

Characteristics of Leaf Stomata and Their Relationship with Photosynthesis in *Saccharum officinarum* Under Drought and Silicon Application

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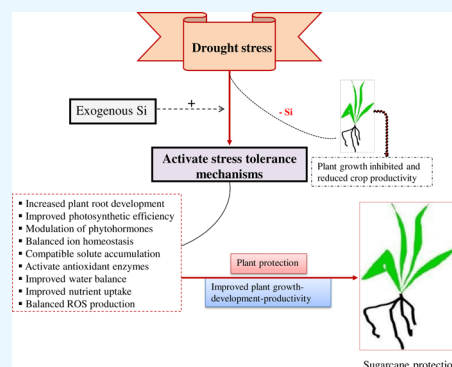
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ABSTRACT: Silicon (Si) plays an important role in the sustainable agriculture industry. The increasing demand for crop production with a significant reduction of synthetic chemical fertilizers and pesticide use is a big challenge nowadays. The use of Si has been proven to be an environmentally sound way of enhancing crop productivity by facilitating plant growth and development through either a direct or indirect mechanism, especially in tropical and subtropical regions. In particular, it has been investigated for its role in water stress management. The aim of the current experiment was to examine the protective role of Si in the photosynthetic capacity of different leaf segments and the ultrastructure of sugarcane (*Saccharum officinarum*) plants under water stress. Sugarcane cv. GT 42 plants were supplied with 0, 100, 300, and 500 mg L⁻¹ Si and exposed for 60 days under each stress condition such as 100–95, 55–50, and 35–30% of field capacity. For the photosynthetic responses, each leaf was observed and separated into three equal parts (base, middle, and tip). We used intact leaves and were able to assess leaf photosynthetic responses. Under moderate and severe stress conditions, applied Si increased the photosynthesis (base, ~16–143%; middle, 20–66%; and tip leaf part, 41–71%), transpiration rate (base, 15–97%; middle, 26–68%; and tip leaf part, 6–61%), and stomatal conductance (base, 26–137%; middle, 12–70%; and tip leaf part, 7–75%) in sugarcane plants. Ultrastructural examination of sugarcane leaves using scanning electron microscopy showed the remarkable effects on stomata ultrastructure. Silicon increased plant growth development, photosynthetic efficiency, and biomass/yield, and promoted better adaptation of stomata to drought. This study suggests that the application of Si may be used to increase the stress tolerance of sugarcane plants.



1. INTRODUCTION

Drought is one of the major environmental stresses that hinders plant growth, development, and productivity worldwide.^{1,2} It causes a broad range of growth, photosynthetic, metabolic, and ultrastructural variations in plants.¹ It also escalates leaf senescence, chlorosis, and necrosis, and degrades photosynthetic pigments, which in turn reduces photosynthetic efficiency and canopy size, resulting in reduced crop productivity and sometimes in total failure of the crop plants.^{2–4}

Plant stomata are important channels among plants and the environment may play an important role in plant responses to atmospheric variables.^{5,6} In addition, various studies show that stomatal density responds to various environmental variables like extreme temperature,⁷ elevated CO₂ concentration,⁸ salinity,⁹ insufficient water,^{10,11} precipitation change,¹² and plant density.¹³

Photosynthesis is an important physiological process for plant carbon uptake, development, and productivity.^{14,15} It is commonly considered that stomatal limitation, which affects

the substomatal CO₂ content, is the major source of the loss of photosynthetic efficiency under stress conditions.^{15–17} The photosynthetic apparatus of plants appears to be more sensitive to drought.^{18,19} Improving photosynthetic traits is the basis for the enhancement of plant biomass and crop productivity. Water stress triggers closure of stomata, affects the electron transport rate (ETR), aggravates photoinhibition induced by excessive light intensity, and lowers rates of photosynthesis as well as reduction of photosynthetic pigments. All of these variations could further lead to a loss in crop production and affect plant development.^{20–22} Under water stress conditions, Si can enhance soil water-use

