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Effects of silicon on the growth and genetic stability of passion fruit

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ABSTRACT. The objective of this study was to determine the silicon concentration that would provide good growth in passion fruit plants. Passion fruit seeds were sown in polystyrene. After 60 days, when they were approximately 15 cm tall, the plants were transplanted into polyethylene pots containing 1.1 kg Tropstrato® substrate. Treatments consisted of four concentrations (0, 0.28, 0.55, and 0.83 g pot^{-1}) of silicon applied as a silicic acid solution 1%. This solution was applied around the stem of the plants (*drenched*), with the first application being administered 15 days after transplanting. In total, three applications were made at intervals of 15 days. After the last application, the plants were subjected to chemical analysis to determine the silicon concentration and to X-ray microanalysis and flow cytometry. Phytotechnical analyses were performed during the applications. The use of silicon in concentrations of 0.28 and 0.55 g pot^{-1} provides better growth of the passion fruit, and the absorption and deposition of the silicon in the passion fruit leaves are proportional to the availability of this element in the plant. The roots of the passion fruit plant are silicon accumulators, and the DNA stability and amount are preserved in the silicon-treated passion fruit plants.

Keywords: flow cytometry, silicic acid, *Passiflora edulis*.

Silício no crescimento e estabilidade genética de plantas de maracujazeiro

RESUMO. O objetivo foi determinar uma concentração de silício que proporcionasse um bom crescimento de plantas de maracujá. Sementes de maracujazeiro foram semeadas em bandejas de poliestireno, após 60 dias, as plantas com aproximadamente 15 cm de altura, foram transplantadas para vasos de polietileno contendo 1,1 kg de substrato Tropstrato®. Os tratamentos consistiram de quatro concentrações (0; 0,28; 0,55 e 0,83 g $vaso^{-1}$) de silício, na forma de solução de ácido silícico a 1%. Esta solução foi aplicada ao redor do caule das plantas (*drench*), sendo a primeira aplicação realizada 15 dias após o transplantio das plantas. No total, foram realizadas três aplicações, em intervalos de 15 dias. Após a última aplicação, as plantas foram submetidas à análise química de concentração de silício, microanálise de raios-X e citometria de fluxo. As análises fitotécnicas foram realizadas no decorrer das aplicações. O uso do silício nas concentrações 0,28 e 0,55 g $vaso^{-1}$, proporciona melhor crescimento das plantas de maracujazeiro, a absorção de silício e sua deposição nas folhas de maracujazeiro são proporcionais à disponibilidade desse elemento para a planta, o maracujazeiro é uma planta acumuladora de silício nas raízes e a estabilidade da quantidade de DNA é preservada nas plantas de maracujazeiro tratadas com silício.

Palavras-chave: citometria de fluxo, ácido silícico, *Passiflora edulis*.

Introduction

The genus Passiflora is economically important to Brazil, which has 129 known native species from the genus, 83 of which are endemic; these plants can be used as food, medicine and ornaments (Cervi, Azevedo, & Bernacci, 2010).

Brazil is the main producer and consumer of passion fruit, with a planted area in 2011 of 61,631 hectares and an average yield of approximately 923,035 tons (Agrianual, 2014).

Fertilization often increases crop yields due to increased plant vigor (Espindula, Rocha, Souza, Grossi, & Souza, 2010). However, the practice of fertilization should be based on knowledge of the morphological and physiological characteristics of the plant in addition to factors such as the availability of nutrients in the soil and the behavior of these nutrients in the plant (Almeida, Damatto Junior, & Leonel, 2007).

Although silicon (Si) is not considered an essential element for most plants, the benefits of silicate fertilization have been studied and recognized in cultivated species (Epstein, & Bloom, 2006; Ma, & Yamaji, 2008; Richmond, & Sussman, 2003).

