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Yield, nutritional state and silicon accumulation in lettuce cultivars fertilized with calcium silicate

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ABSTRACT

The objective of this work was to evaluate the application of calcium silicate in the yield and nutritional state of lettuce plants and to verify forms of foliar Si accumulation, using scanning electron microscopy (SEM), at the Universidade Estadual de Londrina greenhouse. Experimental design was randomized, in a 4x2 factorial scheme with four replications, including four lettuce cultivars (Lucy Brown, Vanda, Elisa and Romaine) and two doses of silicate calcium (0 and 4000 kg ha^{-1}). Calcium silicate application showed no differences for yield, growth and nutritional state in the assayed plants. Differences among cultivars were probably due to genotypic traits. The Romaine cultivar showed greater aerial part fresh matter, aerial dry matter and height. Lucy Brown and Romaine cultivars showed greater N content. 'Lucy Brown', 'Romaine' and 'Vanda' showed greater K content. The Elisa cultivar showed the greatest Ca, Mg and Zn contents. The Elisa and Romaine cultivars showed the greatest Si content. The scanning electron micrograph revealed that there was no development of siliceous bodies in the lettuce, and Si foliar accumulation occurred sparsely.

Key words: *Lactuca sativa* L., plant nutrition, silicate fertilization, scanning electron microscopy (SEM).

INTRODUCTION

Grown all over the world, lettuce (Lactuca sativa L.) is the most popular among leafy vegetables, standing out for its economic importance, nutritional value and wide acceptance by the population. (Diniz et al., 2006; Souza et al., 2007; FAO 2016). In Brazil, there are six types of cultivars: Butter; American; Boston; Crisp; Mimosa and Romaine.

Silicon (Si) is a beneficial element for plants and its presence may contribute favorably to the performance of several cultures (Zanão Júnior et al., 2010; Lima et al., 2011; Paulino et al., 2013; Teodoro et al., 2015). According to Luz et al. (2006), lettuce plants grown in Si solution show less physiological problems. Ferreira et al. (2010) report that lettuce plants supplied with calcium silicate may show greater physical yield due to the absorption of Si, Ca, K, Mg and other elements present in the product. According to these authors, calcium silicate reduces the percentage of dry matter in senescent and sick leaves, besides affecting N and Mn content.

Absorption capacity and Si accumulation differ among vegetable species and among genotypes of the same species as well (Andrade et al., 2012a). These capacities allow the classification of vegetables according to percentages of SiO2 in the aerial part dry matter, in accumulators, which contains 10% to 15%; intermediaries with contents that vary from 1% to 5% and non-accumulators with contents below 0.5% (Marschner 1995).

The silicate absorbed from the soil solution is hydrous (SiO2.nH2O) and may deposit itself on leaves, seeds, fruits, roots, stem, inside of cells or on the cell wall as silicate bodies with characteristic and microscopic forms and sizes (Piperno and Pearsall 1998; Runge 1999; Alvarez et al., 2005; Andrade et al., 2014). However, according to Andrade et al. (2012b), not all vegetables that accumulate Si in their tissues develop silicate bodies.

Therefore, the objective of this work was to assess the influence of silicate calcium in the yield, growth and nutritional state of four lettuce cultivars. In addition, it evaluates, with the help of a scanning electron microscope (SEM), forms of Si foliar accumulation in the studied plants.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse at Universidade Estadual de Londrina – UEL, from July to September of 2011, in a substrate of the commercial brand Tropstrato Hortaliças HA®. The substrate used in the experiment showed the following chemical characteristics: pH ($CaCl_2$) = 5,5; K = 0,62 cmolc/dm^3; Ca = 21,1 cmolc/dm^3; Mg = 4,6 cmolc/dm^3; Al = 0,05 cmolc/dm^3; H + Al = 5,35 cmolc/dm^3; P = 53,1 mg/dm^3; Si = 73320,00 mg/kg; Dry density = 200 kg/m^3.

