

Can silicon (Si) influence growth, physiology and postharvest quality of lettuce?

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Abstract

Although it is not considered an essential element for plants, silicon (Si) provides benefits for several species, especially grasses, such as increase in yield and resistance to pests and diseases, reducing the effects of salt and water stress, among others. The aim of this study was to evaluate the effect of silicon on the performance of lettuce in hydroponic system. The experiment was carried out in a completely randomized design, with five doses of silicon (0, 2, 4, 6, 8 mM) in the nutrient solution. Shoot fresh and dry mass, gas exchanges, photosynthetic pigments and post-harvest were evaluated. It was observed that from the dose of 2 mM, there was a reduction in most variables. The dose of 0.4 mM $[-(-0.572)/(2*-0.7055)]$ provided a greater increase in shoot fresh mass. The highest photosynthetic rate was at the dose of 3.19 mM. Soluble solids, titratable acidity and pH were higher at the 4 mM dose. Silicon provides better lettuce production, with a dose of 0.4 mM allowing a greater shoot fresh mass, being the most indicated for lettuce cultivation in hydroponic system.

Keywords: *Lactuca sativa* L.; titratable acidity; chlorophyll; photosynthesis; sodium silicate.

Introduction

Lettuce (*Lactuca sativa* L.) is the main leafy vegetable for human consumption worldwide (Sala and Costa, 2012). It is the most traded leafy vegetable in Brazil, presenting an economic and social importance for the country (Freitas et al., 2013). Currently, its production is concentrated around big cities, known as "green belts", due to its fast perishability (Azevedo et al., 2015). Losses (before and after harvest) are estimated at 35 to 40% (Vilela et al., 2003; Tofaneli et al., 2009). These losses are mainly due to sensitivity to handling and loss of turgor after harvest when lettuce is not correctly stored and transported.

Some practices can be adopted to reduce these losses in different production systems. Fertilization is one of these practices and it can directly affect respiration, transpiration, chemical composition, visual characteristics and taste of fruits and vegetables (Galati et al., 2015a). Silicon fertilization (Si) has shown positive responses regarding production increment and postharvest quality in some vegetables such as lettuce (Resende et al., 2007; Galati et al., 2015b), strawberry (Figueiredo, 2010) and tomato (Marodin et al., 2016).

Si is absorbed by the plant as monosilicic acid (H_4SiO_4) and accumulates as hydrated amorphous silica or polymerized silica (SiO_2) in areas of maximum transpiration such as the leaf epidermis. The improvement in postharvest quality with Si may be related to the formation of a cuticle-silica double layer which reduces water loss during the storage period (Korndörfer, 2006; Mitani and Ma, 2005).

This element provides important benefits for plants such as: i) great resistance against pests and diseases once it is deposited on the cell walls of epidermis, ii) reduces the effects of potentially toxic metals, iii) mitigates the effects of saline stress and water deficiency, (iv) increases resistance to lodging and (v) decreases transpiratory flow (Liang et al., 2007; Rodrigues et al., 2011; Bybordi, 2012; Marodin et al., 2016).

Regarding the plant physiology, it has been observed that this mineral increases the liquid photosynthesis of some species, reduces the transpiration rate, acts on the balance of mineral absorption and the regulation of water absorption by the roots (Zhu and Gong, 2014; Sahebi et al., 2015). Despite the benefits of silicon in some crops, there are still few researches dealing with their effects on vegetables, mainly regarding the physiology and post-harvest quality of lettuce. In addition, an optimal dose to be used in hydroponic culture has not been established. Therefore, the aim of this study was to evaluate the effect of silicon on the growth, physiology and postharvest quality of lettuce.

Results

Through the analysis of variance, silicon (Si) doses affected the variables related to growth, postharvest quality, gas exchange and photosynthetic pigments. Regression models

were adjusted to observe the behavior of these variables as a function of Si doses.

Effect of silicon on the biomass accumulation of lettuce

There was an increase in shoot and root fresh mass (SFM and RFM) up to the doses of 0.4 $[-(-0.572)/(2*-0.7055)]$ and 1.4 mM (Figures 1A and C), as well as in the number of leaves (NL) (Figures 2C), which culminated in a greater fresh mass. These variables followed a quadratic model with maximum levels of 0.4 (43.93 g) for SFM and 1.4 mM (22.51 g) for RFM (Figures 1A and C). Shoot and root dry mass (SDM and RDM) were not influenced by the silicon doses (Figures 1B and D).