The beneficial effect of Si on biomass formation in cultivated plants is associated with alterations in the plant structure, allowing, for example, better solar energy collection and reducing lodging. Although the role of Si in plant metabolism is still unknown (Epstein, & Bloom, 2006), this element is postulated to be solubilized in the plant and to play a role in the synthesis of plant defense molecules (Ma, & Yamaji, 2008; Rodrigues et al., 2004). Thus, silicon can indirectly promote plant growth and production, causing an increase in the chlorophyll content of the leaf tissue, altering the plant architecture, making the plants more upright, preventing excessive auto shading, delaying senescence, increasing the structural tissue rigidity and protecting the plants from abiotic and biotic agents (Epstein, & Bloom, 2006; Ma, & Yamaji, 2008; Marschner, 1995).

Few studies exist on the effects of silicon on plant growth, with the majority of publications dealing with the nutritional aspects and beneficial role of this element in biotic stress resistance, with possible evaluations of final productivity of the crop (Laviola, Martinez, Souza, & Alvarez, 2007; Pozza et al., 2009; Reis, Figueiredo, Guimarães, Botrel, & Rodrigues, 2008). Ma and Yamaji (2008) stated that the beneficial effects of Si on plant growth are commonly observed in plants under stress conditions. From a physiological point of view, for the growth and development of plants, silicon has demonstrated beneficial effects on the growth and development of plants in the increased production of various crops (Gomes, Moraes, & Assis, 2008).

A lack of silicon adversely affects DNA synthesis and chlorophyll in diatoms (Werner, 1977; Raven, 1983). However, no reports exist of excess silicon altering the DNA content of plants. In this sense, the flow cytometry technique has gained particular attention because it allows the relative amount of nuclear DNA of plant cells to be estimated rapidly and with high accuracy (Jin et al., 2008; Bairu, Aremu, & Van staden, 2011; Smulders, & Klerk, 2011).

This work was carried out to evaluate the effect of the addition of silicon on the growth, morphology and genetic stability of passion fruit plants.

Material and methods

Passion fruit (*Passiflora edulis* Sims f. *flavicarpa* Deg.) were sown in polystyrene trays and allowed to grow until they reached a size appropriate for transplantation into pots.

After 60 days, the plants, which were approximately 15 cm in height, were transplanted to polyethylene pots that contained 1.1 kg of Tropstrato® substrate. The plants were randomly arranged on a bench in the greenhouse and irrigated daily to meet their water needs.

Treatments consisted of four concentrations (0, 0.28, 0.55, and 0.83 g pot^{-1}) of silicon in the form of 1% silicic acid solution ($SiO_2.XH_2O$) (Pereira, Moraes, Prado, & Dacosta, 2010). This solution was applied around the stems of the plants (*drenched*), with the first application being administered 15 days after transplanting. All three applications were done at intervals of 15 days with the same concentrations. The control pots received water in the same amount. After the last application, the plants were subjected to chemical analysis to determine the concentration of silicon and to X-ray microanalysis and flow cytometry. The phytotechnical analyses were performed in the course of the applications.

Phytotechnical analyses

All experimental plants were evaluated for the number of leaves, plant height (cm), stem diameter (mm), leaf length (mm), web width (mm), aerial part fresh and dry weight (g) and root fresh and dry weight (g). The dry weight of the plant material was obtained after drying in an oven at 60°C for 72 hours to a constant weight.

Silicon concentration

To determine the silicon concentrations, analyses were carried out in the Fertilizer Laboratory of the Federal University of Uberlândia, Institute of Agricultural Sciences. The leaves, stems and roots of 12 plants were collected. The materials were dried in a forced circulation oven at 60°C for 72 hours to a constant weight and ground separately. For the determination of silicon, we used the methodology proposed by Korndörfer, Pereira, and Nolla (2004).

X-ray microanalysis

This analysis was conducted in the Electron Microscopy Laboratory, Department of Plant Pathology, UFLA. Samples from the middle third of 2 leaves from 3 plants were mounted on "stubs" and maintained in a silica gel desiccator for 72 hours for evaporation of all water. Later, the samples were coated with carbon CED 020 Baltec and analyzed in an LEO-EVO scanning electron microscope, following the protocol of Alves (2004).

