Silicon-induced increase in chlorophyll is modulated by the leaf water potential in two water-deficient tomato cultivars

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ABSTRACT

This study aims to explain the effects of silicon on chlorophyll and to measure gas exchange and carbohydrate levels in two *Lycopersicon esculentum* cultivars that are exposed to drought. The experimental design used in this study was a randomised combination of five different water and silicon conditions (control, water deficit + 0.00 μmol Si, water deficit + 0.25 μmol Si, water deficit + 1.00 μmol Si, and water deficit + 1.75 μmol Si) applied to the two cultivars (Super Marmante and Santa Cruz). Parameters measured were gas exchanges, chlorophylls, and total soluble carbohydrates. Silicon at concentrations of 0.25, 1.00, and 1.75 μmol induced a gradual increase in the total chlorophyll levels. A correlation analysis revealed a linear, positive interaction between the leaf water potential and the total chlorophyll ($r = 0.71$; $P < 0.05$). This study confirmed the hypothesis that silicon has a beneficial effect with regard to chlorophyll. Under water-deficient conditions, both cultivars showed an increase in chlorophyll *a* when treated with silicon in addition to changes in the total chlorophyll levels. These results were supported by the change in leaf water potential. In addition, a reduction of the effects of water restriction was also observed in the transpiration rate, the stomatal conductance and in the levels of total carbohydrates.

Keywords: *Lycopersicon esculentum* Mill.; water deficit; Si; photosynthetic pigments

Silicon is assimilated primarily through the plant roots, and its ability to accumulate in tissues varies, depending on the tissue (Liang et al. 2007). Several monocots are considered silicon accumulators and display active absorption through their roots. However, many dicots, such as *Lycopersicon esculentum*, are not accumulators of silicon and display passive absorption (Mitani and Ma 2005).

A reduction of the negative effects of water deficiency has frequently been described in plants treated with silicon. For example, Hattori et al. (2005) investigated the effects of silicon on gas exchange in *Sorghum bicolor* plants. The results obtained by Ahmad and Haddad (2011) with *Triticum aestivum* plants demonstrated that silicon positively influenced antioxidant enzyme activities. In addition, Lobato et al. (2009) reported a maximisation of proline synthesis in *Capsicum annuum* plants treated with silicon.

Silicon application resulted in an increase of chlorophyll and an improvement in the antioxidant system in *Lycopersicon esculentum* plants that were exposed to salt stress, but the regulatory mechanism responsible for this effect was not explained (Al-aghabary et al. 2004). Ávila et al. (2010) investigated the interaction between silicon and nitrogen and reported an increase in the levels of chlorophyll *a* in *Oryza sativa* plants. However, drought typically causes a significant decrease in photosynthetic pigments. Therefore, there is limited information regarding the effects of silicon on chlorophyll levels in water-deficient plants.

This study aims to explain the effects of silicon on chlorophyll and to measure gas exchange and carbohydrate levels in two *Lycopersicon esculentum* cultivars that are exposed to drought.

MATERIAL AND METHODS

Growth conditions. The study was performed at the Institute of Agrarian Sciences (IAS) of the University Federal Rural of the Amazon (UFRA) in Belém, Para, Brazil (01°27'S, 48°26'W). The plants were grown in a greenhouse without environmental control, and the minimum, maximum and median temperatures were 24.1°C, 35.5°C, and 27.4°C, respectively. The relative humidity during the experimental period oscillated between 65% and 93%, and the photoperiod was set to 12 h of light.

Plants, substrate and containers. Seeds of the tomato species (*Lycopersicon esculentum* Mill.) cvs. Super Marmante, a cultivar with economic potential, and Santa Cruz, a traditional cultivar, were used in this study. The substrate was a mix of sand and silica (a neutral material used to maintain the substrate humidity) in a 2:1 proportion, and it was autoclaved at 120°C/atm for 40 min. The container used for plant growth was a 2-L Leonard-type pot.