Plant length and leaf area were not affected by silicon doses either (Figure 2A, B and D), since they presented an increasing linear behavior. The number of leaves followed a quadratic behavior (Figure 2C).

Silicon changing the parameters of gas exchange

For liquid photosynthesis (*A*), stomatal conductance (*gs*) and transpiration (*E*) there was a quadratic adjustment (Figure 3). Si doses provided increases in these parameters up to a maximum point, from 3.19 mM to *A*; 3.12 mM for *gs* and 1 mM for *E* (Figures 3A, B and D).

Ci/Ca ratio presented a linear decreasing behavior as a function of increasing levels of Si (Figure 3C), consuming the internal CO₂ of the intercellular spaces, whereas the instantaneous efficiency of carboxylation (*A*/Ci) presented quadratic behavior (Figure 3E) and increasing linear for the instantaneous water use efficiency (WUE) (Figure 3F).

Influence of Si on photosynthetic pigments

For chlorophyll 'a' and total chlorophylls, the best regression model was the quadratic, with maximum values of 2.08 and 3.23 mg g⁻¹, at doses of 2.12 and 3.62 mM, respectively (Figure 4A and C). For chlorophyll 'b', the linear model was the best one, with higher values at the maximum dose (8.0 mM) (Figure 4B). For the carotenoids, the increasing linear model was the best one, with the highest values at 8.0 mM (Figure 4D).

Si changing parameters related to lettuce postharvest quality

Regarding the parameters related to the postharvest quality of lettuce, silicon doses increased soluble solids, titratable acidity and pH, with highest means for the dose of 4 mM (Figures 5A, C and D). Vitamin C decreased with increasing doses of silicon (Figure 5B). SS/AT ratio also increased with the presence of silicon (Figure 5E) and the external appearance of the plants was not influenced by this element (Figure 5F).

Discussion

Small concentrations of silicon in the nutrient solution was beneficial for lettuce growth, since it provided an increase in fresh root and shoot mass (Figure 5A and C). This result may be related to a higher water content in the plant, making it more turgid due to the presence of Si. A greater turgescence

for longer periods is important for lettuce plants since it increases shelf life for the species. It is important to note that this increase is only possible up to a certain point (1.40 mM for RFM and 0.40 mM for SFM), where the accumulation of silicon in the plant does not lead to accumulation of fresh biomass. This result was also verified by Resende, Yuri and Souza (2007), who verified quadratic behavior for total and commercial fresh mass working with different doses of silicon and planting times of American lettuce.

The fact that there was no increase in dry mass with increasing silicon doses in the nutrient solution can be explained by a possible competition between ions in the absorption channels. This is because the presence of the H₂SiO₄⁻ anion in the solution may reduce the absorption of other anions such as sulfate (SO₄²⁻) and phosphate (H₂PO₄⁻). Sulphate is essential for the formation of amino acids such as methionine and cysteine. In situations of sulfate deficiency, the formation of these amino acids is reduced, and the proteins are not formed. Therefore, the plants cannot assimilate N in the proteins (Cantarella, 2007). These nutrients important for cell growth and are directly related to the accumulation of dry mass (Malavolta, 2006). According to Marschner (2012), such competition can be influenced by the transport properties of each element and the difference in the concentration of ions in the solution. This fact would explain why the plants did not convert the fresh mass into dry, even with an increase in the availability of silicon in the solution.

Thus, although silicon increases the turgescence and fresh mass of the plants, it did not contribute to the increase of dry mass production when compared to the control (Figure 5 B and D). Luz et al. (2006) observed lower fresh shoot mass in lettuce plants grown in nutrient solution with 1.5 mmol L⁻¹ Si. However, the use of Si did not affect the final production of lettuce, since the plants reached the commercial size and also presented a lower incidence of burning leaves, justifying the beneficial action of this element for the crop.