Flow cytometry

Flow cytometry was performed at the Plant Tissue Culture Laboratory of the Agriculture Department of UFLA. Approximately 30 mg of leaf samples was collected, along with the same amount of tomato leaf biomass (*Solanum lycopersicon*)

(reference standard with a quantity of 1.96 pictograms (pg) of DNA), with the samples being crushed in a petri dish containing 1 mL Marie nuclei extraction buffer (Dolezel, Binarova, & Lucretti, 1989). The extract containing the nuclei was subsequently stained with 25 µL mL^{-1} of propidium iodide and placed in the equipment; 5,000 nuclei analyses were performed on each sample. Histograms were obtained on a FacsCalibur® cytometer (Becton Dickinson) with the Cell Quest program (Dickinson, 1998). The DNA content (pg) of the plants was obtained with the following equation: DNA content (pg) = the G1 peak position the sample position/G1 peak of the standard x pg. Three repetitions were conducted, and statistical analysis was performed using the WinMDI 2.8 program (Trotter, 2000).

Experimental design and statistical analysis - The design was completely randomized with 4 treatments and 20 repetitions/treatment. All data were submitted to an analysis of variance using the statistical program SISVAR (Ferreira, 2011), followed by data regression or the Scott-Knott test.

Results and discussion

Analysis phytotechnical

No interaction was observed between the treatments and the three application times; therefore, the factors were studied separately. For the regression analysis results, no model fit the curve, and we used the statistical tests to better explain the results.

An increase was observed in the stem diameter with increasing silicon concentrations, with the largest diameter, 2.33 mm, being attained with 0.21 g silicon pot^{-1} and a reduction occurring at higher concentrations (Figure 1a). Similar results were obtained for the plant height, with the maximum height, 9.82 cm, being attained at a concentration of 0.28 g silicon pot^{-1} (Figure 1b).

These results agree with those of Prado and Natale (2005), whose work with calcium silicate and passion fruit also showed that the application of silicon increased the height and stem diameter quadratically.

Similar results were obtained by Prado and Natale (2004), who observed while working with the application of chrome iron slag to passion fruit that a silicon application of rate of 333.4 g kg^{-1} increased the stem diameter and plant height quadratically.

This positive relation between height and stem diameter was also observed by Ferri (1985) and indicates the great morphophysiological importance of vegetative characteristics, as reflected in a practical way in the growth and differentiation of the plant. In addition, several studies have shown that fertilization with silicon can have a positive influence on the plant growth and productivity (Sávio, Silva, Teixeira, & Borém, 2011).

Increasing silicon concentrations resulted in a proportional increase in the fresh and dry leaf biomass and fresh stem biomass (Figure 1c, d and g), reaching a fresh leaf biomass of 12.09 g at levels of 0.66 g silicon pot^{-1}, a dry weight of 2.08 g with 0.57 g pot^{-1} and a fresh stem biomass of 5.29 g at a concentration of 0.60 g silicon pot^{-1}. At higher concentrations, a downward trend in values was recorded for these three variables.

A linear decrease for root fresh and dry biomass was observed with the increasing silicon concentrations (Figure 1e and f). These results agree with those obtained by Ribeiro et al. (2011), who applied calcium silicate to coffee plants and found that the highest calcium silicate dose (6 mg ha^{-1}) provided a reduction in coffee plant root systems without compromising the functionality and development of the plant aerial parts.

Among the benefits related to silicon fertilization are the shoot and root dry matter increase and the importance of silicon for the growth and development of plants (Epstein, 1994). Prado and Natale (2005) observed a quadratic increase in passion fruit plant shoot and roots dry matter in response to increased calcium silicate concentrations.

The same authors, while assessing steel slag in passion fruit plants, observed a quadratic increase in the shoot and root dry matter (Prado & Natale, 2004). Similar data were obtained in the present experiment with respect to the dry and fresh weight of the leaves and stems, thereby showing the positive effect of silicon on plant growth. However, a decrease was observed in the fresh and dry biomass of the root, which may be due to the production of photoassimilates being directed to vegetative production, thus expressing the higher plant growth. Therefore, the production of assimilates for the root was lower, which resulted in lower fresh and dry biomass.

