultivars, the cv. Irene was superior to the cv. Elba in all other nutrient solutions. Such superiority was equal to 23.3, 27.4 and 25.0%, in the solutions S3, S4 and S5, respectively (Figure 2A).

Both cultivars showed reduction in the number of leaves when fertigated with saline nutrient solution (S2), with losses of 23.33 and 20.49% in the cv. Elba and Irene, respectively (Figure 2A). However, both cultivars exhibited positive responses to the enrichment of the saline nutrient solutions with $Ca(NO_3)_2$.

Leaf area (LA) was 19.08% higher in the cv. Elba in comparison to the cv. Irene when both were fertigated with S1. On the other hand, the cv. Irene was superior in the other nutrient solutions, with largest difference between the cultivars in the solutions S2 (80.92%) and S4 (91.55%). The use of saline nutrient solution (S2) reduced LA in both cultivars, causing losses of 61.11 and 13.04% in Elba and Irene, respectively (Figure 2B).

Still referring to the variable LA, the extra addition of $Ca(NO_3)_2$ reduced the deleterious effect of salt stress on both cultivars, with more expressive effect on the cv. Irene, in which the solutions enriched with $Ca(NO_3)_2$ led to higher LA compared with the standard nutrient solution (S1), especially S4, which was superior to S1 by 70.16% (Figure 2B).

The cultivars did not differ for the variable fresh weight in the standard nutrient solution, but differed in the other nutrient solutions, with superiority of the cv. Irene, especially in S3 and S4, in which its fresh weight was 110.25 and 163.81% higher compared with the cv. Elba, respectively (Figure 3A).

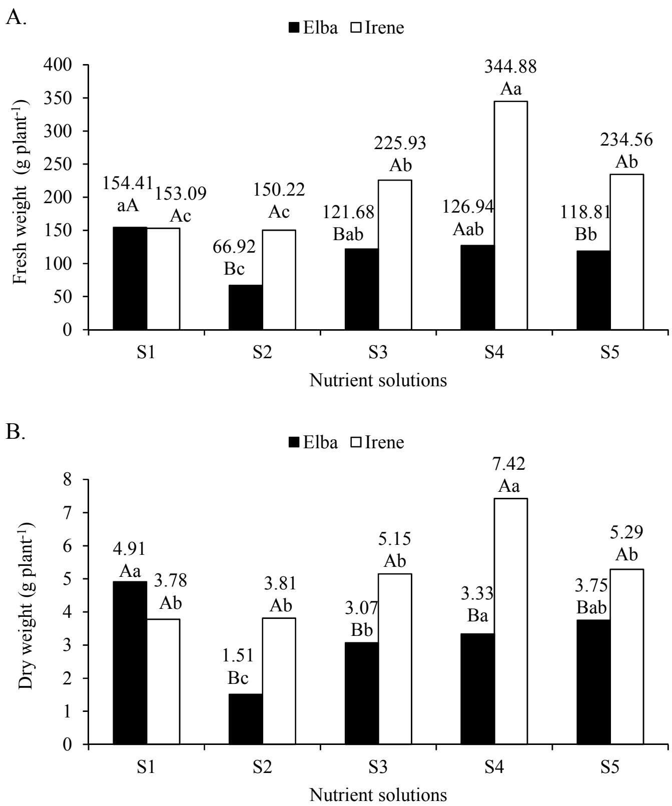


Figure 3. Fresh weight (A) and dry weight (B) of lettuce cultivars produced in coconut fiber and fertigated with saline nutrient solutions enriched with calcium nitrate (Means followed by the same letters, uppercase for cultivars and lowercase for nutrient solutions, do not differ by Tukey test at 0.05 probability level). [S1: standard nutrient solution; S2: S1 + NaCl (28.48 mmol L^{-1}); S3: S2 + $Ca(NO_3)_2$ (6.89 mmol L^{-1}); S4: S2 + $Ca(NO_3)_2$ (9.15 mmol L^{-1}); S5: S2 + $Ca(NO_3)_2$ (11.43 mmol L^{-1})]

