Morpho-anatomical and physiological alterations of passion fruit fertilized with silicon

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Abstract – The objective of this work was to evaluate the effect of silicon fertilization on gas exchange, leaf anatomy, and ultrastructural characteristics of passion fruit (Passiflora edulis). The treatments comprised four concentrations of silicon (0, 0.28, 0.55, and 0.83 g per pot) at 1% silicic acid solution (SiO2·XH2O). This solution was applied around the stems of the plants. The first application was made 15 days after seedlings were transplanted. In total, three applications were made at 15-day intervals. The pots that constituted the control treatment received water in the same amount. After the final application, the plants were subjected to analyses of gas exchange, anatomical changes, and ultrastructural characteristics. The use of silicon promotes anatomical changes in passion fruit seedlings, such as increased adaxial epidermis thickness, reduced palisade parenchyma, and increased polar diameter/equatorial diameter ratio, which is related to stomata functionality. The concentrations of 0.55 and 0.83 g silicon per pot provide higher rates of photosynthesis, of transpiration, and stomatal conductance. The concentration of 0.83 g silicon per pot results in the greatest deposition of silicon in the abaxial epidermis of leaf surface.

Index terms: Passiflora edulis, photosynthetic rate, silicic acid, ultrastructural features.

Introduction

The passion fruit occupies a prominent place in fruit growing, even when compared to other tropical fruits with a higher tradition of consumption. Its participation in the horticultural market is guaranteed – it is perfectly adapted to this segment, which values high value-added products (Meletti et al., 2010). The national average productivity is 12-15 tons per hectare, with potential to produce 30-35 tons per hectare (Nogueira Filho et al., 2010). However, this potential is usually not reached due to phytosanitary problems that affect the crop. Therefore, it is necessary to identify techniques that minimize such damages, as the case of silicate fertilization, mainly regarding the production of seedlings.
The beneficial effects of silicon can be divided into physical and physiological. The physical benefits include accumulation of silicon in the cell walls, which improves the architecture of the plants, reduces water loss through the stomata, and hinders the penetration of pathogens and insects (Botelho et al., 2005). The physiological benefits are little studied, but many studies report that silicon may favor the increase in the photosynthetic rate (Ferraz et al., 2014), photosynthetic pigment contents (Ávila et al., 2010), and productivity (Paulino et al., 2013).

It is known that the leaf is an organ with high plasticity. Improvement in the interception of light can influence the anatomy of the leaf. Studies show that the application of silicon leads to changes in the leaf anatomy of several species such as orchids (Soares et al., 2012), strawberry (Braga et al., 2009), and coffee (Botelho et al., 2009). Other studies report the efficiency of silicon in the increase in the photosynthetic rate. Furthermore, the positive effect of silicon on the anatomy of anthurium (Dias et al., 2014) and banana (Luz et al., 2012) has been demonstrated.

The objective of this work was to evaluate the effect of silicon fertilization on gas exchange, leaf anatomy, and ultrastructural characteristics of passion fruit.

**Materials and Methods**

Passion fruit seeds (*Passiflora edulis* Sims) were sown in polystyrene trays containing Tropstrato substrate, in which they remained for 60 days. The seedlings, approximately 15 cm tall, were transplanted to polyethylene pots containing 1.1 kg of Tropstrato substrate and arranged randomly on the bench where they were irrigated daily. There was no additional fertilization. Treatments consisted of concentrations of silicon 0, 0.28, 0.55, and 0.83 g per pot in the form of silicic acid solution (SiO₂·XH₂O) at 1% (Pereira et al., 2010).

This solution was applied around the stem of the plants (drench). The first application was performed 15 days after transplant. In total, three applications were conducted at 15 day-intervals. The control vases received water in the same amount. After the final application, analyses of gas exchange, anatomical changes, and ultrastructural characteristics of plants were performed.

