

# RESEARCH PAPER 8 OPEN ACCESS



# Improving soybean drought tolerance via silicon-induced changes in growth, physiological, biochemical, and root characteristics

Malik Muhammad Abdullah<sup>a</sup>, Ejaz Ahmad Waraich<sup>a</sup>, Muhammad Ahmad<sup>a</sup>, Saddam Hussain<sup>a</sup>, Hafiz Naeem Asghar<sup>b</sup>, Arslan Haider<sup>c</sup>, Usman Zulfiqar od Zahoor Ahmad<sup>e</sup>, Walid Soufan<sup>f</sup>, PV Vara Prasad<sup>g</sup>, and Ivica Djalovic<sup>h</sup>

<sup>a</sup>Department of Agronomy, University of Agriculture, Faisalabad, Pakistan; <sup>b</sup>Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan; <sup>c</sup>Department of Botany, University of Agriculture, Faisalabad, Pakistan; <sup>d</sup>Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur, Pakistan; <sup>e</sup>Department of Botany, University of Central Punjab Constituent Punjab College, Bahawalpur, Pakistan; <sup>e</sup>Department of Plant Production, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia; <sup>e</sup>Department of Agronomy, Kansas State University, Manhattan, KS, USA; <sup>h</sup>Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad, Serbia

#### **ABSTRACT**

Drought-induced osmotic stress is a significant constraint to soybean growth and yield, necessitating the development of effective mitigation strategies. Silicon acts as an important strategy to mitigate the negative stress effects of drought stress. The study was aimed to evaluate the potential of soil-applied silicon in alleviating drought stress in soybean. Two field capacities were tested: control (85% FC) and drought (50% FC), with four silicon application rates (0, 100, 200, and 300 kg ha<sup>-1</sup>) applied at sowing. Drought stress significantly affected the morphological parameters in soybean as plant height, leaf area, and water potential were reduced by 25%, 20%, and 36%, respectively, while root length increased as compared to control-85% FC. However, drought stress reduced root density, surface area, and biomass as compared to control-85% FC. Additionally, drought reduced photosynthetic rates, chlorophyll a and b levels, and stomatal conductance, while increasing malondialdehyde and hydrogen peroxide. The natural plant defense system was upregulated, with increased activity of phenolics, soluble proteins, and antioxidant enzymes like catalase, superoxide dismutase, and peroxidase. However, silicon applications, especially at 200 kg ha<sup>-1</sup>, significantly alleviated the negative effects of drought stress by improving morphophysiological and biochemical traits in soybeans. Compared to the control, Si<sub>200</sub> increased plant height, root length, photosynthetic rate, and water potential by 22%, 39%, 23%, and 17%, respectively, as compared to control. Furthermore, silicon reduced malondialdehyde and hydrogen peroxide levels by 21% and 10%, enhancing plant resilience. Silicon supplementation also boosted biochemical attributes, with total soluble proteins, phenolics, and antioxidant enzyme activities increasing by 30%, 55%, 19%, 24%, and 31%, respectively, under drought conditions. In crux, silicon at 200 kg ha<sup>-1</sup> effectively mitigated the effects of drought stress in soybean, becoming a more sustainable approach to sustain crop yield and food security.

#### Silicon improved the seedling Drought stress decreased the growth & physiology growth and physiology Repolarization of Depolarization of membrane membrane Chloroplast Inhihit wate uptake Silicon application Oxidative Increase stress caused by photosynthesis ruptured Drought mitochondrial membrane mitochondria (Lipid Reduced Silicon peroxidation RWC ncreasing gas decreased of drought attributes Increased root Proliferated **Drought stress** drought stress root system

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CONTACT Malik Muhammad Ahmad ahmad ahmad ahmad ahmad bajwa516@gmail.com abpartment of Agronomy, University of Agriculture, Faisalabad 38040, Pakistan; Usman Zulfiqar auman.zulfiqar@iub.edu.pk abpartment of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan; Ivica Djalovic aivica.djalovic@ifvcns.ns.ac.rs all Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad, Serbia

#### 1. Introduction

Soybean (Glycine max L.) is a vital oilseed crop known for its adaptability to various climatic conditions. In Pakistan, soybean production is low as Pakistan produces approximately 1 million tonnes of soybean from an area of 0.2 m ha.<sup>2</sup> The current climate patterns, categorized by unpredictable rainfall patterns and limited water availability pose a significant threat to the world's food security.3-6 Furthermore, the increasing severity of abiotic stresses, including drought stress, driven by climate change, has become a major challenge in agricultural systems, 3,7,8,9 Drought stress significantly affects the yield potential of soybean crops in various parts of the world. 10-12 Drought negatively affects crop growth and yield as it significantly reduces the photosynthetic rate at the mechanistic level as a type of polygenic stress.<sup>8,13</sup> This includes thylakoid electron transport disruptions, stomatal conductance disruptions, CO<sub>2</sub> assimilation, and Calvin cycle disruptions. 14,15 Furthermore, the length and severity of the water shortage as well as the stage of growth at which plants are exposed to it such as the seedling, vegetative, or reproductive stages - also determine how severe the drought is and how it will affect crop performance. 14,16,17 At the crop level, the quality of traits of soybean seeds are essential indicators of their high market value, and these are influenced by moisture availability and soil fertility of soybean. Furthermore, drought stress interacts with key metabolic activities; impairs sink potential by altering the C:N ratio and significantly affecting the qualitative composition.<sup>8,18</sup> The imbalanced physiological interplay among tissue moisture, photosynthetic pigments, antioxidant enzymes, and the generation of reactive oxygen species (ROS), during episodes of drought adversely affects the overall crop output. 10,14,17 Consequently, one of the major challenges facing agriculture is to enhance crop yield or minimize crop losses while considering the effects of climate change and economic constraints. <sup>19,20,76</sup> Drought stress has emerged as a significant constraint affecting crop growth and development, particularly given the prevailing climatic conditions. Climate change models consistently project more frequent and severe droughts, posing a significant threat to the yields of key crops. 21,22 Specifically, soybean is susceptible to the detrimental effects of drought stress. The availability of water plays a crucial role in determining the overall performance and productivity of soybean. 17,23 Therefore, it is crucial to employ strategies to mitigate drought stress in field crops and support efforts to ensure adequate productivity even under drought conditions.

