Silicate nutrition and its biological mechanisms involved in water use efficiency in sugarcane and energy cane crops under water deficit

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Abstract

Sugarcane and recently energy cane strengthen the world's renewable bioenergy production capacity. Notwithstanding, climate change has resulted in droughts that limit their growth. One of the strategies to attenuate water deficit damage in these crops concerns the contribution of silicate nutrition to physiology. This strategy is likely to increase water use efficiency, thus favoring the sustainability of these crops. However, studies on this issue are still incipient. This study assesses whether Si applied via fertigation and foliar spraying in the seedling production phase and as a complement after transplanting to the soil is efficient in attenuating water deficit effects in sugarcane and energy cane. The study further elucidates the physiological and biochemical mechanisms involved in this process. For this, the authors conducted two experiments, one with sugarcane and the other with energy cane. The treatments consisted of a 2 x 2 factorial scheme, with absence (-Si) and presence of Si (+Si) applied via fertigation and foliar spraying; and two water regimes: 70% (no deficit) and 30% (severe water deficit) of the soil water retention capacity. The treatments were arranged in randomized blocks with 5 replications. Silicon supply occurred during the formation phase of pre-sprouted seedlings and during their transplanting to pots filled with samples of Entisols (Quartzipsamments). In the latter, water regimes were induced from 7 to 160 days after transplanting. Severe water deficit reduced the water content and water potential of plants, inducing oxidative stress and impairing gas exchange and photosynthetic water use efficiency, with a consequent reduction in plant growth. Silicon supply via fertigation in association with foliar spraying in the seedling formation phase with complementation after transplanting was efficient in increasing the accumulation of the element in the plants. Silicon was effective in attenuating severe water deficit damage until initial culm formation through mechanisms that maintain water and physiological balance by favoring the antioxidant defense system in sugarcane and energy cane plants.

1. Introduction

Drought is one of the factors that most limit agricultural production ¹. This condition has worsened recently, with estimates of future increase due to climate change ², reducing sugarcane yield. This effect is important because the sugarcane growing area has expanded and currently occupies more than 100 countries ³ for being a source of sustainable bioenergy, helping to reduce greenhouse gas emissions ⁴. In this scenario, irrigated cultivation of sugarcane has expanded, especially in top producing countries such as Brazil ⁵, China ⁶, and India ⁷. Thus, reducing consumption and increasing the economic use of water to ensure adequate resources and sustainable agricultural production is of great importance ⁸.

A strategy to reduce water deficit damage in crops would be the use of silicon (Si). Silicon is the most abundant element in soil after oxygen, as silicon dioxide (SiO₂) comprises 50-70% of the soil mass ⁹. In the solution, however, the available Si is in the form of monosilicic acid (H₄SiO₄) at low concentration (~1 mmol L⁻¹) ¹⁰. Silicon is a beneficial element absorbed in the form of H₄SiO₄, which remains in the same form in the xylem sap and reaches the leaves through the transpiration gradient ¹¹. It concentrates in leaf

tissue in polymerized form as amorphous and biogenic silica, constituting 90% of the absorbed Si, being contained in Si-cellulose structures present in the cell wall ¹².

The beneficial effects of Si on plants under water deficit prevent leaf water imbalance, as deposits of this element on cell walls reduce water loss by transpiration ¹³. Silicon also induces an osmoregulatory response by modifying the proline content that adjusts root hydraulic conductivity ¹⁴, and stimulates the activity of enzymes such as aquaporin ¹⁵, increasing water absorption. The role of Si in regulating enzymatic ¹⁶ and nonenzymatic ¹⁷ antioxidant defense systems favor plant tolerance to drought damage. Thus, by decreasing oxidative stress, Si preserves and increases photosynthetic pigments ¹⁸ and maintains high photochemical efficiency ¹⁹. This should increase the duration of active green leaves and, consequently, photosynthetic rate and plant growth. However, further studies are necessary to prove this hypothesis.

A high uptake of the element is important for obtaining a biological benefit of Si in the plant. This can occur with the use of calcium silicate; however, it needs to be incorporated into the soil at high doses (1000 kg ha⁻¹ of Si) to favor its dissolution ²⁰. Another way would be from the use of high solubility sources (potassium silicate or sodium silicate). Soluble sources allow use via fertigation, which could reduce Si polymerization rates in the soil. Application includes low doses of this element per area, but which guarantee its adequate absorption by the plant. This is because fertigation allows the use of a solution with concentrations below 3 mmol L⁻¹ Si, avoiding the occurrence of polymerization ²¹. Previous studies have shown the efficiency of Si fertigation in optimizing the absorption of this element in forage plants ²² and in pre-sprouted sugarcane seedlings ¹⁷. However, there is a lack of studies in plant cane, especially in phases with high relative growth rates, which can occur between 84 and 135 days after transplanting ⁷ depending on genotype and growing conditions ²³.

Studies on Si focus on sugarcane and address physiological parameters only, without demonstrating whether there are additive effects of biochemical mechanisms. As far as we know, there are no reports on energy cane plants. However, water deficit induces oxidative stress, increasing the production of reactive oxygen species (ROS) ²⁴. In addition, there are indications of increased planting of sugarcane and energy cane in areas with greater occurrence of water deficit, which require a greater need for supplementary irrigation ⁵. This strengthens the importance of using Si while collaborating with the rational use of water for irrigation, which is a finite resource ²⁵.

It is thus evident that the effects of Si application via fertigation in association with foliar spraying during the production of pre-sprouted seedlings with complementation after their transplanting to the soil have not yet been described. The same can be said for the effects of Si application on the initial formation of sugarcane and energy cane culms. However, it is important to evaluate two strategies involving Si optimization in crops. One would be the use of this element in its soluble form via fertigation as mentioned above. The other would be the use of Si from the formation of pre-sprouted seedlings as well as after transplanting to the soil. These strategies could maximize the biological benefits of this element

in the plant to mitigate severe water deficit that normally occurs in the field for sugarcane and energy cane, but further studies are needed.

Therefore, it is important to verify the following hypotheses: i) Si fertigation in association with foliar spraying in the seedling formation phase, with complementation after transplanting ($< 20 \text{ kg ha}^{-1} \text{ Si}$), provides high accumulation of this element in the plants; ii) Si promotes benefits by inducing enzymatic (catalase (CAT), superoxidase dismutase (SOD), and ascorbate peroxidase (APX) and nonenzymatic (phenols and proline) antioxidant defense mechanisms, reducing oxidative stress, which would favor physiological aspects including increased water content and use efficiency, pigments, photosynthesis, and consequently tillering, number of green leaves, and dry mass production of the two species under study; iii) Si is efficient in attenuating severe water deficit damage up to the initial formation of culms of sugarcane and energy cane.

To test these hypotheses, we conducted two experiments, one with sugarcane and the other with energy cane. The aim was to evaluate whether Si applied via fertigation in association with foliar spraying in the seedling production phase with complementation after transplanting to the soil is efficient in attenuating water deficit effects in sugarcane and energy cane, as well as to elucidate the physiological and biochemical mechanisms involved.

