



Silicon fertilization increases gas-exchange and biomass by silicophytolith deposition in the leaves of contrasting drought-tolerant sugarcane cultivars under well-watered conditions

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Abstract

Purpose Silicon (Si) fertilization provides benefits to sugarcane. However, information remain scarce about the relationship between Si fertilization, gas exchange responses, biomass and silicophytolith accumulation in contrasting drought tolerant sugarcane cultivars under well-watered conditions

Methods Sugarcane cultivars (drought-tolerant and drought-sensitive) were grown in pots containing soil with low available Si and were treated (at rates equivalent to 0, 250, 500, 750, and 1000 kg ha⁻¹ Si) with Si as silicate. The silicophytolith contents, morpho-type descriptions, Si concentrations and gas exchange

were evaluated in the top visible dewlap leaves. Stalk length and stalk biomass were also evaluated.

Results The silicophytolith, Si contents, net CO₂ assimilation rate (A), plant transpiration (E), stomatal conductance (gs) and electron transport rate (ETR) of leaves and fresh biomass and length of stalks increased linearly as functions of the Si application rate, independent of cultivar. RB86-7515 showed the highest stalk length, fresh stalk and green leaf biomass, relative water content, and water potential, while RB85-5536 showed superior values for A, E, gs, and ETR.

Conclusions Si fertilization improved photosynthesis, transpiration, stalk length, and stalk biomass production in sugarcane. The highest silicophytolith content was reflected in a diversity of silicified cells, which may favor a higher photosynthesis and biomass. The increase of silicification in stomata

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complexes and trichomes with Si may be associated to a higher Si availability and transpiration. Contrasting drought-tolerant cultivars showed similar silicification and gas exchange responses with Si. Considering these benefits, Si should be included in the fertilization program of sugarcane.

Keywords *Saccharum* spp · Soil · Silicate · Absorption · Silica · Plant nutrition

Introduction

Silicon (Si) is one of the main elements in the Earth's crust (28%) and, consequently, in soils (Wedepohl 1995), but low Si concentrations are available for plant uptake in most agricultural areas in tropical regions (Camargo et al. 2014; Camargo and Keeping 2021; Haynes 2017). Sugarcane is an economically important crop for biofuel production, and it is planted in those areas (Ferreira et al. 2017). Sugarcane is also considered a Si-accumulating crop (Epstein 2009), and Si concentrations in plants are variable, depending on the cultivar (Camargo et al. 2010; Deren et al. 1993) and other factors, such as soil water availability, transpiration, Si availability and soil properties (Camargo and Keeping 2021; Haynes 2017). As sugarcane is a multiyear crop, Si fertilization may contribute to the Si supply after Si extraction and decrease Si levels (Camargo et al. 2013b), especially in soils with low available Si, over time (Camargo and Keeping 2021).

Silicon is taken up by plant roots as monosilicic acid (H_4SiO), and is deposited as amorphous silica (SiO_2), mostly in epidermal cells (Epstein 2009; Fernández Honaine and Osterrieth 2012; Gomez Coe and Osterrieth 2014; Ma and Yamaji 2006; Sangster et al. 2001). Si fertilization provides yield increases (Camargo et al. 2014; Camargo and Keeping 2021; McCray and Ji 2018) and reduces damage caused by biotic and abiotic stresses in sugarcane (Camargo et al. 2014; Camargo et al. 2020; Majumdar and Prakash 2020). Physiological aspects, such as increased chlorophyll, water relative contents and water potential levels, have already been reported to be associated with improvements in sugarcane yield from plants grown with and without stress under Si fertilization (Bezerra et al. 2019; Camargo et al. 2019; Elawad et al. 1982; Teixeira et al. 2020; Verma

et al. 2020). Nevertheless, the gas exchange responses of Si fertilization of sugarcane have not been fully elucidated, and further research on this subject could contribute to a better understanding of the role played by Si in improving biomass production.

Few studies on the enhancement of photosynthesis, transpiration, and stomatal conductance with Si fertilization have been reported in sugarcane (Bokhtiar et al. 2012; Verma et al. 2020). For instance, silicate application to the soil increased gas exchange responses and Si deposition in sugarcane leaves (Bokhtiar et al. 2012). In general, a higher Si availability in soils or substrates is associated with higher Si accumulation in plants, which in turn may have some role in physiological aspects, such as photosynthesis or transpiration processes (Ma and Takahashi 2002; Henriet et al. 2006). In particular, it has been shown that silica accumulation in sugarcane leaves is conditioned by the type of soil and climate because of differences in Si availability and transpiration rate (De Tombeur et al. 2020). These authors also identified the location of the silica accumulation in tissues, and they found that there was an increase in the silicification of stomata, long cells and fragments of tissue according to an increment of Si available in soils. They also hypothesize that “the formation of large multicellular silica deposits could be crucial to explain the increase in leaf erectness under Si fertilization” (De Tombeur et al. 2020), which in turn would promote a higher productivity. In rice plants, Si fertilization has already been demonstrated to improve leaf position and relative light intensity (Ando et al. 2002). The improvements in gas exchange responses in sugarcane with Si supply could be associated with the deposition of Si in plant tissues due to an increase in the strength of these tissues, especially in the leaves, and, consequently, enhanced plant structure (Raven 1983; Yamamoto et al. 2012; Kido et al. 2015; De Tombeur et al. 2020). However, the role of Si deposition in increasing the mechanical strength of plant tissues requires further study (Bauer et al. 2011; Cooke and Leishman 2016; De Tombeur et al. 2020). A study about fertilization and silicophytolith accumulation under controlled conditions would improve the comprehension about these associations in sugarcane.