Experimental design and treatments. The experimental design was a randomised combination of five different water and silicon conditions (control, water deficit + 0.00 µmol Si, water deficit + 0.25 µmol Si, water deficit + 1.00 µmol Si, and water deficit + 1.75 µmol Si) applied to the two cultivars (Super Marmante and Santa Cruz), resulting in a total of 10 treatments. This experiment was assembled with 6 replicates for a total of 60 experimental units with 1 plant in each unit.

Plant conduction and silicon treatment. Five seeds were placed in each pot, and each pot was limited to 1 plant after seed germination. The control and deficit + 0.00 µmol Si treatments received macronutrients and micronutrients from the nutritive solution used by Schwarz (1995), with the following modifications: 5.0 µmol KNO_3, 5.0 µmol $Ca(NO_3)_2 \cdot 4\ H_2O$, 2.0 µmol $NH_4H_2PO_4$, 1 µmol $MgSO_4 \cdot 7\ H_2O$, 50.0 µmol KCl, 12.5 µmol H_3BO_3, 2.0 µmol $MnSO_4 \cdot H_2O$, 2.0 µmol $ZnSO_4 \cdot 7\ H_2O$, 0.5 µmol $CuSO_4 \cdot 5\ H_2O$, 0.5 µmol $NaMoO_4 \cdot 5\ H_2O$, and 100.0 µmol $NaEDTAFe \cdot 3\ H_2O$ in a total volume of 1.5 L without silicon addition. The deficit + 0.25 µmol Si, deficit + 1.00 µmol Si, and deficit + 1.75 µmol Si treatments received macronutrients and micronutrients from the above nutritive solution and silicon from sodium metasilicate ($Na_2SiO_3 \cdot 9\ H_2O$) as described in Liang et al. (2006) and adapted at the Laboratory of the Physiology Advanced (LFVA). The solutions were applied to plants for 45 days, were changed at 09:00 h over 5-day intervals and had their pH adjusted to 6.0 ± 0.1 via the addition of HCl or NaOH. On the 45th day, plants from the deficit + 0.00 µmol Si, deficit + 0.25 µmol Si, deficit + 1.00 µmol Si, and deficit + 1.75 µmol Si groups were subjected to a period of 5 days without the nutritive solution to simulate drought. After this period, plants were physiologically and biochemically analysed.

Leaf water potential and gas exchange. The leaf water potential (Ψ_w) was measured in fully expanded leaves under light between 05:00 and 06:00 h using an analogue plant moisture system (Skye Instruments, Llandrindod Wells, UK).

The transpiration rate and stomatal conductance were evaluated using a steady-state porometer (LICOR, Lincoln, USA) in fully expanded leaves, under light in the middle 3rd of the main branch. The gas exchange was evaluated between 10:00 and 12:00 h in all of the plants in the experiment. The photosynthesis-active radiation oscillated between 830 and 1020 $\mu mol/m^2/s$ (at 12:00 h) during the physiological evaluations.

Measuring photosynthetic pigments. The levels of photosynthetic pigments were measured using 25 mg of leaf tissue. The samples were homogenised in the dark in the presence of 2 mL of 80% acetone (Nuclear), and the homogenate was centrifuged at 5.0 g for 10 min at 5°C. The supernatant was removed, and the chlorophyll fractions *a*, *b*, and total were quantified using a spectrophotometer (Femto, São Paulo, Brasil) according to the methodology of Lichtenthaler (1987).

Leaf sample preparation. The leaves were harvested and placed in a 70°C oven with forced air circulation at 96 h. The dried leaves were ground, and the powder was stored in a glass container in the dark at 15°C until biochemical analyses were performed.

Determination of total soluble carbohydrates. To determine the quantity of total soluble carbohydrates, 20 mg of leaf powder was incubated with 2.0 µL of 80% ethanol at 95°C for 20 min and centrifuged for 5 min at 5.0 g and 20°C. The supernatant was then removed, and the quantification of the total soluble carbohydrates was performed in reactions containing 1.250 µL of 100% H_2SO_4, 70 µL of 15% phenol, 580 µL H_2O, and 100 µL of extract for a total volume of 2.0 µL. Measurements were taken at 490 nm (Dubois et al. 1956) using glucose (Sigma chemicals, São Paulo, Brasil) as a standard.