The acceptance of the hypotheses of this study enables understanding the synergistic mechanisms between Si and the physiological and biochemical aspects of sugarcane and energy cane. This would allow using the benefits from mineral nutrition of plants with Si in favor of more efficient use of water, with environmental and agronomic gains, by favoring the more sustainable establishment of these species even in drought regions.

2. Material And Methods

2.1 Plant material and growing conditions

Two experiments were carried out simultaneously in a greenhouse at the UNESP from January to September 2019. The temperature and relative humidity inside the greenhouse were recorded daily with the aid of a thermohygrometer (U23-001, Sigma Sensors, Brasil) (S 1).

Pre-sprouted seedlings of two species were used: *S. officinarum* L. (sugarcane - variety RB 966928 - experiment I) and *S. spontaneum* L. (energy cane - variety VX2 - experiment II)). The variety of energy cane used was obtained from Vignis® and classified as type II due to the high fiber content ($>28\%$) and low sugar content in the form of sucrose ($<6\%$) ²⁶.

The experiments were carried out in two stages. The first consisted of the formation of seedlings cultivated in pots filled with inert substrate. During this period, part of the seedlings received treatments with Si from 10 to 70 days after sprout emergence, while others did not receive Si. The second phase consisted of transplanting these seedlings to pots filled with samples of a Entisols (Quartzipsamments).

During this period, part of the seedlings received treatments with Si up to 80 days after transplanting and the application of water regimes took place from 7 to 160 days after transplanting with the completion of the experiments.

2.2 Treatments and experimental design

Treatments were arranged in a 2 x 2 factorial scheme in both experiments. The first factor consisted of whether or not Si was supplied: absence (-Si) and presence of Si (+Si). Si was applied via fertigation and foliar spraying during seedling formation and also after transplanting. Applications were combined with two soil water regimes: 70% (no deficit - control) and 30% (severe water deficit - WD) of the soil water retention capacity (WRC), which were applied only after the transplanting the crops. The plots were arranged in randomized blocks with 5 replications.

The pre-sprouted seedlings were produced in the first phase of the experiment. To this end, budded setts (5 ± 0.5 cm) were planted in seedling production trays filled with fine vermiculite. The nutrient solution used was Hoagland and Arnon ²⁷ with a change in iron concentration to $368 \mu\text{mol L}^{-1}$ in the source of Fe-EDDHA, as indicated by Cavalcante et al. ²⁸. To avoid salinization of the substrate, the concentration of the nutrient solution during the first week of cultivation was maintained at 25% dilution and increased to 50% on the second week until the end of the seedling formation phase. To determine the volume of solution to be applied, a saturation test was previously carried out, with a volume of 10 mL per cell sufficient to saturate the substrate, avoiding loss by leaching. The pH value of the solution was adjusted at 5.5 ± 0.2 with the use of solution of Hydrochloric acid (HCl) or Sodium hydroxide (NaOH), both at 1 mol L^{-1} .

The source of soluble Si used was sodium and potassium silicate stabilized with sorbitol (113.4 g L^{-1} of Si, 18.9 g L^{-1} of K_2O , 100 mL L^{-1} of sorbitol and pH 11.8) at the concentration of 2.5 mmol L^{-1} . This concentration of Si used does not induce polymerization as it only starts at a concentration greater than 3 mmol L^{-1} ²¹. Fifteen Si applications were performed at 4-day intervals, starting 10 days after full emerged sprouts (DAE) (S 1), with application of 10 mL per seedling via fertigations in substrate (to induce root uptake) and 1.5 mL per seedling via foliar spraying (to induce foliar uptake). The pH value of the Si solution was adjusted at 5.5 ± 0.2 . The amount of potassium present in the Si source was balanced in the treatments without the element, using a 1 mol L^{-1} potassium chloride solution with root application and foliar spraying. In each condition of Si supply (+Si and -Si) 50 seedlings of each species were used, selecting those with the most uniform length and diameter for later transplanting.

After fertilization with Si, at 70 DAE, the second phase of the experiment started. On that occasion, 20 dm^3 pots (surface area = 962 cm^2) were filled with samples of Entisols (Quartzipsamments) ²⁹ collected from the superficial horizon (Ap). The chemical analysis of the soil was carried out for fertility purposes, according to the method described by Raij et al. ³⁰. The following results were found: pH (CaCl_2): 4.3, organic matter: 9 g dm^{-3} , P (res): 2 mg dm^{-3} , B: $<0.12 \text{ mg dm}^{-3}$, Cu: 0.2 mg dm^{-3} , Fe: 9 mg dm^{-3} , Mn: 1.7 mg dm^{-3} , Zn: 0.4 g dm^{-3} , Ca: 3

mmol_c dm⁻³, Mg: 1 mmol_c dm⁻³, K: 0.3 mmol_c dm⁻³, H+Al: 16 mmol_c dm⁻³, Sum of bases (Ca+Mg+K) (SB): 4 mmol_c dm⁻³, Cation exchange capacity (CEC): 20.3 mmol_c dm⁻³ and Base saturation (V: SBx100/CEC): 21%. Si content was 1 mg dm⁻³, determined by using calcium chloride at 0.01 mol L⁻¹ as an extractor according to the method of Korndörfer et al. ³¹.

Thirty days before the transplanting of the seedlings, limestone was applied (relative power of total neutralization: 125%, CaO: 48%, MgO: 16%) to raise the V to 60%, and the soil sample was properly mixed and sustained at 70% WRC to induce the limestone reaction. After this period, fertilizer was applied to the soil using 250 mg dm⁻³ of N and K and 150 mg dm⁻³ of P in the sources of ammonium sulfate, potassium chloride and triple superphosphate, respectively. The triple superphosphate was applied in a single dose and incorporated into the soil volume. The application of N and K was carried out via fertigation in five doses of 50 mg dm⁻³ starting four days after transplanting with seven-day intervals. It was also applied 5 mg dm⁻³ of Zn in the form of zinc sulfate and 2 mg dm⁻³ of B as boric acid via fertigation, applied in a single dose in the first application of fertilizers N and K.

In the transplant, the seedlings had six fully developed leaves and the cut was made at 30 cm from the sheath of the first newly developed leaf, that is, almost one-third of the leaves were removed. This is a common practice seedling nurseries with the objective of decreasing the loss of water vapor by transpiration when transplanting them to soil ³². Then, the seedlings were transplanted to pots filled with soil and sustained in 70% WRC for seven days and then subjected to soil water regimes.

After transplanting, five more fertigations with Si were carried out with soil application and foliar spraying at 20, 35, 50, 65 and 80 days after transplanting (S 1). The application of Si via fertigation was performed by simulating a 5 mm irrigation blade of silicate solution, with 481 mL of solution being applied via roots per pot considering an area of 962 cm². The concentration of Si used was 2.5 mmol L⁻¹, corresponding to 33.7 mg of Si per pot, equivalent to 3.5 kg ha⁻¹ of Si per application. The foliar spraying was carried out using a manual sprayer out to ensure coverage of the leaf, without any runoff. The pH value of the fertigated solution was adjusted to 5.5±0.2. The amount of potassium present in the Si source was equilibrated in plants that did not receive solution of the element, using a 1 mol L⁻¹ potassium chloride solution with application via root and foliar spraying.