Si deposition as silicophytolith is commonly observed in epidermal plant tissues and vascular cells, suggesting that their distribution is related to plant transpiration (Ma and Takahashi 2002; Piperno

2006; Strömberg et al. 2016; Trembath-Reichert et al. 2015). However, the differences in the rate of silicification of certain cells or the presence of specialized phytolith-forming cells (or morphotypes) could be related to the specific functions of some silicophytoliths (Strömberg et al. 2016; Kumar et al. 2017a, b; Fernández Honaine et al. 2021). Therefore, the association of Si deposition as silicophytoliths in sugarcane, including the morphology and localization in the tissues, as well as modifications in physiological aspects, especially transpiration and photosynthesis under controlled conditions, warrants further study to determine the role played by Si in the biomass production of sugarcane. Additionally, Si uptake is variable among sugarcane cultivars (Camargo et al. 2010; De Camargo et al. 2017; Camargo and Keeping 2021; Deren et al. 1993), and at present, silicophytolith deposition and morphotypes have not been shown to be a function of the Si application rate in drought-contrasting sugarcane cultivars.

This study aims to determine a) whether Si fertilization rates impact Si concentration, silicophytolith contents and their morphotypes and physiological responses in leaf tissues, including photosynthesis, transpiration, and biomass production, in two drought-contrasting sugarcane cultivars under well-watered conditions and b) whether silicophytolith contents, morphotypes, and physiological responses in leaf tissues are similar between drought-contrasting sugarcane cultivars with increased Si fertilization. We hypothesized that Si fertilization will increase total silicophytolith content, and this increment will be due to a higher silicification of specific type of epidermal cells, which could be associated to alterations in photosynthesis, transpiration, and biomass production in sugarcane, even without water deficit conditions. We also hypothesized that Si fertilization will provide similar silicification in the leaf tissues, gas exchange responses and biomass production in drought-contrasting sugarcane cultivars, even under well-watered conditions. The experiment was conducted using Si rates applied in soil with low plant-available Si concentration with two sugarcane cultivars (drought-tolerant and drought-sensitive cultivars). The best period for sugarcane growth was chosen with higher sunlight and temperature, and the water was maintained at 100% soil humidity during all experiments.

Material and methods

Sugarcane cultivars

Drought-tolerant (RB86-7515) and drought-sensitive RB85-5536 sugarcane cultivars, according to Medeiros et al. (2013) and Vital et al. (2017), were used in this experiment.

Soil characteristics

Samples of Typic Quartzipsamment (85% total sand; 12.5% clay; 2.5% silt) were collected from areas without previous fertilization to fill the pots. The Si contents were 2.8 mg kg⁻¹ Si extracted by 0.01 mol L⁻¹ CaCl₂ and 13.8 mg kg⁻¹ Si extracted by 0.5 mol L⁻¹ acetic acid. The soil chemical characteristics, performed according to Rajj et al. (1997), showed: organic matter concentration=5.0 g kg⁻¹; pH CaCl₂=4.5; P resin=4.0 mg dm⁻³; K, Ca, and Mg exchangeable concentrations=0.3, 3, and 1 mmol_c dm⁻³, respectively; cation exchangeable capacity (CEC)=22.0 mmol_c dm⁻³; basis saturation (SB)=19%; and aluminum saturation=19%.

Experimental procedure

An experiment with sugarcane was conducted in pots (polyethylene, 20 L) under greenhouse conditions at Piracicaba (22° 42' 30" S; 47° 38' 01" W), SP, Brazil, during October 2018 and April 2019. This period corresponds to a one-year sugarcane planting system (known as "one-year sugarcane") and to favorable temperature and humidity to promote high accumulation of sugarcane biomass, and it is used in some regions of commercial plantations. The average of minimum and maximum temperatures, respectively, were 17.5 °C, and 31.5 °C during the experiment.

A factorial experiment was arranged in a randomized complete block design with eight replications. Factors included two sugarcane cultivars (RB86-7515 and RB85-5536) and five Si application rates equivalent to 0, 250, 500, 750, and 1000 kg ha⁻¹. The source of Si was Ca-Mg silicate containing 105 g kg⁻¹ Si; 262 g kg⁻¹ Ca, and 56.8 g kg⁻¹ Mg. As silicate is a source of Ca and Mg, it was necessary to use lime (286 g kg⁻¹ Ca and 60 g kg⁻¹ Mg) and/or MgCl₂ (11 g kg⁻¹ Mg) to equilibrate the levels of these

nutrients in all treatments; therefore, the effect was only that of Si.

The soil samples were collected from areas of native vegetation, air-dried, and sifted through a 5-mm screen. Soil samples and silicate, lime and/or $MgCl_2$ were homogenized inside plastic bags before filling the pots, which were incubated for 11 weeks to provide adequate time soil for chemical reactions of the materials.

Sugarcane stalks were collected from commercial field areas. Single-budded setts were planted in plastic pots with sand soil to obtain similar plants regarding height and root system. Fertilization with 180 kg ha^{-1} P_2O_5 (simple superphosphate), 30 kg ha^{-1} N (ammonium sulfate), and 100 kg ha^{-1} K_2O (KCl), according to Raij et al. (1997), was used in soil samples before transplanting. One sugarcane plant was planted in each pot (20 L) on October 26, 2018. Superficial fertilization with 30 kg ha^{-1} N and 100 kg ha^{-1} K_2O was provided to plants 30 days after transplanting. Micronutrients were also applied at rates equivalent to 1.82 kg ha^{-1} B, 1.82 kg ha^{-1} Cu, 1.82 kg ha^{-1} Mn, 7.26 kg ha^{-1} Fe, 0.36 kg ha^{-1} Mo, and 0.73 kg ha^{-1} Zn, provided by ConMicros® (Conplant, Campinas, SP, Brazil) Manual irrigation was performed as a function of soil moisture data collected from puncture tensiometers in each pot according to De Camargo et al. (2017). Soil humidity was maintained close to 100% soil field capacity for all treatments during the entire experiment.

The gas exchange, physiological traits, plant length, stalk and plant length, and fresh biomass of stalks, and green leaves were evaluated in this experiment.