To reduce the losses brought on by drought, several tactics have been employed, including the use of crop varieties that are tolerant to the condition and enhanced irrigation management, strategic fertilization, and organic amendments. <sup>24–28</sup> Reaching the maximum yield may also be facilitated by efficient management techniques and access to sufficient, high-quality irrigation water. From the practical perspective, farmers prefer to use certain amendments (biostimulants, soil conditioners) to mitigate the limitations affecting their productivity; usually encountering multiple stressors like drought and other associated effects; for example, degraded soils. These amendments, may improve water holding capacity, and enhance soil

fertility and productivity by improving eco-physiological resilience. Thus, manipulating plant nutrition emerges as a highly promising and practical approach to regulating crop growth and yield under abiotic stresses. 21,29,30 It has been demonstrated that micronutrient treatments improve plant resilience to drought and raise antioxidant concentrations, which lowers ROS toxicity. 22,78 Silicon has attracted a lot of interest over the last 20 years because of its capacity to increase plant resistance to a variety of abiotic stressors.<sup>31</sup> Silicon has demonstrated great promise in enhancing crop quality, yield, and plant growth through its impact on photosynthetic activity, nitrogen assimilation, and resilience against stresses. 32,77 The presence of silicon is particularly notable in cell walls, intercellular gaps, and lumens, with accumulation occurring both above and below the cuticular layers, contributing to the numerous health benefits it provides when applied to crops.<sup>33</sup> The silicon content in the soil plays a pivotal role in determining its accumulation in plant tissues and its subsequent effects on plant physiology. 34 The silicon content varies significantly among plant species, with wheat (Triticum aestivum L.) reported to absorb 50-150 kg ha<sup>-1</sup>, rice (Oryza sativa L.) 150-300 kg ha<sup>-1</sup>, and sugarcane (Saccharum officinarum L.) 300-700 kg ha<sup>-1.35</sup> The application of silicon has been demonstrated to enhance morphophysiological attributes by positively influencing foliar chlorophyll content and physiological attributes.<sup>36</sup> Additionally, silicon-treated crops exhibit reduced concentrations of flavonoids, glutathione, and lipid peroxidation, while increasing ascorbate content.<sup>37</sup> Moreover, silicon supplementation was reported to enhance drought stress tolerance by upregulating antioxidant activities and maintaining high water potential.<sup>38</sup>

The applications of silicon in various crops under abiotic stresses have been well documented, but its specific impact on soybean under water-limited conditions, particularly through improvements in morphophysiological and biochemical attributes, is underexplored. We hypothesized that silicon soil applications may improve soybean drought stress tolerance by activating plant defense mechanisms by upregulating the antioxidant and osmolyte-induced defense. The objectives were to, i) evaluate the impact of silicon on the growth and physiological response of soybean under drought stress, and ii) examine the silicon-induced stress tolerance against drought by affecting root characteristics and biochemical responses.

# 2. Materials and methods

# 2.1. Experiment plan

Seeds of the "Faisal soybean" variety were acquired from the Ayub Agriculture Research, Institute Faisalabad, and an experiment was conducted in the wirehouse Department of Agronomy, University of Agriculture, Faisalabad in 2023. Plastic pots measuring 35 × 25 cm were used, filled with 5 kg of sieved, and cleaned sand after removing contaminants. Ten soybean seeds were maintained in each pot, and at 12 days after sowing (DAS) (formation of side shoots-BBCH scale code-12),

the thinning was done to maintain five seeds per pot.<sup>39</sup> The completely randomized design (CRD) was used to experiment with factorial arrangements and three replications. The experiment comprised two factors; drought stress and silicon application. The treatments were i) Field capacity (FC);  $FC_1 = 85\%$ FC and FC<sub>2</sub> = 50% FC, ii) silicon (Na<sub>2</sub>SiO<sub>3</sub>) applications; NSi= control-no silicon,  $Si_{100} = 100 \text{ kg ha}^{-1}$ ,  $Si_{200} = 200 \text{ kg ha}^{-1}$ , and  $Si_{300} = 300 \text{ kg ha}^{-1}$  which was applied at sowing. Silicon was applied at sowing to ensure silicon is available when root systems are actively developing, enhancing root architecture and water absorption. As is highly soluble, ensuring a steady supply of bioavailable silicon throughout the crop's early growth phase. A modified Hoagland solution was used to meet the crop's nutritional needs.<sup>21</sup>

# 2.2. Determination of field capacity of soil and maintenance of drought stress

The experiment comprised a total of 24 pots, which were divided into two sets, including normal-85% FC (12 pots) and drought stress-50% FC (12 pots). Drought stress was applied 20 days after sowing (DAS) (formation of side shoots-BBCH scale code-20). 39 To stop the sand from evaporating, the lid was put on, and the pot was left to drain until its weight stabilized. The weight of the water-saturated sand, plus the pot and lid was used as the "wet weight" (WW). Drought stress was maintained by using the standard gravimetric method. 40 Water was withheld until the pot weight reached a threshold level (85% and 50% F.C) and then re-watered to reach the pot at its initial weight. The experimental area was visited every 12 hours and irrigation water was applied to maintain the required field capacity levels.

The crop attributes, gas exchange parameters, biochemical parameters, seedling growth parameters, and root parameters were measured at 32 DAS, 33 DAS, 37 DAS, and 40 DAS, respectively.

# 2.3. Measurements

## 2.3.1. Seedling growth attributes

The characteristics related to seedling growth were evaluated by selecting two randomly chosen plants from each replication. The plant's height and the length of its roots were measured using a meter rod, and the findings were averaged. Using an electrical balance, the fresh weight of the root and shoot was ascertained (Kern 440 49A, Balingen, Germany). To measure the dry weight of the samples, they were subjected to oven drying (Shimadzu AW 320, Japan) and dry weight was measured with electrical balance (Kern 440-49A, Germany).

#### 2.3.2. Root attributes

To analyze and visualize the root data, WINRHIZO'S Companion Software was used to record root traits. Roots were carefully uprooted and cleaned using distilled water and tissue paper under consistent conditions to reduce variability. The cleaned roots were placed on a flatbed scanner and scanned with MINRHIZO. The root length was measured by averaging all the lengths. The data was used and software was set to measure the root surface area for all fine and coarse roots. The fresh and dry weight of roots was measured by using a digital weighing balance. The root mass was used to calculate the root density which is the ratio of root weight over soil volume. The root volume was calculated by using the detected root diameter and root length with MINRHIZO, while the root projected area was calculated based on the scanned images and the software's analysis of pixel areas occupied by roots.