For the parameters related to gas exchange, it was verified that as the liquid photosynthetic rate (*A*) increased, there was an increase in the stomatal conductance (*gs*), which represents a greater opening of the stomatal pore for CO₂ entry and, consequently, higher transpiration rate (*E*). However, *A*, *gs* and *E* increased with the increasing doses of Si until reaching a maximum point (Figures 3A, B and D), and from that point on, there was a decrease in all these parameters.

Si provided an increase in chlorophyll 'a' production up to 2.09 mM (Figure 8A), which may have triggered an increase in light capture in photosystem II (PSII). This fact may have triggered a greater excitation of the FSII reaction center, with more electrons being transported to reduce NAD⁺ to NADPH and to ATP production, both used in the biochemical phase of photosynthesis to reduce CO₂ to carbohydrates (Marengo and Lopes 2009). Thus, this increase in gas exchange may have been stimulated by the increase in the synthesis of chlorophyll 'a'. With the increase of photosynthesis, more CO₂ entered the sub-static chamber and, therefore, higher Ci and *gs*, as the stomata remained more open for CO₂ entry, which also provided a higher transpiration rate (*E*) for the plant.

The decrease in the Ci/Ca ratio presented a linear behavior with the increase in Si doses. This reduction may be related

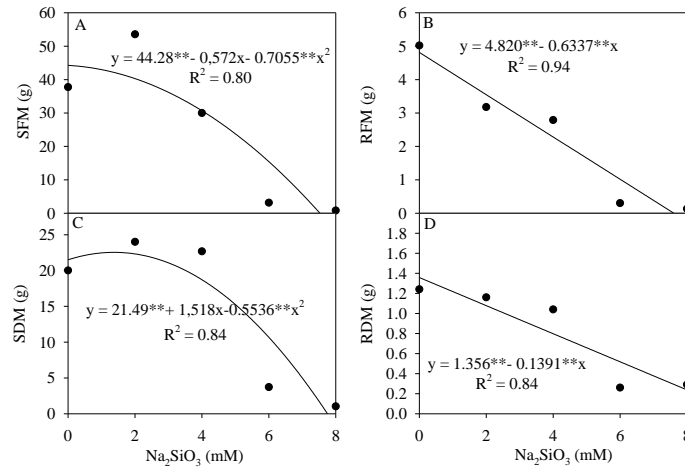


Fig 1. Lettuce plants cultivated in nutrient solution with different doses of sodium silicate (Na_2SiO_3). A: Shoot fresh mass (SFM), B: shoot dry mass (SDM), C: root fresh mass (RFM) and D: root dry mass (RDM). ** and * - Significant at 1% and 5%, by Student's t-test.

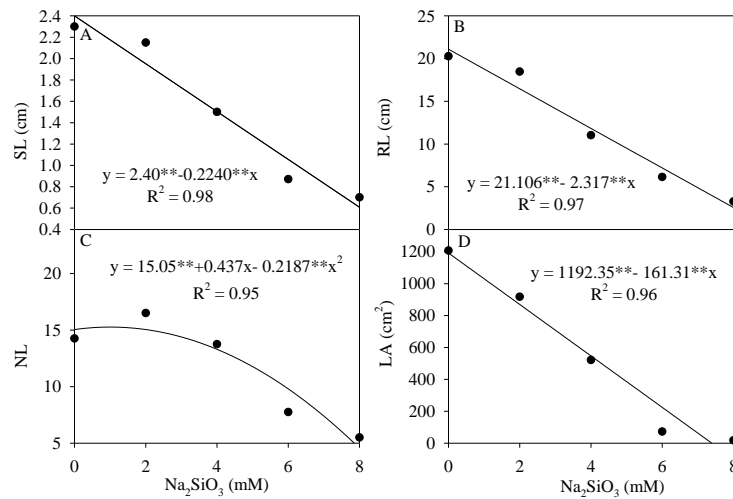


Fig 2. Lettuce plants cultivated in nutrient solution with different doses of sodium silicate (Na_2SiO_3). A: shoot length (SL), B: root length (RL), C: number of leaves (NL) and D: leaf area (LA). ** and * - Significant at 1% and 5%, by Student's t-test.