Both cultivars were affected by the salinity of nutrient solutions, but showed different responses. Fresh weight decreased in the cv. Elba when saline nutrient solution (S2) was used, with loss of 56.66% compared with the value obtained in the standard nutrient solution (S1). Additionally, it was observed that nutrient solution enrichment with $Ca(NO_3)_2$ reduced the deleterious effect of salinity. For the cv. Irene, salinity had no significant effect on fresh weight (S2) and there was a positive response to the extra application of $Ca(NO_3)_2$. Highest values were obtained in the solutions S3, S4 and S5, which were respectively 67.11, 118.75 and 53.22% higher than the fresh weight obtained in the standard nutrient solution (Figure 3A).

Positive effect of Ca supplementation on fresh weight production has been found by other authors, working with other vegetables such as chicory (Tzortzakis, 2010) and spinach (Turhan et al., 2013). In addition, attention should also be paid to the increase of nitrogen concentration in the nutrient solution, due to competition in the absorption between nitrate and chloride, so that an increase in the nitrate concentration in the root zone can inhibit a greater uptake of chloride by the plant.

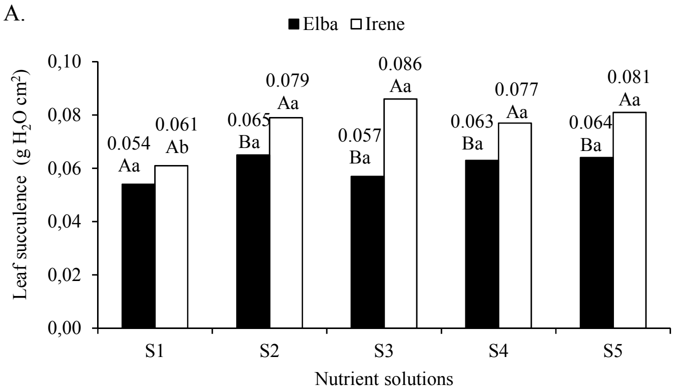
As observed for the variable fresh weight, there was also no significant difference in dry weight (DW) between the cultivars when fertigated with the standard nutrient solution (S1). However, in the other nutrient solutions, the cv. Irene was superior to the cv. Elba, especially in S2 and S4, in which its DW was 152.32 and 122.82% higher, respectively (Figure 3B).

Regarding the effect of the nutrient solutions on DW, the saline nutrient solution (S2) caused reduction of DW only in the cv. Elba, equal to 69.25% in comparison to the DW obtained in the solution S1. In addition, nutrient solution enrichment with $Ca(NO_3)_2$ reduced the effect of salinity, although there were losses of 37.47% (S3), 32.18% (S4) and 23.62% (S5). For the cv. Irene, there was no significant response to the addition of NaCl in the nutrient solution (S2). Moreover, this cultivar showed positive response to the extra addition of $Ca(NO_3)_2$, with increments of 36.24, 96.29 and 39.95% in the solutions S3, S4 and S5, respectively (Figure 3B).

These data demonstrate that the cv. Irene is more tolerant to nutrient solution salinity and has higher demand for $Ca(NO_3)_2$ to obtain maximum biomass production. Other authors have already observed that the cv. Elba is sensitive to salinity in hydroponic cultivation NFT (Santos et al., 2011).

Such reduction in plant growth due to salt stress can be explained by photosynthetic limitations, resulting from stomatal closure and, simultaneously, lower CO_2 assimilation (Taarit et al., 2010). In addition, it may be associated with the energetic expenditure involved in the synthesis of organic solutes, necessary for the processes of compartmentalization and regulation of the transport of ions (Mendonça et al., 2007).

The cultivars did not differ significantly for the variable leaf succulence (LS) in the standard nutrient solution. The cv. Irene was superior to the cv. Elba in the other solutions, especially in S3 (50.78%). In addition, the solutions had no effect on the LS of the cv. Elba, which showed mean value of 0.06 g H_2O cm^{-2} of leaf. On the other hand, the use of saline nutrient solution increased LS in the cv. Irene (Figure 4A).