The gas exchange – photosynthetic rate (A), internal carbon (Ci), transpiration (E), stomatal conductance (Gs), and relation internal carbon/external carbon (Ci/Ca) – was evaluated using an infrared gas exchange analyzer (IRGA, model LI-6400, LI-COR, Nebraska, USA). To evaluate these variables, fully expanded leaves were selected on six plants per treatment, starting at 8:00 a.m. The photosynthetically active photon flux density was set in the equipment chamber at 1,000 μmol m⁻² s⁻¹.

Samples from the middle third of the second fully expanded leaf were collected from 4 different plants per treatment and previously fixed in FAA (70% formaldehyde – glacial acetic acid –, 70% ethanol) for 72 hours, and subsequently stored in 70% ethanol (v/v) for the anatomical analyses (Johansen, 1940). The cross sections were obtained with an LPC table microtome and paradermal sections by hand using a steel blade. The sections underwent clarification with sodium hypochlorite (1–1.25% active chlorine), triple washing in distilled water, and staining with either safrablau solution (0.1% astra blue and 1% safranin) for the cross sections or 1% safranin for the paradermal sections. The materials were subsequently mounted on semipermanent slides with glycerinated water following the methodology described by Kraus & Arduin (1997). The slides were observed and photographed under an Olympus BX 60 optical microscope coupled to a Canon A630 digital camera. The images were analyzed in UTHSCSA Image J image analyzing software (Schneider et al., 2012) with 6 replicates for each variable analyzed. The thickness of the epidermis of the adaxial and abaxial face, mesophyll, main nervure, and palisade and spongy parenchyma were measured. For the characterization of the stomata, analysis was performed on the stomatal density (number of stomata per mm²), polar diameter (PD), equatorial diameter (ED) and the PD/ED ratio obtained in Olympus CBB and Ken-a-vision 2100 microscopes.

Samples of the middle third of 4 leaves were fixed in Karnovsky solution (Karnovsky, 1965), post-fixed in osmium tetroxide (OsO₄), and dehydrated in increasing solutions of acetone (30, 50, 70, 90, and 100%) for the ultrastructural characteristics analyses. Thereafter, they were subjected to critical point drying, using liquid CO₂ as a transition liquid (Robards, 1978). They were later covered with gold (20 nm), and analyzed by
scanning electron microscopy LEO-EVO (Carl Zeiss, Germany) following the Alves (2004) protocol.

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

Results and Discussion

There were significant differences between treatment groups in photosynthetic rate, transpiration, and stomatal conductance (Table 1). Silicon concentrations of 0.28, 0.55, and 0.83 g per pot showed higher photosynthetic rate and transpiration compared to the control. It is known that silicon fertilization leads to better plant architecture due to morphological and physiological adaptations, leading to more upright leaves and more efficient light interception, which is reflected in the increased photosynthetic rate (Deren et al., 1994). The increase in plant transpiration may have occurred because the stomatal opening is directly linked to photosynthetic rate and transpiration. At the same time, the plant absorbs CO₂ for photosynthesis; thereby, it loses water to the atmosphere in the transpiration process (Pinto et al., 2012).

The concentration of 0.28 g per pot increased the net photosynthetic rate by 48.82% compared to the control, followed by the concentrations of 0.55 and 0.83 g per pot, which increased the rate by 43.25% and 39.30%, respectively. Pinto et al. (2012) investigated the influence of silicon on cocoa, and also found no significant differences in internal carbon concentration (Ci). According to Table 1, the concentrations of 0.55 and 0.83 g per pot led to higher stomatal conductance when compared to the 0.28 g per pot concentration and control treatment, but the concentration 0.28 g per pot led to greater stomatal conductance when compared to control.

According to Paiva et al. (2005), stomatal conductance regulates gas exchange and, therefore, has a direct relationship with the photosynthetic process and the consequent growth and development of plants. The results of the present study show the direct relationship between stomatal conductance and photosynthesis. The treatments that resulted in the highest photosynthetic rate also had the highest stomatal conductance. These results also show the influence of silicon on gas exchange, since this element led to an increase in most of the analyzed variables.