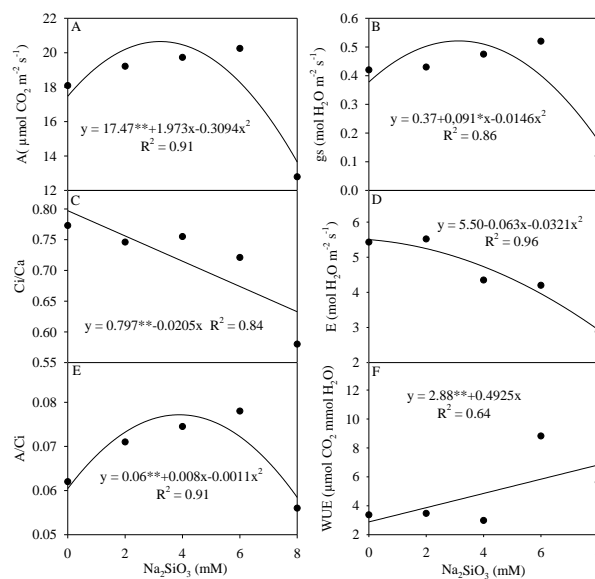


Fig 3. Lettuce plants cultivated in nutrient solution with different doses of sodium silicate (Na_2SiO_3). A: liquid photosynthesis (A), B: stomatal conductance (gs), C: ratio of internal to atmospheric CO_2 (Ci/Ca), D: transpiration (E), E: instantaneous carboxylation efficiency (A/Ci) and F: water use efficiency (WUE). ** and * - Significant at 1% and 5%, by Student's t-test.

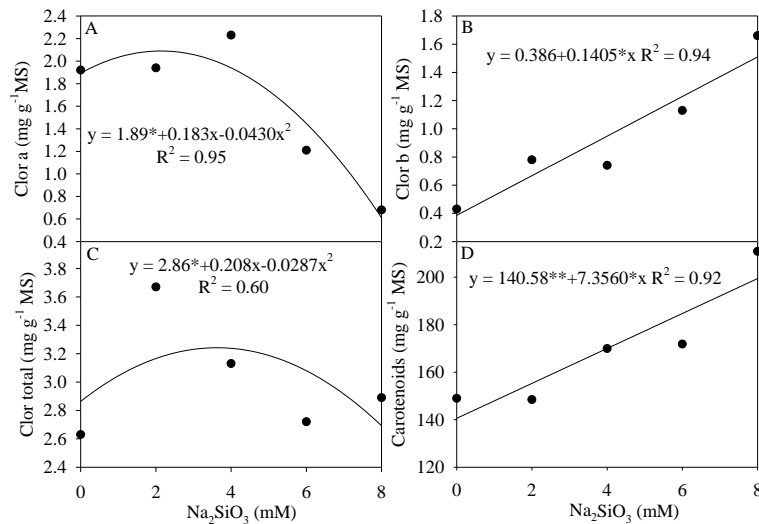


Fig 4. Lettuce plants cultivated in nutrient solution with different doses of sodium silicate (Na_2SiO_3). A: chlorophyll a (Clor 'a'), B: chlorophyll b (Clor 'b'), C: total chlorophyll and D: carotenoids. ** and * - Significant at 1% and 5%, by Student's t-test.