## 2.3.3. Plant water relation parameters

The uppermost, third, and fully developed, leaves of each treatment were collected for water potential.<sup>3</sup> Between 8:00 and 10:00 am, the sampling was done and water potential was measured with a pressure chamber (Scholander-type).<sup>41</sup> To measure the leaf osmotic potential, the same samples were frozen for 10 days at -20°C. Crushing the frozen samples released the cell-sap, collected in a glass-tube, and injected into the osmometer using a syringe to measure osmotic potential (USA, Wescor-5500). To estimate the relative water contents, the fresh weight of the samples was first determined using a digital scale, and then these samples were soaked in distillate water for 24 hours. Tissue paper was used to dry the samples, and turgid weight was measured. The samples were dried at 65°C and dry weight was taken. The relative water contents were measured with the formula of Mullan and Pietragalla. 42 The amount of irrigation water and the difference in soil moisture in the pot between the start of the treatment (the first harvest) and the end of the harvest were combined to estimate the water use per plant (cm<sup>3</sup>). Dry mass (g) means the total dry mass obtained from leaves, stems, and roots. Water use efficiency (kg m<sup>-3</sup>) of plants was estimated as dry mass to water use ratio.

## 2.3.4. Leaf exchange parameters

To determine the plant gas exchange, a portable photosynthetic system (IRGA-CI-340, Camas, WA, USA) was employed<sup>3</sup> at 22.4°C and 27.9°C outside temperature, 99.9 K Pa atmospheric pressure, 6.0 and 8.9 m bar vapor pressure of water to inside the chamber's walls, 403.3 mmol m<sup>-2</sup> s<sup>-1</sup> air flow rate per unit area, 352 mol 5 mol<sup>-1</sup> ambient carbon dioxide, and the average temperature of the leaf was between 28.5°C and 32.5°C. The gas exchange measurements were conducted following the methodology outlined by Farquhar et al.<sup>43</sup>

## 2.3.5. Chlorophyll contents

Fresh soybean leaf samples were collected to determine the chlorophyll concentration, following the methods described by Arnon,<sup>44</sup> and Davies.<sup>45</sup> For the analysis, approximately 0.1 g of freshly chopped leaf sample was mixed with acetone (10 Ml, 80%) and then centrifuged at 12,000 rpm for 5 min. at 0°C. The absorbance was measured on a spectrophotometer at wavelengths of 663 nm and 645 nm, respectively (Hitachi, 220, Japan).

## 2.3.6. Enzymatic antioxidants activities

The method was suggested by Chance and Maehly, 46 to assess peroxidase and catalase activity. After the preparation of the solution, the phosphate cold buffer (1.9 mL), H<sub>2</sub>O<sub>2</sub> (1 mL), and

plant extract (1 mL) were put into a cuvette. The decomposition of H<sub>2</sub>O<sub>2</sub> was followed by monitoring the decrease in absorbance at 240 nm using a spectrophotometer (IRMECO U2020 UV-visible). The decrease in the rate of absorbance is used to calculate CAT activities. The activities of POD involved using a reaction mixture containing the enzyme extract (50 μL), hydrogen peroxide (100 μL), buffer (750 μL), and guaiacol (100 µL) were placed in a cuvette. The reaction is initiated by adding H2O2, and the formation of oxidized product, tetraguaiacol, is monitored with a spectrophotometer by measuring the increase the absorbance at 470. The POD activities were calculated based on the rate of change in absorbance over time. The SOD activity was identified using Stagner and Popovic,<sup>47</sup> techniques. In plastic cuvettes, distilled water (400 µL) was added first, then potassium phosphate buffer  $(250 \,\mu\text{L})$ , triton X solution  $(0.1 \,\text{mL})$ , NBT solution  $(50 \,\mu\text{L})$ , plant sample (0.5 mL), L-methionine solution (0.1 mL), and riboflavin solution (50 μL) were added and mixed. Every solution was poured into a cuvette at that point. To start the reaction, they were exposed to light for 15 minutes. Then, at 560 nm, the absorbance of each sample and the blank sample were measured using a spectrophotometer.

## 2.3.7. Osmolytes production

Total soluble proteins were calculated using the Bradford, 48 bioassay. The material was first homogenized, then to separate the pellet and centrifuged at 12,000 rpm for 15 minutes. A test tube containing 100 microliters of the extract was filled after the samples were vortexed and 5 mL of Bradford reagent was added. Following that, absorbance was measured at 595 nm. Phenolic contents were measured by following the Folin-Ciocalteu-technique. 49 Thoroughly mixed the Folin-Ciocalteu reagent, 7.5 mL of deionized water (7.5 mL), and a sample (0.5 mL) or series of gallic acid standards (0.5 mL) of the aqueous extract were carefully mixed. Before adding sodium carbonate, the supernatant (1.5 mL; 20%) was left for 10 minutes at room temperature. The mixes were then placed in a water bath and cooked for 20 minutes at 40°C before being immediately cooled. At 755 nm, the absorbance was measured.

## 2.3.8. Stress indicators

Among the stress indicators malondialdehyde (MDA) was measured by following the mechanism of Cakmak and Horst.<sup>50</sup> The supernatant was prepared by homogenizing trichloroacetic acid and centrifuged. The supernatant along with thiobarbituric acid (TBA) and TCA was heated for 30 minutes at 95°C and cooled in an ice bath. The absorbance was measured at 532 nm by using a spectrophotometer. Heath and Packer<sup>51</sup> proposed a method to measure H<sub>2</sub>O<sub>2</sub>. The reaction mixture was prepared containing supernatant, potassium iodide, and phosphate buffer. The buffer was incubated, and the results in the formation of a yellowish complex. The absorbance of this complex was measured at 390 nm. The amount of H<sub>2</sub>O<sub>2</sub> was measured by using the standard curve.

## 2.3.9. Statistical analysis

Experimental data were analyzed by analysis of variance techniques using the statistical software Statistix 8.1. The means were compared at a 5% confidence interval by using the LSD test (least significant difference).<sup>52</sup> The graphs of the collected data were created using Microsoft Excel Worksheet version 2016. The correlation matrix and polar heatmap were created by Using R-studio (v4.3.3).

## 3. Results

Analysis of variance had shown the significant ( $p \le 0.05$ ) impact of drought and silicon applications on root and shoot growth, plant physiology, and biochemical attributes in soybean. The interaction of silicon × drought stress was significant for root fresh and dry weight, root projected area, root density, root surface area, leaf area, stomatal conductance, and total soluble proteins.

## 3.1. Morphological attributes

Plant growth attributes were significantly ( $p \le 0.05$ ) reduced under drought stress-50% FC as compared to control-85% FC (Table 1). Plant height (PH) and leaf area (LA) in soybean were reduced under drought by 25% and 20% as compared with

Table 1. Impact of silicon applications on plant growth attributes in soybean grown under drought stress.