The experimental design was totally randomized, with four lettuce cultivars, two dosages of calcium silicate (0 and 4000 kg ha^{-1}), with four replications. The following cultivars were tested: Lucy Brown (American group); Vanda (Crisp-loose group); Elisa (Bibb) and Romaine (Romaine group). Calcium silicate was of the brand Vetec® (CaO content of 14-18%, SiO2 content of 60-68%), and foliar accumulation forms were established by SEM.

Seedlings, obtained in Styrofoam trays and without the previous application of fertilizers, were transplanted, 23 days after seeding (d.a.s.), to plastic vases with capacity for four liters, with one plant per vase. Irrigation took place periodically and sufficiently keep the substrate with adequate humidity for the plants development. Prior to transplantation, all vases were fertilized with calcium silicate (0 and 4000 kg ha-1) and with 0.3 kg de 4-14-8 (N-P-K) for each 25 kg of the used substrate. At 35 days after transplantation, all plants received top dressing in the 312.5 kg ha^{-1} dosage of urea.

Plants were harvested when they reached commercial size, at 73 d.a.s. Moments prior to being harvested, foliar samples were removed to be prepared for the SEM analysis. After the harvest, the aerial part fresh matter (APFM), plant height (PH commercial head diameter (CHD) were measured. Next the aerial part of the plants were submitted to drying in a greenhouse at 65 ºC until constant to assess aerial part dry matter (APDM) , which occurred after 96 hours of drying.

After the APDM assessment, samples were grounded in a Wiley-type mill for foliar Si content, for macro and micronutrients analysis. Samples nitroperchloric digestion was realized to determine Si contents, which after 24 hours were evaluated by plasma atomic emission spectroscopy. Macro and micronutrients foliar contents were determined by the Carmo et al. (2000) methodology.

Foliar samples collection to be analyzed by the SEM occurred as follows: one plant per cultivar and treatment was used to obtain the samples. Three leaves with no signs of deterioration were collected from each plant. These leaves were located in the inferior third, medium third and superior third of the assessed plants. Three samples were removed from each leaf (two from the extremities of the superior and inferior third and another form the medium third). After the collection, samples were placed in an antifade solution with 5% of formaldehyde 90% of ethyl alcohol at 70% and 5% of acetic acid (FAA), where they remained for 24 hours at room temperature. After the antifade period, the materials were dehydrated in ethanol ascending series (70%, 80%, 90%, 100%) and dried at the critical point in CO_2 (Bal Tec CPD 030) for ultrastructural analysis. Next, the samples were glued to the stubs with both adaxial and abaxial surfaces turned upwards and covered with carbon, using the Sputter Coater Bal-Tec SCD 050. To detect Si accumulation, the samples were analyzed by the energy dispersive spectroscopy (EDS-Oxford), software INCA, coupled to FEI Quanta SEM 200.

To evaluate plants yield, growth and nutritional state, data related to the assay were submitted to an analysis of variance by the Sisvar software, and the means compared among themselves by the Tukey test at 5% of probability.

RESULTS AND DISCUSSION

The analysis of variance showed no significant differences for cultivar variation source for the MFPA, MSPA, Height characteristics. Calcium silicate application, as well as interaction with cultivars brought no significant differences to the assessed agronomic characteristics (Table 1).

Table 1. Analysis of variance of data related to the aerial part fresh matter (APFM), aerial part dry matter (APDM) , commercial head diameter (CHD) and height of lettuce cultivars treated with 0 and 4000 kg ha-1 of calcium silicate.

Variation source	GL	APFM	APDM	CHD	Height
Cultivar	3	6193.46*	326.13*	9.61ns	109.65*
Dosages	1	276.13ns	0.13ns	3.78ns	0.28ns
Cultivar*Dosages	3	3947.46ns	5.46ns	9.28ns	1.05ns
Residue	24	1527.46	11.21	4.45	1.42
CV (%)		11.73	15.90	4.52	4.84

*significant at 5% of probability (p< .05)
ns not significant (p>= .05)

Fertilizer supply did not increment production, since lettuce crops do not accumulate silicon (Ferreira et al., 2010). These same authors found no significant differences when evaluating yield and size of lettuce cultivars treated with calcium silicate dosages. However, Carvalho et al. (2002) working with tomato, a crop that also does not accumulate Si, observed that silicate fertilization promoted greater fruit production and quality. Luz et al. (2006), assessing the effect of Si on lettuce cultivars performance, observed that treated plants were smaller when compared to non- treated plants. According to these authors, the Si accumulated on epidermal cells and stomata walls are found in the form of monosilicic acid, and, when the plant starts to lose water, this element goes under polymerization. This way, the stomata lose their walls flexibility, remained closed, thus reducing plant development.

