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Silicon-Mediated Improvement in Drought and Salinity Stress Tolerance of Black Gram (*Vigna mungo* L.) by Modulating Growth, Physiological, Biochemical, and Root Attributes

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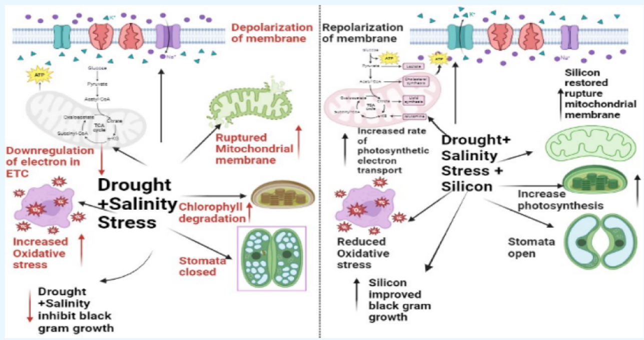
ABSTRACT: Water is a precious commodity for plant growth and metabolism; however, its scarcity and saline sand conditions have a drastic effect on plant growth and development. The main objective of the current study was to understand how silicon (Si) application might help Black gram (*Vigna mungo* L.) against the negative impacts of salt stress and drought. The treatments of this study were: no silicon = 0 mg/kg; silicon = 40 mg/kg; control = no stress; drought stress = 50% field capacity (FC); salinity = 10 dSm^{-1}; drought + salinity = 10 dSm^{-1} + 50% field capacity (FC). The findings showed that the application of silicon in the sand significantly affected growth indices such as leaf area (LA), shoot fresh weight (SFW), shoot dry weight (SDW), and shoot length (SL). Root length (RL) increased significantly up to 55.9% in response to drought stress. Applying Si to the sand increased the root length (RL) by 53.9%. In comparison to the control, the turgor potential of leaves decreased by 10.3% under salinity, while it increased by 44.7% under drought stress. However, the application of silicon to the sand significantly improved the turgor potential of leaves by 98.7%. Under both drought and salt stress, gas exchange characteristics and photosynthetic pigments dramatically decreased. Applying 40 mg/kg silicon to sand improved the gas exchange characteristics, protein contents, and photosynthetic pigments of plants under drought and salt stress, such as levels of chlorophyll (*a*, and *b*) increased by 18% and 26%, respectively. Under control conditions, the hydrogen peroxide (H_2O_2) concentration was lower but increased during periods of drought and salinity stress. The concentrations of peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) were decreased by salt and drought stress and increased by sand application of silicon at a rate of 40 mg/kg. Application of silicon at 40 mg/kg sand rate improved the growth and development under control and stress conditions. Overall, this study provides an extensive understanding of the physiological mechanisms underlying the black gram's ability to withstand under salt stress and drought stress by application of Si which will serve as a roadmap for future cellular research.



■ INTRODUCTION

Abiotic stress including drought has a detrimental effect on biotic life globally.[1] Drought has multiple dimensions, making it difficult to forecast and track its effects.[2] As per UNCCD COP-15, the effects of drought stress are on the rise and have impacted around 2.3 billion people, leading to a loss of over $120 billion in socio-economic terms.[3] Pakistan is one of the areas listed in the study that has been impacted by water stress during the past two years (2020−2022). The agricultural land of Pakistan was affected by water scarcity up to 11% in 2014 and is expected to have an average debt of 31% by 2025. Compared to other provinces in Pakistan, Sindh and Baluchistan are particularly vulnerable to drought.[4] Around 41% of the earth's surface is made up of dry zones, which are identified by low average yearly precipitation to potential evapotranspiration ratios.[5]

It has been discovered that drought stress negatively affects germination as well as other growth phases.[6] Drought has a significant impact on the germination of seeds in a variety of crops, which is an important stage in the life cycle of plants.[7] This effect is most noticeable in the way that dry seeds absorb water. For the process of imbibition of seeds and subsequent germination, the sand moisture content is crucial. A specific amount of moisture is necessary for the germination of the

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seeds and subsequent growth. On the other hand, the lack of moisture either entirely prevents or severely retards the development of seedlings. By slowing the pace at which seeds absorb water during the germination process, water stress lowers the rate and percentage of germination. It has been discovered that reactive oxygen species (ROS) cause oxidative stress in plants, which disrupts several physiological functions.[8,9]

It often faces abiotic challenges such as salinity, dehydration, and other conditions, though it grows well in semiarid habitats. Increased sand salinity due to global climate change could pose a serious danger to lentil production worldwide.[10] According to data from the FAO Land and Plant Nutrition Management Service, salinity affects more than 6% of the world's land. Forty-five million hectares (19%) of the 230 Mha of irrigated land currently under cultivation are damaged by salt, while 32 million hectares (2%) of the 1500 Mha agricultural land are affected by salt to varied degrees. Salinity affects irrigated lands, and as a result, 1.5 Mha of agricultural land is lost annually, and these lands are no longer suitable for cultivation. In 2050, 50% of cultivated lands will be in danger of disappearing if the present conditions continue.[11] Sand salinity is caused by an increase in the amount of groundwater with high amounts of salt, ineffective irrigation and drainage systems, and excessive fertilizer use.[12] Salinity suppresses physiological and morphological traits in grain legumes, resulting in a yield drop of 12–100%.[13] Additionally, it has an impact on reproductive growth, reducing the quantity of flowers and pollen production and leading to smaller grain pods. In salinity-affected sands, where germination and seedlings are most susceptible to salt stress, poor stand establishment poses a barrier to sustainable crop legume production.[14] Because salt stress causes osmotic stress on developing seeds or toxicity of Na^+ and Cl^- ions, it lowers and delays seedling germination, resulting in insufficient growth and biomass production.[15]

