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

Krishan K. Verma, # Xiu-Peng Song, # Yuan Zeng, Dong-Mei Li, Dao-Jun Guo, Vishnu D. Rajput, Gan-Lin Chen, Anatoly Barakhov, Tatiana M. Minkina, and Yang-Rui Li*

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.\(^3,4\)

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 CO\(_2\) 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 CO\(_2\) 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
efficiency, root growth zone, and uptake of nutrients and consequently enhance the crop yield.\textsuperscript{15,23}

Silicon has been commonly recognized as a fertilizer, biostimulating plant protection under atmospheric environmental variables.\textsuperscript{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 (SiO$_2$·nH$_2$O) called phytoliths.\textsuperscript{27,28} In particular, the connection of silicon with tolerance to environmental stresses has been studied extensively in various crops.\textsuperscript{15,24–31} Silicon might be associated with physiologic and molecular mechanisms in plants\textsuperscript{32,33} and potentially alleviates the detrimental impact of water stress, a severe abiotic stress.

Sugarcane (\textit{Saccharum officinarum} L.) is a major cash crop and is cultivated in the arid and semiarid areas of the world. In relation to geoponics, 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.\textsuperscript{5,15,20,21} The synergy of water and fertilizers or the combined use of both is an important measure to save water in agricultural crops.\textsuperscript{34–36}

However, knowledge about how Si modulates the photosynthetic capacity in \textit{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).
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 12–32% 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 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 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 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.

2.2. Correlation of Photosynthesis and Transpiration with Stomatal Conductance. Leaf stomatal conductance to water vapor ($g_s$) was positively correlated with photosynthesis ($P_N$, $R^2$) and transpiration rate ($E$, $R^2$) 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. $R^2$ and $R^2_p$ are $R$ square values on the chart of linear and polynomial regression types, respectively ($n = 5$).

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). The addition of Si enhanced stomatal aperture size, regardless of normal and limited water supply (Figure 3C,D,E,F,G,H). The increase in response to water stress was observed to cause reductions in the stomatal density and aperture size in sugarcane plant leaves. The enhanced stomatal density and aperture size
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 μm$^2$ 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 (100–500 mg L$^{-1}$) and limited water (55–50 and 35–30%...
through stomatal closure.\textsuperscript{15,53} Ming et al.\textsuperscript{43} and Chen et al.\textsuperscript{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.\textsuperscript{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 at leaf gas exchange. Although the stomatal aperture has a significant link with \( P_{30} \), \( E_a \), and \( g_\text{s} \) of crop plants,\textsuperscript{6,58,59} in this study, the leaf stomatal appearance was reduced with limited water irrigation, which is similar to the results reported by Xu and Zhou.\textsuperscript{6} Yang et al.\textsuperscript{59} and Meng et al.\textsuperscript{60} Stomatal density was also negatively associated with the length of stomata on various watering levels in \textit{Platanus acerifolia} and \textit{Leymus chinensis}.\textsuperscript{61} 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.\textsuperscript{52,63}

Dynamic adjustments to the opening degree of stomatal pores are linked to regulation of \( g_\text{s} \) in the short term, allowing plants to immediately reduce water loss according to atmospheric conditions.\textsuperscript{64} Over a longer duration, anatomical adaptations, i.e., variations to SS and SD, can modify the range of \( g_\text{s} \) by altering the higher \( g_\text{s} \). 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 \( g_\text{s} \) due to a smaller SS has been linked to higher water conservation, as demonstrated for plants subjected to water deficit.\textsuperscript{65} Growth during insufficient soil moisture capacity has been shown to cause a reduction in SS in various plant species/cultivars,\textsuperscript{66–69} but the effect on SD is less consistent.\textsuperscript{6,67–70} In response to variations in water availability, leaf morphology and ultrastructure can vary considerably.\textsuperscript{71} Specifically, with respect to stomatal numbers, variations in atmospheric variables that influence mature leaf \( g_\text{s} \) will have lasting effects on the stomatal differentiation of newly developing leaves.\textsuperscript{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.\textsuperscript{73,74}

Water deficit may initially inhibit plant development, significantly decreasing leaf area expansion,\textsuperscript{75,76} although stomatal density is closely linked to leaf growth and development. Casson and Hentherington\textsuperscript{71} reported that stomata morphology or size directly affected the photosynthetic CO\textsubscript{2} 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,
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\(\cdot\)SiO\(_2\)) was used as a source of Si. The