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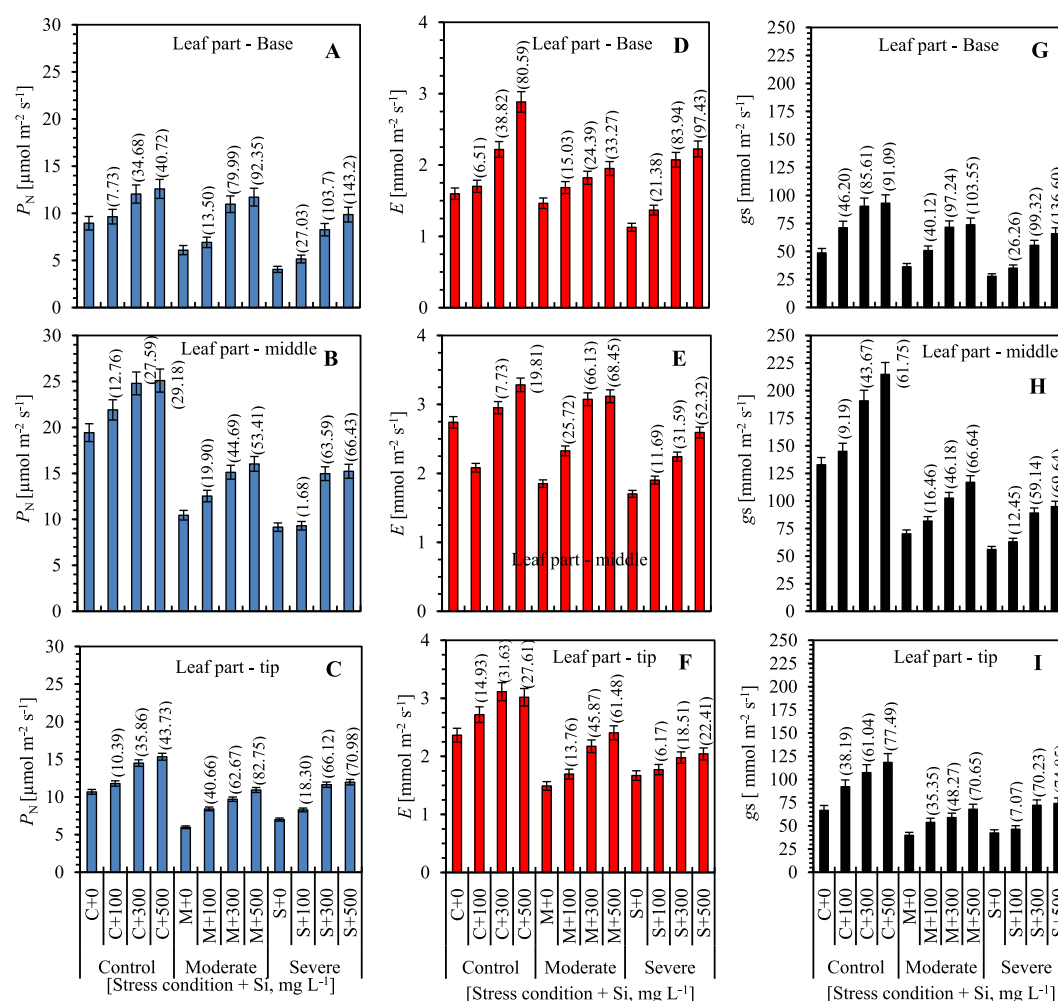


Figure 1. Changes in photosynthesis (P_N), transpiration (E), and stomatal conductance (g_s) in the base (A, D, G), middle (B, E, H), and tip (C, F, I) parts of sugarcane leaves under well-watered and limited water supplies with different silicon concentrations at 60 days after the stress condition. Five independent biological replicates are shown for each leaf part. Parenthesis values indicate percentage gain in Si-amended soil of various irrigation levels. Vertical bars indicate the standard error (SE) ($n = 5$).

efficiency, root growth zone, and uptake of nutrients and consequently enhance the crop yield.^{15,23}

Silicon has been commonly recognized as a fertilizer, biostimulating plant protection under atmospheric environmental variables.^{15,24–26} Silicon is absorbed by root hairs through an active uptake by a transpiration stream, being later transported as monosilic acid to the plant tissues, where it is polymerized as solid amorphous silica bodies ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$) called phytoliths.^{27,28} In particular, the connection of silicon with tolerance to environmental stresses has been studied extensively in various crops.^{15,24,28–31} Silicon might be associated with physiologic and molecular mechanisms in plants^{32,33} and potentially alleviates the detrimental impact of water stress, a severe abiotic stress.

Sugarcane (*Saccharum officinarum* L.) is a major cash crop and is cultivated in the arid and semiarid areas of the world. In relation to geonics, water scarcity is one of the major factors that limits sugarcane productivity, while the utilization of fertilizers exerts a significant impact on photosynthetic performance and yield.^{6,15,20,21} The synergy of water and fertilizers or the combined use of both is an important measure to save water in agricultural crops.^{34–36}

However, knowledge about how Si modulates the photosynthetic capacity in *S. officinarum* “GT 42” during drought

remains elusive. Although the importance of this element to crop plants is still debated, there have been beneficial impacts on our understanding of the uptake of Si in plants. The purpose of this study was to assess the responses of stomata morphology to different water irrigation levels with Si amendment and to evaluate the importance of stomatal conductance with photosynthesis and transpiration rate in sugarcane based on a greenhouse experiment.