Table 1 presents the significant difference observed for the dry biomass of the stem, and the length and width of the leaf. Concentrations of 0.28, 0.55, and 0.83 g silicon pot^{-1} provided higher dry stem biomass with respect to the control, which reflects the results found for the fresh stem biomass. The silicon accumulates in the stem support and sustaining tissues, substantially strengthening the plant structure (Plucknett, 1971).

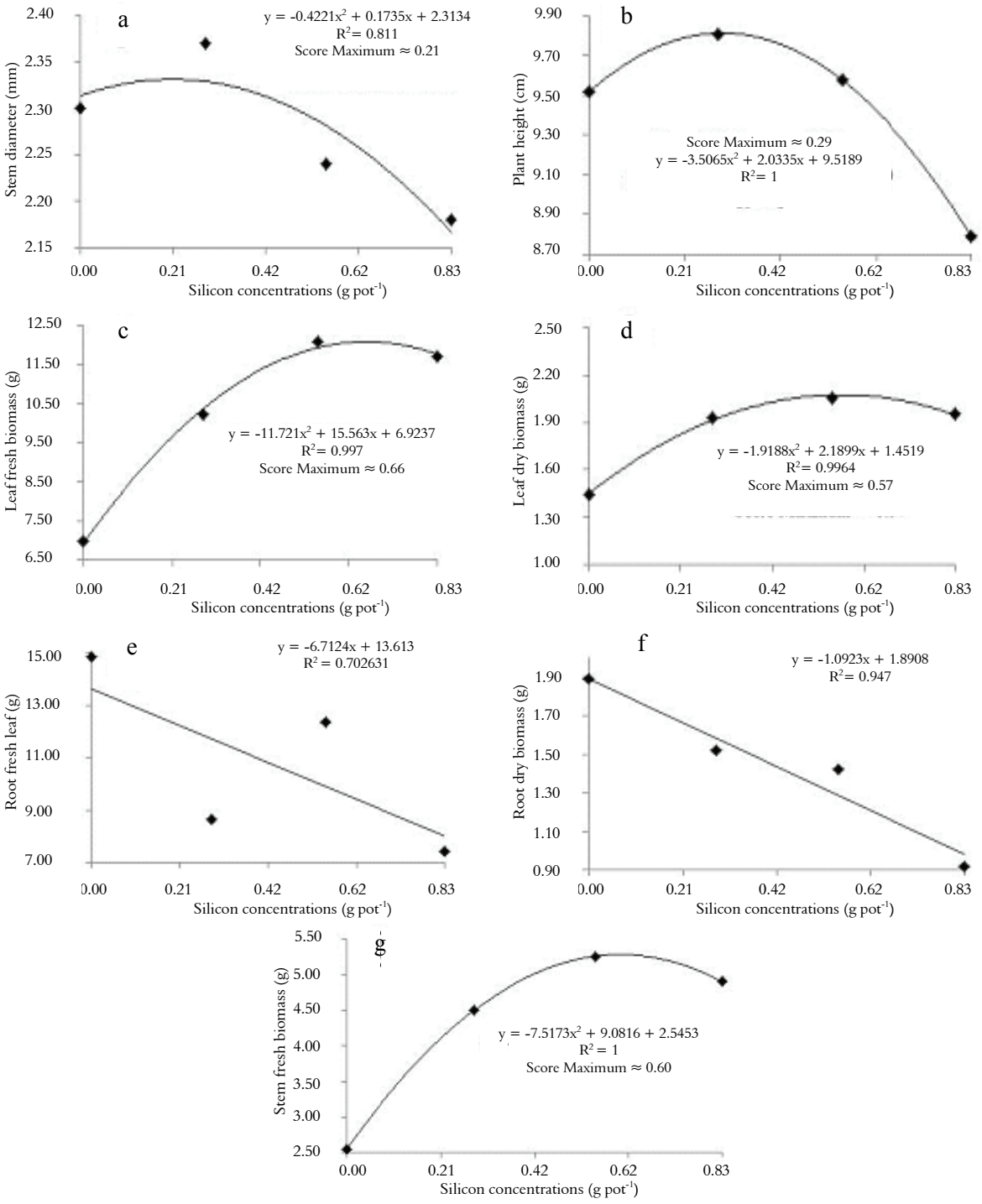

Figure 1. Phytotecnical characteristics of passion fruit (Passiflora edults Sims f. flavicarpa Deg.), submitted to different silicon concentrations.