Gas exchange evaluations

The gas exchange measurements were between 9 a.m. and 11 a.m. in the top visible dewlap (TVD) leaves of sugarcane using a portable infrared CO_2 analyzer (Li-6400XT, Li-Cor, NE, EUA). These evaluations were performed at 138, 145 and 152 days after transplanting (DAT), a period considered to have intense vegetative growth due to favorable temperature during the summer in south-central São Paulo state. Irradiance was adjusted at $1600 \mu\text{mol m}^{-2} \text{ s}^{-1}$ during measurements, which is an average of the sunlight in the summer of this region. The net CO_2 assimilation rate (A), stomatal conductance (gs), plant transpiration (E),

and electron transport rate (ETR) were evaluated. The intrinsic water-use efficiency (iWUE) was calculated by $iWUE = A/g$.

Physiological traits evaluations and Si content

Physiological evaluations were performed on the top visible dewlap (TVD) leaves on the same day of harvest because physiological evaluations are destructive analyses. The contents of chlorophyll *a* (*Chla*) and *b* (*Chlb*) were evaluated using extracts obtained from the immersion of sugarcane leaf discs (0.245 cm^2) in 2 mL dimethylformamide solvent (Lichtenthaler and Buschmann 2001). The absorbance (A) was read using a spectrophotometer at 664 and 647 nm. The quantification of chlorophyll was calculated using the following equations of Porra et al. (1989): $Chla = 12.00 A_{663.8} - 3.114 A_{646.8}$; $Chl b = 20.78 A_{646.8} - 4.88 A_{663.8}$.

The relative water content (RWC) was obtained using leaf discs (0.245 cm^2) collected from TVD leaves, which were measured for fresh weight (FW), saturated weight (SW), and dry weight (DW). For SW, leaf discs were maintained in deionized water for 24 h and were subsequently oven-dried at $80 \text{ }^\circ\text{C}$ for 48 h to obtain DW. Finally, RWC was estimated using the following equation: $RWC = [(FW - DW) / (SW - DW)] \times 100$.

Leaf water potential was determined between 12 p.m. and 2 p.m. using a Scholander pressure chamber (Soil Moisture Equipment, Santa Barbara, CA, USA). Electrolyte leakage (LE) was determined in a solution with 10 leaf discs from the leaves and 10 mL of deionized water. The electrical conductivity of the solution was measured at $25 \text{ }^\circ\text{C}$ for 24 h (X_i) and at $60 \text{ }^\circ\text{C}$ for 3 h (X_f) using a conductivity meter (TEC-NOPON, model mCA150, Tecnal, Brazil). The LE (%) was determined by the following equation (Campos and Thi 1997): $(X_i/X_f) \times 100$.

After physiological evaluations were performed, TVD leaves were collected, washed in water, dried, and then ground, and the Si concentration was determined according to the methodology of Elliott and Snyder (1991) modified. For Si extraction, 0.100 g of dry and ground leaves and 2 mL of H_2O_2 solution (50%, v/v) were added to 100 mL polyethylene tubes that were previously washed with 0.1 M NaOH and distilled water. After this solution was shaken for a few seconds (vortex shaking), 3 mL of NaOH (1:1)

was added. Tubes were covered with a plastic cap and autoclaved for one hour at 1.5 atm and 123 °C. Then, 45 mL of distilled water was added to increase the volume to 50 mL, and the extract was transferred to a plastic bottle and remained at rest for 12 h. For Si determination, a 1 mL aliquot of the extract supernatant was transferred to a plastic cup, 19 mL of distilled water was added, 1 ml of HCl (1:1) and 2 ml of ammonium molybdate were added, and the samples were shaken. After 5 min, 2 mL of oxalic acid was added with stirring. The reading was performed in a spectrophotometer (410 nm).

Biometric evaluations

The plant length was measured from the base to the top visible dewlap (TVD) leaf, and the stalk length was measured from the base to leaves at harvest. The diameter was measured in the center part of the stalk. Next, sugarcane plants were harvested, divided into green leaves and stalks, and the fresh weight was obtained.

Silicophytolith content and morphotype description

Three TVD leaves from 42-day-old plants of all treatments were collected for silicophytolith analyses. Silicophytolith extraction followed the Labouriau calcination technique (Labouriau 1983). The leaves were first placed in an ultrasound bath for 15–20 min and washed with distilled water to remove mineral contaminants. The samples were dried at 60 °C for 24 h, weighed (initial weight) and charred at 200 °C for 2 h. Later, the samples were boiled in a 5 N HCl solution for 10 min, washed with distilled water and filtered with ashless filter paper until no more chloride ions were detected. Finally, the material was ignited at 800 °C for 2.5–3 h. and weighed (final weight). Silicophytolith content was calculated as a percentage dry weight, following this formula: silicophytolith content = (final weight/initial weight)*100.

To analyze the silicophytolith morphotypes produced by the two cultivars under two different and extreme Si treatments (0 and 1000 kg ha⁻¹ Si), the ashes obtained after the calcination technique were mounted in immersion oil and observed under an optical microscope (Zeiss Axiostar Plus microscope) at 400× magnification. At least 250 silicophytoliths were counted and the morphologies were classified

according to International Code for Phytolith Nomenclature 2.0 (ICPT 2019). It was calculated the percentage of each morphology in each slide.

Finally, some leaf fragments were clarified with 50% (w/v) sodium hypochlorite solution and mounted in oil immersion for microscope observation at 400× magnification (Fernández Honaine et al. 2019) in order to relate the silicophytolith morphologies with the tissue origin. This technique allows the observation in situ of the cells that became silicified without destroying the tissues.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) to evaluate the effects of factors (cultivar and Si rates) and interactions among them by the F test. The results for the two cultivars were compared using Tukey's test, while Si rates were analyzed by linear and polynomial regression at a confidence level of 0.05 ($P < 0.05$). Statistical Analysis System software (SAS®, Cary, NC, USA) was used to perform the statistical analysis.