The seeds of the self-pollinating *Vigna mungo* (L.) grain have a high protein content. Through the uptake of atmospheric nitrogen fixed by legume crops, it modifies the physical characteristics and enhances the fertility of the sand. Globally eaten, black gram comes in fourth place in terms of acreage and output in India, behind green gram, pigeon pea, and chickpea.[16] About 20% of the world's pulse supply is derived from the black gram, an Indian legume crop that grows in a short amount of time (90–120 days).[17] Around the world, India is noted for having the highest production and consumption of black gram, making it the third most important pulse.[18] Due to its high nutritional content, it is also utilized in cosmetics, nutraceuticals, medical preparations, and sustainable agricultural systems. Due to some factors, including stress, black gram production has not increased during the past ten years.[19] Typical characteristics of drought stress include its gradual onset, elusive nature, lack of a single indication, difficulty in quantifying, and projected 50% worldwide decline in crop yield.[20]

To combat drought and other abiotic challenges, plants can use a variety of defense strategies.[21] Plants produce antioxidants as one of their defensive strategies against the damaging effects of drought.[22] Furthermore, a variety of proteins and amino acids that are produced by plants are involved in their defense mechanisms.[2] Application of silicon (Si), is one of the many techniques that is crucial in both biotic and abiotic stressors.[23] Silicon is the most prevalent element in Earth's outermost layer and is well-known for enhancing plant performance in stressful environments.[24] Additionally, it has been demonstrated that applying exogenous silicon increases the relative water content and promotes the growth of seedlings.[25] Applications of silicon boost photosynthetic rate, antioxidant defense system in crops.[26] By upregulating the physiological mechanism of the plant, silicon treatment under drought enhances root growth in plants.[27] Applications of silicon in various crops under abiotic stressors have been reported that plants exposed to salt stress need to use more energy within their cells in order to keep their cytosolic K^+ concentration high and their Na^+ concentration low. Applying silicon can reduce the amount of Na^+ that builds up in the roots and/or shoots.[28] It was suggested that the main mechanism of silicone-enhanced salt tolerance in this species is the decrease of both Na^+ and Cl^- levels but the increase of K^+ in salt-stressed barley roots, with Na^+ and K^+ being more uniformly distributed across the entire root section.[28]

On the other hand, very little is known about the combined effects of salinity and drought stress in the context of black gram under silicon applications. Enhancing the yield and area under cultivation of black gram requires an understanding of the biochemical and root morphological responses to silicon treatment under salinity and drought stress. This study hypothesized that the application of silicon may help plants in alleviating the deteriorating effects of drought and salt stress. Keeping in view the above-mentioned facts, the current study was conducted with following objectives

- To study the effects of salinity and drought stress on growth, physiology, biochemical, and root attributes of black gram.
- To determine the ameliorative role of sand applied silicon to improve the morpho-physiological and root attributes of black gram under drought and salinity stress

■ MATERIALS AND METHODS

Crop Husbandry. An experiment was conducted in the wirehouse of the old Botanical Garden at the University of Agriculture, Faisalabad. Seed of the *Vigna mungo* variety (Bittale-2017 and Noor-2019) were acquired from the Ayub Agricultural Research Institute, Faisalabad. The treatments of this study were: no silicon = 0 mg/kg; silicon = 40 mg/kg; control = no stress; drought stress = 50% FC; salinity = 10 dSm^{-1}; drought + salinity = 10 dSm^{-1} + 50% FC. The experiment was conducted under a completely randomized design (CRD) and three replications. To do this, black gram was planted in 16 cm by 11 plastic pots that were filled with 1.5 kg of sand in each pot. With mean maximum temperatures of 35 and 44 °C and mean minimum temperatures of 30 and 32 °C, humidity level 26 and 36% during the summer seasons of 2022–23. The study area had a subtropical climate; pots were kept under direct sunlight (Figure 1).

The field capacity was estimated, and irrigation was foregone in order to prolong the drought. The required moisture content was maintained on a weight basis. Two days before the salt stress was imposed, the last treatments were applied. Specifically, 48 h later, Hoagland solution was used to apply salinity stress every day for a month at two different concentrations (0 and 10 dSm^{-1} NaCl). To reduce EC and pH fluctuations throughout the salinity stress application, the pots' culture media were rinsed 5 days apart with tap water. Nearly 10 viable seeds of the same weight and size were planted in each of the 24 pots. Half of the pots were treated

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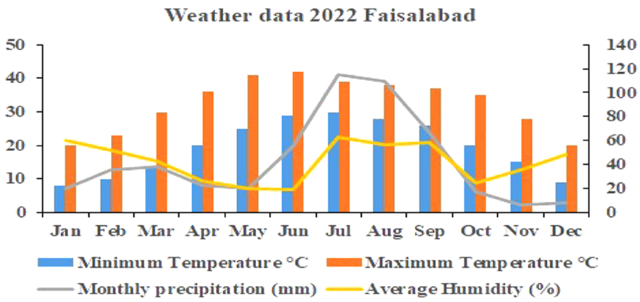

Figure 1. Accumulated environmental conditions, including maximum and minimum temperature (°C), relative humidity (%), and rainfall (mm), for experimental year 2022.

with silicon (Na_2SiO_3) at a concentration of 40 mg/kg of sand; the remaining half was left untreated in the face of salinity and drought. The total silicon content in sodium silicate is 8.72%. By changing 40 mg of silicon to grams, 0.04 g of sodium silicate was taken. As a result, 1 kg of sand required 0.458 g of sodium silicate to provide 40 mg of silicon. Five plants remained in each pot following the 14-day thinning period. Observations regarding growth, water relations, gaseous exchange, and biochemical attributes were observed following the application of drought and salinity stress.