2. RESULTS

2.1. Leaf Gas Exchange Measurement. To characterize sugarcane leaf positions (base, middle, and tip parts), photosynthetic traits were measured. The silicon fertilization influenced photosynthesis (P_N) (Figure 1A–C). The highest P_N was observed in the middle part of the leaf (Figure 1B) as compared to the base and tip leaf parts under control, moderate, and severe water stress with Si. Maximum photosynthetic rates were obtained at 40.7, 27.6, and 43.7% of control, 92.4, 53.4, and 82.8% of moderate, and 143.2, 66.4, and 70.9% of severe water stress conditions with 500 mg L^{-1} Si supplied as compared to 0–300 mg L^{-1} Si with different irrigation levels (Figure 1A–C).

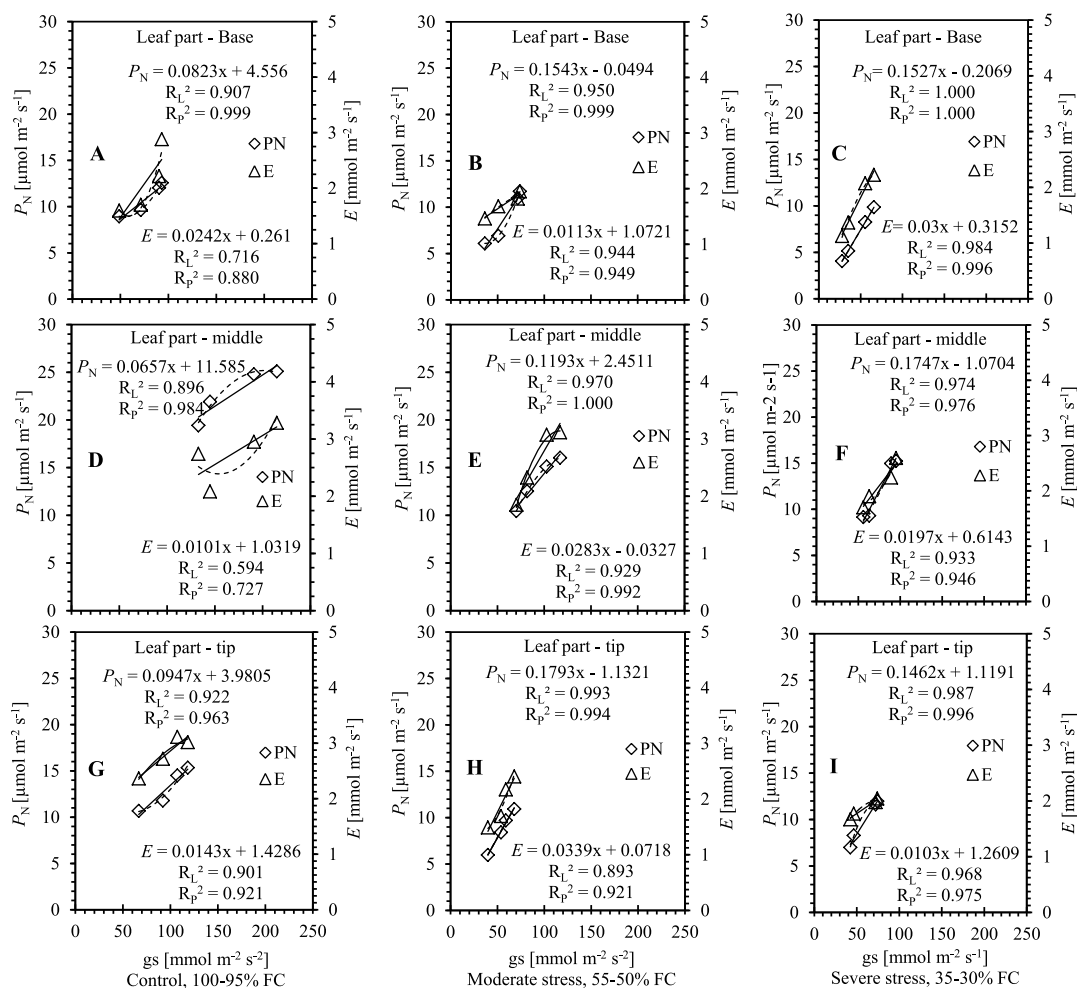


Figure 2. Correlations of stomatal conductance (g_s) with photosynthesis (P_N , A–I) and transpiration rate (E , A–I) in different leaf parts (base, A–C; middle, D–F; and tip, G–I) of sugarcane plants under control (A, D, G; 100–95% FC), moderate water stress (B, E, H; 55–50% FC), and severe water stress (C, F, I; 35–30% FC), respectively. R_L^2 and R_P^2 are R square values on the chart of linear and polynomial regression types, respectively ($n = 5$).