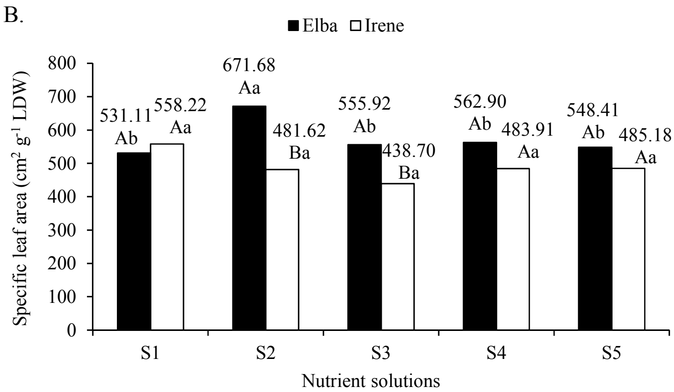


Figure 4. Leaf succulence (A) and specific leaf area (B) of lettuce cultivars produced in coconut fiber and fertigated with saline nutrient solutions enriched with calcium nitrate (Means followed by the same letters, uppercase for cultivars and lowercase for nutrient solutions, do not differ by Tukey test at 0.05 probability level). [S1: standard nutrient solution; S2: S1 + NaCl (28.48 mmol L^{-1}); S3: S2 + $Ca(NO_3)_2$ (6.89 mmol L^{-1}); S4: S2 + $Ca(NO_3)_2$ (9.15 mmol L^{-1}); S5: S2 + $Ca(NO_3)_2$ (11.43 mmol L^{-1})]

Succulence is a characteristic with important anatomical and physiological implications in stressed plants and is directly related to the accumulation of salts in the tissues (Aquino et al., 2007). Leaf succulence allows the regulation of the concentration of salts in the leaf tissues and directly depends on the absorption, transport and accumulation of ions, possibly contributing to reducing the effect of salts on plant growth (Larcher, 2000).

The cv. Elba showed higher specific leaf area (SLA) in the solutions S2 and S3, being higher in 28.29 and 21.08%, respectively (Figure 4B). Figure 4B also shows that the cultivars responded differently to the addition of NaCl in the nutrient solution; SLA increased in the cv. Elba in S2, but did not affect SLA in the cv. Irene

SLA represents the ratio between leaf area and leaf dry weight. Hence, plants with lower SLA exhibit thicker leaves. In some plants subjected to salt stress conditions, leaf mesophyll thickness increases due to the increment in the number and length of palisade cells and in the number of palisade and spongy cell layers (Parida et al., 2004). According to Ottow et al. (2005), such increase in leaf succulence is due to the increment in the number and volume of the cells, thus causing a dilution in the leaf contents of sodium and chloride. Hence, it is considered as an acclimatization to the saline environment.

Since $Ca(NO_3)_2$ contains in its composition very similar contents of Ca and N (19% of Ca and 15.5% of N), it is not possible to separate the individual effect of these nutrients. Therefore, plant response was attributed to their joint effect. Nitrogen is a macronutrient found in organic compounds such as amino acids and nucleic acids, and participates in various physiological processes in the plant life cycle, such as ionic absorption, photosynthesis, respiration and cell multiplication and differentiation (Malavolta, 2006).

Due to the importance of Ca and N for plant development, some authors found that their deficiency leads to significant reduction in lettuce growth (Almeida et al., 2011; Tischer & Siqueira Neto, 2012). The positive effect of saline nutrient solution enrichment with $Ca(NO_3)_2$ is due to the probable increment of Ca^{2+} and NO_3^- contents in the lettuce leaf tissue, since the excess of Na^+ and Cl^- ions in the leaf tissue, resulting from the NaCl addition in the nutrient solution, causes reduction in the leaf contents of Ca^{2+} and NO_3^- (Paulus et al., 2012; Soares et al., 2016; Cova et al., 2017).

4. Conclusion

The cv. Irene (Crisphead) is more tolerant to nutrient solution salinity compared with the cv. Elba (Curly).

Nutrient solutions enriched with 50 and 100% of $Ca(NO_3)_2$ promoted better performance of the cultivars Elba and Irene, respectively, fertigated with saline nutrient solution.

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