No difference was observed between the control and the concentration 0.28 g silicon pot^{-1}, which showed greater leaf length and width relative to the other treatments. The leaves are the most important part of the plant with respect to the amount of CO_2 fixed by photosynthesis, a physiological process that results in dry matter accumulation (Ritchie, Hanway, Thompson, & Benson, 1994).

The growth can be measured by the amount of accumulated dry matter, which is directly related to the amount of light absorbed and increases as the leaf area increases (Gomes et al., 2008). However, the amount of dry matter produced depends not only on the amount of radiation received by the plant but also the efficiency in the use of such energy (Shibles, & Weber, 1966). Thus, an increase

in the leaf area does not always mean an increase in the amount of dry matter produced by the plants (Gomes et al., 2008). The results obtained in this article show that, despite the control having larger leaf area than the other treatments, the leaf and stem dry weight were higher at concentrations of 0.28, 0.55 and 0.83 g silicon pot^{-1} compared to the control.

Table 1. Dry stem biomass, number, length and width of passion fruit (*Passiflora edulis* Sims f. *flavicarpa* Deg.) leaves submitted to different concentrations of silicon.

Silicon concentrations (g pot^{-1})	Stem dry biomass (g)	Number of leaf	Leaf legth (mm)	Leaf width (mm)
Control	0.81 b	9.97 a	78.83 a	41.92 a
0.28	1.32 a	10.08 a	79.62 a	43.64 a
0.55	1.38 a	9.75 a	69.89 b	38.63 b
0.83	1.12 a	9.87 a	74.48 b	40.33 b
CV	29.38	12.33	18.72	17.07

*Means followed by the same lowercase letters in the same column belong to the same group, by Scott-Knott test ($p \leq 0.05$).

X-ray microanalysis

A linear increase was observed in the percentage of silicon in the passion fruit leaves as the concentrations of the applied silicon increased (Figure 2). Silicon is a mineral element, which is polymerized after being absorbed by the plants and accumulates in the cell wall of the epidermis (Jarvis, 1987).

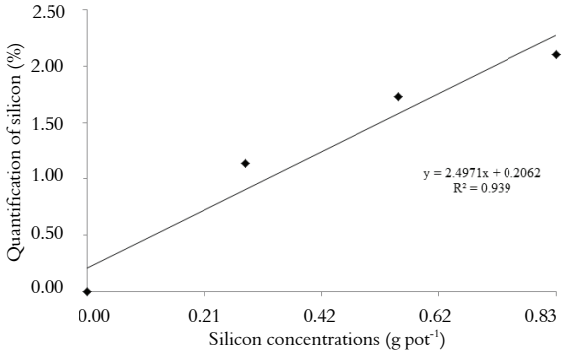

Figure 2. Quantification of silicon via X-ray microanalysis in passion fruit leaves (*Passiflora edulis* Sims f. *flavicarpa* Deg.) subjected to different concentrations of silicon.

In the silicon mapping of the abaxial epidermis of passion fruit leaves, the control did not show silicon, whereas the other treatments showed an increasing presence of silicon (Figure 3). The silicon polymerization on the lower surface of the leaf, a process called silicification, is common in grasses (Lux, Luxová, Hattori, Inanaga, & Sugimoto, 2002) and can occur in dicotyledonous plants, such as coffee (Pozza et al., 2004).