With reference to the transpiration rate (E , Figure 1D–F), an increase in Si induced the highest E in the base, middle, and tip parts of the sugarcane leaves at all of the irrigation levels. The highest enhancement of E was measured in the 500 mg L⁻¹ Si treatment in the base part (80.6, 33.3, 97.4%), middle part (19.8, 68.5, 52.3%), and tip part (27.6, 61.5, 22.4%) of the leaves in the control and limited water, while in the 100 and 300 mg L⁻¹ Si applications, only a slight increase was observed (nearly 7–39, 15–24, and 21–84% in the base part; 8–20, 26–66, and 12–32% in the middle part; and 15–32, 14–46, and 6–19% in the tip part) as shown in Figure 1.

Silicon supply also positively influenced the stomatal conductance in the base, middle, and tip parts of leaves in the limited water irrigation treatments (Figure 1G–I). In terms of water stress tolerance, as compared with the control without Si, the Si treatments applied led to a great increase in the maximum g_s in the moderate (~103.6, 66.6, and 70.7%) and severely stressed plants (136.6, 69.6, and 74.9%) at 500 mg L⁻¹, while only a slight increase was found in the 100 mg L⁻¹ Si-treated plants in different leaf segments of the normal and stressed plants.

2.2. Correlation of Photosynthesis and Transpiration with Stomatal Conductance. Leaf stomatal conductance to water vapor (g_s) was positively correlated with photosynthesis

(P_N , R_L^2 0.896–1.000, R_P^2 0.963–1.000) and transpiration rate (E , R_L^2 0.594–0.984, R_P^2 0.727–0.996) in different leaf segments, i.e., base, middle, and tip parts (Figure 2A–I). Leaf transpiration rate was enhanced with increasing stomata aperture. Responses of photosynthesis and transpiration rate to limited water supply (Figure 2B,C,E,F,H,I) showed a significant correlation with stomatal conductance, which indicated that upregulated leaf g_s was closely associated with P_N and E in the base, middle, and tip segments of sugarcane plant leaves. However, the correlation of leaf area expansion with g_s was also significant, suggesting that the leaf area expansion may be closely associated with the occurrence of stomatal guard cells during various water irrigation levels.

2.3. Effect of Limited Water Irrigation and Si on Stomata. Scanning electron microscopic observations found that stomata morphology in different irrigation levels with Si was amended as shown in Figure 3. Under severe water stress, stomata aperture size was decreased as compared with normal irrigation (100–95% FC) (Figure 3A,B,E,F). The addition of Si enhanced stomatal aperture size, regardless of normal and limited water supply (Figure 3C,D,G,H). Stomatal alterations in response to water stress were observed to cause reductions in the stomatal density and stomatal aperture size in sugarcane plant leaves. The enhanced stomatal density and aperture size