Growth Parameters. Three plants from each experimental unit were selected randomly to measure the growth parameters. The leaf area of the plants was determined manually. Using a scale, the length of the shoot was measured from the sand's surface to the tip. Next, the entire plant was carefully taken out of the dirt, and its roots and shoots were divided using scissors after the pots had been filled with water. Using a digital weighing balance, the shoots and roots' weights were determined. Following a 72 h oven drying process at 65 °C for shoot and root samples, the dry weight of the plant was determined using a digital scale.

Chlorophyll Content. The immature seedlings were used to gather samples and were weighed. They were then transferred to an Eppendorf tube and macerated with 3.0 mL of 80% acetone. The material was then centrifuged for seven min at 12 000 rpm. Finally, measurements at wavelengths of 470, 645, 652, and 663 nm were conducted using the ELISA plate reader.[29]

$$\text{Chl. a} = 12.9(\text{Ab663}) - 2.69(\text{Ab645}) \times V/1000 \times W$$

$$\text{Chl. b} = 22.9(\text{Ab645}) - 4.68(\text{Ab663}) \times V/1000 \times W$$

$$\text{TotalChl.} = \text{Chl. a} + \text{Chl. b}$$

$$\text{Carotenoidscontent}(\mu\text{g/g}) = A \times V(\text{mL}) \times 10^4 / A \times P(\text{g})$$

where "A" stands for absorbance (nm), "V" for extracted volume (mL), and "W" for fresh leaf tissue weight (g).

Water Relations. The youngest and fully developed leaves of black gram plants were taken from each treatment in order to calculate the leaf water potential. ELE-International, Tokyo, Japan's RIMAD-2 Scholander-type pressure chamber was used in accordance with the protocol outlined by Ahmad et al. (2017).[30] The modified approach Karrou and Maranville (1995).[31] was employed to determine the relative water content (RWC). The turgid weights of samples (0.5 g) were measured after being saturated in 100 mL of distilled water for 24 h at 4 °C in the dark. The samples were then oven-dried for 48 h at 65 °C, and their dry weight was noted. The following formula is used to calculate the relative water content (RWC) of plant tissue in units of percentage:

$$\text{RWC} = (\text{FM} - \text{DM})/(\text{TM} - \text{DM}) \times 100$$

Where, FM, DM, and TM were the fresh, dry, and turgid weights, respectively.

Biochemical Parameters. Activities of Antioxidant Enzymes The updated procedures by Chance and Maehly (1955) were employed to measure the activity of catalase and peroxidase.[32]

Using a cooled mortar and pestle, the samples were ground in 2 mL of 0.1 M phosphate buffer (pH 7.8). Centrifugation at 12 000 rpm for 15 min came next, and 0.2 mL of sample, 1 mL of 0.05 M phosphate buffer (pH 7), 1 mL of H_2O_2, and 0.8 mL of distilled water were added to create the reaction mixture. Last, absorbance was measured every 30 s for 3 min at 240 nm.

Using a cooled mortar and pestle, 2 mL of 0.1 M phosphate buffer (pH 7.8) was used to macerate the seedlings. Centrifugation at 12 000 rpm for 15 min then add, 0.2 mL of extract, 1 mL of 0.1 M phosphate buffer (pH 6.1), 0.5 mL of H_2O_2, 0.5 mL of guaiacol, and 0.8 mL of distill water were added to create the reaction mixture. Last, absorbance was measured for 3 min at intervals of 15 seconds at 470 nm.

$$\text{POX} = (\text{final} - \text{initial}) \times 3 \times 1000/26.6 \times 3 \times 1 \times 0.2 \times \text{freshweight} \quad (1)$$

Histochemical Examination of H_2O_2 and O_2 in Leaves Using DBT and NBT and 3,30-diaminobenzidine (DAB) staining techniques were used to identify the production of superoxide anion (O^{2-}) and H_2O_2, respectively.

The Spitz and Oberly, 2001 modified procedure was employed to note the superoxide activity.[33] In order to macerate the seedlings, 2 mL of 50 mM phosphate buffer (pH 7) was added. For 30 min, the mixture was centrifuged at 13 000 rpm. A 50 μL amount of extract, 2.15 mL of 50 mM phosphate buffer (pH 7.8), 0.2 mL of methionine, 0.2 mL of NBT, 0.2 mL of EDTA, and 0.2 mL of riboflavin solution were added to create a reaction mixture. The samples were put below fluorescent light for 10 min after the test tubes were shaken. The test tubes were subsequently covered with a black cloth, and all lights were turned off. At 560 nm, the reaction mixture's absorbance was finally detected.

Total Soluble Protein. Utilizing a modified technique, the total soluble protein content was measured.[34] A 5.0 mL aliquot of 10 mM phosphate buffer (PPB) (pH 7) containing 4% polyvinylpyrrolidone (PVP) was used to macerate the seedlings. For 15 min, this mixture was centrifuged at 12 000 rpm. After addition of 20 μL of extract and 980 μL of Bradford reagent, the reaction mixture was ready to be incubated for 15 min at room temperature. At 595 nm, the value was finally measured.