The soil water regimes were determined on the basis of the microporosity values obtained by the tension table method with a 60 cm water column. To this end, undisturbed soil samples were collected using a volumetric ring with volume (v) equal to 98.125 cm³, subsequently the samples were saturated with water for 24 h, then placed on the tension table for 72 h and mass was determined (a); after that, the samples were dried in an oven at 110°C for 24 h and the mass was determined again (b). The total microporosity (Mi): ((a-b)/v) found was equivalent to 100% WRC ³³. However, the ideal water condition was 70% of this value, because it allows 70% of the micropores to be filled with available water and the remaining 30% with air, maintaining gaseous exchange of the roots ³⁴. Thus, the water regimes in the soil were: no water

deficit (control) (70% WRC) and severe water deficit (WD) (30% WRC) condition for sugarcane as indicated by Teixeira et al. ³⁵.

Irrigation management was carried out daily so that the soil moisture, within each treatment, the minimum possible variation, that would be insignificant to alter the biological response of the plant. Thus, the mass of soil used to fill each pot was strictly controlled, in addition, the mass of the pots was also defined to discount from the total mass. From this, the mass restoring method was used, considering the water losses by evaporation of the soil and transpiration of the plant, controlled daily by weighing the pots. This ensured that the plants were maintained at the levels of WRC proposed in the treatments, as adjustments were made in all pots. The same irrigation frequency was used for both species and for the irrigation treatments, that is, all were weighed daily.

At the time of water replacement, the 70% WRC pots were approximately 60% and the 30% WRC pots had 25%. It is justified that water variation in the pots of 70% WRC was greater because the losses by transpiration were greater due to plant growth. In addition, irrigations have always carried out at the same time of day (at 5 p.m.). Preliminary tests were carried out and the water loss was more accentuated between the period of 2–5 p.m. hours, therefore, although there was variation, it only occurred during a period of 3 h a day.

The application of Si in treatments with water deficit was terminated 160 days after transplanting the seedlings to pots with soil (initial culm formation) and the plants were evaluated as described below.

2.3 Analyses

2.3.1 Quantum efficiency of Photosystem II (Fv/Fm)

The quantum efficiency of PSII were performed between 8 a.m. and 1 p.m., on the first fully developed leaf, using a portable fluorometer (Os30P+, Opti-Sciences Inc., USA) ³⁶.

2.3.2 Chlorophyll and carotenoid content

Five leaf discs of 15 mg were collected in the middle third of the leaf blade of the first fully developed leaf. The readings were performed on a spectrophotometer (DU640, Beckman, USA) at 663 nm for Chlorophyll a (Chl *a*), 647 nm for Chlorophyll b (Chl *b*) and 470 nm for carotenoids, according to the methodology proposed by Lichtenthaler ³⁷. The content was determined on the basis of fresh matter.

2.3.3 Photosynthetic parameters

Photosynthetic parameters were assessed in the middle third of the first fully developed leaf, avoiding the midrib. Gas exchange parameters were measured with a portable photosynthesis analyzer (LcPro-SD, ADC BioScientific Ltd., Hoddesdon, UK). Data were collected in the morning, between 9 and 11 a.m. Gas exchange measurements were taken at a constant light intensity of 1,800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ emitted by a blue-

red LED light source, under natural CO₂ conditions (403-428 ppm). Leaf temperature was maintained at 30±0.5°C.

Net photosynthesis rate (A), leaf transpiration (E), stomatal conductance (Gs) and intracellular CO₂ concentration (Ci) were determined after stabilization (3-5 min). The instantaneous Water use efficiency (WUE) was calculated by the ratio between A and E and the Instantaneous carboxylation efficiency (ICE) was calculated by the ratio between A and Ci.

2.3.4 Electrolyte leakage index

Ten leaf discs (26.4 mm² each) were collected from the first fully developed leaf in two periods (at 5 a.m. and 2 p.m.). The discs were emerged in deionized water for 2 h and the Electrical conductivity (EC₁) of the solution was read using a conductivity meter (AK51, Akso, Brazil). The samples were autoclaved at 121°C for 20 min, and final Electrical conductivity (EC₂) was determined after cooling. The electrolyte leakage index was determined considering the formula: $EC_1/EC_2 \times 100$ ³⁸.

2.3.5 Leaf water potential (Ψ_w)

Leaf water potential (Ψ_w) was determined in the middle third of the blade of the second fully developed leaf with a Scholander pressure chamber (3000F01, Soil Moisture Equipment, USA). Pressure was applied until exudation occurred because of the cut made on the leaf petiole ³⁹. The measurements were evaluated between 5 a.m. and 1 p.m.

2.3.6 Relative water content

Ten discs (26.4 mm²) were collected from the first fully developed leaf in two periods (at 6 a.m. and 2 p.m.). The discs were immediately weighed to determine Fresh mass (FM). Subsequently, the samples were rehydrated in deionized water for 6 h to obtain Turgid mass (TM) and dried in a forced air circulation oven (TE-394/3-MP, Tecnal, Brazil) at 80°C for 24 h, to determine Dry mass (DM). The values were determined by the equation proposed by Barrs and Weatherley ⁴⁰: $[(FM-DM)/(TM-DM)] \times 100$.

2.3.7 Phenols content

Total phenols content were extracted using 0.1 g of fresh second fully developed leaf collected, which were immediately weighed and immersed in concentrated methanol and sustained in the dark for 3 h. After extraction, a colorimetric reaction of total phenols was induced with the 2 N Folin-Ciocalteu reagent, allowing reacting for three minutes, and 20% sodium carbonate, allowing reacting for two hours. In the end, the absorbance was read on a spectrophotometer (B442, Micronal, Brazil) at a wavelength of 765 nm, while the content was determined using a standard curve with gallic acid, expressed as gallic acid equivalent (GAE) 100 g⁻¹ ⁴¹.

2.3.8 Proline content

Proline content was determined in samples from the third fully developed leaf that were collected and immediately stored in a freezer at -80°C. The ninhydrin reaction was used following the method proposed by Bates et al.⁴². Proline concentration was measured at 520 nm in a spectrophotometer (Beckman DU 640). The values were estimated using a standard curve, being expressed in $\mu\text{mol g}^{-1}$ of FM.

2.3.9 Hydrogen peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) content was determined in samples of the third fully developed leaf that were collected and immediately stored in a freezer at -80°C. The reaction with potassium iodide, following the method of Alexieva et al.⁴³. The material was read on a spectrophotometer (DU640, Beckman, USA) at 390 nm, and H_2O_2 content was determined using a standard curve. The results were expressed in $\mu\text{Mol g}^{-1}$ of FM.