Treatments	Plant height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Root surface area (cm²/ plant)	Root volume (cm³/plant)	Root density (cm/cm <sup>3</sup> soil)	Root projected area (cm²/plant)
Silicon										
NSi-no silicon	16.6c	3.34c	0.48b	19.0d	1.10d	0.12d	27.8d	0.32d	1.28d	10.2d
Si <sub>100</sub>	20.0b	3.53b	0.54ab	22.5c	1.20c	0.14c	33.5c	0.35c	1.53c	11.0c
Si <sub>200</sub>	24.0a	3.67a	0.62a	30.2a	1.30a	0.16a	44.8a	0.46a	2.01a	14.3a
Si <sub>300</sub>	20.6b	3.50b	0.58a	26.7b	1.24b	0.15b	37.2b	0.41b	1.68b	11.9b
LSD ( <i>p</i> ≤0.05)	2.96	0.09	0.08	1.37	0.02	$9.89E^{-03}$	1.33	0.01	0.05	0.29
Drought										
Control-85% FC	22.6a	4.04a	0.64a	26.8a	1.29a	0.16a	39.8a	0.42a	1.77a	13.5a
Drought stress-50% FC	18.0b	2.99b	0.47b	22.4b	1.13b	0.12b	31.9b	0.35b	1.47b	10.2b
LSD ( <i>p</i> ≤0.05)	2.09	0.06	0.05	0.97	0.01	$6.95E^{-03}$	0.94	0.01	0.04	0.21
Interaction										
Silicon × Drought stress	ns	ns	ns	ns	***	***	***	***	ns	***

ns = significant, \*\*\* = significant ( $p \le 0.05$ ), NSi = control-no silicon, Si<sub>100</sub> = 100 kg ha<sup>-1</sup>, Si<sub>200</sub> = 200 kg ha<sup>-1</sup>, and Si<sub>300</sub> = 300 kg ha<sup>-1</sup>. Values having same letters or without lettering do not differ significantly ( $p \le 0.05$ ) by the least significant difference test, for a parameter.

control. Among the growth parameters, drought stress reduced the shoot fresh weight (SFW) and shoot dry weight (SDW) by 27% and 33% as compared to the control. However, silicon applications played an important role in ameliorating the negative effects of drought stress. Results have revealed that PH and LA were improved by 22% and 14% compared with no silicon applications. The highest soybean growth attributes were observed at silicon applied at 200 kg ha<sup>-1</sup>, followed by 300, and 100 kg ha<sup>-1</sup>, respectively (Table 1). Results showed that SFW and SDW were improved by 11 and 14% under silicon soil applications as compared to control. Applications of 200 kg ha<sup>-1</sup> silicon improved PH, LA, SFW, and SDW by 33%, 19%, 15%, and 22%, respectively compared with the control.

## 3.2. Root attributes

Results have shown a significant ( $p \le 0.05$ ) role of drought stress and silicon applications on root attributes in soybean (Table 1). Root length (RL) was reduced by 20%, while root surface area (RSA), root density (RD), root volume (RV), root projected area (RPA), root dry weight (RDW), and root fresh weight (RFW) are decreased by 20%, 17%, 15%, 24%, 26%, and 12% as compared to control. However, silicon applications played an ameliorative role in combating drought stress. Root attributes were improved by silicon applications in soybean drought stress. Furthermore, RSA, RD, RV, RPA, RDW, and RFW were improved by 37%, 36%, 26%, 22%, 21%, and 13% under silicon application in soybean relative to no silicon applications. In addition, the highest improvement in root attributes was noted when silicon was applied at 200 kg ha<sup>-1</sup>

followed by 300, and 100 kg ha<sup>-1</sup>, respectively. Applications of 200 kg ha<sup>-1</sup> silicon improved the RSA, RD, RV, RPA, RDW, and RFW by 61%, 57%, 43%, 30%, 32%, and 17%, respectively as compared to control.

# 3.3. Leaf gas exchange parameters

The gas exchanges in soybean plants were significantly ( $p \le 0.05$ ) affected by drought and silicon applications (Figure 2). According to results, the photosynthetic rate (Pn), stomatal conductance (gs), intercellular  $CO_2$  concentration (Ci), and transpiration rate (Tr) were reduced by 21%, 34%, 30%, and 25% in soybean, respectively as compared to control-no stress. However, soil-applied silicon improved Pn, gs, Ci, and Tr by 23%, 51%, 12%, and 13%. Among silicon levels, the maximum improvement in gas exchange parameters was observed at 200 kg ha<sup>-1</sup> followed by 300 kg ha<sup>-1</sup>, and 100 kg ha<sup>-1</sup>. The Pn, gs, Ci, and Tr were improved by 34%, 79%, 19%, and 19% at 200 kg ha<sup>-1</sup> silicon applied relative to control-no silicon (Figure 1).

## 3.4. Water relations

Results have shown a significant ( $p \le 0.05$ ) role of drought stress and silicon applications on plant water status in soybeans. Significant ( $p \le 0.05$ ) effect of silicon application and drought stress in water relations for soybean (Figure 1). Drought stress led to reduced relative water content (RWC), water potential (WP), turgor potential (TP), and osmotic potential (OP) by 20%, 36%, 24%, and 13% in soybean, respectively, as compared to control. Though, soil-applied silicon enhanced RWC, WP, TP, and OP by 29%, 17%, 15%, and

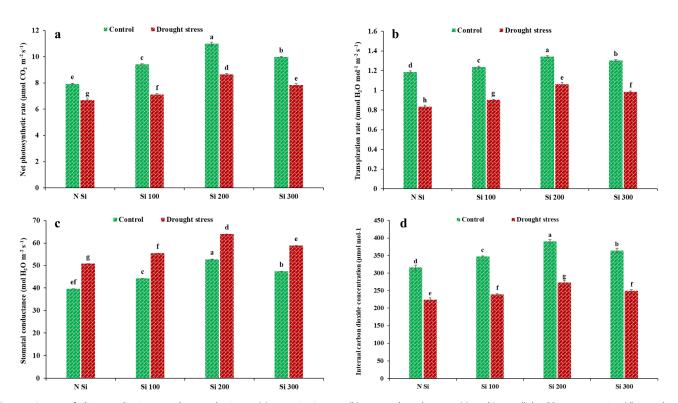


Figure 1. Impact of silicon applications on photosynthetic rate (a), transpiration rate (b), stomatal conductance (c), and Intercellular  $CO_2$  concentration (d) in soybean grown under drought stress (50% FC). NSi (control-no silicon),  $Si_{100}$  (silicon applied at 100 kg ha<sup>-1</sup>),  $Si_{200}$  (silicon applied at 200 kg ha<sup>-1</sup>), and  $Si_{300}$  (silicon applied at 300 kg ha<sup>-1</sup>).