Total Phenolics. A 0.5 g sample was obtained, macerated in 5 mL of 80% ethanol, and then centrifuged for 20 min at 10 000 rpm. A 0.5 mL portion of plant sample, 2.5 mL of 10% Folin-Ciocalteu Reagent (FCR), and 2.5 mL of 7.5% NaHCO3 were added to create the reaction mixture. After that, this mixture spent 45 min in a water bath at 45 °C. The absorbance was measured at 765 nm following incubation. Lastly, gallic acid was used to create the conventional graph.[35]

Root Analysis Parameters. We used WinRhizo software and an Epson scanner to scan roots in order to evaluate

Table 1. Effect of Silicon Treatment and Stress Conditions on Growth Attributes of the Black Gram[a]

	treatments	shoot.length (cm)	shoot.fresh weight (g)	shoot.dry weight (g)	root.fresh weight (g)	root.dry weight (g)	leaf.area (cm^2)
no silicon	control	19.41 ± 0.54a	0.46 ± 0.01b	0.17 ± 0.008a	0.45 ± 0.01a	0.03 ± 0.001b	4.39 ± 0.05a
	drought stress	18.53 ± 0.64a	0.33 ± 0.01cd	0.12 ± 0.005bc	0.50 ± 0.02a	0.03 ± 0.002b	3.20 ± 0.008b
	salinity	13.60 ± 0.50b	0.26 ± 0.01e	0.08 ± 0.005c	0.09 ± 0.005b	0.01 ± 0.0005c	2.4 ± 0.04cd
	salinity + drought	10.83 ± 0.08c	0.24 ± 0.008e	0.08 ± 0.005c	0.08 ± 0.005b	0.008 ± 0.005c	1.98 ± 0.07d
silicon (40 mg/kg)	control	19.06 ± 0.65a	0.55 ± 0.02a	0.19 ± 0.01a	0.45 ± 0.02a	0.03 ± 0.001b	4.14 ± 0.18a
	drought stress	19.1 ± 0.76a	0.36 ± 0.008c	0.12 ± 0.008b	0.49 ± 0.03a	0.04 ± 0.002a	3.33 ± 0.07b
	salinity	13.77 ± 0.41b	0.30 ± 0.01de	0.12 ± 0.005bc	0.09 ± 0.006b	0.008 ± 0.005c	3.15 ± 0.04b
	salinity + drought	12.43 ± 0.41bc	0.28 ± 0.01de	0.10 ± 0.006bc	0.11 ± 0.008b	0.01 ± 0.0005c	2.64 ± 0.09c

[a]Values (mean ± standard error), LSD = least significant difference; values sharing the same case letter or without lettering for a parameter do not differ significantly ($p \leq 0.05$) by the LSD test. No silicon = 0 mg/kg; silicon = 40 mg/kg; control = no stress; drought stress = 50% FC; salinity = 10 dSm^{-1}; drought + salinity = 10 dSm^{-1} + 50% FC.

Table 2. Effect of Silicon Treatment and Stress Conditions on Photosynthetic and Biochemical Attributes of Black Gram[a]

	treatments	chlorophyll a	chlorophyll b	total chlorophyll	SOD	POD	CAT
no silicon	control	1.27 ± 0.02ab	0.82 ± 0.02a	0.36 ± 0.01b	19.22 ± 0.62e	2.01 ± 0.11f	11.21 ± 0.41f
	drought stress	0.67 ± 0.02c	0.69 ± 0.02a	0.24 ± 0.01de	24.67 ± 1.20de	3.14 ± 0.13d	26.41 ± 0.42d
	salinity	0.36 ± 0.01d	0.41 ± 0.01b	0.23 ± 0.01de	30.44 ± 1.22cd	3.51 ± 0.08cd	31.72 ± 1.46bc
	salinity + drought	0.18 ± 0.005e	0.36 ± 0.01b	0.21 ± 0.00e	35.36 ± 1.82bc	3.96 ± 0.08b	36.6 ± 0.91b
Silicon (40 mg/kg)	control	1.39 ± 0.01a	0.78 ± 0.03a	0.44 ± 0.01a	25.36 ± 0.75d	2.56 ± 0.05e	18.59 ± 0.77e
	drought stress	1.22 ± 0.03b	0.76 ± 0.04a	0.42 ± 0.00a	33.79 ± 1.21bc	3.69 ± 0.03bc	31.08 ± 0.85cd
	salinity	1.22 ± 0.04b	0.82 ± 0.02a	0.29 ± 0.01c	37.94 ± 1.15bc	3.92 ± 0.03bc	42.72 ± 1.44a
	salinity + drought	1.28 ± 0.04ab	0.70 ± 0.03a	0.27 ± 0.01cd	43.06 ± 1.58a	4.51 ± 0.09a	47.7 ± 1.62a

[a]Values (mean ± standard error), LSD = least significant difference; values sharing the same case letter or without lettering for a parameter do not differ significantly ($p \leq 0.05$) by the LSD test. No silicon = 0 mg/kg; silicon = 40 mg/kg; control = no stress; drought stress = 50% FC; salinity = 10 dSm^{-1}; drought + salinity = 10 dSm^{-1} + 50% FC.