Data analysis. An analysis of variance was performed, and when significant differences were present, a Scott-Knott test with a 5% level of error probability was used. The standard errors were calculated for all of the evaluated treatments. The correlation analysis was performed using the Pearson parametric method.

RESULTS AND DISCUSSION

Silicon-induced interference on gas exchange. Water deficit caused a significant reduction in gas exchange in both cultivars (Figure 1A). An exogenous application of silicon at concentrations of 0.25, 1.00, and 1.75 μmol proportionally attenuated the negative symptoms induced by the water restriction.

The application of increasing levels of silicon alleviated the effects of the water deficit as a result of the silicon increasing the water retention in the water-deficient plants. In addition, silicon accumulation in the cell wall reduced water loss from transpiration, and silicon improved the water utilisation of the soil, likely due to a reduction in evapotranspiration.

The transpiration rates of the two water-deficient cultivars were reduced (Figure 1B), with the slowest transpiration rates being observed in the treatment groups water deficit + 0.00 μmol Si (Super Marmante cultivar) and water deficit + 0.25 μmol Si (Santa Cruz cultivar). The results revealed that water deficit decreased the transpiration rate, which is linked to a stomatal mechanism. Because the stomata are normally closed when water is limited (Pereira et al. 2006), the low-silicon conditions reduced the loss of water from the plant to the environment (Castro Neto 2003). Further, the addition of increasing levels of silicon decreased the negative effects induced by the water deficit, thereby increasing the transpiration rate.

Water deficiency caused a significant decrease in the stomatal conductance in both cultivars (Figure 1C). The negative effects caused by water deficit were attenuated when higher silicon concentrations were applied. The Super Marmante and Santa Cruz cultivars exhibited the lowest conductance values in the absence of added silicon and the highest conductance values with the addition of 1.75 μmol Si. A decrease in conductance was correlated with water availability, and the water potential results supported these data. An increase in silicon levels reduces these effects because of the silicon deposition in the epidermal walls of the leaves (Melo et al. 2003), causing the formation of a double layer of cuticular silicon. Depending on its thickness, the double layer also reduces the transpiration rate through the stomata (Ma et al. 2001). Lobato et al. (2009) observed similar results, namely that silicon caused a reduction in the stomatal conductance in *Capsicum annuum* plants that were subjected to water deficit.

Silicon-induced increase of photosynthetic pigments. Water deficiency significantly reduced the levels of chlorophyll *a* relative to the control in the Santa Cruz cultivar but not in the Super Marmante cultivar (Figure 2A). The addition of silicon to the treatment groups water deficit + 0.25 μmol Si, water deficit + 1.00 μmol Si, and