2.3.10 Lipid peroxidation

Lipid peroxidation was determined in samples from the third fully developed leaf that were collected and immediately stored in a freezer at -80°C. The estimate of the content of substances reactive to thiobarbituric acid, as described by Heath and Packer⁴⁴. Malondialdehyde (MDA) concentration was determined on a spectrophotometer (DU640, Beckman, USA) at 535 and 600 nm. The data were calculated using an extinction coefficient of $1.55 \times 10^{-5} \text{ mol}^{-1} \text{ cm}^{-1}$ ⁴⁵, and were expressed in nMol g^{-1} of FM.

2.3.11 Antioxidant enzymes

For enzymatic analysis, protein extracts were initially obtained as described by Gomes-Junior et al.⁴⁶ using samples from the third fully developed leaf that were collected and immediately stored in a freezer at -80°C. They were macerated in liquid N_2 and homogenized in potassium phosphate buffer containing ethylene diaminetetraacetic acid, dithiothreitol and polyvinylpolypyrrolidone. The homogenate was centrifuged, and the supernatant was stored in aliquots at -80°C. The concentration of total proteins was determined according to the method of Bradford⁴⁷.

2.3.11.1 Catalase (CAT, EC 1.11.1.6)

Catalase activity was determined using protein extracts with the method described by Kraus et al.⁴⁸ with the modifications of Azevedo et al.⁴⁹. It was determined by monitoring the degradation of H_2O_2 at 240 nm for 1 min. The results were expressed in $\mu\text{mol min}^{-1} \text{ mg}^{-1}$ of protein.

2.3.11.2 Superoxide dismutase (SOD, EC 1.15.1.1)

Superoxide dismutase activity was determined according to Giannopolitis and Ries⁵⁰, based on the measurement of inhibition of the photochemical reduction of blue nitro chloride tetrazolium, carried out in

a reaction chamber illuminated by a 15W fluorescent lamp, at 25°C. The measurement was performed using a spectrophotometer at 560 nm, and the results were expressed in SOD U mg⁻¹ protein.

2.3.11.3 Ascorbate peroxidase (APX, EC 1.11.1.11)

Ascorbate peroxidase activity was determined by monitoring the oxidation rate of ascorbate at 290 nm, following the method of Moldes et al. ⁵¹. The results were expressed in nMol of ascorbate min⁻¹ mg⁻¹ of protein.

2.3.12 Number of tillers, number of green leaves, percentage of dry leaves and leaf area

The number of tillers and number of green leaves were determined by counting. The percentage of dry leaves was obtained from the ratio of the number of dry leaves by the total number of leaves (dry + green), multiplied by 100. Leaf area was measured using a leaf area meter (L-3100, LiCor, USA).

2.3.13 Dry mass production

The plants were separated into leaves and culms, then washed in running water with a detergent solution (0.1% v/v), HCl solution (0.3% v/v) and deionized water. The plant material was dried in a forced air circulation oven (TE-394/3-MP, Tecnal, Brazil) (65±5°C) to constant mass, in order to obtain the dry mass values of each part of the plant.

2.3.14 Si analyses

Si content in the leaves and culms were determined by extracting the element according to the methodology described by Kraska and Breitenbeck ⁵² and reading was performed using a spectrophotometer (B442, Micronal, Brazil) at 410 nm, as indicated by Korndörfer et al. ³¹. Si accumulation in the leaves and culms of the plants was calculated on the basis of Si content and dry mass.

2.4 Statistical analysis

The experiments were analyzed independently for both species. To normalize the variance, the percentage data of electrolyte leakage index, percentage of dry leaves and relative water content, was transformed to the arc sine = . The data underwent bidirectional analysis of variance by the F-test (p<0.05) after satisfying the premises of normality (Shapiro-Wilks W test) and homogeneity of variances (Bartlett test).

Factorial analysis was used for testing the main effects of silicon (Si) supply and soil water regime (WD) and their interactions (Si × WD), where N was 20 experimental units, in each experiment. The means were compared by Tukey's test at 5% probability using the SAS® statistical software (Cary, NC, USA).

3. Results

In sugarcane, Si application in the seedling formation phase with complementation after transplanting increased the accumulation of this element in the leaves and culms by 155 and 280% in the controlled water regime, and by 119 and 400% in the water deficit regime, respectively (Fig. 1a, c). In energy cane, +Si also increased the accumulation of this element in leaves and culms by 155 and 358% in the controlled water regime, and by 125 and 225% under water deficit, respectively, in relation to -Si (Fig. 1b, d).

Figure 1

Sugarcane had higher Si accumulation in the culms than in the leaves of plants that received Si fertigation in association with foliar spraying (Fig. 1a, c). On the other hand, energy cane plants that received Si application had greater accumulation of the element in the leaves than in the culms (Fig. 1b, d). The severe water deficit regime reduced Si accumulation in the leaves and culms of sugarcane under both Si supply conditions (Fig. 1a, c). In energy cane, water deficit in relation to the controlled water regime decreased Si accumulation in the leaves and culms of plants under +Si, and only in the leaves of plants under -Si (Fig. 1b, d).

The Ψ_w assessed at dawn (at 5 a.m.) decreased under water deficit in sugarcane (Fig. 2a) and energy (Fig. 2c) cane plants grown under -Si in relation to the controlled water regime. However, Si supply to the plants increased Ψ_w in the water deficit condition in relation to -Si, even remaining similar to that of plants of both species grown under adequate water conditions (Fig. 2a, c). When Ψ_w was measured in the hottest period of the day (at 1 p.m.), the imposition of water deficit decreased this parameter in relation to the controlled water regime, in both +Si and -Si, for both species (Fig. 2b, d).

Figure 2

In -Si, water deficit decreased relative leaf water content in the assessments at 6 a.m. and 2 p.m. in sugarcane and energy plants in relation to the adequate water regime. Silicon enrichment in plants of both species reduced leaf water content losses in the assessments at 6 a.m. and 2 p.m. in the severe water deficit regime, with values even similar to those of unstressed plants, except for the assessment at 6 a.m. in sugarcane (Fig. 2e-h).

The quantum efficiency of PSII, expressed by the F_v/F_m values assessed at 8 a.m. and 1 p.m., was lower in the condition of severe water deficit in plants of both species that did not receive Si supply in relation to the controlled water condition (Fig. 2i-l). Silicon application in relation to its absence increased the efficiency of PSII in sugarcane and energy cane plants under water deficit, being similar to that of plants under controlled water regime in the two evaluation periods (Fig. 2i-l).

In -Si, water deficit in relation to the controlled water regime increased the cellular electrolyte leakage index in the assessments at 5 a.m. and 2 p.m., for both species. However, Si supply in relation to its absence prevented an increase in electrolyte leakage index in sugarcane and energy cane plants under

severe water deficit in the two evaluation periods (Fig. 2m-p). This result is similar to that of sugarcane (Fig. 2m, n) and energy cane (Fig. 2o, p) plants treated without water deficit.

In sugarcane plants grown under water deficit and -Si, Chl *a* content remained unchanged, and Chl *b* content increased in relation to the values of plants grown under controlled water condition and -Si (Fig. 3a, b). Moreover, Chl *a* and Chl *b* contents increased in sugarcane plants grown without Si application under severe water deficit in relation to adequate water regime (Fig. 3c, d). Notwithstanding, the supply of the element increased Chl *a* and Chl *b* contents in plants of both species under severe water deficit in relation to -Si in the same water regime (Fig. 4a-d).