There was no significant difference between treatments for anatomical characteristics, mesophyll, spongy parenchyma, main nervure and abaxial epidermis. As for the characteristics equatorial diameter, stomata polar diameter and PD/ED ratio, there was significant difference between treatments (Table 2).
Concentrations 0.0 (control) and 0.28 g per pot showed stomata with greater equatorial diameter in relation to the other treatments. The 0.0 (control), 0.28, and 0.55 g per pot concentrations showed stomata with higher polar diameter in relation to the 0.83 g per pot concentration. The concentrations 0.28, 0.55, and 0.83 g per pot showed higher PD/ED compared to the control (Table 2).

Khan et al. (2002) reported that the polar and equatorial diameter ratio (DP/DE) is associated with guard cells and that it constitutes an important characteristic feature of the stomata. An elliptical shape (higher DP/DE) is characteristic of more functional stomata. Alternatively, a rounded shape (smaller DP/DE) is associated with stomata that do not have appropriate function. However, the type and condition of cultivation may modify these results. Elliptical stomata may lead to a higher CO₂ uptake, causing higher photosynthetic potential and reduced transpiration rate (Castro et al., 2009). Thus, in the present study, the stomata may be more functional in the presence of silicon. This is important because the plants, in the presence of silicon, have higher photosynthetic rates in relation to the control.

Rossatto et al. (2009) also showed that variations in the size and frequency of stomata indicate the ability of plants to rearrange these structures of the epidermis in response to environmental modifications, causing a greater contribution of the stomata to photosynthetic rate and transpiration, in an adequate manner.

A decrease in stomatal density was observed (Figure 1 A), reaching the minimum of 43.68 at the concentration of 0.50 gram of silicon per pot. Although this decrease in stomatal density has been previously reported, it indicates that it is not the quantity that is important, but the quality of these stomata. Thus, despite the low number of stomata, the silicon concentrations led to increased function of stomatal passion fruit plants compared to the control treatment.

Similarly, there was a decrease in the thickness of the palisade parenchyma until a minimum of 64.84 µm, corresponding to a concentration of 0.51 g per pot (Figure 1 B). There was an increase in the thickness of the adaxial epidermis to a maximum of 61.21 µm, corresponding to a concentration of 0.50 g per pot (Figure 1 C).

Braga et al. (2009) used silicon sources in vitro, such as potassium silicate or calcium silicate and sodium silicate, in their study on strawberry seedlings, and observed that silicon favored an increase in the thickness of the palisade parenchyma in the seedlings obtained by micropropagation. These results were not consistent with those of the present study.

Zanetti et al. (2016) found no difference in the thickness of palisade parenchyma in cacao plants fertilized with different concentrations of silicon. The results obtained with regard to the thickness of the palisade parenchyma were similar to those obtained by Luz et al. (2012), in which the use of the sodium silicate source in vitro led to lower thickness of this tissue in relation to the control in banana seedlings obtained by micropropagation.

Thus, the behavior of silicon can vary according to the species and cultivation environment. In most species studied, silicon causes a positive effect on plant characteristics. However, in some species, this element may be responsible for promoting some anatomical changes.

In the present study, silicon led to an increase in the thickness of the adaxial epidermis. Epstein (2001)

### Table 2. Anatomical characteristics of passion fruit (*Passiflora edulis*) leaves submitted to different silicon concentrations: mesophyll, spongy parenchyma (SP), main nervure (MN), abaxial epidermis (ABE), stomata equatorial diameter (SED), stomata polar diameter (SPD), and ratio SPD/SED(1).