Sonobe et al. (2009) showed that Si concentrations of 50 mg.L-1, besides causing accumulation, the element was efficient in reducing hydric stress of sorghum plants, increasing stomatal conduction, photosynthetic and transpiration rate and dry matter when compared to non-treated plants. Table 2 shows the differences found in cultivars regarding MFPA, MSPA and height. These differences may be due to the different leaves architecture and arrangement, and photosynthetic efficiency.

Table 2. Comparison among lettuce cultivars in relation to aerial part fresh matter (APFM), aerial part dry matter (APDM), commercial head diameter (CHD) and height.

Cultivars	APFM (plant)	APDM (g/plant)	CHD (cm)	Height (cm)
Lucy Brown	340.00 b	15.75 c	45.44 a	22.25 c
Vanda	353.00 b	22.50 b	46.81 a	23.88 b
Elisa	213.75 c	16.50 c	46.31 a	22.31 bc
Romaine	425.50 a	29.50 a	48.06 a	30.06 a
CV (%)	11.54	15.58	4.51	4.76
DMS	52.88	4.51	2.89	1.61

Means followed by lower case letters in the column do not differ among themselves at 5% of significance by the Tukey test.
M.S.D – Minimum significant difference for means in the columns.

The 'Romaine' shoed 425.50 g/plant and 29.50 g/plant of MFPA and MSPA, respectively, standing out in relation to the others. This was possibly due to this cultivar greater size in relation to the others. In regards to DCC, results varied from 45.44 to 48.06 cm, and the cultivars showed no significant differences among themselves. As for the height criterion, the Romaine cultivar stood out in relation to the others, possibly due to its typical more open foliar arrangement and architecture, which probably promoted greater height growth during the vegetative phase. 'Lucy Brown', on the other hand, which shows a foliar arrangement that closes and tends to have a cabbage-like aspect, showed lower height (22.25 cm), together with 'Elisa' (22.31 cm).

The analysis of variance of the macro and micronutrients data and source of cultivar variation showed significant differences for N, K, Ca, Mg, Zn and Si contents. However, calcium silicate dosages and the cultivar x dosages interaction effects were not significant (Table 3).

Table 3. Analysis of variance of macro and micronutrients contents data of different lettuce cultivars treated with 0 and 4000 kg ha-1 calcium silicate.

Fonte de Variação	GL	N	P	K	Ca	Mg
Cultivar	3	40.53*	0.65ns	98.39*	122.17*	17.33*
Dosages	1	10.06ns	0.09ns	0.17ns	0.11ns	2.95ns
Cultivar*Dosages	3	2.35ns	0.38ns	22.83ns	14.26ns	0.6ns
Residue	14	9.13	0.22	26.4	12.84	1.65
CV (%)		12.19	6.78	7.28	20.05	19.15
	GL	Cu	Zn	B	Mn	Si
Cultivar	3	0.72ns	739.15*	3.89ns	5428.58ns	28194.78*
Dosages	1	0.64ns	1.27ns	24.85ns	2346.7ns	567.16ns
Cultivar*Dosages	3	0.95ns	42.58ns	4.33ns	4454.26ns	315.2ns
Residue	14	0.75	102.48	6.4	1710.98	92.09
CV (%)	32.58	16.66	7.13	27.84	23.25	

*significant at 5% of probability (< .05)
ns not significant (p>= .05)

Table 4 shows that "Romaine" and "Lucy Brown" had higher values, with 28.44 and 24.75 g/kg^{-1} of N, respectively. Based on P, contents varied from 6.47 to 7.16 g/kg^{-1}. In regards to K content, the Lucy Brown, Vanda and Romaine cultivars showed greater values, with 75.00; 67.67 and 73.00 g/kg^{-1}, respectively. 'Elisa" showed greater Ca and Mg com content with 24.22 g/kg^{-1} and 9.19 g/kg-1, respectively. Macronutrients content found in the assessed cultivars showed decreasing values in the K>N>Ca>P>Mg order, with the exception of 'Elisa' that showed Ca value greater than the N and Mg value greater than the P. Aquino et al. (2007) observed the extraction of nutrients from lettuce plants following such decreasing order K>N>Ca>P>Mg>S. Therefore, potassium is the nutrient with more content in lettuce plants.