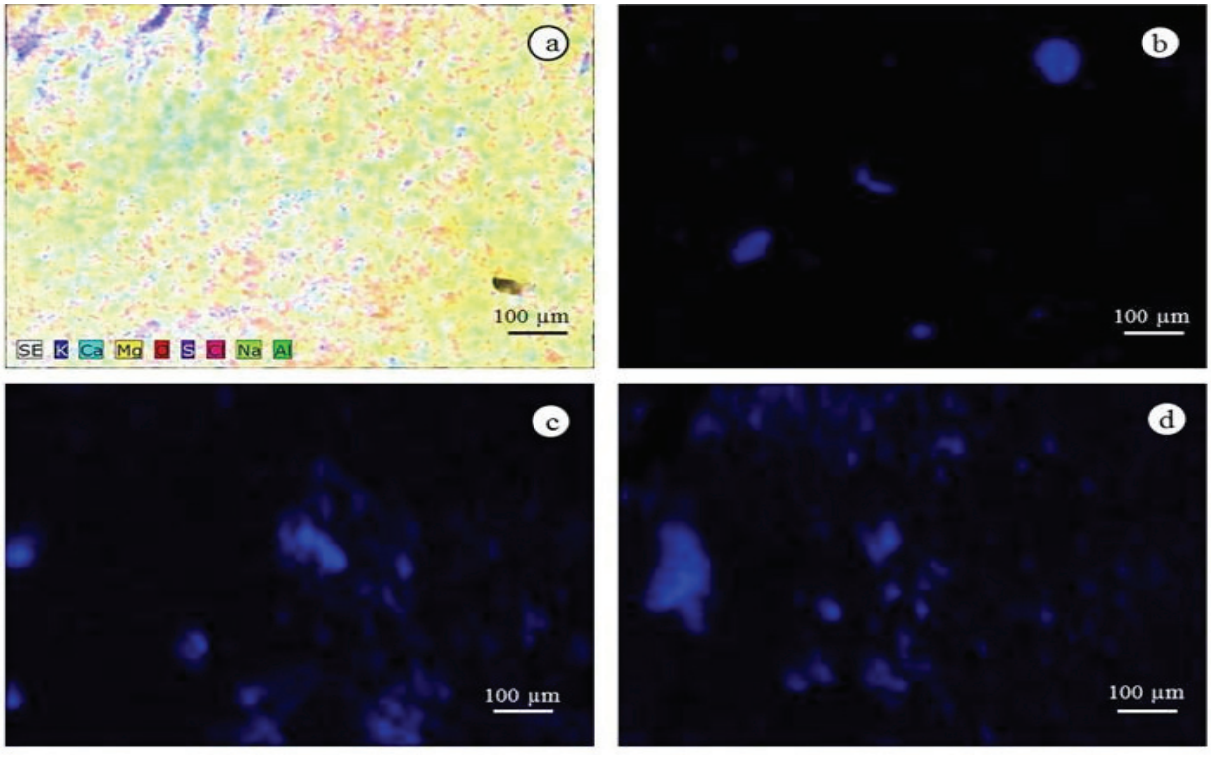

Figure 3. Silicon mapping on the abaxial epidermis of *Passiflora edulis* Sims f. *flavicarpa* Deg. leaves, showing the presence of silicon (in blue). a) Control showing mapping of various elements and absence of silicon; b) 0.28 g silicon pot^{-1}; c) 0.55 g pot^{-1}; d) 0.83 g pot^{-1}.

Silicon concentration

As shown in Table 2, no significant difference was observed among treatments for the silicon content in the stem; however, a significant difference did exist in the leaf and root. The concentration of 0.83 g silicon pot^{-1} represents a higher silicon content in the leaf compared to other treatments, and the concentrations of 0.28 and 0.55 g silicon pot^{-1} did not differ but showed higher silicon content in the leaf compared to the control. The concentrations of 0.55 and 0.83 g silicon pot^{-1} resulted in a higher silicon content in the root compared to the other treatments, and the 0.28 g silicon pot^{-1} treatment showed silicon content higher than observed in the control.

Tabela 2. Chemical analysis of silicon percentage in leaf, stem and root of passion fruit (*Passiflora edults* Sims f. *flavicarpa* Deg.) submitted to different silicon concentrations.