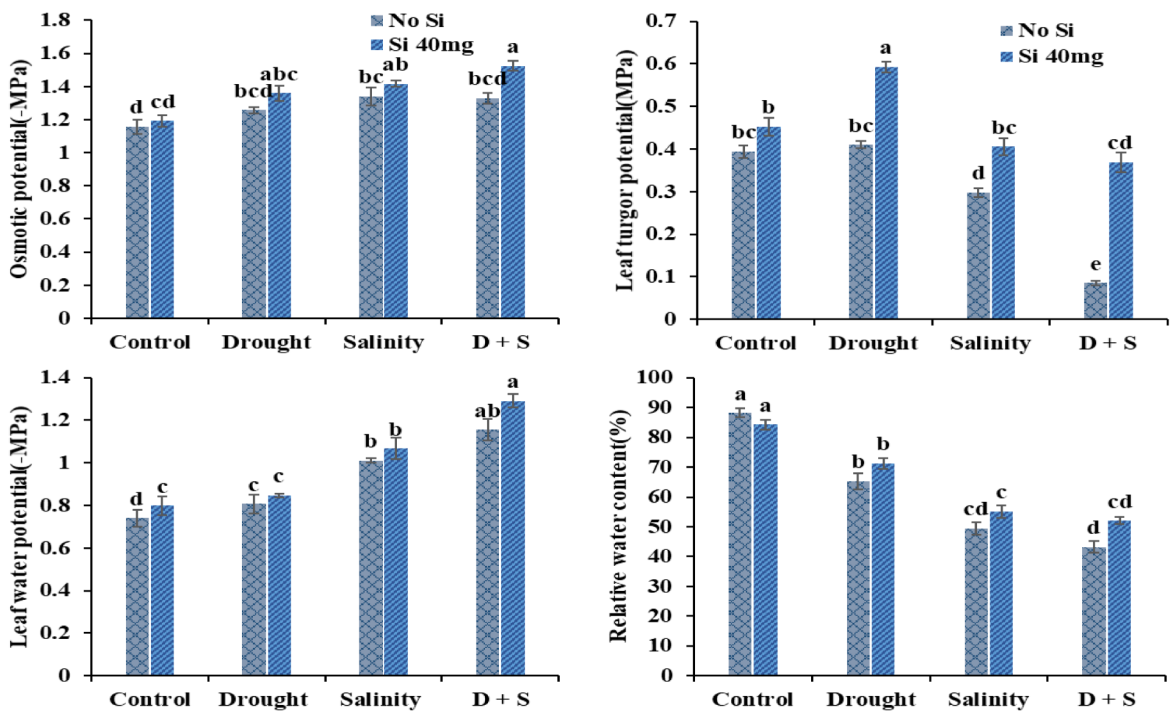

Figure 2. Impact of silicon (T0 = control, T1 = drought stress, T2 = salinity stress (EC= 10 dSm^{-1}), T3= drought + salinity stress) on water potential (−MPa), osmotic potential (−MPa), relative water content (%), and turgor potential (Mpa) on black gram. Error bars above means indicate the ± SE. Means sharing the same letter in both varieties do not differ significantly at $p \leq 0.05$. No silicon = 0 mg/kg; silicon = 40 mg/kg; control = no stress; drought stress = 50% FC; salinity = 10 dSm^{-1}; drought + salinity = 10 dSm^{-1} + 50% FC.

characteristics linked to roots, such as root length, surface area, projected area, and root volume.

Statistical Analysis. Three factors were used in the Complete Randomized Design (CRD) structure of this experiment. At the 5% probability level, the treatment means

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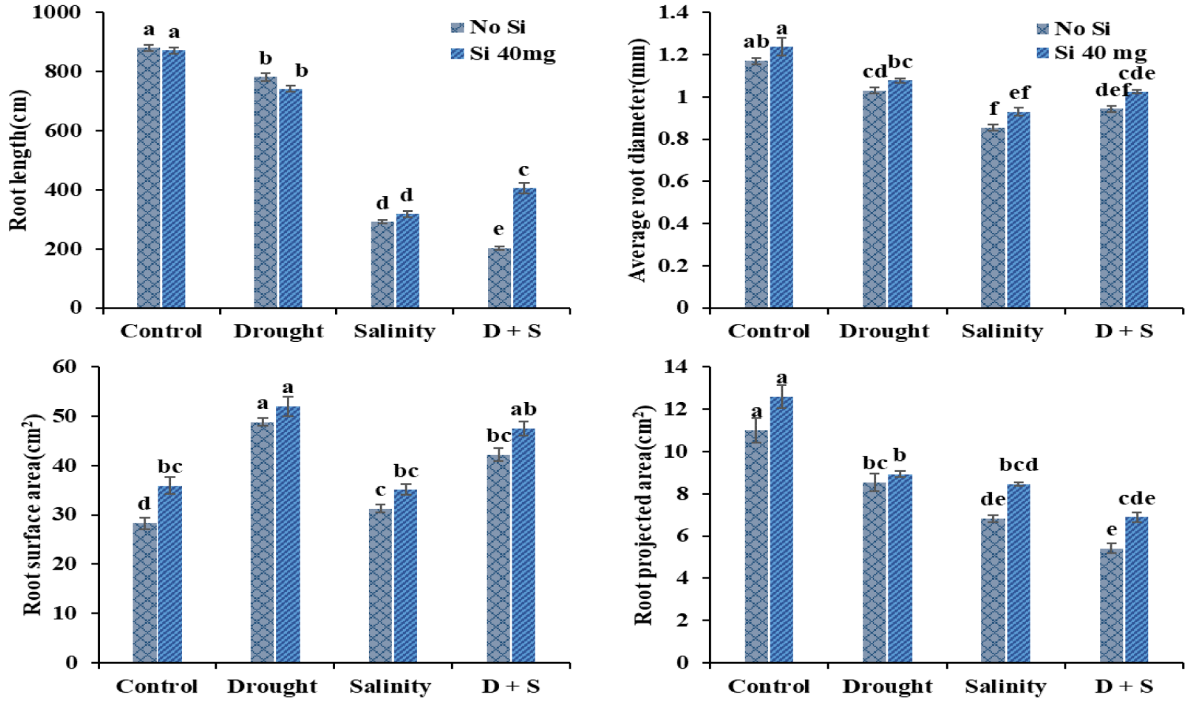

Figure 3. Impact of silicon (T0 = control, T1 = drought stress, T2 = salinity stress (EC= 10 dSm^{-1}), T3= drought + salinity stress, on root projected area (cm^2), surface area (cm^2), average root diameter (mm) and root volume (cm^3) and estimated root tips on black gram. Error bars above means indicate the ± SE. Means sharing the same letter in both varieties do not differ significantly at $p \leq 0.05$. No silicon = 0 mg/kg; silicon = 40 mg/kg; control = no stress; drought stress = 50% FC; salinity = 10 dSm^{-1}; drought + salinity = 10 dSm^{-1} + 50% FC.

were compared using Tukeýs HSD all-pairwise comparison test. Following the conclusion of the research trial, the data collected from plant samples were statistically analyzed and graphically represented using the following software: Microsoft Excel (Version 2016) (Microsoft Corporation, Redmond, WA, USA), R-studio (Version 4.3.3), Originpro (2022), and Statistix 8.1 (Analytical Software, Statistix, Tallahassee, FL, USA).