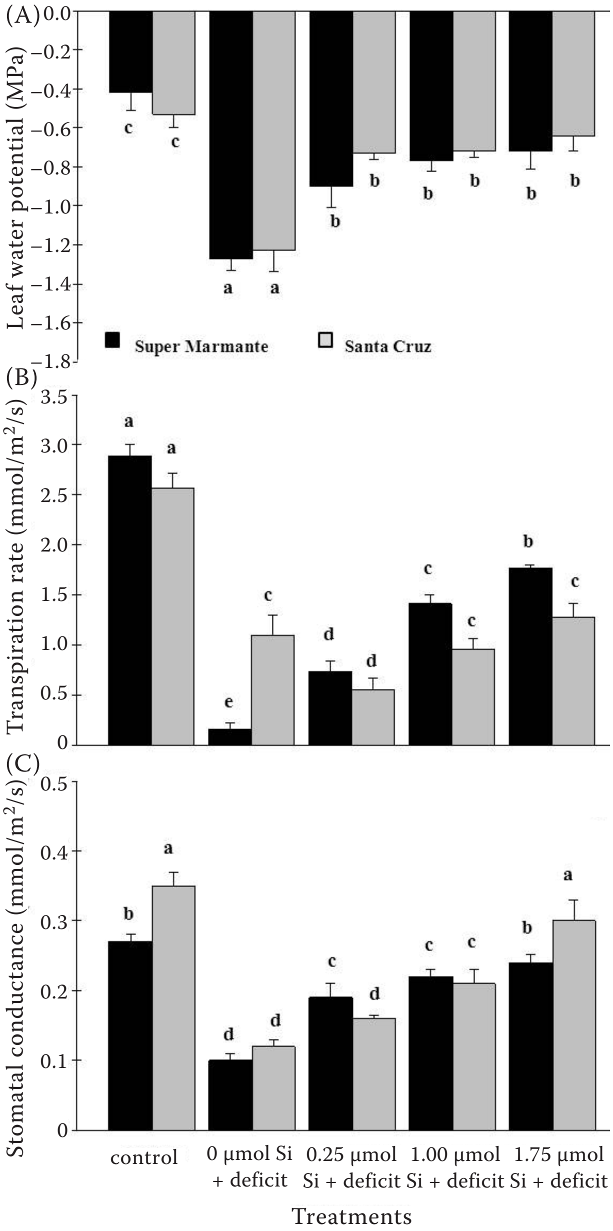

Figure 1. Leaf water potential (A), transpiration rate (B), and stomatal conductance (C) in two tomato cultivars treated with silicon and exposed to water deficiency. Means followed by the same letter are not significantly different by the Scott-Knott test at 5% of probability. The bars represent the mean standard error

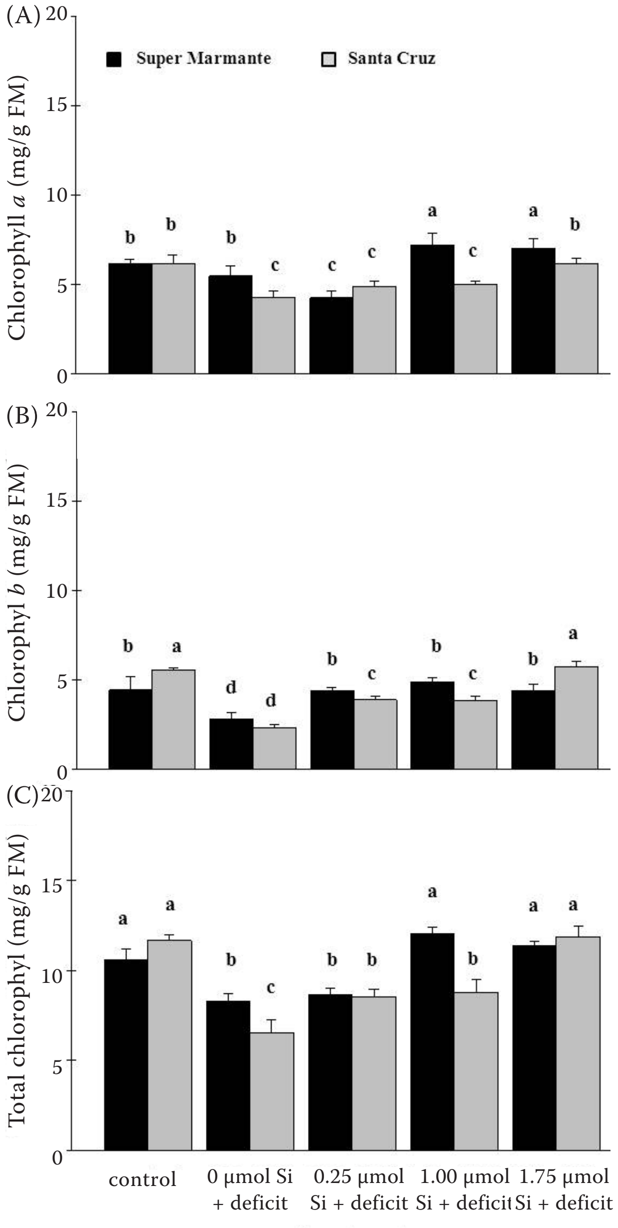

Figure 2. Chlorophyll *a* (A), chlorophyll *b* (B), and total chlorophyll (C) in two tomato cultivars treated with silicon and exposed to water deficiency. Means followed by the same letter are not significantly different by the Scott-Knott test at 5% of probability. The bars represent the mean standard error

water deficit + 1.75 µmol Si resulted in an increase in the levels of chlorophyll *a* in both cultivars.