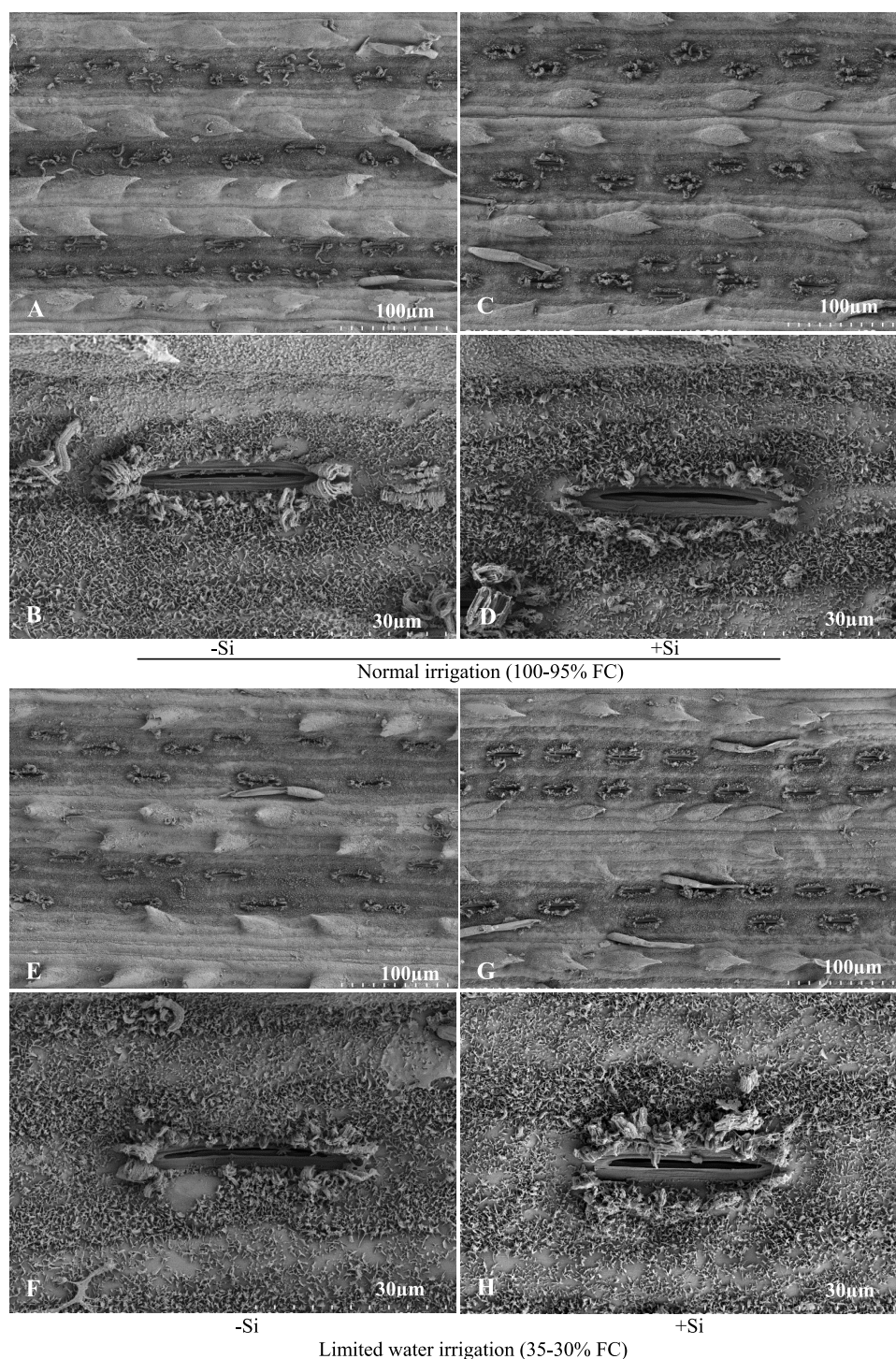


Figure 3. Scanning electron micrographs of stomata ultrastructure in the epidermis of sugarcane leaves in normal (A, B) and limited water supply treatments without Si (E, F) or with Si supplementation for normal (C, D) and limited water (G, H) at 500 mg L^{-1} Si. The scale bars for the micrographs were $30 \mu\text{m}$ (B, D, F, and H) and $100 \mu\text{m}$ (A, C, E, and G), respectively.

were monitored in control and stressed plants with Si application. The average stomatal density and stomatal aperture size of each treatment were found to be 218, 232, 179, and 183 mm^2 and 29.92, 35.70, 29.09, and $29.51 \mu\text{m}^2$ for control, control with Si, stress, and stress with Si application, respectively (Figure 4).

Plant growth and biomass traits were significantly inhibited under limited water supply as compared with the normal irrigation. Insufficient water supply reduced noticeably leaf area

expansion, shoot, root, and plant dry masses of sugarcane plants (data not shown). However, the inhibition of plant development by water stress was mitigated by silicon application. Leaf area expansion was significantly different in the treatments with different Si concentrations. Actually, it was augmented significantly when Si application was increased from 0 to 500 mg L^{-1} . The fresh and dry biomasses of the sugarcane plants subjected to the combined treatments with Si ($100\text{--}500 \text{ mg L}^{-1}$) and limited water (55–50 and 35–30%