Figure 3

Comparing with the controlled water regime, in plants that did not receive Si, the imposition of water deficit to the soil also increased carotenoid content in energy cane, although this variable did not change in sugarcane under these conditions. However, plant nutrition with the beneficial element increased the content of this pigment in both species under water restriction in relation to those grown under controlled water regime (Fig. 3e, f).

The water deficit condition decreased A in relation to the controlled water regime in sugarcane and energy cane plants that did not receive Si. However, under water deficit, Si supply reduced A losses in the plants, with values even similar to those of plants of both species under controlled water conditions (Fig. 4a, c).

Figure 4

Moreover, Gs increased in plants of both species grown without Si fertilization under water deficit in relation to water sufficiency. Notwithstanding, Si supply decreased the Gs of sugarcane and energy cane plants under severe water deficit, being similar to that of plants without water stress (Fig. 4b, d).

Regarding -Si, plants grown under water deficit had higher E than plants grown under controlled water conditions. However, Si enrichment in plants decreased the E of both species under water deficit in relation to the absence of application of the element. This Si-induced decrease reduced transpiration rates in sugarcane, while energy cane plants had values similar to those of unstressed plants (Fig. 4e, g).

In addition, Ci decreased in sugarcane and energy cane plants under water deficit in relation to those under the controlled water regime in -Si. However, Si supply increased the Ci of sugarcane plants under water deficit in relation to those that did not receive the element. This increase was enough for their Ci concentration to equal that of unstressed plants (Fig. 4f, h).

The WUE of plants grown without Si application decreased in sugarcane and energy cane plants under water deficit in relation to plants grown in an adequate water regime. However, WUE benefited from Si supply in plants under water deficit, increasing by 123 and 15.2% in sugarcane and energy cane, respectively, in relation to plants under -Si (Fig. 4i, k).

Plants of both species grown without Si supply had lower ICE in the water deficit regime than in the controlled water regime. However, nutrition with Si increased ICE in plants under water deficit in relation to -Si, being even similar to that of plants of both species without water stress (Fig. 4j, l).

Absence of Si supply increased H_2O_2 content (Fig. 5a, b) and lipid peroxidation, expressed by the MDA content (Fig. 5c, d), in plants of both species grown under severe water deficit in relation to the controlled water regime. However, Si supply decreased H_2O_2 content and lipid peroxidation in plants of both species under water deficit (Fig. 5a-d). The Si-induced decrease in H_2O_2 and MDA made these concentrations similar to those of unstressed plants in sugarcane (Fig. 5a, c, d), but with lower H_2O_2 in energy cane (Fig. 5b).

Figure 5

Proline content increased with water deficit under both Si supply conditions in sugarcane and energy cane plants in relation to unstressed plants. However, Si supply accentuated the increase in proline content in plants under water deficit, being higher than that of plants of both species grown under -Si (Fig. 5e, f).

Cultivation of sugarcane and energy cane plants under severe water deficit decreased total phenols content regardless of Si nutrition in relation to cultivation under the controlled water regime. However, Si supply decreased the losses in total phenols content in plants of both species under water deficit (Fig. 5g, h).

Furthermore, SOD activity decreased with the imposition of severe water deficit in sugarcane and energy cane (Fig. 6a-b) plants that did not receive Si fertilization. Still, Si supply totally reversed this decrease in energy cane plants (Fig. 6b), and partially reversed it in sugarcane (Fig. 6a), as +Si plants had higher SOD activity under water deficit than -Si plants. However, SOD activity increased even in unstressed sugarcane plants in relation to -Si plants in the same water regime (Fig. 6a).

Figure 6

Cultivation of sugarcane and energy cane plants under severe water deficit decreased CAT activity in relation to the controlled water regime. However, Si supply via fertigation in association with foliar spraying increased CAT activity in plants of both species in relation to -Si, with values similar to those of unstressed plants (Fig. 6c-d).

In relation to the controlled water regime, APX activity increased in plants under severe water deficit and without Si supply only in energy cane, remaining unchanged in sugarcane. Silicon fertilization intensified the increase in APX activity in plants of both species under water deficit in relation to unstressed plants (Fig. 6e-f).

In the absence of Si, severe water deficit reduced the number of green leaves of both species. However, nutrition with Si provided a beneficial effect that increased the number of green leaves of plants under

water deficit in relation to -Si (Fig. 7a, b), with sugarcane showing values similar to those of plants grown in the controlled water regime (Fig. 7a).

Figure 7

The percentage of dry leaves decreased in sugarcane plants under water deficit and without Si supply, remaining unchanged in energy cane plants under the same conditions in relation to the controlled water regime. However, nutrition with Si decreased the percentage of dry leaves in plants of both species under water deficit in relation to the -Si condition (Fig. 7c, d).

In the absence of Si supply, the number of tillers decreased for both species in the severe water deficit regime in relation to water sufficiency. However, fertigation with Si increased the number of sugarcane and energy cane tillers under water deficit (Fig. 7e, f), with sugarcane showing values even similar to those of plants under adequate moisture (Fig. 7e). In energy cane plants, the beneficial effect of Si in increasing tillering also occurred in plants under the controlled water regime (Fig. 7f).

Leaf area also decreased in plants of both species under severe water deficit, regardless of Si nutrition, in relation to plants under an adequate water regime (Fig. 7g, h). However, in relation to the -Si condition, Si application to seedlings with complementation after transplanting increased the leaf area of sugarcane and energy cane plants grown under water deficit (Fig. 7g, h).

The dry mass of leaves (Fig. 8a, b), culms (Fig. 8c, d), and shoots (Fig. 8e, f) decreased with the cultivation of plants under severe water deficit until initial culm formation in both species. However, Si supply partially reduced these losses, increasing the dry mass of leaves, culms, and shoots by 28, 78, and 48% in sugarcane, and by 30, 52, and 45% in energy cane, respectively (Fig. 8a-f).

Figure 8

4. Discussion

Water deficit is one of the main environmental stresses limiting plant growth and consequently reducing agricultural production¹. This study evidenced the harmful effect of water deficit as cultivation under a water regime of 30% WRC for a period of 7 to 160 days after transplanting impaired biochemical and physiological variables, decreasing plant growth in both sugarcane and energy cane. The sensitivity of energy cane to water deficit had not yet been reported despite the current use of this crop in the sugar-alcohol sector to expand electricity cogeneration. This use stems from the higher energy conversion of this crop in relation to sugarcane, due to its greater dry mass production and high fiber content⁵³. These characteristics can provide greater tolerance to environmental stress damage²⁶.

However, the results of this study evidence that severe water deficit, which has been a common scenario in regions with an unstable water regime⁵⁴, affects energy cane growth even at a more advanced stage of development, *i.e.*, initial culm formation.