| Silicon (g per pot)(2) | Mesophyll (µm) | SP (µm) | MN (µm) | ABE (µm) | SED (µm) | SPD (µm) | SPD/SED |
|------------------------|----------------|---------|---------|----------|----------|----------|---------|
| 0.0                    | 319.04a        | 166.07a | 587.60a | 47.14a   | 28.09a   | 38.92a   | 1.38b   |
| 0.28                   | 318.91a        | 146.31a | 580.09a | 46.27a   | 27.41a   | 39.03a   | 1.43a   |
| 0.55                   | 302.77a        | 126.92a | 652.60a | 45.81a   | 24.94b   | 37.39a   | 1.50a   |
| 0.83                   | 336.20a        | 161.25a | 628.35a | 50.69a   | 24.37b   | 34.73b   | 1.43a   |
| CV(3) (%)              | 13.90          | 25.74   | 9.70    | 11.12    | 6.65     | 5.86     | 7.32    |

(1) Means followed by equal letters do not differ by the Scott-Knott test at 5% probability. (2)Silicic acid. CV, coefficient of variation.

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showed that, when accumulated by plants, silicon causes anatomical changes in their tissues, such as the appearance of thicker epidermal cells due to the deposition of silicon.

Some authors have reported an increase in the epidermis due to the application of silicon in some species such as banana (Luz et al., 2012), strawberry (Braga et al., 2009), and orchid (Soares et al., 2012).

With respect to characterization of the tissues, in all treatments it was observed that passion fruit leaves have an unstratified epidermis. The dorsiventral mesophyll has a layer of palisade cells, occupying approximately one third of the thickness of the mesophyll. The spongy parenchyma consists of six to seven layers, with the presence of braciform cells and convex main nervure, in accordance with Beraldo & Kato (2010) (Figure 2).

In relation to the stomata, they are classified as anomocytic stomata because they are bordered by two or four subsidiary cells. The stomata are distributed on the surface of the abaxial epidermis, and the leaves are classified as the hypostomatic type (Beraldo & Kato, 2010) (Figure 3).

By means of scanning electron microscopy (SEM), a more significant deposition of epicuticular wax on the abaxial epidermal surface of passion fruit subjected to treatment with silicon was observed when compared to the control (Figure 4).

Polymerization of silicon, called the silicification process, on the lower surface of the leaf is common in grasses (Lux et al., 2002) and can occur in dicotyledonous plants, such as coffee (Pozza et al., 2004). According to Pozza et al. (2004), this occurs due to the increase in the cuticle in the lower surface of leaves treated with silicon. This increase is mainly due to the more developed epicuticular wax layer. These authors concluded that this process is important to prevent the viral pathogenesis processes, such as the germination and penetration of fungi, and to facilitate the accumulation of antifungal substances in the cuticle.

In view of the above, silicon, although not an essential element, should be considered a beneficial element as it contributes to the improvement in the anatomical and physiological attributes of passion fruit seedlings. Treatments that allow changes in plant morphology and physiology are extremely important, especially in the initial phase of seedling production, because when they are brought to the field, they will be much better

Figure 1. Density of stomata (A), thickness of palisade parenchyma (B), and thickness of adaxial epidermis (C) of passion fruit (Passiflora edulis) leaves subjected to different silicon (silicic acid) concentrations.
Figure 2. Photomicrographs of cross sections of *Passiflora edulis* main nervure and leaf, subjected to different silicon (silicic acid) concentrations.
Figure 3. Photomicrographs of paradermal sections showing stomata in abaxial face of *Passiflora edulis* leaves, subjected to different silicon (silicic acid) concentrations.

Figure 4. Electron micrographs of paradermal sections showing the abaxial stomata of *Passiflora edulis* with deposition of epicuticular wax (arrows). Leaves were subjected to different silicon (silicic acid) concentrations.
adapted to the abiotic and biotic stresses in the field, culminating in higher yields of this culture.

**Conclusions**

1. Silicon fertilization promotes anatomical changes in passion fruit seedlings, including an increase in adaxial epidermis thickness, a reduction in palisade parenchyma, and an increase in the PD/ED ratio, which is related to stomata functionality.

2. The concentrations 0.55 and 0.83 g of silicon per pot lead to higher photosynthetic rates, transpiration, and stomatal conductance of passion fruit seedlings.

3. The concentration 0.83 g of silicon per pot leads to greater deposition of silicon in the abaxial epidermis leaf surface.

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