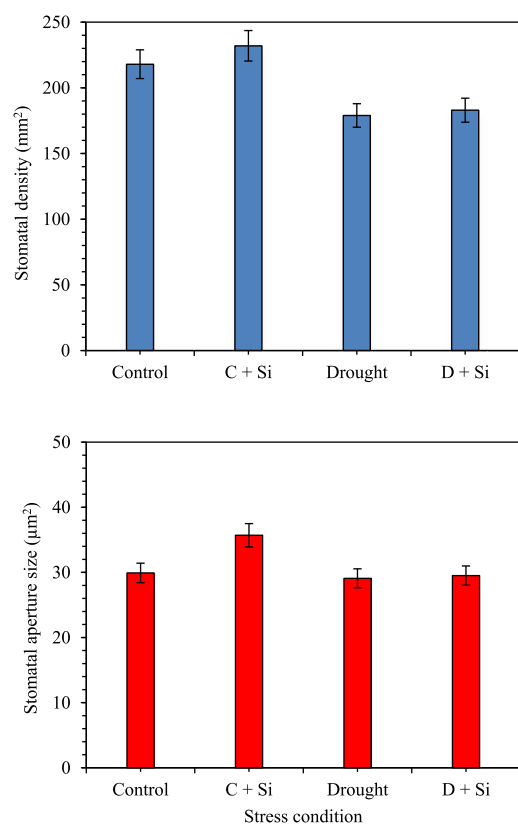


Figure 4. Effect of stomatal density [SD, number of stomata per unit area, (A)] and stomatal aperture size [guard cell area, based on guard cell pair length and width, (B)] in sugarcane plants subjected to drought and silicon application. Average values \pm SE of 10 biological replicates. C = control, D = drought, and Si = silicon level (500 mg L⁻¹).

FC) were clearly enhanced compared to the control plants without Si application (data not shown). The application of Si (500 mg L⁻¹) with limited water led to better plant performance than application with 100–300 mg L⁻¹ Si.

3. DISCUSSION

Plant leaves are the main organs for photosynthetic CO₂ assimilation, and leaf area determines light harvesting, affects photosynthetic performance, and the accumulation of photosynthetic products.^{37,38} This study found that for all of the irrigation levels, leaf area expansion was larger in the treatments with Si than those without Si supply. In this experiment, Si fertilizers can guarantee a relatively higher photosynthetic performance under the limited water irrigation. Under water stress conditions, the significant impact of silicon on plant water status has been broadly investigated in many agricultural crop plants such as *Sorghum bicolor*,^{39,40} *Triticum aestivum*,⁴¹ *Zea mays*,⁴² *Oryza sativa*,⁴³ *Cucumis sativus*,²⁹ *Kentucky bluegrass*,⁴⁴ *Brassica napus*,⁴⁵ *Helianthus annuus*,⁴⁶ *Cicer arietinum*,⁴⁷ *Glycine max*,⁴⁸ *Medicago sativa*,⁴⁹ and *Solanum lycopersicum*.⁵⁰ Under water stress, the plant's initial response is to exclude the minimum water potential by adjusting its water maintenance between root water uptake and water loss in leaves.^{51,52} The plants can decrease leaf water loss by controlling the exhalation of water vapor through the stomata and also by reducing their leaf area expansion.¹⁵ When plants initially begin to experience water deficit, they reduce the leaf water loss mainly by reducing the transpiration rate

through stomatal closure.^{15,53} Ming et al.⁴³ and Chen et al.⁵⁴ reported that the leaf transpiration rate was increased by silicon when the plants were facing water shortage. Numerous scientific reports published on insufficient water for plants have been shown to be compatible with upregulated leaf transpiration rates by Si amendment.^{15,28,41,54–57}

In this study, stomata ultrastructure was destroyed in the severely water-stressed plants but stabilized considerably with the addition of silicon. Therefore, silicon may upgrade or enhance the rate of stomatal conductance by protecting the stomatal aperture from destruction. The number of stomata was similar in different leaf segments when considering both surfaces, i.e., adaxial and abaxial. This indicates that stomata density does not contribute to the changes observed in leaf gas exchange. Although the stomatal aperture has a significant link with P_N , E , and g_s of crop plants,^{6,58,58} in this study, the leaf stomatal appearance was reduced with limited water irrigation, which is similar to the results reported by Xu and Zhou,⁸ Yang et al.,⁵⁹ and Meng et al.⁶⁰ Stomatal density was also negatively associated with the length of stomata on various watering levels in *Platanus acerifolia*⁶¹ and *Leymus chinensis*.⁸ However, the stomatal length was increased in the water-stressed plants, whereas its width decreased. Nevertheless, various impacts of environmental variables on stomata morphology may depend on plant genotypes.^{62,63}

Dynamic adjustments to the opening degree of stomatal pores are linked to regulation of g_s in the short term, allowing plants to immediately reduce water loss according to atmospheric conditions.⁶⁴ Over a longer duration, anatomical adaptations, i.e., variations to SS and SD, can modify the range of g_s by altering the higher g_s .^{65,66} Changes in the size and density of stomata may arise due to genetic factors and/or plant growth and development against various environmental variables. Loss in g_s due to a smaller SS has been linked to higher water conservation, as demonstrated for plants subjected to water deficit.⁶⁷ Growth during insufficient soil moisture capacity has been shown to cause a reduction in SS in various plant species/cultivars,^{6,67–69} but the effect on SD is less consistent.^{6,67–70} In response to variations in water availability, leaf morphology and ultrastructure can vary considerably.⁷¹ Specifically, with respect to stomatal numbers, variations in atmospheric variables that influence mature leaf g_s will have lasting effects on the stomatal differentiation of newly developing leaves.⁷² Our results regarding improvement in stomatal ultrastructure due to exogenous Si application against water stress are shown in Figure 4. The optimum level of Si application maintained/upgraded the stomatal functions by enabling plants to reopen their stomata against stressed conditions, suggesting a significant role of Si in stomatal regulation.^{1,73,74}