Silicon concentrations (g pot^{-1})	Leaf (%)	Stem (%)	Root (%)
Control	0.27 c	0.08 a	0.51 c
0.28	0.30 b	0.05 a	0.61 b
0.55	0.31 b	0.06 a	0.73 a
0.83	0.36 a	0.05 a	0.77 a
CV	8.60	38.60	10.18

*Means followed by the same lowercase letters in the same column belong to the same group, by Scott-Knott test ($p \leq 0.05$).

The presence of silicon in the control can be explained by the abundance of silicon in nature, where it is ubiquitous and is found even in water (Luz et al., 2006).

These results agree with those obtained by Ferreira, Botelho and Faria (2013), who studied the development of plum trees treated with silicon and found a positive linear effect for the leaf content due to the application of silicon doses.

Silicon accumulates in the leaf, so a lower amount of this element was found in the plant stem. However, higher levels were found in the root compared to the leaf, which has not been verified yet in other experiments of the application of silicon to passion fruit. According to Oliveira and Castro (2002), the average silicon content of the roots is lower compared with the stem and leaves, but in some cases, for example, in soybean, the silicon content in the root is greater than in the leaves. Fawe, Menzies, Cherif, and Belanger (2001) suggested that root silicon plays a role in the signaling network and can induce systemic resistance in other organs.

In the present study, the passion fruit was observed to be an intermediate accumulator of silicon in the roots and not an accumulator in the leaf and stem because, according to Ma, Miyake and Takahashi (2001), at relations above 1.0, plants are considered accumulators; between 1.0 and 0.5, they are considered intermediate; and under 0.5, they are nonaccumulators.

Flow cytometry

For a further elucidation of the effects of Si on this species, flow cytometry analysis was also performed.

These analyses have advantages over conventional genomics methods such as chromosome counts and other cytogenetic analysis because of the technical ease and speed (Chen, Hou, Zhang, Wang, & Tian, 2011; Nguyen et al., 2003).

No significant difference was observed in the DNA content among the treatments (Table 3); therefore, the application of the silicon did not modify the DNA content, which is important because the silicon maintains the genetic stability of the plants.

Tabela 3. Quantifications of DNA in passion fruit leaves (*Passiflora edults* Sims f. *flavicarpa* Deg.) analyzed by flow cytometry.

Silicon concentrations (g pot^{-1})	Leaf (%)	Stem (%)	Root (%)
Control	0.27 c	0.08 a	0.51 c
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0.55	0.31 b	0.06 a	0.73 a
0.83	0.36 a	0.05 a	0.77 a
CV	8.60	38.60	10.18

*Means followed by the same lowercase letters in the same column belong to the same group, by Scott-Knott test ($p \leq 0.05$).

The flow cytometry technique is useful for distinguishing differences in the DNA content of plants maintained under stressful conditions, such as during micropropagation or when being subjected to chemical treatments. Many economically important species, such as *Vitis vinifera* (Yang et al., 2008; Prado et al., 2010), *Gossypium hirsutum*, (Jin et al., 2008), *Musa* spp. (Msogoya, Grout, & Roberts, 2011; Escobedo-GraciaMedrano, Maldonado-Borges, Burgos-Tan, Valadez-González, Ku-Cauich, 2014), *Passiflora* spp. (Silva et al., 2011), *Elaeis guineensis* (Madon, Heslop-Harrison, Schwarzacher, & Hashim, 2012), *Coffea arabica* (Clarindo, Carvalho, & Mendonça, 2012), *Prunus cerasus* (Vujović, Cerović, Ružić, 2012) and *Saccharum* spp (Nogueira, Pasqual, & Scherwinski-Pereira, 2013), have had alterations to their DNA content evaluated by flow cytometry.

The plants treated with silicon in this study showed no alteration in DNA content. This result is important because fertilization with silicon can occur at concentrations as high as 0.83 g per pot, which causes no harmful effect on the plant genome. However, due to the lack of studies on cytogenetics and silicon, more research in the area is needed.