The reduction in chlorophyll *a* in both cultivars was a consequence of water restriction, leading to the production of peroxidative enzymes that are associated with the degradation of chlorophyll *a* in the thylakoid membrane (Gandul-Rojas et al. 2004). Silicon proportionately increased the levels of chlorophyll *a* in the water-deficient cultivars, indicating the synthesis of new pigments and the maintenance of previously existing chlorophyll *a*. Donegá (2009) also concluded that silicon use improved the plant architecture and increased photosynthesis. The deposition of silicon in the cell wall also increases tissue resistance and promotes better-performing plants due to leaf position and their interception of light (Lana et al. 2003).

The water-deficient cultivars showed a reduction in chlorophyll *b* (Figure 2B) compared to the control plants. The water deficiency decreased the chlorophyll *b* levels due to water restriction in the soil. The addition of silicon at concentrations of 0.25, 1.00, and 1.75 µmol, however, caused the levels of chlorophyll *b* to increase. Chlorophyll *b* was positively affected by the addition of silicon, and this effect was related to the accumulation of Si in the epidermal cells located in the shoot. Here, Si indirectly protected the photosynthetic apparatuses and consequently decreased the damage caused by water restriction.

Water deficiency caused a significant decrease in the total chlorophyll in both cultivars (Figure 2C), but the addition of silicon at concentrations of 0.25, 1.00, and 1.75 µmol induced a gradual increase in the total chlorophyll levels. A correlation analysis revealed a linear, positive interaction between the leaf water potential and the total chlorophyll ($r = 0.71$; $P < 0.05$) (Figure 3).

The treatment with 1.75 µmol silicon maintained the levels of total chlorophyll under water-deficient conditions. Similar levels were obtained with the control plants from both cultivars, in which the control represents the ideal conditions for the plant. This result can be explained by the relationship between the leaf water potential and the total

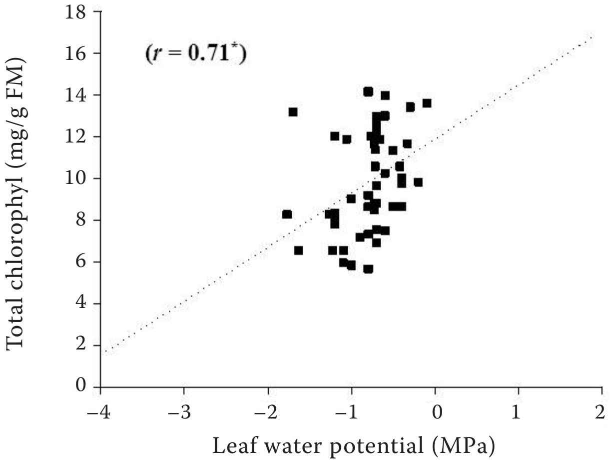

Figure 3. Relationship between leaf water potential and total chlorophyll in two tomato cultivars treated with silicon and exposed to water deficiency. The bars represent the mean standard error and the asterisks (*) indicate significance at 0.05 probability level