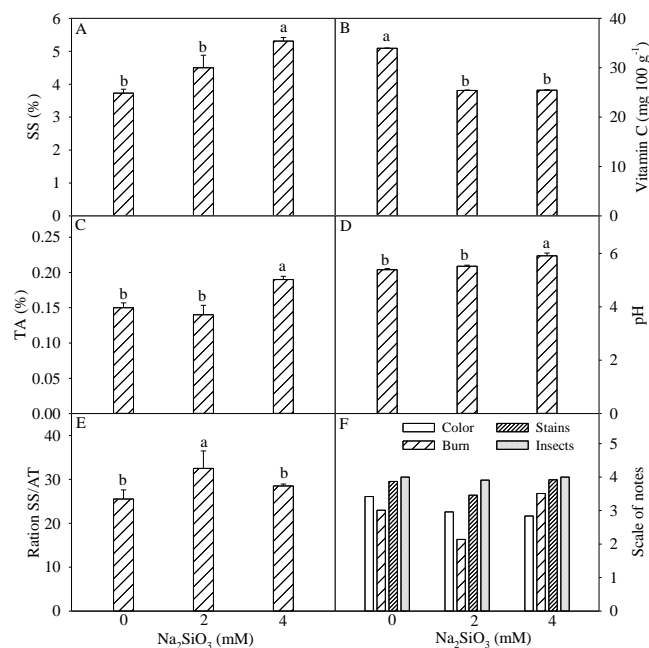


Fig 5. Lettuce plants cultivated in nutrient solution with different doses of sodium silicate (Na_2SiO_3). A: Soluble solids (SS), B: vitamin C, C: titratable acidity (TA), D: pH, E: soluble solids and titratable acidity ratio (SS/AT) and F: external appearance. Means followed by different lowercase letters show differences among each other by the Scott-Knott's test ($p \leq 0.05$). The bars represent the mean standard error.

to a possible diffusive limitation, since the results obtained show that it was not caused by a biochemical limitation of the process. This limitation is mainly due to the increased resistance to CO_2 entry. As the stomata are closing, there is less CO_2 entering leaf. If the photosynthetic machinery in the chloroplast is not compromised and remains intact, CO_2 continues to be fixed, even with the stoma closing. In this case, the amount of internal CO_2 decreases, with a reduction in Ci/Ca ratio due to the lower input of CO_2 .

There was a reduction in Ci/Ca ratio that may be explained by a limitation of diffusive order, since the results demonstrate it was not caused by a biochemical limitation of the process. This limitation of diffusive order is mainly due to the increased resistance to CO_2 entry. As the stomata are

closing, less CO_2 enter the leaf. If the photosynthetic machinery in the chloroplast is not compromised and remains intact, the CO_2 continues to be fixed even with the stomata closing. In this case, the amount of internal CO_2 decreases, reducing Ci/Ca ratio.

In wheat plants treated with Si at concentrations of 0 and 2 mM, there were increases in gas exchange (A , g_s and E) as a function of silicon addition (Rios et al., 2014). This result corroborates with the one found in this study, since it was also verified increase in gas exchanges provided by Si at concentrations close to 2 mM (2.47 to 3.19 mM). Another study conducted by Abdalla (2011) using diatomite (Si source) in soil under water stress showed an increase in

photosynthetic rate and stomatal conductance in lupine plants.

Most studies that evaluated gas exchanges using Si, the plants were under water deficit. Even so, the application of silicon allowed an increase in gas exchange (A , E and g_s). In this research, plants were also in good conditions of water supply and an increase in these parameters was verified until reaching an optimum point. This fact reinforces the importance of silicon on variables related to gas exchanges, since either under drought or in good water supply conditions, there were increases in gas exchange with the use of this element, reinforcing its beneficial effect.

In corn seedlings, Gao et al. (2005) observed that the addition of silicon decreased leaf transpiration and water flow rate in the xylem vessels resulting in higher water use efficiency. A possible cause for these results may be related to Si deposition on the cell wall of the root, which could affect the properties of xylem vessels and the transport of water or solute. These results corroborate with those found in this work, where WUE (A/E) was greater as Si doses were increased. Likewise, Gao et al. (2005) observed that the Si application increases the WUE in corn under drought stress, reducing the transpiration rate of the leaves indicating that Si influences the stomatal movement of the plants.

The use of silicon resulted in increased chlorophyll 'a' and total chlorophyll for lettuce plants (Figures 4A and C). It has also been observed in wheat (Rios et al., 2014) and tomato plants (Rodrigues et al., 2016) under dry conditions. This may be related to the increase in characteristics such as gas exchange, water absorption potential and reduction of oxidative stress. Reducing oxidative stress, the production of reactive oxygen species (ROS), which cause damage to proteins, nucleic acids and lipids, is reduced, increasing the synthesis of pigments. Rios et al. (2014) verified increased levels of chlorophyll 'a' and 'b' in wheat plants that were in the presence of Si.

An increase in carotenoids with doses of Si was observed (Figure 4D). Besides acting as light-receiving pigments, carotenoids protect chlorophyll from excess radiation, neutralizing the action of free radicals that damage cells (Marengo and Lopes, 2009). Si provides an increase in the production of antioxidant compounds that may be enzymatic and/or non-enzymatic, such as carotenoids. The increased content of non-enzymatic antioxidant compounds play an important role in reducing oxidative stress in plants. Studies show that Si has an influence on gas exchange increase or decrease. To date, further studies are necessary to understand how Si acts in plants, especially in their physiology.