In regards to Cu, B and Mn contents, the cultivars showed values that vary from 2.20 to 2.97 g/kg^{-1}; 34.81 to 36.60 g/kg^{-1} and 111.97 to 179.40 g/kg^{-1}, respectively, and with no statistically difference among themselves. However, 'Elisa' showed greater Zn content in relation to the others. As for Si, the Elisa and Romaine cultivars showed contents of 208.95 and 166.43 g/kg^{-1}, respectively, standing out in relation to the others. (Table 5). Si content was influenced probably by plants genetics and not by the calcium silicate in the substrate.

Table 4. Macronutrients content (g/kg-1) in the assessed lettuce cultivars.

Cultivars	N	P	K	Ca	Mg
Lucy Brown	24.75 ab	6.47 a	75.00 a	17.87 b	6.47 b
Vanda	23.43 b	6.75 a	67.67 ab	15.29 b	5.81 b
Elisa	22.53 b	7.16 a	66.67 b	24.22 a	9.19 a
Romaine	28.44 a	7.13 a	73.00 ab	14.11 b	5.42 b
CV (%)	12.04	6.82	6.87	19.00	18.17
DMS	4.93	0.77	8.01	5.61	2.02

Means followed by the same lower case letters in the column do not differ among themselves at the 5% level of significance by the Tukey test.
MSD. – Minimum Significant Difference for the means in the columns.

Table 5 - Micronutrients and silicon content (mg/kg-1) in the assessed lettuce cultivars.

Cultivars	Cu	Zn	B	Mn	Si
Lucy Brown	2.97 a	55.92 b	35.59 a	111.97 a	79.90 b
Vanda	2.20 a	56.67 b	34.81 a	137.23 a	66.54 b
Elisa	2.61 a	77.24 a	36.60 a	179.40 a	208.95 a
Romana	2.88 a	53.16 b	35.00 a	165.77 a	166.43 a
CV (%)	32.10	16.16	6.74	27.02	22.05
DMS	1.41	16.22	3.95	66.34	47.54

Means followed by low case letters in the column show no difference among themselves at 5% level of significance by the Tukey test.
M.S.D. – Minimum significant difference for the means in the columns.

The silicone translocates itself in vegetables by mass flow and accumulates mainly in the maximum transpiration areas. Therefore, the foliar architecture of "Romaine" and "Elisa" may have provided greater transpiration rates, contributing to this element's greater content.

Voogt and Sonneveld (2001), working with lettuce in hydroponic cultivation with constant flow of nutritious solution with Si, observed that the element absorption by the lettuce was small; however, Si application reduced Mn toxicity.

Luz et al. (2006) report that the application of Si in lettuce plants influenced foliar content. In a nutritious solution, without application, they noticed that the Uberlândia 10000 cultivar, from the Bibb group showed 0.30% more Si content in relation to the tested cultivars. In regards to nutritious solution with Si, "Uberlândia 1000" once again showed greater Si content; however, it showed no statistical difference when compared to the Mimosa, from the Mimosa group, and Verônica, from the Crisp group. Lettuce plants may present Si in their tissues even when not treated with this element, since Si is abundant in the earth's crust, being present in water used in the experiment's irrigation and crop substrate.

The lettuce shows no Si accumulation in the form of silicon bodies **(Figure 1)**. Ferreira et al. (2009) report that lettuce does not accumulate Si in great amounts. Most plants, mainly the dicotyledonous, are incapable of accumulating high levels of Si in their tissues. The difference in this element accumulation among species may be attributed to the differences in absorption by the roots (Ma and Takahashi 2002).