The cultivation of plants of both species under severe water deficit until the initial culm formation impaired growth because it induced stress, initially caused by the decrease in leaf water content. The lower water content was due to the decrease in $\Psi_{w_{5am}}$ and $\Psi_{w_{1pm}}$ in sugarcane and energy cane plants (Fig. 3a-d). Smaller Ψ_w limits leaf water uptake because it alters the tension of the xylem solution, which is transmitted through continuous water columns to the roots^{13 24}. Along with this, G_s (Fig. 4b, d) increased resulting in higher E (Fig. 4e, g) inducing high water vapor losses, which, added to the limitation in water absorption by the roots, considerably decreased leaf water content (Fig. 2e-h), leading to an imbalance in water status.

Despite the increase in G_s and E , plants under severe water deficit that did not receive Si application had a low internal concentration of CO_2 (Fig. 4f, h) and ICE (Fig. 4j, l) that decreased A (Fig. 4a, c) in both plant species. These results suggest that the water deficit damage in inhibiting photosynthesis in the plants under study during initial culm formation is predominantly due to nonstomatal limitations. Some authors reported this effect in plants under severe stress⁵⁵ due to the decrease in the content of total soluble protein and photosynthetic pigments. This has led to photochemical inefficiency⁵⁵ while decreasing the activity of the enzymes phosphoenolpyruvate carboxylase (PEPcase) and ribulose-1,5-bisphosphate carboxylase (Rubisco)⁵⁶. However, although the present study has proven photochemical inefficiency (Fig. 3i-l), the content of photosynthetic pigments such as chlorophylls (Fig. 3a-d) and carotenoids (Fig. 3e, f) was not affected. This suggests limitations in the action of enzymes linked to photosynthetic metabolism caused by increased oxidative stress.

Photosynthetic inhibition contributed to the increase in ROS production due to excess light energy in the chloroplasts, induced by the decrease in CO_2 assimilation rate⁵⁷. The increase in H_2O_2 content (Fig. 5a, b) evidenced ROS accumulation in plants under severe water deficit in -Si, destabilizing cell membranes and thus leading to lipid peroxidation, as verified by the higher MDA content (Fig. 5c, d)⁵⁸. This condition was also evidenced by increased electrolyte leakage index (Fig. 2m-p), which is a physiological parameter that estimates the integrity of cell membranes⁵⁹. The imbalance in cellular homeostasis, which aggravated ROS accumulation, also compromised the integrity of enzymes, damaging the secondary antioxidant defense system of plants.

The antioxidant defense system of plants comprises enzymatic and nonenzymatic antioxidant systems⁶⁰. Superoxide dismutase acts in the first line of enzymatic elimination of ROS, catalyzing the conversion of O_2^- to H_2O_2 within the cell²⁴. In this study, SOD activity decreased in plants of both species under severe water deficit and without Si supply (Fig. 6a, b). Antioxidant enzymatic defense also includes H_2O_2 detoxification, which occurs through the action of APX and CAT that regulate cellular redox levels by breaking down H_2O_2 molecules⁶⁰. Thus, the activity of these enzymes has been analyzed to assess the tolerance of plants grown under drought conditions²⁴.

This study evidenced that severe water deficit decreased CAT activity only in sugarcane (Fig. 6c, d), and increased APX activity only in energy cane (Fig. 6e, f). These results demonstrate that sugarcane has a

less efficient enzymatic antioxidant detoxification mechanism than energy cane, that is, the first is more sensitive to water deficit. Ascorbate peroxidase activity was also higher in sorghum plants under water deficit ¹⁴.

In addition to the effects on the action of the enzymatic defense system, severe water deficit affected the content of phenols and proline that act on the nonenzymatic antioxidant defense system. Proline content increased with the imposition of water deficit in both species (Fig. 5g, h), confirming the stress condition. Previous studies report the accumulation of free amino acids in plants under drought ^{14 17 61} as a strategy to raise osmotic pressure, therefore acting as an osmoregulatory mechanism ⁶².

Water deficit also decreased phenols content (Fig. 5e, f). Phenols are bioactive substances that act as nonenzymatic antioxidants, interfering with metabolic homeostasis from the direct elimination of active molecular oxygen and H_2O_2 ⁶³. Thus, phenols protect cell tissues from the action of ROS, inhibiting lipid peroxidation and maintaining cell integrity ⁶⁴. In the present study, phenol contents were comparatively lower in plants under water deficit than in plants under an adequate water regime. This decrease helps to justify the high accumulation of H_2O_2 and MDA in both species under study (Fig. 5a-d). The literature mentions similar results in maize plants ⁶¹.

Biological changes caused by oxidative stress in the plant decreased WUE (Fig. 4i, k), indicating losses in the use of available water. This condition affected the development of plants grown under water deficit until initial culm formation, which can be seen from the decrease in leaf area (Fig. 7g, h). This decrease correlates with increased senescence and leaf abscission, evidenced by the decrease in the number of green leaves (Fig. 7a, b) and the high percentage of dry leaves (Fig. 7c, d). The high leaf senescence results from the curling of leaves and the decrease in cell elongation ⁶⁵, which intensified from the high rate of lipid peroxidation, resulting in sharp decreases in tillering (Fig. 7e, f) and dry mass production for both species (Fig. 8a-f). Other authors reported the harmful effects of water deficit in pre-sprouted sugarcane seedlings, but for a short period of 3 days ¹⁷ and 30 days after transplanting ⁶⁶, and with effects restricted to physiological and growth variables.

Thus, although biological mechanisms vary between species, the sensitivity of sugarcane and energy cane to severe water deficit up to the initial stage of culm formation is evident. This condition demonstrates the need to increase the tolerance of these species, given the increase in planting in areas with recurrent water deficit and the occurrence of cyclical water shortages in different cropping regions. Therefore, this study proposed to enrich these species with Si in the seedling formation phase with complementation via fertigation in association with foliar spraying after transplanting as a strategy to increase plant resistance under water deficit.

Silicon supply by fertigation in association with foliar spraying provided high uptake of this element in both species (Fig. 1a-d), demonstrating the feasibility of using it for adding Si in the seedling formation phase with complementation after transplanting. The efficiency of Si supply is due to the proper handling of the solution by using a concentration that favors the permanence of the element in the monomeric

form that is absorbed by the plants ¹¹, that is, below 3 mmol L⁻¹ ²¹. The adequate source of Si also improved its efficiency. This study used soluble Si along with sorbitol, which favors the stability of the monomeric forms of the element. Silicon forms organic complexes when binding with this polyol ⁶⁷, which contributes to its uptake via roots. In addition, sorbitol increases the wetting properties of the solution by decreasing the deliquescence point of the leaf surface ⁶⁸, delaying drying and contributing to the uptake of the element. Thus, Si supply via fertigation in association with foliar spraying was efficient in inducing its absorption by leaves and roots.

The proven efficiency of Si fertigation and foliar spraying reinforces its applicability in the seedling formation phase. This technique is already used in nurseries for fertilizer application ³², allowing the use of low concentrations of the beneficial element at high frequency. In addition, complementation after transplanting is also a possibility given the intensification of the irrigated sugarcane cultivation area and the use of rescue irrigation, which occurs after transplanting seedlings ⁵. Thus, the soil solution can be enriched with Si from its supply via fertigation, increasing the concentration of H₄SiO₄ in the root zone. Fertigation in association with foliar spraying increases Si accumulation in plants, improving its residual effect. The combination attenuates severe water deficit damage for longer periods of time, extending to initial culm formation.