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—phytochelatins, 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. | min. | max. | min. | max. | min. | max. | daylight | sunshine |
| March | 15   | 22   | 82   | 50   | 12   | 12   | 10   | 10   | 12.0     | 2.0      |
| April | 20   | 26   | 81   | 107  | 10   | 10   | 12   | 12   | 12.7     | 3.0      |
| May   | 23   | 31   | 80   | 188  | 12   | 12   | 12   | 12   | 13.2     | 4.6      |
| June  | 25   | 32   | 81   | 216  | 12   | 12   | 12   | 12   | 13.5     | 5.4      |
| July  | 25   | 33   | 82   | 201  | 12   | 12   | 12   | 12   | 13.4     | 6.3      |
| August| 25   | 33   | 82   | 214  | 13   | 13   | 12   | 12   | 12.9     | 6.0      |
| September | 24   | 32   | 78   | 125  | 8    | 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.
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 (lum) 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 (${E_t}$), 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) m⁻² s⁻¹, the leaf chamber (6 cm²) temperature was fixed at 25 °C, and the CO₂ concentration was set to 400 μmol mol⁻¹ by a CO₂ cylinder simulating the current atmospheric CO₂ 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 OsO₄ (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 CO₂ 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.

■ AUTHOR INFORMATION

Corresponding Author
Yang-Rui Li — Key Laboratory of Sugarcane Biotechnology and Genetic Improvement (Guangxi), Ministry of Agriculture and Rural Affairs/Guangxi Key Laboratory of Sugarcane Genetic Improvement/Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530 007, Guangxi, China; Email: liyr@gxaas.net

Authors
Krishan K. Verma — Key Laboratory of Sugarcane Biotechnology and Genetic Improvement (Guangxi), Ministry of Agriculture and Rural Affairs/Guangxi Key Laboratory of Sugarcane Genetic Improvement/Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530 007, Guangxi, China; orcid.org/0000-0002-5501-7905
Xiu-Peng Song — Key Laboratory of Sugarcane Biotechnology and Genetic Improvement (Guangxi), Ministry of Agriculture and Rural Affairs/Guangxi Key Laboratory of Sugarcane Genetic Improvement/Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530 007, Guangxi, China
Yuan Zeng — International Co-operation Division, Guangxi Academy of Agricultural Sciences, Nanning 530 007, Guangxi, China
Dong-Mei Li — Key Laboratory of Sugarcane Biotechnology and Genetic Improvement (Guangxi), Ministry of Agriculture and Rural Affairs/Guangxi Key Laboratory of Sugarcane Genetic Improvement/Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530 007, Guangxi, China
Duo-Jun Guo — Key Laboratory of Sugarcane Biotechnology and Genetic Improvement (Guangxi), Ministry of Agriculture and Rural Affairs/Guangxi Key Laboratory of Sugarcane Genetic Improvement/Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530 007, Guangxi, China; College of Agriculture, Guangxi University, Nanning 530 004, Guangxi, China
Vishnu D. Rajput — Academy of Biology and Biotechnology, Southern Federal University, Rostov-on-Don 344 006, Russia; orcid.org/0000-0002-6802-4805
Gan-Lin Chen — Institute of Biotechnology, Guangxi Academy of Agricultural Sciences, Nanning 530 007, Guangxi, China
Anatoly Barakhov — Academy of Biology and Biotechnology, Southern Federal University, Rostov-on-Don 344 006, Russia
Tatiana M. Minkina — Academy of Biology and Biotechnology, Southern Federal University, Rostov-on-Don 344 006, Russia; orcid.org/0000-0003-3022-0883

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c03820

Author Contributions
K.K.V. and X.-P.S. have contributed equally to this work.

Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are thankful to the Guangxi Academy of Agricultural Sciences, Nanning, Guangxi, China, for providing the necessary facilities for this study. This study was financially supported by the Guangxi R and D Program Fund (GK17195100), the Fund for Guangxi Innovation Teams of Modern Agriculture Technology (gnyxgxcmxt-03-01), and the Fund of Guangxi Academy of Agricultural Sciences (2015YT02).

■ REFERENCES

(1) Cui, G.; Zhao, X.; Liu, S.; Sun, F.; Zhang, C.; Xi, Y. Beneficial effects of melatonin in overcoming drought stress in wheat seedlings. Plant Physiol. Biochem. 2017, 118, 138–149.
Verma, K. K.; Singh, P.; Song, X. P.; Malviya, M. K.; Singh, R. K.; Chen, G. L.; Solomon, S.; Li, Y. R. Mitigating climate change for sugarcane improvement: role of silicon in alleviating abiotic stresses. *Sugar Tech* 2020, 741–749.