The increases in the parameters related to post-harvest of lettuce as SS and SS/TA ratio (Figure 5A and E) are important because they can provide a longer shelf life as well as making the vegetable more palatable. The SS/TA ratio in the concentration of 2 mM of Si increase sugars in relation to the accumulation of acids which may indicate an improvement in flavor. In a study carried out by Galati et al. (2015), a dose of 84 mg L⁻¹ of Si allowed the maintenance of parameters such as titratable acidity and pH with a longer shelf life.

The fact that vitamin C content did not increase (Figure 5B) may be related to the species. For example, in tomatoes treated with different sources and doses of silicon, positive responses regarding vitamin C in fruits have been observed

(Marodin et al., 2016). However, the response to Si can vary according to the species and to the climatic conditions and interactions with other mineral elements (Resende et al., 2007; Camargo, 2016).

Materials and methods

Experimental area

The experiment was carried out from August to October 2016 in a protected environment in the didactic garden of the Federal University of Ceará (UFC), campus Pici, in Fortaleza-CE, Brazil, at 3°43'6" S and 38°32'36" W and average altitude of 14 m. The climate classification is 'As', tropical with dry season (Alvares et al., 2013).

The protected environment was a screened nursery with shading screen (Sombrite) at 30%. The structure dimensions are: high ceiling of 1.8 m, length and width of 8.0 m each. The temperature averages and relative humidity within maximum and minimum shelter were 36.4 °C, 25.4 °C and 78.3% and 25%, respectively (Alvares et al., 2013).

Plant materials and growth conditions

Seeds of Lucy Brown cultivar were placed into phenolic foam with 1.0 cm depth. Two watering can irrigations were performed in the morning and afternoon. Seven days after sowing (DAS), foliar fertilization with nutrient solution at 50% of the ionic strength and electrical conductivity (EC) of less than 2 dS m⁻¹ was performed to meet possible nutritional requirements of the seedlings.

At 10 DAS, the seedlings were transplanted to trays of white polyethylene with 7.0 L of nutrient solution based on the recommendations of Furlani et al. (1998) at 50% of the ionic strength. Twelve seedlings were arranged per tray and each tray corresponded to a treatment, with a determined concentration of sodium silicate (Na₂SiO₃). The doses of Na₂SiO₃ were 0; 2; 4; 6; 8 mM. The best dose was calculated by the maximum point of the second degree function as following:

$[(-b)/(2 * a)]$, where $b = -0.572$; $a = -0.7055$, then; would be: $[(-0.572)/(2 * -0.7055)] = 0.40$ mM.

This phase lasted one week and worked as a nursery to adapt the seedlings. After this period, the seedlings were transferred to pots with 5 L of nutrient solution at 75%, where the different levels of sodium silicate (Na₂SiO₃) were applied again. The "floating" hydroponic system was used (deep pool with static aeration system) and was installed on a wooden bench (0.8 m high, 1.5 m wide and 3.0 m long). An air compressor (type Chang 9000) was used for airing the nutrient solution.

The water type C2S1 was used in the preparation of the nutrient solution, coming from the supply of the *Companhia de Água e Esgoto do Ceará* (Cagece), whose values of electrical conductivity were adjusted with a benchtop conductivity meter with temperature correction. The chemical characteristics of the water were: pH = 7.0; EC = 0.46 dSm⁻¹; Ca²⁺, Mg²⁺, K⁺, Na⁺, Cl⁻ and HCO⁻³ (0.40; 2.30; 0.80; 1.10; 3.20; 1.40 mmol. L⁻¹, respectively).

Due to the evapotranspiration, the replacement of the nutrient solution in the pots with water was performed daily. The pH adjustment of the nutrient solution was performed with citric acid (C₆H₈O₇) or sodium hydroxide

(NaOH), keeping it in the range of 5.5 to 6.5. At 40 DAS, gas exchange analyzes were performed and the plants were harvested and taken to the laboratory for analysis of photosynthetic pigments, growth and postharvest.