The hypothesis can be accepted that the use of fertigation in association with foliar spraying applied to seedlings during their formation, with complementation after sugarcane and energy cane transplanting, allows high Si uptake with longer-lasting residual beneficial effects in the plant, extending up to 160 days of cultivation. This finding has important practical implications as it should expand the use of Si in irrigated sugarcane crops, given the low amount of Si used, close to 20 kg ha⁻¹, in relation to other sources of lower solubility such as calcium silicate, which requires doses above 1000 kg ha⁻¹ ²⁰. Previous reports have already shown the efficiency of Si fertigation in optimizing the uptake of this element in forage plants ²² and pre-sprouted sugarcane seedlings ¹⁷.

Increased Si accumulation in plants induced an important biological response for the survival of plants under severe water deficit, improving cellular water status. Applications maintained the water potential similar to that of plants without water deficit in the first hours of the day, with variation only at times of higher temperature (Fig. 2a, c). This adjustment in leaf water potential facilitated plant water uptake and increased relative leaf water content (Fig. 2e-h). The higher leaf water content correlates mainly with physical changes caused by the deposition of silica that binds to cellulose in the leaf epidermis and below the cuticle ¹¹. This deposition acts as a barrier to water loss, decreasing transpiration, a common fact in Si-accumulating plants ⁶⁹, which was evidenced in the present study (Fig. 4e, g). This is because the absorbed monosilicic acid reaches the leaves through the transpiration gradient ¹¹ and concentrates inducing polymerization as amorphous or biogenic silica being bound to cell wall cellulose, constituting 90% of the absorbed Si ¹².

Plants treated with Si also have higher water content due to the contribution of this element in water uptake by the roots, as it induced an increase in water potential through the osmoregulatory action of proline^{13 14}. Allied to this, the activity of aquaporins was also likely to have increased. Aquaporins are proteins specialized in water uptake by plants, which may have the expression of their genes activated by Si^{15 70}. Thus, it can be stated that plants that received Si were further protected from tissue dehydration by two associated mechanisms: decreased water loss and increased water uptake by the mechanisms discussed above.

Silicon-induced maintenance of cellular hydration decreased photosynthetic damage in plants under water deficit (Fig. 4-l) due to stomatal and nonstomatal changes. Stomatal changes were evidenced by the decrease in Gs (Fig. 4b, d) and E (Fig. 4e, g), possibly by the polymerization of part of the silica on the leaf surface¹¹. However, Ci (Fig. 4f, h) increased despite the decrease in Gs, suggesting the involvement of nonstomatal factors in photosynthetic activation¹⁹.

Nonstomatal factors correlate with biochemical adjustment in the prevention and reduction of oxidative damage in plants. Prevention was evidenced by the maintenance of water status, which avoided losses in the photochemical efficiency of the PSII. This was demonstrated by the highest values of Fv/Fm assessed in the morning (Fig. 2i, k) and at the time of high temperature (Fig. 2j, l) for both species. This increase indicates that Si decreases damage to the PSII reaction centers as most of the solar radiation was being used in the photochemical phase of photosynthesis¹⁹, preventing excessive ROS formation⁵⁶. The increased photochemical efficiency may be due to the higher content of Chl *a* (Fig. 3a, b) and Chl *b* (Fig. 3c, d) and carotenoids (Fig. 3e, f), which collaborate to maintain photosynthetic activity and prevent exposure of chloroplasts at high excitation energy levels⁵⁷. Other authors reported the effect of Si in increasing carotenoids, which are powerful antioxidants that decrease the degradation of different organic compounds in the plant, including chlorophyll⁶¹.

The transfer of light energy from carotenoids to chlorophylls thus increases, favoring the protection of plants against stress⁵⁶. Along with this, the effects of Si can also be due to increased dissipation of ROS formed from the activation of the enzymatic (Fig. 6a-f) and nonenzymatic (Fig. 5e-h) antioxidant defense system of plants.

Superoxidase dismutase activity in Si-nourished plants increased in both water regimes in sugarcane and in the water deficit regime in energy cane (Fig. 6a, b), demonstrating that Si collaborates in active oxygen inactivation by catalyzing O_2^- to H_2O_2 and O_2 ⁶⁰. Previous reports indicate higher levels of SOD activity as a mechanism that increases drought tolerance in sugarcane plants under water deficit conditions⁷¹. Therefore, Si would increase the tolerance of plants under severe water deficit because it stimulates SOD activity. In this sense, sugarcane is more efficient in the use of this biological mechanism as it can obtain this response even under adequate water conditions (Fig. 6a, b). However, despite the formation of H_2O_2 , its content decreased due to the concomitant increase in the activity of the enzymes CAT (Fig. 6c, d) and APX (Fig. 6e, f) that use it as a substrate for the formation of H_2O and O_2 ⁶⁰. These results agree with

those of other authors ¹⁶ who reported that Si application strengthened the antioxidant defense system and decreased lipid peroxidation and oxidative damage in sugarcane plants under water deficit stress.

In addition to activating the enzymatic antioxidant system, Si also provided a beneficial effect on the performance of nonenzymatic antioxidant agents such as proline (Fig. 5g, h) and phenolic compounds (Fig. 5e, f). The increased concentration of proline contributed to maintain the cellular osmotic adjustment, increasing cell water potential by stimulating an increase in cellular homeostasis ¹⁴. Proline can also act as a molecular chaperone, protecting the integrity of proteins and maintaining or increasing the activity of enzymes signaling ROS dissipation ⁷². Thus, the increase in proline content improved the defense system of plants of both species fertilized with Si. The contents of phenolic compounds were also comparatively higher in sugarcane and energy cane plants that received Si supply (Fig. 4e, f). This increase collaborated with the decrease in ROS accumulation ⁶³. Furthermore, the increase in the content of phenols content correlated with the decrease in ROS production in maize plants under water deficit ⁶¹.

The physiological and biochemical mechanisms modified by Si in increasing the tolerance of plants grown under water deficit increased the WUE of plants. This is an important physiological attribute, especially for plants under drought conditions ⁷³. In this study, the water deficit decreased WUE, which was improved by Si supply (Fig. 4i, k). The literature also indicates an increase of WUE by Si in sugarcane plants under water stress, but with the application of the element only via leaves ¹⁹.

The beneficial effect of Si on the biological mechanisms under study attenuated the severe water deficit damage on plants. Its application favored plant growth while decreasing leaf senescence, *i.e.*, percentage of dry leaves (Fig. 7c, d), further increasing the number of green leaves (Fig. 7a, b) and consequently leaf area (Fig. 7g, h), tillering (Fig. 7e, f), and dry mass production of leaves (Fig. 8a, b), culms (Fig. 8c, d), and shoots (Fig. 8e, f). Thus, the second hypothesis of this study was accepted, indicating that the enrichment of plants with Si applied in the seedling formation phase with complementation after transplanting provides a beneficial effect that attenuates water deficit damage until initial culm formation. This occurred due both to the Si-induced mechanism that changes physiological parameters favoring the maintenance of cell hydration and to the biochemical benefits from the increased activity of enzymatic and nonenzymatic antioxidants, decreasing oxidative stress.