■ RESULTS

The results revealed that silicon application significantly ($p < 0.05$) improved the morphological and physiological characteristics under drought and salinity stress in black gram (Tables 1,2 and Figures 2,3 and 4).

Growth Parameters. Statistical analysis showed that application of silicon at 40 mg/kg significantly improved the growth characteristics of black gram under different stress conditions compared to control (Table 1). Drought and salinity stress declined the growth indices of black gram plants and more reduction in morphological characters was recorded in black gram under drought and salinity combined stress where no silicon was added. The root length was improved up to 55.5% under drought stress and 53.9% under combined drought and salinity stress conditions as compared with nonstress conditions. Various growth parameters like leaf area, shoot and root fresh and dry weight, and shoot length were significantly reduced under salt stress up to 42.1, 28.6, and 53.8, 69.1, and 71.2, and 43%, and combined stress up to 52.2, 43.1, 53.8, and 81.8, 77.5, and 45% as compared with nonstress conditions. However, growth attributes leaf area, shoot and root fresh and dry weight, and shoot length were significantly improved under salt stress up to 31.5, 13.5, and 50, 44.7, and 27.2, and 13.5% while under combined stress improved up to

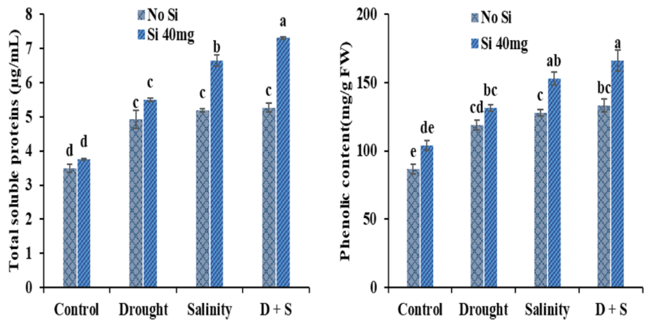

Figure 4. Impact of silicon (T0 = control, T1 = drought stress, T2 = salinity stress (EC= 10 dSm^{-1}), T3= drought + salinity stress, on total soluble proteins (μg/mL) and phenolic contents (mg/g FW), on black gram. Error bars above means indicate the ± SE. Means sharing the same letter in both varieties do not differ significantly at $p \leq 0.05$. No silicon = 0 mg/kg; silicon = 40 mg/kg; control = no stress; drought stress = 50% FC; salinity = 10 dSm^{-1}; drought + salinity = 10 dSm^{-1} + 50% FC.

33.6, 14.7, 33.3, and 41.6, 50, and 14.7% with silicon @ 40 mg application as compared to no silicon application (Table 1). Among the interactions, Si × Stress was significant for root length. Two-way interaction of Si × Stress was highly significant for leaf area, and the interaction of Si × Stress was highly significant for root dry weight of black gram plants.

Photosynthetic and Antioxidant Attributes. Statistical analysis showed that silicon application at 40 mg/kg significantly improved the antioxidant activities and reduction of photosynthetic attributes of black gram under different stress conditions compared to control without Si (Table 2). Drought and salinity stress showed an adverse effect on antioxidants and photosynthetic attributes of black gram plants and more reduction in activity of antioxidants and photosynthetic attributes were observed in plants under drought and salinity combined stress where no silicon was added. The photosynthetic attributes chlorophyll *a* and *b* reduced up to 16.7 and 17.3% while antioxidants SOD, POD, and CAT increased up to 58.3, 74.7 and 129.8% under salt stress and 14.6, 19.9%, and 84% under combined (drought + salinity) stress as compared with nonstress conditions. However, photosynthetic and antioxidant activities improved chlorophyll *a* and *b* up to 14.7 under salt stress and 11.1% under drought stress and antioxidants SOD, POD, and CAT up to 36.9, 17.6, and 34.6% under drought stress while 17.7, 12.1% and 24.6, 13.7, 30.3% under combined (drought + salinity) stress, respectively, compared to plants where no silicon was applied (Table 2). Among the interactions, Si × Stress was significant for chlorophyll *a* and catalase absorbance. Interaction of Si × Stress was highly significant for chlorophyll *b*.

Water Relations. Silicon application of silicon at 40 mg/kg significantly impacted the plant water relations parameters under drought and salinity stress (Figure 2). Drought and salinity stress declined the plant water relations including water potential, osmotic potential, turgor potential, and relative water content, respectively, as compared to control. Water potential and osmotic potential increased up to 33.5, and 27.6%, and leaf relative water content and turgor potential decreased up to 37.5, and 10.3% under salt stress while increased up to 61.6, and 18.7%, and leaf relative water content and turgor potential decreased up to 41, and 18.6% under combined (drought + salinity) stress as compared with control. However, Water potential and osmotic potential, leaf relative water content, and turgor potential were significantly enhanced under drought stress up to 5.5, 8.2, 11.4, and 44.7%, while under combined stress improved up to 11.8 and 14.5, 20.3, and 98.7% with silicon @ 40 mg application as compared to no silicon application (Figure 2).