Water deficit may initially inhibit plant development, significantly decreasing leaf area expansion,^{75,76} although stomatal density is closely linked to leaf growth and development.^{6,59} Casson and Hentherington⁷⁷ reported that stomata morphology or size directly affected the photosynthetic CO₂ assimilation and transpiration rate of plant leaves and that the key factors linked to plant environmental adaptation were two irrelevant indications: adjustment of stomatal motion and optimization of stomatal density and appearance.

Application of irrigation water combined with silicon fertilizers can induce water stress tolerance and sustain sugarcane biomass production under water stress conditions,

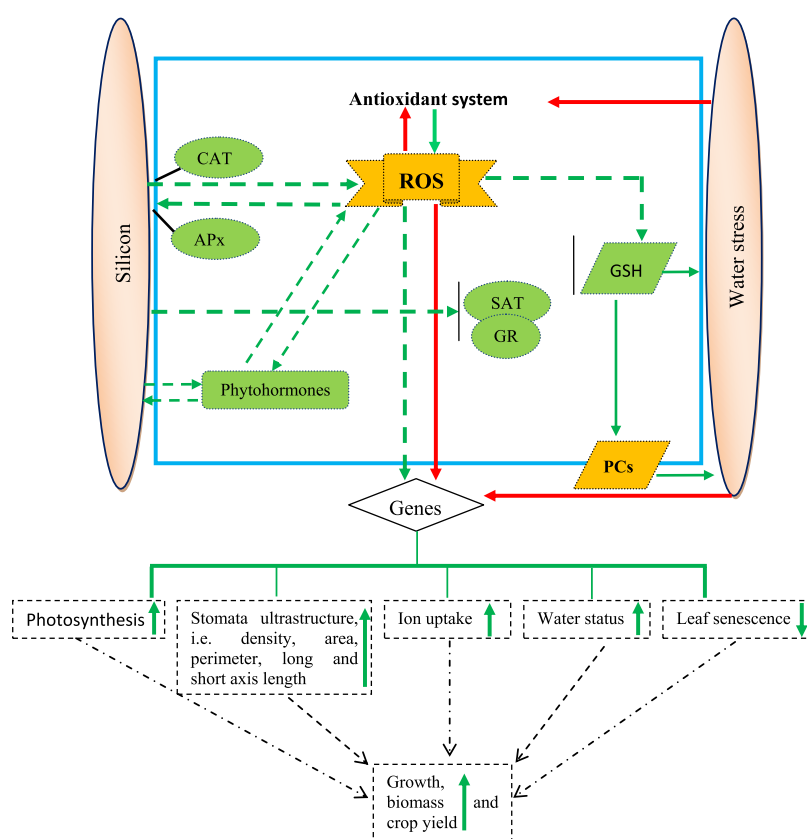


Figure 5. Schematic diagram representing the effects of water stress and defensive system induced by silicon and limited water, underlying sugarcane water stress tolerance. Water stress causes negative impacts by repressing photosynthetic performance, disrupting osmoprotectant status, and negatively influencing water status and ionic balance. Severe water stress can promote the production of reactive oxygen species (ROS). ROS contribute to lipid peroxidation, resulting in oxidative stress that causes growth reduction, biomass loss, and subsequently extreme loss of yield. Conversely, Si shows a protective mechanism against limited water by maintaining or improving water balance, leaf gas exchange, and maintaining ionic balance. As a result, Si improves water stress tolerance by retaining better plant growth and the productivity of stressed plants. CAT—catalase, APx—ascorbate peroxidase, GR—glutathione reductase, GSH—glutathione, PCs—phytochelatin, and SAT—serine acetyltransferase. The dotted arrows indicate possible signaling pathways. The red arrow indicates damage and the green arrow indicates positive effects, respectively.

Table 1. Atmospheric Data during the Experiments

month	average air temperature (°C)		average air humidity (%)	average precipitation (mm)	average rainy days	light (h)	
	min.	max.				daylight	sunshine
March	15	22	82	50	8	12.0	2.0
April	20	26	81	107	10	12.7	3.0
May	23	31	80	188	12	13.2	4.6
June	25	32	81	216	12	13.5	5.4
July	25	33	82	201	12	13.4	6.3
August	25	33	82	214	13	12.9	6.0
September	24	32	78	125	8	12.3	6.2

which is of great importance for sustainable development of sugarcane plants (Figure 5). Thus, appropriate Si fertilization is recommended for agricultural crops to improve photosynthetic performance inhibited by limited water and to facilitate plant establishment under limited water irrigation management.