Results

Gas exchange measurements

The probability level of significance of the F test (p values) for the analysis of variance showed that Si application and sugarcane cultivar influenced ($p < 0.05$) the net CO₂ assimilation rate (A), stomatal conductance (g_s), and plant transpiration (E) taken in the TVD leaves at 138 and 152 days after transplanting (DAT), while no significant effects of treatments on the electron transport rate (ETR) at 152 DAT or gas exchange measurements at 166 DAT were found (Tables 1, 2, and 3). In addition, no interaction between Si and cultivar was found for these gas exchange measurements in these periods.

The measurements of A , g_s , E and ETR at 138 and 152 DAT were increased as a function of Si application rates (Fig. 1). The SP85-5536 cultivar showed the highest Si values of A , g_s , and E in both evaluations, and cultivars did not differ in ETR at 152 DAT (Tables 1 and 2).

Table. 1 Net CO₂ assimilation rate (A), stomatal conductance (gs), plant transpiration (E), and electron transport rate (ETR) taken at 138 days after transplanting (DAT) in leaves of two sugarcane cultivars as a function of the Si application rate

Cultivar	A μmol CO ₂ m ⁻² s ⁻¹	gs mol H ₂ O m ⁻² s ⁻¹	E mmol H ₂ O m ⁻² s ⁻¹	ETR μmol électrons m ⁻² s ⁻¹
RB86-7515				
0	12.21	0.0650	2.12	79.85
250	12.52	0.0760	2.27	88.35
500	13.74	0.0800	2.44	84.53
750	14.66	0.0899	3.05	101.53
1000	17.25	0.1009	3.26	103.55
RB85-5536				
0	16.84	0.0892	3.06	92.83
250	17.08	0.0978	3.17	101.81
500	19.11	0.1163	3.48	103.23
750	19.90	0.1224	3.66	110.68
1000	23.98	0.1538	4.42	142.68
Probability level of significance of the F test (p values)				
Cultivar(C)	0.0001*	0.0001*	0.0001*	0.0007*
Si Rates (R)	0.0005*	0.0001*	0.0001*	0.0004*
C x R	0.9450	0.4644	0.8570	0.3941
Tukey's test				
RB86-7515	14.07 b	0.0823 b	2.63 b	91.56 b
RB85-5536	19.38 a	0.1159 a	3.56 a	110.25 a
MSD	1.81	0.0116	0.32	10.46

*Significant at 5% of probability by F test. MSD=Minimum significative difference: means followed by the different letter in the column differ by Tukey's test at 95% of confidence. *n*=9 repetitions

Physiological trait evaluations

The analysis of variance by F test showed that treatments (cultivar, and Si rates) did not influence the chlorophyll *a* and *b*, carotene contents, while the relative water content (RWC), water potential (Ψ), and electrolyte leakage (EL) in the sugarcane leaves collected at harvest (166 DAT) were influenced only by cultivar (Table 4). In addition, higher values of RWC, Ψ , EL were obtained by Tukey's test for the drought-tolerant cultivar RB86-7515 compared to RB85-5536.

Biometric evaluations and Si content in leaves

The biometric evaluations and Si content in the TVD leaves were influenced ($p < 0.5$) by cultivar and Si rates independently, as shown the analysis of variance by F test (Table 5). The stalk fresh biomass, stalk length, and Si content in the TVD leaves were increased in function of Si rates (Fig. 2). In addition, higher stalk and green leaf fresh biomass and plant

and stalk length were found in RB86-7515, while higher Si contents in the leaves were found in RB85-5536 (Table 5).

Silicophytolith: contents and anatomical localization

The silicophytolith contents were only influence by the Si rates, and no difference between cultivars was found (Table 5). The silicophytolith content in the sugarcane leaves increased as a function of Si fertilization (Fig. 3A).

The analysis of the morphotypes of silicophytoliths produced in sugarcane leaves was carried out only in the treatments without Si (control, zero Si) and high Si in the experiment (1000 kg ha⁻¹ Si). Most of the cells that became silicified belonged to the epidermis (short and long cells, stomata complex, and acute bulbosus), and the results showed differences between Si treatments (Figs. 3B and 4), but not among cultivars (Table S1, S2). Sugarcane leaves with Si showed higher content of silica skeletons (fragments of

Table. 2 Net CO₂ assimilation rate (A), stomatal conductance (gs), plant transpiration (E), and electron transport rate (ETR) taken at 152 days after transplanting (DAT) in leaves of two sugarcane cultivars as a function of the Si application rate

Cultivar	A μmol CO ₂ m ⁻² s ⁻¹	gs mol H ₂ O m ⁻² s ⁻¹	E mmol H ₂ O m ⁻² s ⁻¹	ETR μmol elétrons m ⁻² s ⁻¹
RB86-7515				
0	12.96	0.0669	1.54	70.05
250	15.18	0.0703	2.25	87.90
500	16.29	0.0711	2.45	89.56
750	16.54	0.0728	2.06	80.48
1000	18.70	0.0839	2.72	104.75
RB85-5536				
0	18.08	0.0812	2.61	95.78
250	18.53	0.0832	2.29	79.60
500	17.93	0.0836	2.36	79.49
750	20.39	0.0968	2.74	81.29
1000	21.15	0.1103	3.11	113.78
Probability level of significance of the F test (p values)				
Cultivar(C)	0.0002*	0.0012*	0.0146*	0.5763
Si Rates (R)	0.0176*	0.0525	0.0352*	0.0258*
C x R	0.7280	0.8649	0.1847	0.3461
Tukey's test				
RB86-7515	15.93 b	0.0729 b	2.20 b	86.55 a
RB85-5536	19.21 a	0.0910 a	2.62 a	89.99 a
MSD	1.66	0.0106	0.33	12.24

*Significant at 5% of probability by F test. *MSD*=Minimum significant difference: means followed by the different letter in the column differ by Tukey's test at 95% of confidence. *n*=9 repetitions

silicified tissue composed by short and long cells, stomatal complexes, among others), stomatal complexes, and acute bulbosus (derived from trichomes) and lower contents of bilobates (silica short cells) than control treatment leaves (Fig. 4, Table S1, S2).