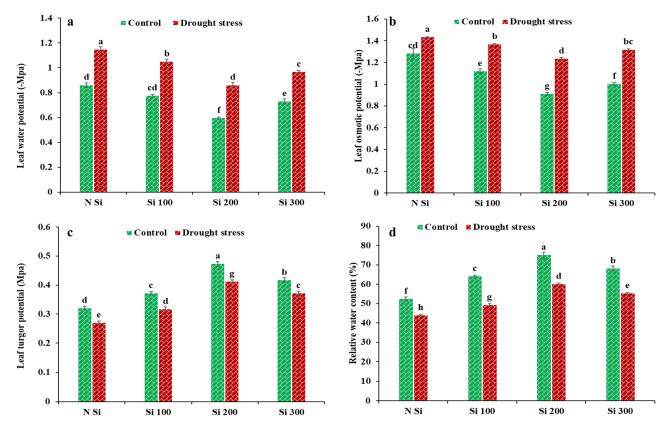


Figure 2. Impact of silicon applications on water potential (a), osmotic potential (b), turgor potential (c), and relative water content (d) in soybean grown under drought stress (50% FC). NSi (control-no silicon), Si<sub>100</sub> (silicon applied at 100 kg ha<sup>-1</sup>), Si<sub>200</sub> (silicon applied at 200 kg ha<sup>-1</sup>), and Si<sub>300</sub> (silicon applied at 300 kg ha<sup>-1</sup>).

33%. Among silicon levels, maximum water relations were observed at 200 kg ha<sup>-1</sup> followed by 300 kg ha<sup>-1</sup>, and 100 kg ha<sup>-1</sup>. In addition, RWC, WP, TP, and OP were improved by 40%, 27%, 21%, and 50% at 200 kg ha<sup>-1</sup> silicon applied relative to control-no silicon (Figure 2).

# 3.5. Water use efficiency (WUE)

Drought and silicon applications significantly ( $p \le 0.05$ ) influenced WUE in soybean plants. Drought reduced WUE by 20%, while soil-applied silicon improved it by 16% compared to the control without silicon. The most notable improvement in WUE was observed with 200 kg ha<sup>-1</sup> of silicon, followed by 300 kg ha<sup>-1</sup> and then 100 kg ha<sup>-1</sup>. Specifically, WUE increased by 25% with 200 kg ha<sup>-1</sup> of silicon compared to the control (Figure 3).

# 3.6. Photosynthetic pigments

Drought stress and silicon applications showed a significant  $(p \le 0.05)$  effect on photosynthetic pigments (Figure 3). Results have shown that drought stress reduced the chlorophyll a (chl a) and chlorophyll b (chl. b) in soybeans by 21% and 16%, respectively relative to control. Through the application of silicon, statistical analysis revealed a significantly higher concentration of chl a and b in the soybean plant. Soybean plants subjected to soil applications of silicon improved the chl a and b contents by 15% and 18% compared with control-no silicon. The highest increase was observed when silicon was applied at 200 kg ha<sup>-1</sup> followed by 300 and at 100 kg ha<sup>-1</sup>, respectively. Silicon applications at 200 kg ha<sup>-1</sup> silicon improved the chl a and chl b by 19% and 25%, respectively, relative to controlno silicon (Figure 3).

## 3.7. Stress indicators

A significant ( $p \le 0.05$ ) effect of silicon application and drought stress on the production of oxidants was observed in soybeans (Table 2). Drought stress increased the malondialdehyde (MDA) production and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as compared to control. However, silicon applications played an ameliorative role in reducing the MDA and H<sub>2</sub>O<sub>2</sub> contents by 21% and 10% relative to control (Table 2). Maximum reduction in MDA and H<sub>2</sub>O<sub>2</sub> activities was observed at 200 kg ha<sup>-1</sup> silicon applications followed by at 300 kg ha<sup>-1</sup>, and 100 kg ha<sup>-1</sup>. The MDA and H<sub>2</sub>O<sub>2</sub> activities were reduced by 42% and 12% at 200 kg ha<sup>-1</sup> silicon applications as compared to control.

## 3.8. Enzymatic antioxidant activity

A significant ( $p \le 0.05$ ) effect of silicon application and drought stress on enzymatic antioxidant activities in soybean was noted (Table 2). Drought stress improved the catalase, superoxide dismutase, and peroxidase activities due to the natural defense system in soybean as compared to control. However, silicon applications upregulated the antioxidant defense system as CAT, SOD, and POD activities were improved by 24%, 30%, and 31% as compared to control (Table 2). Maximum

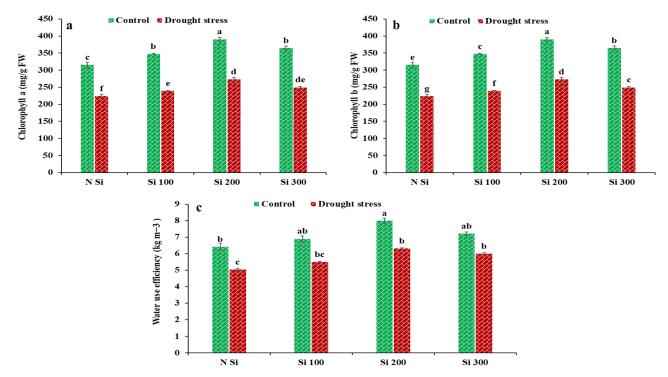


Figure 3. Impact of silicon applications on chlorophyll a (a), chlorophyll b (b), and water use efficiency (c) in soybean grown under drought stress (50% FC). NSi (controlno silicon), Si<sub>100</sub> (silicon applied at 100 kg ha<sup>-1</sup>), Si<sub>200</sub> (silicon applied at 200 kg ha<sup>-1</sup>), and Si<sub>300</sub> (silicon applied at 300 kg ha<sup>-1</sup>).

Table 2. Impact of silicon applications on osmolytes and enzymatic antioxidants attributes in soybean grown under drought stress.