It should be noted that the benefit of Si in increasing the tolerance of plants under water restriction occurred in a relatively long period from 7 to 160 days after transplanting, including the phase of high relative growth rate of the crop. In other words, it indicates the efficiency of production of new dry mass over the existing one, which occurs close to 84 to 135 days after transplanting ⁷. Therefore, the reduction of stress in this stage of high dry mass accumulation rate may even have a beneficial impact on the next phenological stages of the crop.

The research also made it clear that the use of Si under the conditions of this study can enable the use of suboptimal irrigation for the cultivation of these species aiming at saving water with expressive environmental gains as it deals with a finite natural resource.

Further research should be conducted under field conditions so that the use of Si can be expanded for the sustainable cultivation of sugarcane and energy cane crops in different production cycles and in drought areas.

5. Conclusion

Silicon supply via fertigation in association with foliar spraying in the seedling formation phase with complementation after transplanting is efficient in increasing the accumulation of this element in the plants. The presence of Si in the plant attenuates severe water deficit damage until initial culm formation, maintaining water and physiological balance by favoring the antioxidant defense system in sugarcane and energy cane plants.

The present research suggests Si supply in the seedling formation stage and after transplanting to the soil as a complementary strategy in areas with water restriction in the initial formation of culms of sugarcane and energy cane, especially in sandy soils and soils with low Si availability.

Declarations

7. Conflict of Interest

The authors declare that they have no conflict of interest.

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Figures

Figure 1

Accumulation of silicon (Si) in leaves and culms in sugarcane (a, c) and energy cane (b, d) in the absence of Si (-Si) and with Si applied via fertigation associated with foliar spraying (+Si) under adequate water regime (control) and severe water deficit (WD) for a period of 7 to 160 days after transplanting. **: significant at 1% probability by the F-test. Different lowercase letters indicate differences in relation to Si in the same water regime; and different capital letters, in relation to the water regime in the same conditions of Si supply by Tukey's test. Bars represent the standard error of the mean; $n = 5$.

Figure 2

Leaf water potential (Ψ_w), relative water content, quantum efficiency of photosystem II (Fv/Fm) and electrolyte leakage index in sugarcane (a, b, e, f, i, j, m, n) and energy cane (c, d, g, h, k, l, o, p) in the absence of Si (-Si) and with Si applied via fertigation associated with foliar spraying (+Si) under water regime adequate (control) and severe water deficit (WD) for a period of 7 to 160 days after transplantation. ** and *: significant at 1 and 5% probability, respectively; and ^{ns}: not significant by the F-test. Different lowercase letters indicate differences in relation to Si in the same water regime; and different capital letters, in relation to the water regime in the same conditions of Si supply by Tukey's test. Bars represent the standard error of the mean; $n = 5$.

Figure 3

Content of chlorophyll *a* (Chl *a*), *b* (Chl *b*) and carotenoids in sugarcane (a, c, e) and energy cane (b, d, f) in the absence of Si (-Si) and with Si applied via fertigation associated with foliar spraying (+Si) under water regime adequate (control) and severe water deficit (WD) for a period of 7 to 160 days after transplantation. ** and *: significant at 1 and 5% probability, respectively; and ^{ns}: not significant by the F-test. Different lowercase letters indicate differences in relation to Si in the same water regime; and different capital letters, in relation to the water regime in the same conditions of Si supply by Tukey's test. Bars represent the standard error of the mean; *n* = 5. FM = fresh mass

Figure 4

Photosynthesis rate (A), stomatal conductance (Gs), intracellular CO₂ concentration (Ci), leaf transpiration (E), water use efficiency (WUE) and instantaneous carboxylation efficiency (ICE) in sugarcane (a, b, e, f, i, j) and energy cane (c, d, g, h, k, l) in the absence of Si (-Si) and with Si applied via fertigation associated with foliar spraying (+Si) under water regime adequate (control) and severe water deficit (WD) for a period of 7 to 160 days after transplantation. ** and *: significant at 1 and 5% probability, respectively; and ^{ns}: not significant by the F-test. Different lowercase letters indicate differences in relation to Si in the same water regime; and different capital letters, in relation to the water regime in the same conditions of Si supply by Tukey's test. Bars represent the standard error of the mean; *n* = 5.

Figure 5

Hydrogen peroxide (H₂O₂) content, malondealdehyde (MDA) content, proline content and total phenols content in sugarcane (a, c, e, g) and energy cane (b, d, f, h) in the absence of Si (-Si) and with Si applied via fertigation associated with foliar spraying (+Si) under water regime adequate (control) and severe water deficit (WD) for a period of 7 to 160 days after transplantation. ** and *: significant at 1 and 5% probability, respectively; and ^{ns}: not significant by the F-test. Different lowercase letters indicate differences in relation to Si in the same water regime; and different capital letters, in relation to the water regime in the same conditions of Si supply by Tukey's test. Bars represent the standard error of the mean; *n* = 5. FM = fresh mass. GAE = gallic acid equivalent.

Figure 6

Specific activity of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in sugarcane (a, c, e) and energy cane (b, d, f) in the absence of Si (-Si) and with Si applied via fertigation associated with foliar spraying (+Si) under water regime adequate (control) and severe water deficit (WD) for a period of 7 to 160 days after transplantation. ** and *: significant at 1 and 5% probability, respectively; and ^{ns}: not significant by the F-test. Different lowercase letters indicate differences in relation to Si in the same water regime; and different capital letters, in relation to the water regime in the same conditions of Si supply by Tukey's test. Bars represent the standard error of the mean; *n* = 5.

Figure 7

Number of green leaves, percentage of dry leaves, number of tillers and leaf area in sugarcane (a, c, e, g) and energy cane (b, d, f, h) in the absence of Si (-Si) and with Si applied via fertigation associated with foliar spraying (+Si) under water regime adequate (control) and severe water deficit (WD) for a period of 7 to 160 days after transplantation. ** and *: significant at 1 and 5% probability, respectively; and ^{ns}: not significant by the F-test. Different lowercase letters indicate differences in relation to Si in the same water regime; and different capital letters, in relation to the water regime in the same conditions of Si supply by Tukey's test. Bars represent the standard error of the mean; *n* = 5.

Figure 8

Dry mass of leaves, dry mass of culms and shoot dry mass of sugarcane (a, c, e) and energy cane (b, d, f) in the absence of Si (-Si) and with Si applied via fertigation associated with foliar spraying (+Si) under water regime adequate (control) and severe water deficit (WD) for a period of 7 to 160 days after transplantation. ** and *: significant at 1 and 5% probability, respectively; and ^{ns}: not significant by the F-test. Different lowercase letters indicate differences in relation to Si in the same water regime; and different capital letters, in relation to the water regime in the same conditions of Si supply by Tukey's test. Bars represent the standard error of the mean; *n* = 5.

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