Jiang, C.; Cui, Qi; Peng, K.; Xu, D.; Li, C.; Zheng, Q. Melatonin improves antioxidant capacity and ion homeostasis and enhances salt tolerance in maize seedlings. *Acta Physiol. Plant.* 2016, 38, 92.

Naeem, M.; Naeem, M. S.; Ahmad, B.; Ahmad, B.; Ashraf, M. Y.; Ihsan, M. Z.; Nawaz, F.; Athar, H. U. R.; Ashraf, M.; Abbas, H. T.; Abdullah, M. Improving drought tolerance in maize by foliar application of boron: water status, antioxidative defense and photosynthetic capacity. *Arch. Agron. Soil Sci.* 2018, 64, 626–639.

Nilson, S. E.; Assmann, S. M. The control of transpiration insights from Arabidopsis. *Plant Physiol.* 2007, 143, 19–27.

Xu, Z.; Zhou, G. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *J. Exp. Bot.* 2008, 59, 3317–3325.

Beeling, D. J.; Chaloner, W. G. The impact of atmospheric CO2 and temperature change on stomatal density: observations from *Quercus robur* Lammard leaves. *Ann. Bot.* 1993, 71, 231–235.

Woodward, F. L. Stomatal numbers are sensitive to increases in CO2 from pre-industrial levels. *Nature* 1987, 327, 617–618.

Zhao, S.; Chen, W.; Ma, D.; Zhao, F. Influence of different salt level on stomatal character in rice leaves. *Reclaiming Rice Cultiv.* 2006, 6, 26–29.

Zhao, R. X.; Zhang, Q. B.; Wu, X. Y.; Wang, Y. The effects of drought on epidermal cells and stomatal density of wheat leaves. *Inner Mongolia Agric. Sci. Technol.* 2001, 6, 6–7.

Galmés, J.; Flexas, J.; Save, R.; Medrano, H. Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: responses to water stress and recovery. *Plant Soil* 2007, 290, 139–155.

Yang, L.; Han, M.; Zhou, G.; Li, J. The changes of water-use efficiency and stomata density of *Leymus chinensis* along Northeast China Transsect. *Acta Ecol. Sin.* 2007, 27, 16–24.

Zhang, X. Y.; Yang, H. M.; Hou, Z. D.; Wang, G. X. Stomatal density and distributions of spring wheat leaves under different planting densities and soil moisture levels. *Chin. J. Plant Ecol.* 2003, 27, 133–156.

Chaves, M. M.; Flexas, J.; Pinheiro, C. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Ann. Bot.* 2009, 103, 551–560.

Verma, K. K.; Liu, X. H.; Wu, K. C.; Singh, R. K.; Song, Q. Q.; Malviya, M. K.; Song, X. P.; Singh, P.; Verma, C. L.; Li, Y. R. The impact of silicon on photosynthetic and biochemical responses of sugarcane under different salt levels. *Silicon* 2019, 1, 13–19.

Conric, G. Drought stress inhibits photosynthesis by decreasing stomatal aperture—not by affecting ATPsynthesis. *Trends Plant Sci.* 2000, 5, 187–188.

Song, X.; Zhou, G.; Xu, Z.; Lv, X.; Wang, Y. Detection of photosynthetic performance of *Stipa bungeana* seedlings under climatic change using chlorophyll fluorescence imaging. *Front. Plant Sci.* 2016, 7, 1254.

Petras, A.; Grammatikopoulos, G. Drought resistance and recovery of photosystem II activity in a Mediterranean semi-deciduous shrub at the seedling stage. *Photosynthetica* 2009, 47, 284–292.

Zivcak, M.; Kalaji, H. M.; Shao, H. B.; Olsovksa, K.; Brestic, M. Photosynthetic proton and electron transport in wheat leaves under prolonged moderate drought stress. *J. Photochem. Photobiol., B* 2014, 137, 107–115.

Chen, Z. K.; Niu, Y. P.; Ma, H.; Haifee, A.; Luo, H. H.; Zhang, W. F. Photosynthesis and biomass allocation of cotton as affected by deep-layer water and fertilizer application depth. *Photosynthetica* 2017, 55, 638–647.

Li, J. H.; Wang, Y. Y.; Li, N. N.; Zhao, R. H.; Khan, A.; Wang, J.; Luo, H. H. Cotton leaf photosynthetic characteristics, biomass production, and their correlation analysis under different irrigation and phosphorus application. *Photosynthetica* 2019, 57, 1066–1075.
(42) Amin, M.; Ahmad, R.; Basra, S. M. A.; Murtaza, G. Silicon induced improvement in morpho-physiological traits of maize (Zea mays L.) under water deficit. Pak. J. Agric. Sci. 2014, 51, 187–196.