In this work, the tomato (*Lycopersicon esculentum*) was used as the internal standard, having DNA content of 1.96 pg (Figure 4). This standard was chosen because it formed a peak in a channel near that of the sample, without overlapping that of the sample. The closer the two peaks, the lower the experimental error.

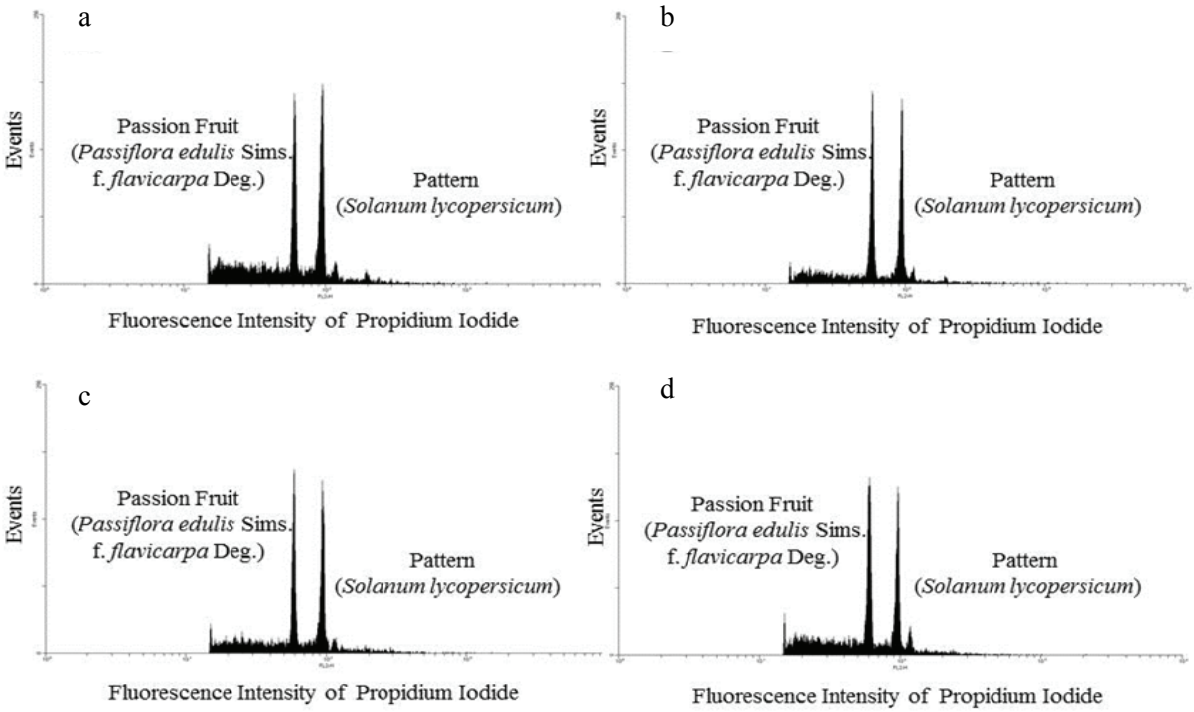

Figure 4. Histograms obtained by cytometric analysis in passion fruit (*Passiflora edulis* Sims f. *flavicarpa* Deg.). a) Control; b) 0.28 g silicon pot^{-1}; c) 0.55 g pot^{-1}; d) 0.83 g pot^{-1}.

Another positive point in this study was that the average coefficient of variation (CV) for the leaf samples ranged between 1.88 and 2.30. These values demonstrate the quality of the results obtained and the reliability of estimated amounts of DNA from the leaves of the passion fruit (Table 3), which can be seen by the thickness of the peaks in Figure 2.

Conclusion

The use of silicon at concentrations of 0.28 and 0.55 g pot^{-1} provides better passion fruit growth.

The absorption and deposition of silicon in the passion fruit leaves are proportional to the availability of this element in the plant.

The passion fruit is an intermediate root silicon accumulator plant.

Genetic stability is preserved in passion fruit treated with silicon.

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