These results shows that the increment of total silicophytolith content in plants subjected to high Si treatment is a consequence of the silicification of other cells than the typical short silica cells (bilobates). In addition, other morphotypes identified were polilobates (Fig. 4) and crosses, which are silicifications deposited in short cells, and elongates derived from the silicification of long cells in the epidermal tissues (Fig. S1).

Discussion

The silicophytolith and Si contents in sugarcane leaves increased linearly as a function of silicate

fertilization. There was a corresponding increase in the net CO₂ assimilation rate or photosynthesis (A), plant transpiration (E), stomatal conductance (gs) and electron transport rate (ETR) of sugarcane as a function of the Si application rate and fresh biomass and length of stalks during the grand growth phase of sugarcane. These responses obtained as a function of Si were not variable across cultivar. An independent effect ($p < 0.05$) of cultivar was shown in stalk length, fresh stalk and green leaf biomass, relative water content (RWC), and water potential (Ψ), with higher values for RB86-7515, while RB85-5536 showed superior values for A, E, gs, ETR, and electrolyte leakage (EL). These results confirmed that Si is beneficial to plant growth processes, such as A, E, and gs, and could be related to an increased silicophytolith and Si concentrations in sugarcane leaves, leading to higher stalk length and stalk biomass, even without stress. Additionally, the analyses of silicophytolith morphotypes showed that higher values of silicified stomatal

Table. 3 Net CO₂ assimilation rate (A), stomatal conductance (gs), plant transpiration (E), and electron transport rate (ETR) taken at 166 days after transplanting (DAT) in leaves of two sugarcane cultivars as a function of the Si application rate

Cultivar	A μmol CO ₂ m ⁻² s ⁻¹	gs mol H ₂ O m ⁻² s ⁻¹	E mmol H ₂ O m ⁻² s ⁻¹	ETR μmol électrons m ⁻² s ⁻¹
RB86-7515				
0	7.64	0.0548	1.26	54.88
250	6.98	0.0566	1.35	57.84
500	8.88	0.0481	1.20	46.71
750	9.50	0.0517	1.25	49.42
1000	10.90	0.0580	1.34	45.99
RB85-5536				
0	13.29	0.0828	2.21	66.38
250	13.66	0.0918	2.02	64.90
500	15.34	0.1346	2.34	66.44
750	14.20	0.1090	2.22	67.38
1000	16.31	0.1091	2.55	79.71
Probability level of significance of the F test (p values)				
Cultivar(C)	0.0001*	0.0001*	0.0001*	0.0001*
Si Rates (R)	0.7849	0.5756	0.8037	0.3872
C x R	0.7082	0.3017	0.7733	0.8911
Tukey test				
RB86-7515	9.37 b	0.0540 b	1.28 b	49.93 b
RB85-5536	14.91 a	0.1050 a	2.27 a	70.38 a
MSD	1.74	0.018	0.286	8.57

*Significant at 5% of probability by F test. MSD=Minimum significative difference: means followed by the different letter in the column differ by Tukey's test at 95% of confidence. n=9 repetitions

Fig. 1 Net CO₂ assimilation rate (A), plant transpiration (E), stomatal conductance (gs) and electron transport rate (ETR) of sugarcane as a function of the Si application rate taken at 138 and 152 days after transplanting (DATs). The linear regressions used the mean values of two cultivars. *Significant by the F test ($p < 0.05$). Values are presented as the mean \pm standard error. n=08 repetitions

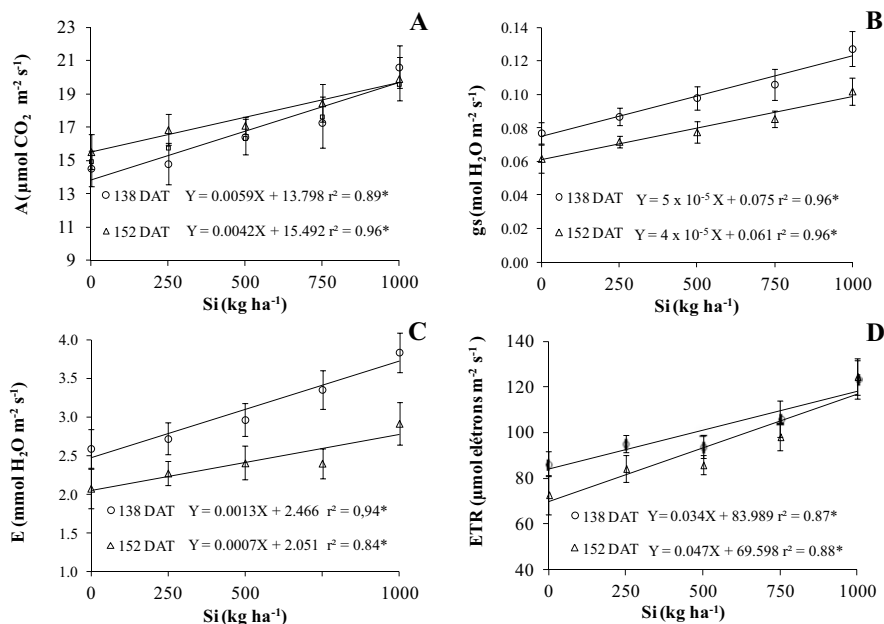


Table. 4 Chlorophylls *a* and *b* (*Chla*, *Chlb*), relative water content (RWC), water potential taken at between 12 a.m. and 2 p.m. (Ψ), electrolyte leakage (EL), and specific foliar mass (SLM), and SPAD values in leaves collected at 166 days after transplanting (DAT) of two sugarcane cultivars as a function of the Si application rate