(43) Ming, D. F.; Pei, Z. F.; Naem, M. S.; Gong, H. J.; Zhou, W. J. Silicon alleviates PEG-Induced water-deficit stress in upland rice seedlings by enhancing osmotic adjustment. J. Agron. Crop Sci. 2012, 198, 14–26.

(44) Saud, S.; Li, X.; Chen, Y.; Zhang, L.; Fahad, S.; Hussain, S.; Sadiq, A.; Chen, Y. Silicon application increases drought tolerance of Kentucky Bluegrass by improving plant water relations and morpho-physiological functions. Sci. World J. 2014, No. 368694.

(45) Habibi, G. Silicon supplementation improves drought tolerance in canola plants. Russ. J. Plant Physiol. 2014, 61, 784–791.

(46) Gunes, A.; Pilbeam, D. J.; Inal, A.; Coban, S. (2008) Influence of silicon on sunflower cultivars under drought stress, i.e., growth, antioxidant mechanisms, and lipid peroxidation. Commun. Soil Sci. Plant Anal. 2008, 39, 1885–1903.

(47) Gunes, A.; Inal, A.; Bagic, E. G.; Pilbeam, D. J. Silicon-mediated changes of some physiological and enzymatic parameters symptomatic for oxidative stress in spinach and tomato grown in sodic-B toxic soil. Plant Soil 2007, 290, 103–114.

(48) Shen, X. H.; Zhou, Y. Y.; Duan, L. S.; Li, Z. H.; Eneji, A. E.; Li, J. M. Silicon effects on photosynthesis and antioxidant parameters of soybean seedlings under drought and ultraviolet-B radiation. J. Plant Physiol. 2010, 167, 1248–1252.

(49) Liu, H. X.; Guo, Z. G. Forage yield and water use efficiency of alfalfa applied with silicon under water deficit conditions. Philipp. Agric. Sci. 2013, 96, 370–376.

(50) Shi, Y.; Zhang, Y.; Han, W.; Feng, R.; Hu, Y.; Guo, J.; Gong, H. Silicon enhances water stress tolerance by improving root hydraulic conductance in Solanum lycopersicum. J. Front. Plant Sci. 2016, 7, 196.

(51) Lii, D. T.; Maurel, C. Aquaporins in a challenging environment: molecular gears for adjusting plant water status. Plant Cell Environ. 2005, 28, 85–96.

(52) Verslues, P. E.; Agarwal, M.; Katiyar-Agarwal, S.; Zhu, J.; Gray, J. E. Genetic manipulation of stomatal density influences stomatal size, plant growth and tolerance to restricted water supply across a growth carbon dioxide gradient. Philos. Trans. R. Soc., B 2012, 367, 547–555.

(53) Chen, D.; Wang, S.; Yin, L.; Deng, X. How does silicon mediate plant water uptake and loss under water deficiency? Front. Plant Sci. 2018, 9, 281.

(54) Chen, W.; Yao, X.; Cai, K.; Chen, J. Silicon alleviates drought stress of rice plants by improving plant water status, photosynthesis and mineral nutrient absorption. Biotechnol. Adv. 2011, 29, 67–76.

(55) Hattori, T.; Inanaga, S.; Araki, H.; An, P.; Morita, S.; Luxova, M.; Lux, A. Application of silicon enhanced drought tolerance in Sorghum bicolor. Plant Physiol. 2005, 132, 459–466.

(56) Sonobe, K.; Hattori, T.; An, P.; Tsuji, W.; Eneji, E.; Tanaka, K.; Inanaga, S. Diurnal variations in photosynthesis, stomatal conductance and leaf water relation in Sorghum grown with or without silicon under water stress. J. Plant Nutr. 2009, 32, 433–442.

(57) Kang, J.; Zhao, W.; Zhu, X. Silicon improves photosynthesis and strengthens enzyme activities in the C-3 succulent xerophyte Zygophyllum xanthoxylosum under drought stress. J. Plant Physiol. 2016, 199, 76–86.

(58) Mattielli, L.; Riano-Pachon, D. M.; Martins, M. C. M.; de Cruz, L. P.; Bassi, D.; Marchiori, P. E. R.; Ribeiro, R. V.; Labate, M. T. V.; Labate, C. A.; Menossi, M. Physiological and transcriptional analysis of developmental stage along sugarcane leaf. BMC Plant Biol. 2015, 15, 300.