Roots Analysis. Statistical analysis showed that silicon application at 40 mg/kg significantly improved the root morphological parameters of black gram under different stress conditions compared to control no Si (Figure 3). Drought and salinity stress negatively affected the root attributes of root attributes surface area, projected area, and root average diameter. The surface area reduced and root projected area and root average diameter reduced up to 45, 24.79 and 45.3% under drought stress while 32.3, 17.2 and 32.8% under combined (drought + salinity) stress conditions as compared with nonstress conditions. The surface area root projected area and root average diameter improved up to 12.2, 23.8 and 8.9% under salt stress while 12.4, 27.2 and 8.4% under combined (drought + salinity) stress conditions as compared with nonstress conditions, respectively, compared to plants where no silicon was applied (Figure 3). Two-way interaction between Si × Stress was significant for root surface area and estimated root tips. Interaction of Si × Stress was highly significant for the root volume.

Organic Osmolytes. Statistical analysis showed that silicon application at 40 mg/kg significantly improved the total soluble protein and phenolic contents of black gram under different stress conditions compared to control no Si (Figure 4). Drought and salinity stress negatively affected the total soluble protein and increased the phenolic contents under drought and salinity combined stress where no silicon was applied. The total soluble protein reduced and phenolic contents increased up to 49.1% and 60% under salt stress while 50.9 and 46.2% under combined (drought + salinity) stress in comparison with nonstress conditions. However, total soluble protein and phenolic contents increased up to 27.7% and 19.4% under salt stress while 38.8 and 24.7% under combined (drought + salinity) stress compared to plants where no silicon was applied (Figure 4).

Heatmap Analysis. Two-way heatmap with a dendrogram was drawn to observe the role of silicon on various parameters of black gram (*Vigna mungo* L.) under drought and salinity stress conditions (Figure 5). The observations were divided

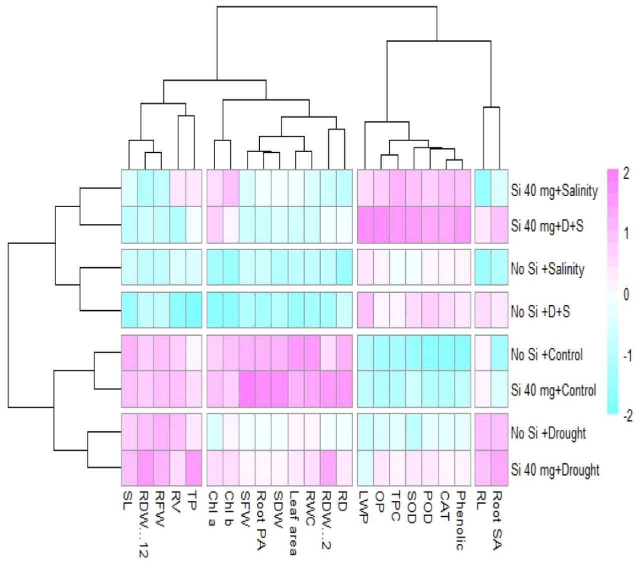

Figure 5. Heatmap with dengrogram between morpho-physiological, water relations, gaseous exchange and biochemical attributes of black gram. No silicon = 0 mg/kg; silicon = 40 mg/kg; control = no stress; drought stress = 50% FC; salinity = 10 dSm^{-1}; drought + salinity = 10 dSm^{-1} + 50% FC. (SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, LA: leaf area, Chl. a: chlorophyll a, Chl. b: chlorophyll b, Total Chl.: total chlorophyll, Car: carotenoids, SOD: superoxide dismutase, POD: peroxidase, CAT: catalase, Pn: Net photosynthetic rate, Ci: internal carbondioxide concentration, TR: transpirational rate, SC: stomatal conductance, RWC: relative water contents, LTP: leaf turgor potential, LWP: leaf water potential, RL: root length, RSA: root surface area, RPA: root projected area, RD: root density, RV: root volume).

into four groups according to how similar they were during different treatment phases, and the relationships between the groups were shown by colored squares. The color (Navy blue) exhibited a strong positive association, while the color (Maroon) exhibited a strong negative correlation for various observations, impacted by silicon under drought and salinity stress (Figure 5). Heatmap has clustered into four groups. In the first group, Root surface area (RSA) and root length (RL) were clustered. These parameters are strong positively correlated with drought (50% FC) and silicon (40 mg) and weakly correlated, at drought + salinity. Under (no silicon) and drought + salinity stress conditions, the above-mentioned attributes showed weak correlation while strong negatively correlated under control (no silicon) and salinity stress (10 dSm^{-1}) conditions, respectively. This group demonstrated that the application of silicon (40 mg) improved the concentration of root surface area and root length which mitigated the adverse effects of oxidative damage caused by drought and combined stress. The second group included phenolic content catalase (CAT) peroxidase (POD), superoxide dismutase

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(SOD), total protein contents (TPC), osmotic potential (Op), and leaf water potential (LWp) that were strongly positively correlated at salinity (10 dSm^{-1}) and combined drought + salinity stress and silicon (40 mg) while weakly correlated at salinity (10 dSm^{-1}) and drought + salinity and silicon (no silicon) while strongly negatively correlated at control (no stress) and control (no silicon) and weakly negatively correlated at control (no silicon) and drought stress (50% FC). These observations showed that under drought and salinity stress CAT, SOD, and POD activities of black gram improved by the application of silicon. The third group contained (Root density, root dry weight, relative water content, leaf area, shoot dry weight, root projected area, shoot fresh weight, and chlorophyll *a* and *b*). These attributes were strongly positively correlated at control (no stress) and silicon (40 mg) while showing a negative correlation at salinity (10 dSm^{-1}) and drought + salinity and silicon (no silicon). These findings showed that growth attributes and photosynthetic pigments of pea varieties improved by the application of silicon. In the fourth group, turgor potential, root volume, root fresh and dry weight, and shoot length were clustered. These parameters were strongly positively correlated at (50% FC) and silicon (40 mg), while strongly negatively correlated at drought stress (50% FC) and control (no silicon) (Figure 5).