Treatments	Total soluble phenolics (mg g <sup>-1</sup> FW)	Total soluble proteins (mg g <sup>-1</sup> FW)	Peroxidase (Unit g <sup>-1</sup> FW)	Catalase (Unit g <sup>-1</sup> FW)	Superoxide dismutase (Unit g <sup>-1</sup> FW)	Malondialdehyde content (µmol g <sup>-1</sup> FW)	$H_2O_2$ (μmol g <sup>-1</sup> FW)
Silicon							
NSi-no silicon	4.38d	19.0d	25.0d	37.8d	45.2d	5.24a	0.76a
Si <sub>100</sub>	5.07c	24.7c	29.3c	42.7c	49.8c	5.00b	0.70b
Si <sub>200</sub>	6.30a	34.1a	37.3a	51.5a	58.3a	2.99d	0.67c
Si <sub>300</sub>	5.76b	29.8b	32.6b	46.6b	53.1b	4.13c	0.69b
LSD $(p \le 0.05)$	0.19	2.08	2.03	2.22	1.43	0.23	0.01
Drought							
Control-85% FC	4.77b	23.8b	25.4b	38.7b	46.0b	0.36b	0.58b
Drought stress-50% FC	5.99a	30.08a	36.3a	50.6a	57.2a	8.24a	0.83a
LSD $(p \le 0.05)$	0.13	1.47	1.43	1.55	1.01	0.32	0.01
Interaction							
Silicon $\times$ Drought stress	ns	***	ns	ns	ns	***	ns

ns = significant, \*\*\* = significant ( $p \le 0.05$ ), NSi = control-no silicon, Si<sub>100</sub> = 100 kg ha<sup>-1</sup>, Si<sub>200</sub> = 200 kg ha<sup>-1</sup>, and Si<sub>300</sub> = 300 kg ha<sup>-1</sup>. Values having same letters or without lettering do not differ significantly ( $p \le 0.05$ ) by the least significant difference test, for a parameter.

improvement in enzymatic antioxidant activities was observed at 200 kg  $ha^{-1}$  silicon applications followed by 300, and 100 kg  $ha^{-1}$ . The CAT, SOD, and POD activities were improved by 36%, 44%, and 49% at 200 kg  $ha^{-1}$  silicon applications as compared to control.

## 3.9. Osmolytes production

Results have shown a significant ( $p \le 0.05$ ) effect of silicon application and drought stress on osmolyte production was measured in soybeans (Table 1). Drought stress applications showed an improvement in the total phenolics content and total soluble proteins under the natural defense system in soybean to combat the negative impacts of drought stress. However, applications of silicon enhanced the total phenolic content and soluble protein accumulations by 30% and 55%

compared to the control (Table 2). The highest improvement in osmolyte production was observed at 200 kg ha<sup>-1</sup> silicon applications followed by 300, and 100 kg ha<sup>-1</sup>. Total phenolics content and total soluble protein productions were improved by 44% and 79% at 200 kg ha<sup>-1</sup> silicon applications as relative to control-no silicon.

#### 3.10. Correlation matrix

The results revealed a strong correlation between morphological and physicochemical parameters in soybeans grown under drought stress (Figure 4). The growth parameters showed a strong positive association with relative water content, photosynthetic rate, and chlorophyll contents. Root analysis showed a strong positive correlation with the water status of plants showing a strong correlation with crop growth. Under drought



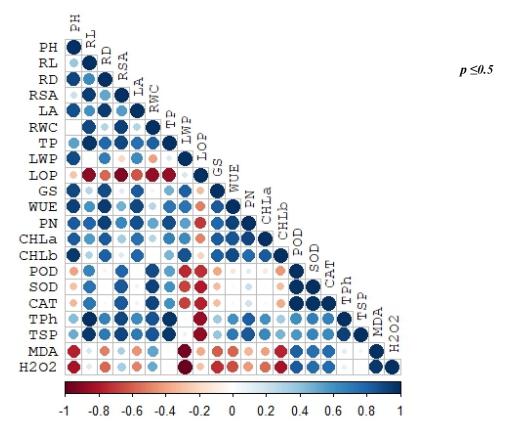


Figure 4. Correlation analysis among different parameters of soybean grown under drought stress and silicon applications. Root architecture (root length (RL), root-fresh weight (RFW), root-dry weight (RDW), root surface area (RSA), root volume (RV), root density (RD), and root projected surface area (RPA), shoot length (SL), shoot fresh weight (SFW) and shoot dry weight (SDW), water relations (water potential (WP), relative water content (RWC), osmotic potential (OP), and turgor potential (TP)), gas exchange parameters (photosynthetic rate (pn), inter stomatal CO<sub>2</sub> concentration (Ci), stomatal conductance (gs), and transpiration rate (tr)), osmolytes (total soluble phenolics (TPh) and total soluble proteins (TSP)) and antioxidant activities (catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and Malondialdehyde (MDA)) of soybean plants grown under well-watered (85% field capacity) and drought stress (50% field capacity). NSi (control-no silicon), Si<sub>100</sub> (100 kg  $ha^{-1}$ ),  $Si_{200}$  (200 kg  $ha^{-1}$ ), and  $Si_{300}$  (300 kg  $ha^{-1}$ ).

stress enzymatic antioxidants and osmolyte production showed a strong correlation with plant morpho-physiological attributes in soybean. Additionally, a significant correlation between various antioxidants and osmolyte activity was found.

# 3.11. Heatmap

A heatmap with a dendrogram was used to evaluate the impact of silicon on the morphophysiological and biochemical attributes of soybean under drought stress. The observations were organized based on their characteristics throughout various treatment phases, with connections between them illustrated by colored squares. When silicon was applied under drought, the color purple displays a strong positive correlation whereas the color blue-green displays a negative correlation of various findings. Three groups have formed in the heatmap. Root attributes, osmolyte accumulation, and antioxidant activities were grouped in the first group. The observed attributes are strongly positive correlation with drought stress (50% FC) and silicon (200 and 300 kg ha<sup>-1</sup>) and poorly correlated, at silicon (100 kg ha<sup>-1</sup>) under drought (50% FC) stress. While under control (85% FC) conditions, above mentioned attributes showed a strong negative correlation at control and N Si (No silicon) and weakly correlated at control and silicon (100 kg ha<sup>-1</sup>).

demonstrating that the addition of silicon (300 kg ha<sup>-1</sup>) reduced the effects of oxidative damage by increasing the concentration of organic osmolytes and antioxidants. These parameters are strongly positively correlated at drought (50% FC) and No Si, and weakly correlated at drought (50% FC) and silicon (100 kg ha<sup>-1</sup>). Conversely, a strong inverse relationship was observed between control (85% FC) and N Si (No silicon), and a weakly negative correlation between control (85% FC) and silicon. The second group of water relation attributes consists of leaf water potential and leaf osmotic potential (100 kg ha<sup>-1</sup>). This observation showed that under drought stress leaf water potential of soybean improved by the application of silicon. The third group is the largest group containing root attributes (RV, RPA, RSA, RD, and RFW) and gaseous exchange attributes stomatal conductance and net photosynthetic rate were clustered. It has been shown that the mentioned attributes are strongly positively correlated to control (85% FC) and silicon (200 and 300 kg ha<sup>-1</sup>) while showing a negative correlation at drought (50% FC) No silicon. These findings showed that the application of 300 kg ha<sup>-1</sup> proved beneficial in increasing, gas exchange, and root attributes of soybean. The fourth group is water relation attributes (WUE), growth attributes (SL, SFW, SDW, and RDW), and chlorophyll a were clustered. These parameters are strongly positively correlated at