(59) Yang, J.; Jonathan, W.; Zhu, Q.; Peng, Z. Effect of water deficit stress on the stomatal frequency, stomatal conductance and abscisic acid in rice leaves. Acta Agron. Sin. 1995, 21, 533–539.

(60) Meng, L.; Li, L.; Chen, W.; Xu, Z.; Liu, L. Effect of water stress on stomatal density, length, width and net photosynthetic rate in rice leaves. J. Shenyang Agric. Univ. 1999, 30, 477–480.

(61) Zhang, H.; Wang, X.; Wang, S. A study on stomatal traits of Platanus acerifolia under urban stress. J. Fudan Univ. 2004, 43, 651–656.

(62) Maherali, H.; Reid, C. D.; Polley, H. W.; Johnson, H. B.; Jackson, R. B. Stomatal acclimation over a subambient to elevated CO2 gradient in a C3/C4 grassland. Plant Cell Environ. 2002, 25, 557–566.

(63) Liu, S.; Liu, J.; Cao, J.; Bai, C.; Shi, R. Stomatal distribution and character analysis of leaf epidermis of jujube under drought stress. J. Anhui Agric. Sci. 2006, 34, 1315–1318.

(64) Farquhar, G. D.; Sharkey, T. D. Stomatal conductance and photosynthesis. Annu. Rev. Plant Physiol. 1982, 33, 317–345.

(65) Franks, P. J.; Beerling, D. J. Maximum leaf conductance driven by CO2 effects on stomatal size and density over geologic time. Proc. Natl. Acad. Sci. U.S.A. 2009, 106, 10343–10347.

(66) Dow, G. J.; Bergmann, D. C.; Berry, J. A. An integrated model of stomatal development and leaf physiology. New Phytol. 2014, 201, 1218–1226.

(67) Doheny-Adams, T.; Hunt, L.; Franks, P. J.; Beerling, D. J.; Gray, J. E. Genetic manipulation of stomatal density influences stomatal size, plant growth and tolerance to restricted water supply across a growth carbon dioxide gradient. Philos. Trans. R. Soc., B 2012, 367, 547–555.

(68) Sun, Y.; Yan, F.; Cui, X.; Liu, F. Plasticity in stomatal size and density of potato leaves under different irrigation and phosphorus regimes. J. Plant Physiol. 2014, 171, 1248–1255.

(69) Zhao, W.; Sun, Y.; Kjelgren, R.; Liu, X. Response of stomatal density and boundary exchange in leaves of maize to soil water deficit. Acta Physiol. Plant. 2015, 37, 1704.

(70) Clifford, S. C.; Black, C. R.; Roberts, J. A.; Stronach, I. M.; Singleton-Jones, P. R.; Mohamed, A. D.; Azam-Ali, S. N. The effect of elevated atmospheric CO2 and drought onstomatal frequency in groundnut (Arachis hypogaea L.). J. Exp. Bot. 1995, 46, 847–852.

(71) Peña-Rojas, K.; Aranda, X.; Joffre, R.; Fleck, I. Leaf morphology, photochemistry and water status changes in resprouting Quercus iler during drought. Funct. Plant Biol. 2005, 32, 117–130.

(72) Miyazawa, S.-I.; Livingston, N. J.; Turpin, D. H. Stomatal development in new leaves is related to the stomatal conductance of mature leaves in poplar (Populus trichocarpa × P. deltoides). J. Exp. Bot. 2006, 57, 373–380.

(73) Li, C.; Tan, D. X.; Liang, D.; Chang, C.; Jia, D.; Ma, F. Melatonin mediates the regulation of ABA metabolism, free-radical scavenging, and stomatal behaviour in two Malus species under drought stress. J. Exp. Bot. 2015, 66, 669–680.

(74) Li, H.; Chang, J.; Chen, H.; Wang, Z.; Gu, X.; Wei, C.; Zhang, Y.; Ma, J.; Yang, J.; Zhang, X. Exogenous melatonin confers salt stress tolerance to watermelon by improving photosynthesis and reoxid homeostasis. Front. Plant Sci. 2017, 8, 1–9.

(75) Chaves, M. M.; Maroco, J. P.; Pereira, J. S. Understanding plant responses to drought-from genes to the whole plant. Funct. Plant Biol. 2003, 30, 239–264.

(76) Gazanchian, A.; Hajheidari, M.; Sima, N. K.; Salekdeh, G. H. Proteome response of Elymus elongatum to severe water stress and recovery. J. Exp. Bot. 2006, 58, 291–300.

(77) Casson, S. A.; Hetherington, A. M. Environmental regulation of stomatal development. Curr. Opin. Plant Biol. 2010, 13, 90–95.