Cultivar	<i>Chla</i> $\mu\text{g cm}^{-2}$	<i>Chlb</i> $\mu\text{g cm}^{-2}$	Carotene $\mu\text{g cm}^{-2}$	WP -MPa	RWC %	EL %
RB86-7515						
0	35.74	9.11	9.60	3.1	81.41	26.21
250	29.61	7.75	8.49	2.3	76.01	28.16
500	35.17	7.52	8.91	2.2	74.57	27.45
750	25.16	6.07	7.12	2.4	82.16	25.02
1000	32.86	8.06	8.94	1.7	77.66	24.79
RB85-5536						
0	23.96	5.58	7.03	6.1	87.68	29.68
250	32.84	8.50	8.68	4.9	78.66	30.72
500	33.75	8.49	9.04	3.5	81.34	26.80
750	36.29	9.16	9.07	3.7	82.08	28.98
1000	36.92	9.75	9.93	2.6	78.37	27.86
Probability level of significance of the F test (p values)						
Cultivar(C)	0.1253	0.0718	0.5154	0.0002	0.0372	0.0013
Si Rates(R)	0.5302	0.049	0.9855	0.2171	0.1201	0.0653
C x R	0.3401	0.1205	0.4212	0.4952	0.466	0.2230
Tukey test						
RB86-7515	40.72 a	7.70 a	8.61 a	2.32 b	78.36 a	26.33 b
RB85-5536	32.75 a	8.29 a	8.75 a	4.15 a	81.63 b	28.81 a
<i>MSD</i>	1.36	0.65	0.44	0.90	3.05	1.37

*Significant at 5% of probability by F test. *MSD*=Minimum significant difference: means followed by the different letter in the column differ by Tukey's test at 95% of confidence. $n=9$ repetitions

complexes, bilobates and acute bulbosus were produced in leaves subjected to high Si fertilization, with no differences between cultivars.

The Si concentrations in the top visible dewlap (TVD) leaves, stalk biomass production and stalk length of sugarcane were increased by Si application rates. These results were in agreement with a few studies using silicate as a source of Si in field conditions (Camargo et al. 2014; Keeping et al. 2013) and pots (Bezerra et al. 2019; Camargo et al. 2013a), which applied the same quantities of Ca and/or Mg in all treatments. Drought-sensitive RB85-5536 showed the highest Si concentration in the leaves compared to RB86-7515, independent of Si application rate. This genotypic difference in Si uptake between sugarcane cultivars is in agreement with some studies (Bezerra et al. 2019; Camargo et al. 2010; De Camargo et al. 2019; Camargo and Keeping 2021; Deren et al. 1993). Additionally, the characteristics of fast growth and rusticity of RB86-7515 (Medeiros et al. 2013) can explain the superior values of stalk biomass, green leaf biomass, and plant and stalk length compared to RB85-5536.

Si application to soil also improved the gas exchange responses (A, E, gs, ETR) of sugarcane leaves, corroborating previous studies (Bokhtiar et al. 2012; Verma et al. 2020). On the other hand, the plants in this study also demonstrated the highest silicophytolith contents with silicate fertilization, in agreement with studies on other plants (Faisal et al. 2012; Hartley et al. 2015; Henriet et al. 2006; Jones and Handreck, 1967; Ma and Takahashi 2002), including sugarcane (Bokhtiar et al. 2012; Ramouthar et al. 2016; De Tombeur et al. 2020). Although the silicophytolith content was determined at 42 DAT in the TVD leaves, Si deposition frequently started at the first developmental stage and continued during the remainder of the plant's life cycle (Alexandre et al. 2019; Fernández Honaine et al. 2013, 2016; De Tombeur et al. 2020). Therefore, the silicophytolith contents and their morphotypes could be used to explain the gas exchange response results, which were based on the same type of leaves (TVD).

The detailed analysis of silicophytolith morphotypes showed that higher values of silicified stomatal

Table. 5 Fresh biomass of stalk and green leaves, length of plant and stalks, leaf area, and Si contents on the top visible dewlap (TVD) leaves of two sugarcane cultivars as a function of the Si application rate at 166 days after transplanting (DAT)

Cultivars	Fresh biomass		Length		Si-TVD g kg ⁻¹	Silicophytolith in the leaves %
	Stalk	Green leaf	Plant	Stalk		
	g	g	cm	cm		
RB86-7515						
0	277.71	127.57	215.29	77.50	4.75	1.0
250	273.43	128.07	220.83	72.88	5.86	1.2
500	316.43	147.36	226.14	85.25	7.23	1.4
750	325.41	139.64	235.71	86.00	9.23	1.4
1000	355.50	139.50	234.00	84.88	11.36	2.6
RB85-5536						
0	256.50	128.88	218.00	79.38	6.98	0.6
250	262.92	106.71	204.14	73.14	7.84	1.0
500	269.88	107.88	204.25	74.38	9.31	1.3
750	274.56	119.63	209.38	78.75	9.81	1.6
1000	288.14	115.00	214.57	83.43	12.34	2.1
Probability level of significance of the F test (p values)						
Cultivar(C)	0.0001*	0.0001*	0.0001*	0.0400*	0.0003*	0.2428
Si Rates(R)	0.004*	0.3477	0.1027	0.0010*	0.0001*	0.0001*
C x R	0.4264	0.0505	0.5884	0.0919	0.5863	0.4735
Tukey test						
RB86-7515	309.69 a	136.43 a	226.39 a	81.30 a	7.69 b	1.52 a
RB85-5536	270.40 b	115.62 b	210.07 b	77.81 b	9.26 a	1.32 a
MSD	15.25	8.23	6.29	3.32	0.79	0.89

*Significant at 5% of probability by F test. MSD=Minimum significative difference: means followed by the different letter in the column differ by Tukey's test at 95% of confidence. $n=9$

complexes, silica skeletons, elongates and acute bulbosus were produced in leaves subjected to high Si fertilization, with no differences between cultivars. The increased silicification in acute bulbosus in relation to Si availability is in agreement to Meunier et al. (2017), who also did not find a relation with water stress and consider that these cells may act as a reservoir of excess Si. Instead, Takeda et al. (2013) associated the presence of silica in trichomes with an improvement of photosynthesis, due to a higher absorption of far-infrared light. Lastly, a higher accumulation of silica resulting in multicellular deposits (silica skeletons) may favor the erectness of leaves, also promoting photosynthesis (De Tombeur et al. 2020). As a consequence, the beneficial effects of Si fertilization on the gas exchange responses could be associated with the higher silicophytolith content due to enhancement of the position of leaves (De Tombeur et al. 2020; Kido et al. 2015; Yamamoto et al. 2012; Zanão et al. 2010).