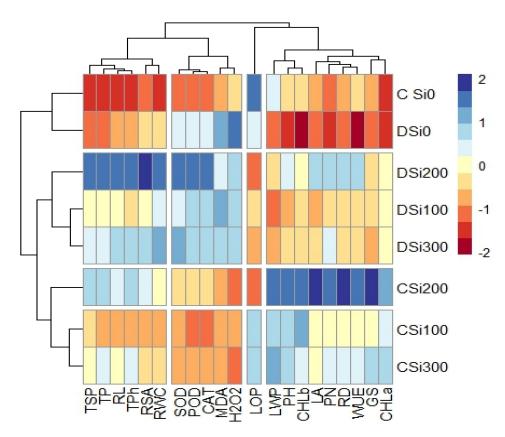


Figure 5. Heatmap of various attributes of soybean, including transpiration rate (TR), inter stomatal CO<sub>2</sub> concentration (Ci), shoot fresh weight (SFW) and shoot dry weight (SDW), root-dry weight (RDW), leaf area (LA), chlorophyll a (chl a), shoot length (SL), water use efficiency (WUE), root density (RD), stomatal conductance (gs), root projected surface area (RPA), root surface area (RSA), turgor potential (TP), root volume (RV), root-fresh weight (RFW), chlorophyll b (chl b), photosynthetic rate (pn), relative water content (RWC), water potential (WP), osmotic potential (OP), catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD), hydrogen peroxide (H<sub>2</sub> O2) and malondialdehyde (MDA), root length (RL), and total soluble proteins (TSP) of soybean plants grown under well-watered (85% field capacity) and drought stress (50% field capacity). NSi (control-no silicon), Si<sub>100</sub> (100 kg ha<sup>-1</sup>), Si<sub>200</sub> (200 kg ha<sup>-1</sup>), and Si<sub>300</sub> (300 kg ha<sup>-1</sup>).

control ((85% FC) and Silicon (200 kg ha<sup>-1</sup>), and weakly correlated at drought (50% FC) and silicon (100 kg ha<sup>-1</sup>) while strong negative correlation at drought (50% FC) No silicon and weakly negative correlated at control (85% FC) and silicon (100 kg ha<sup>-1</sup>). This observation showed that under drought stress water relation attributes (WUE), growth attributes, and chlorophyll an attribute of soybean improved by the application of silicon (Figure 5).

#### 4. Discussion

The evaluation of drought-induced damage on morphophysiological, and biochemical attributes in soybean plants, as well as the identification of strategies in alleviating the deleterious effects of drought stress, is of critical importance. The study has reported how soybean have responded to drought-stressed conditions and whether the applications of silicon could have alleviated the negative effects caused by drought stress. Our results showed that silicon enhanced plant growth by modifying cellular and metabolic mechanisms and improving the plant defense system in soybean under normal and drought-stressed conditions (Tables 1 and 2).

The natural defense system of plants is a vital mechanism in adapting biologically to drought stress. However, prolonged drought stress can lead to reduced water uptake, impeding growth and development in soybean. The negative effects of drought stress on PH, RFW, RDW, SFW, and SDW were observed in this study (Table 1), highlighting the detrimental effects on soybean growth attributes. Conversely, the application of silicon was able to restore plant growth characteristics, alleviating the damages caused by drought stress (Table 1). Similar findings have been reported in various crop species, including wheat,<sup>53</sup> rice,<sup>54</sup> maize,<sup>55</sup> and soybean,<sup>55,56</sup> where silicon supplementation improved morphological attributes under stressed conditions. This positive effect can be attributed to silicon's ability to enhance the mechanical strength of plants, thereby increasing their resistance to drought stress. Notably, the optimal concentration of silicon in this study was found to be 200 kg ha<sup>-1</sup>, which resulted in increased growth attributes in soybean drought stress. Ruppenthal et al.,<sup>57</sup> further emphasized the important role of silicon applications in restoring soybean growth and development under drought stress.

Drought stress considerably affected the root growth in soybean as reported in the results. The results showed the significant effects of drought stress on root attributes in soybean compared to the control without drought stress (Table 1). Drought stress reduces the plant growth attributes due to the impairment in cell division and elongation, which are crucial for establishing proper root architecture. To sustain plant morphology, plants allocate resources to promote the development of their underground parts, enabling them to access sufficient moisture for normal physiological mechanisms.<sup>58</sup> In the current experiment, various parameters were measured to assess the sensitivity of soybean roots to drought stress. The results demonstrated the detrimental effect of drought stress on root architecture. However, the application of silicon in the soil was able to support plant growth under drought stress, serving as a mechanism to enhance resilience against drought stress. Silicon application positively influenced root attributes in soybean plants, leading to longer root length, root volume, and root density even under drought (Table 1). These findings aligned with a study by Shao et al.,<sup>59</sup> which demonstrated that silicon applications can enhance root attributes and improve drought stress tolerance in soybean plants.

One of a plant's most vital functions and the cornerstone of their potential to grow, develop, and reproduce is photosynthesis. 60,15 This fascinating phenomenon encompasses several physicochemical processes at the photosynthetic apparatus in addition to the intricate gas exchange ( $CO_2$  and  $O_2$ ) process through leaf stomata. <sup>32,15,60</sup> In our study, the stomatal conductance diminished under drought stress where available water was scarce to meet the requirements of evapotranspiration (Figure 1). Bheemanahalli et al.<sup>61</sup> Results have revealed a reduction in plant water status, which is related to stomatal opening and closing. The lower tissue water content resulted in reduced stomatal conductance and photosynthesis (Figure 1). Exogenous silicon increased gas exchange parameters under drought stress, and the effect was greatest at a concentration of 200 kg ha<sup>-1</sup> (Figure 1). This might be because cellulose and silicon are applied to the epidermal cell walls to form a thicker silica gel layer, which reduced water loss. 56,62 Furthermore, the transpiration losses from the leaf surface may be decreased due to silicon depositions and the thickness of leaves that improve the water status of plant leaves. 63 Sharma et al. 64 revealed that stomatal conductance is a crucial factor in photosynthesis under drought stress. The addition of silicon improved transpiration and photosynthesis while preventing the decline of stomatal conductance brought on by excessive water loss, thus enhancing greater resilience during the photosynthetic processes.<sup>65</sup> Specifically, after absorption and transportation in plants, silicon can enter the chloroplasts, raising foliar chlorophyll levels, ensuring that CO<sub>2</sub> is available for carbon assimilation, improving photosynthetic efficiency, thereby maintaining the overall water balance within the plant.66