Silva and Bohnen (2001) assessed the accumulation of macro and micronutrients by rice crops grown in a complete nutritious solution, with and without the addition of powder Si, in the form of $SiO2$, observed that Si in a nutritious solution reduced B and P contents in the roots and B, Ca, Fe and Mn contents in the husk. However, there was no significant effect of Si on the macro and micronutrient contents on the stalk, leaves and grains.

When analyzing the effect of Si on Zn toxicity minimization on the growth and mineral nutrition of *Eucalyptus urophylla* plants, Pinto et al. (2009) concluded that the addition of Si minimized the negative effect of excessive Zn on growth. However, it influenced only slightly nutrient contents assessed in the tissues (P, K, Ca, Mg and S), although it promoted a more efficient use of P, Ca, Mg and S by the analyzed plants.

Results from this study showed that the response to calcium silicate application in the crop substrate regarding the agronomic characters and the macro and micronutrients assessed is much more the result of genotypic differences rather than silicate fertilization.

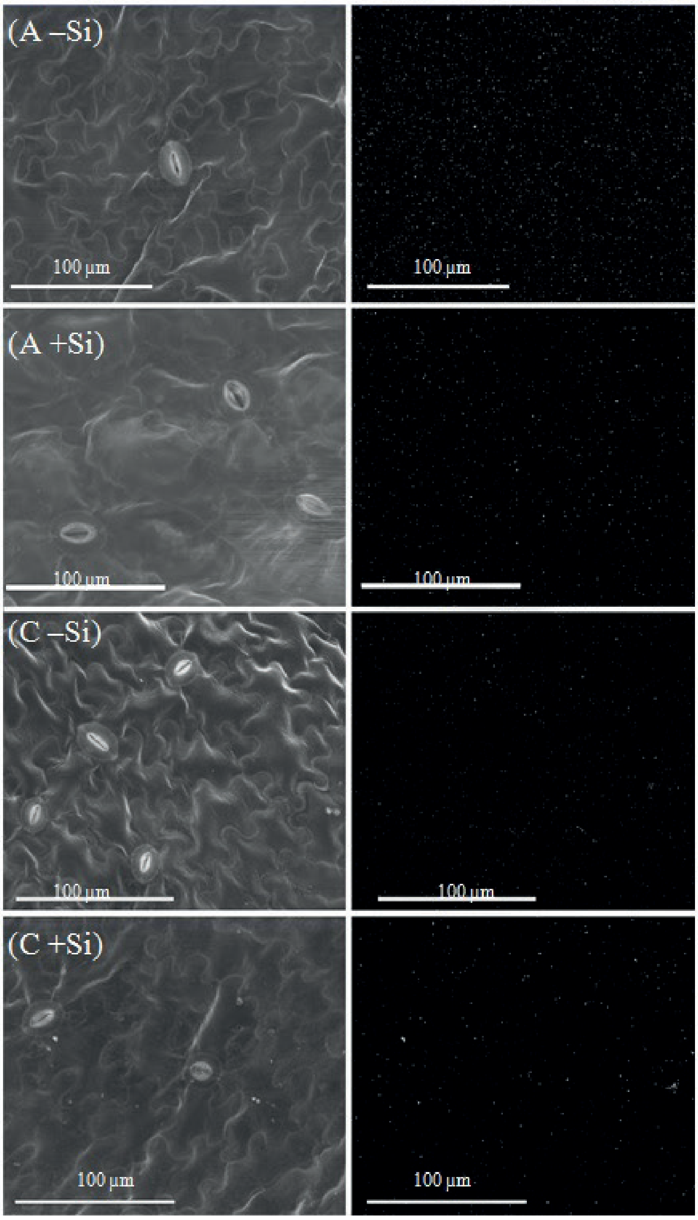

Figure 1 – Scanning electron micrographs illustrating the silicon mapping (to the right) on the adaxial surface of the lettuce leaves (to the left). (A –Si) 'Lucy Brown' with no calcium silicate application; (A +Si) 'Lucy Brown' with the application of 4000 kg ha^{-1} of calcium silicate; (C –Si) 'Elisa' with no application of calcium silicate; (C +Si) 'Elisa' with the application of 4000 kg ha-1 of calcium silicate.

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