Moreover, the straighter leaves of plants with Si fertilization improved sunlight interception (Ando et al. 2002; Zanão et al. 2010), leading to increased photosynthesis. As photosynthesis was intensified along with high carbon demand without water limitation (100% water holding capacity of soil), plant transpiration also increased, as well as the silicophytolith content, nutrient uptake by mass flow, and nutrient uptake by transport channels, which are dependent on the energy generated from this process. These results may indicate that the highest silicophytolith content observed in leaves of plants supplied with Si could be not only the consequence of a higher Si availability in the substrate (Meunier et al. 2017), but also due to the increment in plant transpiration, as suggested for other plants (Jones and Handreck 1967; Ma and Takahashi 2002; Trembath-Reichert et al. 2015).

Additionally, most stomata were open in the sugarcane leaves supplemented with Si, as shown by the increased stomatal conductance, even

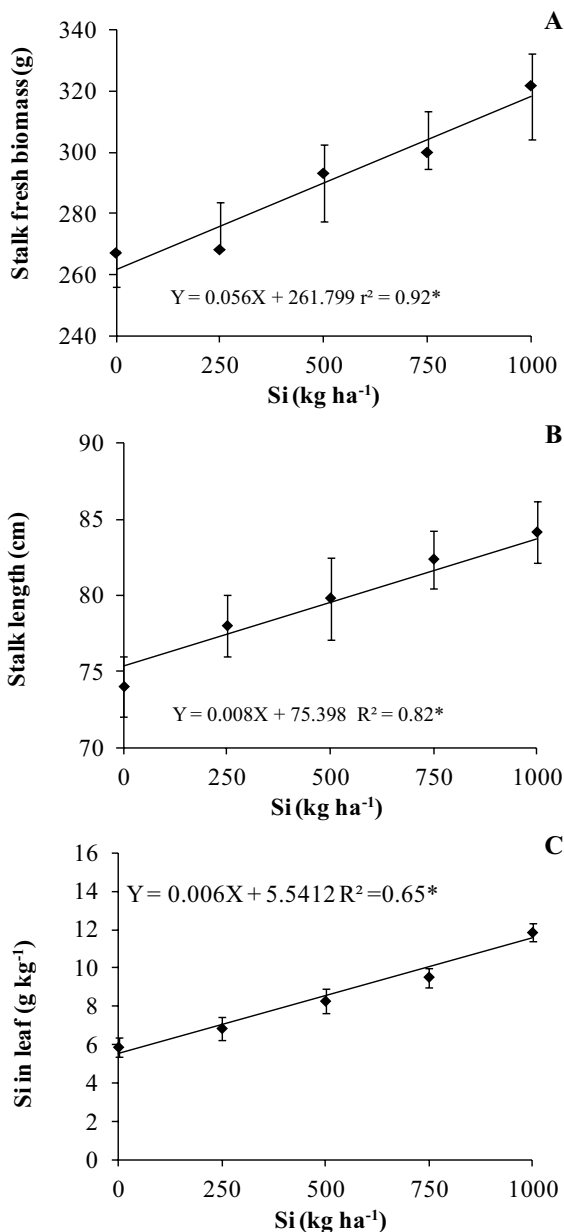


Fig. 2 Stalk fresh biomass (A), stalk length (B), and Si concentration (C) in TVD leaves of sugarcane as a function of the Si application rate. The linear regressions used the mean values of two cultivars. *Significant by the F test ($p < 0.05$). Values are presented as the mean \pm standard error. $n = 08$ repetitions

with intensification of transpiration caused by photosynthesis, because water limitation was not imposed. These results are opposed to those found by Vandeger et al. (2020) who described

a reduction in the stomatal conductance in plants subjected to a Si treatment. They also observed that Si deposits occur in stomatal guard cell and this may contribute to a decrease of stomatal opening capacity. In our study, the increased stomatal conductance under high Si availability was also accompanied with a higher deposition of silica in stomata complexes. It can be proposed that in our study silicophytolith accumulation in stomata also affects stomatal movement, but in this case, the deposit allows the stomata to remain open, increasing the gas exchanges. However, more studies are needed in order to understand the effect of the silica deposition in stomata in physiological processes.

Furthermore, silicification is a passive process that is a consequence of transpiration or senescence in several cells (Alexandre et al. 2019; Kumar and Elbaum 2018; Kumar et al. 2017a, b). However, the silicification of bilobates (short cells) commonly occurs by metabolic control, and there has not yet been any report describing the specific role of bilobates in plants (Strömberg et al. 2016). Therefore, the highest silicophytolith content observed in bilobates under a high Si application rate in this study could be interpreted as a mechanism for secreting excess silica; however, this process was not associated with a specific function in sugarcane leaves.

In contrast to gas exchange measurements, physiological aspects in the sugarcane leaves collected at 166 DAT were only influenced by cultivar. The relative water content (RWC) and water potential (Ψ) showed values superior to RB86-7515, while electrolyte leakage (EL) values were found in RB85-5536. These results were in agreement with the rusticity of RB86-7515 (Medeiros et al. 2013), leading to the highest RWC and Ψ and the lowest EL. In addition, the absence of stressful conditions could justify the lack of influence of Si on the physiological aspects of sugarcane during the experiment. Most studies have shown that Si effects are potentialized under environmental stress. For example, Si fertilization promoted alterations in Ψ (Ahmed et al. 2014; Chen et al. 2011; Ming et al. 2012; Shen et al. 2010), decreased EL (Agarie et al. 1998; Shen et al. 2010), and increased RWC (Gong et al. 2005; Kaya et al. 2006), chlorophyll content, and photosynthesis (Gong et al. 2005) in plants grown under water deficit compared to well-watered conditions.