4. EXPERIMENTAL SECTION

4.1. Site Description and Sugarcane Growing Conditions. Sugarcane seedcane stalks of cultivar GT 42 were kindly provided by the Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, Nanning, Guangxi, China. Sugarcane stalks were cut in one bud per segment and budded in trays containing vermiculite. The 60-day old plants were

transplanted in 3.5 L plastic pots filled with 70% of fertile topsoil and 30% of organic manure and regularly watered with the same water volume. The soil at the experimental site was silty-clay soil and it was top-dressed with N, P, and K fertilizers, following the farmer's standard practices. Fungicides were applied according to standard practice. The pots were randomly distributed into three irrigation groups such as normal (100–95% of FC), moderate stress (55–50% of FC), and severe stress (35–30% of FC) conditions, with 10 biological replicates per treatment. Subsequently, the fertilization of sugarcane plants was done with 200 mL of silicon at the concentrations of 0, 100, 300, and 500 mg L⁻¹. Calcium metasilicate (CaO·SiO₂) was used as a source of Si. The

solution was applied directly to the soil. The watering of the sugarcane plants was done manually in each pot. The substrate elements were quantified before treatment, pH 5.95, organic carbon 0.74%, P 9.20 mg kg⁻¹, K 2.74, Ca 4.2, Mg 1.5, and Na 0.085 cmol (+) kg⁻¹, respectively. The availabilities of Cu, Fe, Zn, and Mn were 0.86, 12.3, 1.32, and 18.7 mg kg⁻¹. Soil water percentage was noted using the Soil Moisture Meter (Top Instrument Co. Ltd. Zhejiang, China) from 12 to 15 cm of the soil layer. The average ambient air temperature (°C), relative air humidity (%), precipitation (mm), and light (h) data were monitored inside the greenhouse from March 2019 to September 2019 (Table 1).

4.2. Photosynthetic Responses. A portable photosynthesis system (Li-6800, Li-COR Biosciences, Lincoln, NE) was used to observe the net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) in the functional top visible dewlap leaf (leaf + 1) of the main stem. In line with the natural light intensity, temperature, and humidity between 9:00 and 11:00 in Nanning, Guangxi, the light intensity provided by the red and blue LED light source was adjusted to 1000 μmol (photon) $\text{m}^{-2} \text{s}^{-1}$, the leaf chamber (6 cm^2) temperature was fixed at 25 °C, and the CO_2 concentration was set to 400 μmol mol^{-1} by a CO_2 cylinder simulating the current atmospheric CO_2 level.

4.3. Scanning Electron Microscopic Examination. Fresh leaves (leaf + 1, middle segment) were obtained and prepared for scanning electron microscopy (SEM) to observe stomata ultrastructure. Photosynthetically fully matured leaves were used for SEM at 60 days after the application of Si and limited water. The middle segment of the leaves was sectioned into small pieces (near 1 mm). To inhibit entry of air bubbles, the samples were fixed in glutaraldehyde (2.5%, 24 h) and further fixed in a sodium sulfide solution (0.5%, pH 7.2, 30 min) and subsequently rinsed with phosphate buffer (0.1 M, pH 7.2) thrice (15 min intervals). The samples were then fixed in OsO_4 (1%) in phosphate buffer (0.1 M, pH 7.2) for 12 h (4 °C) and then dehydrated with increasing concentrations of ethanol series (30, 50, 70, 96, and 100%). After dehydration, the samples were embedded in LR white and polymerized (60 °C, 24 h). The leaf samples were seared under CO_2 using a critical-point drying instrument. Then, the samples were gold sputter-coated with a JFC-1600 metal sputtering equipment and imaged with a JMS-6490 (Japanese Electronics Companies). The leaf stomata characteristics, i.e., stomatal density (SD) and stomatal aperture size (SS), were determined. These factors are known to be affected by the maturity of the leaf, leaf position, leaf surface (abaxial or adaxial), and various stresses. The stomatal characteristics were analyzed by NIS Element 7.0 software.

4.4. Data Analysis. All of the data were subjected to analysis of variance according to a completely randomized block design using GraphPad Prism 5.00 statistical software for Windows (GraphPad Software, San Diego, California). The Tukey test was used to compare the means. Correlation coefficients among photosynthetic capacity and stomatal conductance were calculated to examine the relationship.

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Notes

The authors declare no competing financial interest.

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