Gas exchange analyzes

Gas exchange evaluations were performed on the third pair of fully expanded leaves using the IRGA (Infra-Red Gas Analysis) model, LI-6400XT, LiCor, USA. The following variables were evaluated: stomatal conductance (g_s - mol H_2O $m^{-2} s^{-1}$), liquid photosynthesis (A - μmol CO_2 $m^{-2} s^{-1}$), ratio of internal to atmospheric CO_2 (C_i/C_a), transpiration (E - mol H_2O $m^{-2} s^{-1}$), instantaneous carboxylation efficiency (A/C_i) and instantaneous water use efficiency (WUE - μmol CO_2 mol H_2O^{-1}), calculated by the ratio between A and E . These evaluations were carried out from 08:00 to 11:00 AM on a clear day, with artificial lighting of $1.200 \mu mol$ $m^{-2} s^{-1}$ in the equipment evaluation chamber to maintain the homogeneous environmental conditions.

Photosynthetic pigments analysis

The contents of chlorophyll 'a', 'b', total chlorophylls and carotenoids were determined by the method described by Wellburn (1994).

Growth analysis

For the growth analysis, the following variables were analyzed: shoot and root length (SL and RL) - with the aid of a graduated ruler, in cm; number of leaves (NL) - determined by the single count of the fully expanded leaves; leaf area (LA) in cm^2 determined from the use of a LI-3100 leaf area integrator (area meter, Li-Cor, Inc. Lincoln, Nebraska, USA); shoot and root fresh mass (SFM and RFM) - made from the separation of root and shoot and both parts weighed in a precision scale with four decimal places; shoot and root dry mass (SDM and RDM) - samples of both parts were placed in a forced air oven at $65^\circ C$ until reaching a constant mass, then the samples were weighed on a precision scale, in g plant $^{-1}$.

Postharvest analysis

Soluble solids (SS) - reading performed in a refractometer (mod. 103, with a scale from 0 to 32%), from 1.0 g of the samples of the macerated leaves in mortar, transferring 2 to 3 drops to the prism of the refractometer, with the results expressed in % (Instituto Adolfo Lutz, 2005).

Vitamin C - determined by titration with DPI solution (2.6 dichlorophenolindofenol 0.02%) to light pink staining using 1.0 g of leaf macerated in mortar, expressed as mg ascorbic acid per 100 g of leaf (Strohecker and Henning, 1967).

Titrateable acidity (TA) - determined from the use of 1.0 g of lettuce leaf macerated in mortar, using phenolphthalein as indicator at 1% and titration with 0.1 N sodium hydroxide (NaOH), expressed as % of citric acid (Instituto Adolfo Lutz, 2005).

SS/TA ratio - determined by the ratio of total soluble solids and titrateable acidity.

pH - determined by maceration of 1.0 g of the leaf diluted in 30 ml of distilled water using a digital glass membrane potentiometer (Instituto Adolfo Lutz, 2005).

External appearance - was based on the tolerance limits for leaf color, burned edge, presence of some insects and leaf spots, with the following scores: 3.1- 4.0 (optimum); 2.1 - 3.0 (good); 1.1 - 2.0 (regular); 0 - 1 (bad) (Morais et al., 2011).

Statistical Analysis

The experiment was performed in a completely randomized design with four replicates and five treatments composed by different doses of silicon (0; 2; 4; 6; 8 mM), with the plot consisting of a pot with two lettuce plants. Sodium silicate was used as silicon source (63% of SiO_2 and 18% of Na_2O). 'Lucy Brown' from the American group was used with seeds presenting 99.9% purity and 95% germination. The results were submitted to analysis of variance (Snedecor's F test; $p \leq 0.05$). Later, regression models were adjusted for the silicon doses and the choice of each model was made based on the significance of the parameters through t-Student's test and the coefficient of determination ($R^2 \geq 0.70$). Mean values of the postharvest variables were compared by the Scott-knott's test at 0.05. Only the first three doses of silicon provided plant material with acceptable quality for the results analysis.

Conclusion

Silicon use results in improvements on fresh mass production, postharvest quality and in the physiological aspects of lettuce. Doses from 0.40 mM are the most recommended for lettuce in hydroponic system, for resulting greater increase of fresh mass of the shoot.

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