It was observed that drought stress reduced the levels of chlorophyll content in soybean plants (Figure 3). The accumulation of chlorophyll degrading enzymes amplified under unfavorable conditions leading to a reduced chlorophyll content. This decline in chlorophyll can be attributed to the detrimental effects of drought stress.<sup>67</sup> Conversely, the application of silicon treatments proved beneficial in mitigating these effects and increasing the pigment contents in soybean plants (Figure 3). Among the silicon treatments, Si<sub>200</sub> demonstrated the most significant improvement in chlorophyll a and chlorophyll b compared to Si<sub>100</sub> and Si<sub>300</sub> (Figure 3). Due to drought stress, changes in chl a and b were similar, and silicon improved both components to some extent, particularly in drought stress. By promoting the formation of antioxidants and osmoprotectants, silicon guards against the degradation of chlorophyll pigments reducing photosynthetic injury under drought stress.<sup>68</sup> Shen

et al.<sup>69</sup> stated that silicon-induced photosynthesis and an increase in total chlorophyll content may rescue soybean seedlings from the adversities of drought stress.

The production of oxidants during exposure to abiotic stresses can damage plant tissues by reducing the production of photosynthetic pigments and altering various physiological attributes. Plants adopt several survival mechanisms under drought stress, including physicochemical, and molecularmechanisms. A significant modulation in the antioxidant defense system as activities of SOD, POD, and CAT increased in soybean under drought stress (Table 2). The plant defense system was improved by the silicon applications under drought stress and reduced the accumulation of MDA and H<sub>2</sub>O<sub>2</sub>. The possible reason is that the activities of SOD detoxify the O<sub>2</sub> into H<sub>2</sub>O<sub>2</sub>.<sup>70</sup> Catalase has a role in imparting drought stress by scavenging different ROS especially H<sub>2</sub>O<sub>2</sub>.<sup>71</sup> Increased POD activity due to silicon application enabled the plants to avoid oxidative stress. 70 As a result, silicon addition increased POD activity, indicative of the manufacture of suberin and lignin which create a physical barrier against drought stress (Table 2). The amount of soluble protein and total phenolics in soybean were dramatically increased in the current study with silicon treatment (Table 2). These elevated accumulations were especially pronounced with Si applied at 200 kg ha<sup>-1</sup> under drought stress at an early stage of growth.

Dehydration of tissues is the key effect of drought stress on plants. The natural defense of plants improves osmolyte accumulation to support the osmoregulation of cells by increasing cell water content, lowering osmotic potential, and improving water uptake capacity to protect themselves from droughtinduced damage. The intensity of drought stress can be reflected by the organism's internal plant osmotic regulatory chemicals.<sup>71</sup> Numerous studies have shown that under drought stress, enzymatic activity increases, and oxidative damage decreases (Ishaq et al. 2023). The addition of silicon enhanced the levels of chl a and b, PN, RWC, SOD, POD, CAT, and total phenolics while decreasing lipid peroxidation (MDA) and ROS production (H<sub>2</sub>O<sub>2</sub>). Silicon helps plants retain and absorb the maximum water possible from the soil, improving the water content of soybean leaves. Silicon's reduction of oxidative damage under stress is incompatible with the enhancement of antioxidative defense (CAT, POD, SOD, TSP, and total phenolics). Additionally, osmolytes are necessary osmoprotectants and play a key role in osmotic regulation in soybean plant cells.<sup>72</sup> Silicon application can boost antioxidant activity, decrease ROS, and increase protein content in leaves.<sup>29,73</sup>

The correlation matrix showed a substantial positive correlation between leaf pigments and plant growth parameters and a strong negative correlation between antioxidant enzyme activities and osmolytes (Figure 4). In response to drought stress, an antioxidant defense system was activated to scavenge ROS, however, this essential protective function will also lower physiological activities and growth and causing a negative correlation. 74,75 Conversely, silicon treatment enhanced the antioxidant activities, leading to increased ROS scavenging and restoring growth. As a result of improved antioxidant enzymatic activities and osmolytes, the morphological characteristics, water relations in soybean leaf pigments, and photosynthetic efficiency, have improved in response to the silicon treatments.



#### 5. Conclusion

It was demonstrated that drought stress significantly and adversely affected the growth of soybean. The lack of water affected various aspects of plant development, including height, biomass, gas exchange parameters, water relations, and foliar chlorophyll content. However, the soil applications of silicon were able to enhance the tolerance of soybean plants undergoing drought stress by upregulating the antioxidant defense system and osmolyte synthesis by silicon applications (200 kg ha<sup>-1</sup>). Applications of silicon also help plants maintain their chlorophyll content and plant water status ensuring the stomatal regulation and photosynthetic rate leading to improve crop growth and development. The study concluded that silicon applications may be a sustainable approach to enhancing resilience in soybean plants, providing an appropriate insight for farmers in drought-prone regions. This work bridges the gap between Si physiology and its practical application, paving the way for future research to optimize its use in sustainable agriculture.

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## **ORCID**

Usman Zulfiqar (b) http://orcid.org/0000-0003-3820-1476

## **Author contributions**

Conceptualization, M.M.A., E.A.W., and M.A.; methodology, M.M.A., S.H., and H.N.A.; software, A.H., U.Z., and S.H; validation, U.Z., Z.A., and W.S.; formal analysis, I.D., P.V.V.P., and W.S.; investigation, U.Z., M.A., and A.H.; resources, P.V.V.P., and I.D.; data curation, M.M.A., and E.A.W.; writing - original draft preparation, M.M.A., E.A.W., M. A., S.H., U.Z.; writing - review and editing, H.N.A., A.H., Z.A., I.D., P. V.V.P., W.S.; project administration, E.A.W.; funding acquisition, I.D., and P.V.V.P. All authors have read and agreed to the published version of the manuscript.

## Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Ethics approval and consent to participate

All methods were performed in accordance with the relevant guidelines and regulations. We have obtained permissions to collect plant material and seedlings.

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