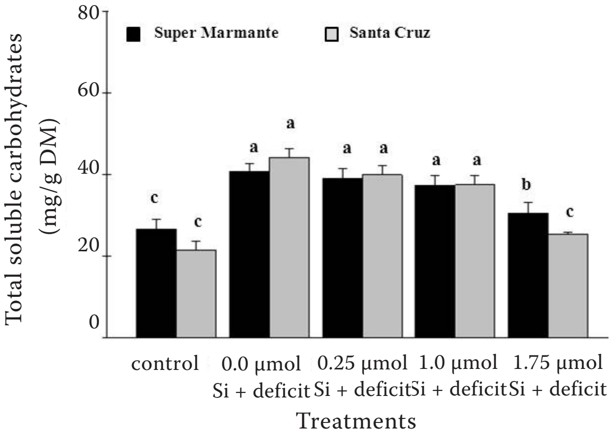

Figure 4. Total soluble carbohydrates in two tomato cultivars treated with silicon and exposed to water deficiency. Means followed by the same letter are not significantly different by the Scott-Knott test at 5% of probability. The bars represent the mean standard error

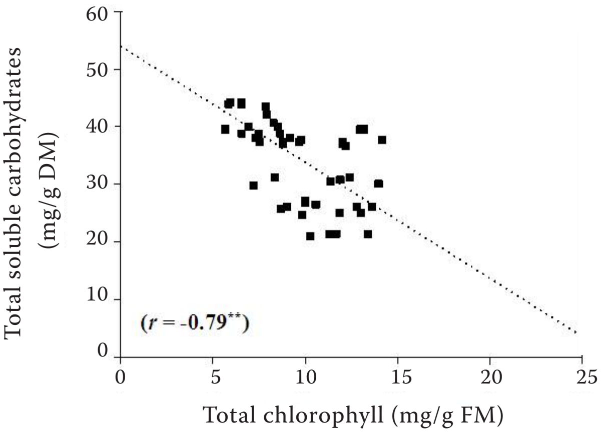

Figure 5. Relationship between total chlorophyll and total soluble carbohydrates in two tomato cultivars treated with silicon and exposed to water deficiency. The bars represent the mean standard error and the asterisks (**) indicate significance at 0.01 probability level

chlorophyll because adequate water in the leaf tissue maintains the stability of the chloroplasts in addition to other functions, such as absorption and energy transport.

Water-deficient plants that were treated with silicon showed an increase in the total chlorophyll. Correspondingly, a decrease was observed in the plants exposed to a water deficit in the absence of silicon, likely because of the decrease in nitrogen absorption, an essential element necessary for the formation of chlorophyll. Silicon treatments were shown to cause changes to nitrogen metabolism (Watanabe et al. 2002). Water is responsible for the conduction of nitrogen and other nutrients during their absorption through the root system.

Silicon-induced changes in carbohydrates. Plants undergoing water restriction, namely the treatment groups water deficit + 0.00 μmol Si, water deficit + 0.25 μmol Si, and water deficit + 1.00 Si μmol, showed a statistically equal increase in total soluble carbohydrates (Figure 4). A correlation analysis revealed a negative relationship between total chlorophyll and total soluble carbohydrates ($r = -0.79$; $P < 0.01$) (Figure 5).

The application of silicon caused a reduction in the levels of total soluble carbohydrates. This effect was likely due to the silicon protecting the photosynthetic apparatus (Harter and Barros 2011) and an adequate formation of the carbon skeleton, resulting in a reduction of the negative effects of the water deficit. In conditions with adequate water, such as in the control treatments, the plant has a lower amount of total soluble carbohydrates in its leaves due to higher rates of carbon translocation from the leaf to the drains (Kingston-Smith et al. 1999). However, carbon accumulation occurs under water restriction because of an osmotic adjustment by the plant (Oliveira Neto et al. 2009). Also, the increase of carbohydrate levels in leaves is related to a low transference of carbohydrates to the receptor organs. The relationship observed between total chlorophyll and total carbohydrates shows that changes to the total chlorophyll levels affect the carbon skeleton synthesis and translocation during water deficiency.

This study confirmed the hypothesis that silicon has a beneficial effect with regard to chlorophyll. Under water-deficient conditions, both cultivars showed an increase in chlorophyll *a* when treated with silicon in addition to changes in the total chlorophyll levels. These results were supported by the change in leaf water potential. In addition, a reduction of the effects of water restriction was also observed in the transpiration rate, the stomatal conductance and in the levels of total carbohydrates.

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