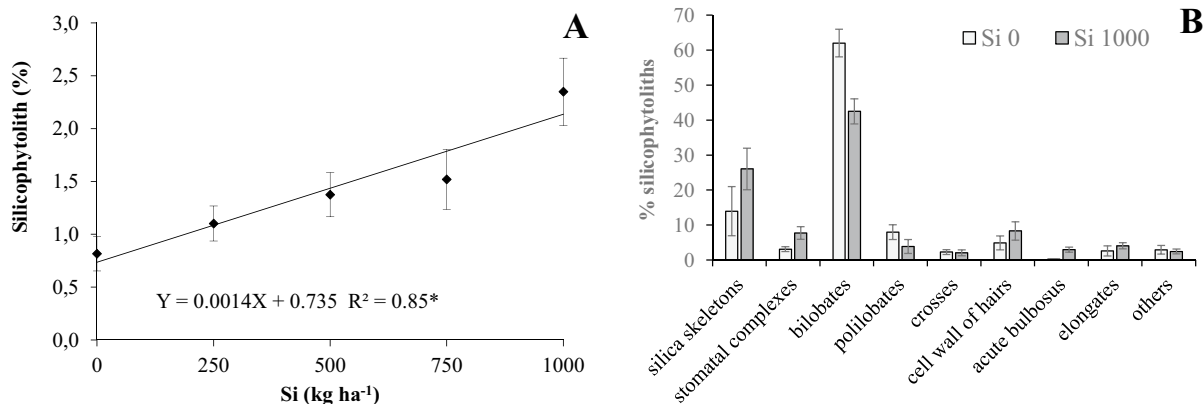
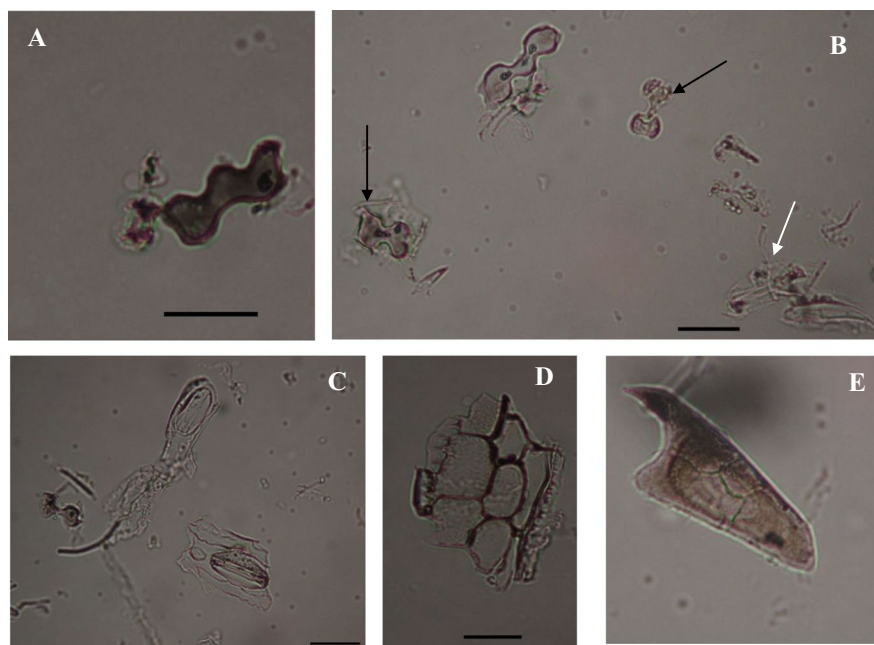


Fig. 3 **A** Silicophytolith content (% dry weight) as a function of the Si application rate. **B** Percentage of the main silicophytolith morphologies described in leaves subjected to different Si treatments (0 and 1000 kg ha⁻¹ Si). The linear regression

applied to silicophytolith content used the mean values of two cultivars. *Significant by the F test ($p < 0.05$). Values are presented as the mean \pm standard error. $n = 03$ repetitions

Fig. 4 Silicophytoliths obtained after the calcination technique in the sugarcane leaves of drought-tolerant (RB86-7515) and drought sensitive (RB85-5536) cultivars grown without Si (**A, B**) and with Si application (**C, D, E**). **A** Polilobate in cultivar RB86-7515. **B** Polilobate, bilobates (black arrow) and stomata (white arrow) in cultivar RB86-7515. **C** Stomatal complex and bilobate in cultivar RB86-7515. **D** Silica skeleton in cultivar RB85-5536. **E** Acute bulbosus in cultivar RB85-5536. Bar 25 μ m. (silicophytoliths $n = 3$)



Finally, this study showed that Si fertilization provided benefits in sugarcane plants independent of cultivars grown under well-watered conditions. Enhanced photosynthesis, transpiration, and silicophytolith contents of leaves as a function of Si fertilization resulted in increased stalk length and stalk biomass production. Moreover, Si supply in low Si plant-available content soil provided higher silicification in the stomatal complexes, acute bulbosus

(trichomes) and lower in short cells (bilobates) compared to those plants without Si application.

Conclusions

Si fertilization improved photosynthesis, transpiration, stalk length, and stalk biomass production in sugarcane. The highest silicophytolith content was

reflected in a diversity of silicified cells, which may favor a higher photosynthesis and biomass. The increase of silicification in stomata complexes and trichomes with Si may be associated to a higher Si availability and transpiration. Contrasting drought-tolerant cultivars showed similar silicification and gas exchange responses with Si. Considering these benefits, Si should be included in the fertilization program of sugarcane.

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Author's contributions MSC planned, designed, and carried out the experiment, analyzed and interpreted the data, and was responsible for the manuscript preparation. MHF, MO, MLB analyzed, and interpreted the phytolith data. NGB carried out the experiments, and prepare all plant samples to chemical analysis. VMS took all physiological measurements. MAS interpreted the physiological data. MSC, MHF, MO, MLB authors commented on draft and approved the final version of the manuscript.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest.

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