Principal Component Analysis (PCA). The PCA analysis manifested those results of PCA 1 and PCA 2 representing 83.2% of the accumulative variations, each with 66.3% and 16.9%, respectively. However, morphological, photosynthetic, organic osmolytes, water relation, and root attributes of both varieties Karan and Dilkash were substantially different under different silicon (0 mg/kg; silicon = 40 mg/kg); drought stress (control = no stress; drought stress = 50% FC) and salinity stress (salinity = 10 dSm^{-1}; drought + salinity = 10 dSm^{-1} + 50% FC) treatments. A very close relationship was observed among various photosynthetic, morphological, and organic osmolytes, with enzymatic antioxidants, total phenolics, osmotic potential, and leaf water potential, while root length and root surface area showed close relationships among themselves. In our study, silicon application proved to be more effective in mitigating drought stress by improving morphological attributes such as shoot length, root dry weight, photosynthetic attributes, and organic osmolytes parameters while reducing the negative impacts of root length, root surface area in black gram plants (Figure 6).

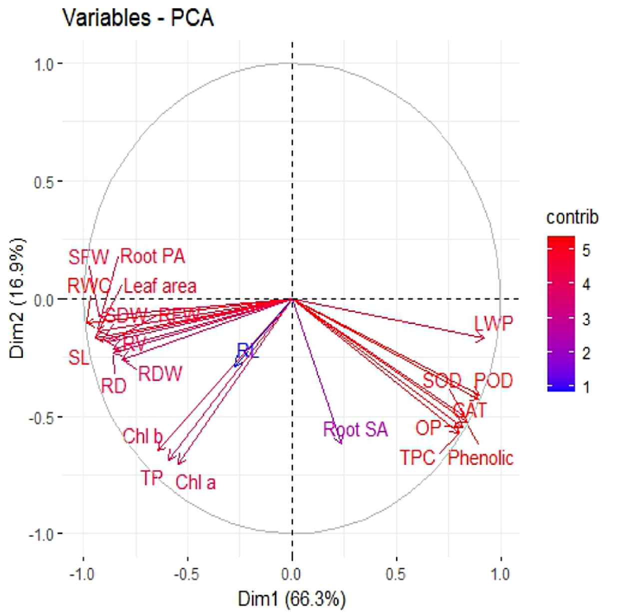

Figure 6. Principal component analysis between morpho-physiological, water relations, gaseous exchange, and biochemical attributes of black gram. No silicon = 0 mg/kg; silicon = 40 mg/kg; control = no stress; drought stress = 50% FC; salinity = 10 dSm^{-1}; drought + salinity = 10 dSm^{-1} + 50% FC.

■ DISCUSSION

The main force behind crop growth and development is the environment, which is steadily getting worse due to rising carbon dioxide levels, rising pollution levels, and the consequent effects of climate change. The majority of arable land is experiencing issues, including salinity and drought, due to climate change and the widespread use of inorganic fertilizers. Stress from salinity and drought has a significant impact on crop growth, especially black gram. This study found that silicon application has a mitigating effect on stressed black gram plants, drought and salinity stress had a negative impact on the growth and development of black grams, including a decline in morphological attributes, a reduction in leaf water status, and adverse impacts on biochemical parameters (Tables 1, 2 and Figures 2, 3, and 4).

The present study's results, which showed reduced growth indices in black gram cultivars grown under saline and drought stress, are in line with these findings. This study's exogenous protectant, silicon, is well-known for encouraging crop development in a variety of situations. In the current investigation, silicon application under salinity and drought stress was also shown to promote growth (Table 1). The most frequent adverse environmental stressors that restrict black gram growth and development, according to the results, were salt and drought. This is mostly due to osmotic stress and cellular sodium toxicity. According to Baghalian et al. (2011), there is usually a drop in the fresh and dry weights of plant components when there is insufficient water.[36] Numerous plant species have shown decreased development in sand salinity conditions, according to several researches.[37−39] (Rahman et al. 2017; Akram et al. 2017; Siddiqui et al. 2017). Due to salinity and drought stress, the current study also found that black gram growth was inhibited (Table 1).

It was discovered that the degree of growth decreased as the salinity treatment levels increased. Growth retardation, independent of plant type, is a critical factor in determining the degree of damage caused by salt, as noted by Acosta-Motos.[40] In many crops, including salt-tolerant plants, lowering the leaf area is a crucial adaptive technique for managing salt stress. This results in a decrease in the plant's assimilatory unit and a decrease in consumption of water. The drop in growth indices can be attributed to physiological reactions like stomatal behavior, ion balance, mineral nutrition, and photosynthetic efficiency.[41] According to Fareed et al., the main reason for decreased growth, biomass, and yield is the suppression of cell division and elongation under salt stress.[42] The use of Si under stress conditions improved plant development by restoring cell membranes and acting as a defense against tissue damage.[43] They can use less water and be better able to withstand droughts with the use of Si fertilizer.[44] Other studies suggest that silicon (Si) may contribute to the synthesis of a variety of compounds in plants, where it increases the water-retention capacity of the

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plant by forming a "double layer" of cuticle and silica in regions that are not physiologically active.[45−47] (Table 1). The photosynthetic pigments chlorophyll *a*, chlorophyll *b*, total chlorophyll levels, and carotenoids were found to considerably diminish the efficiency of the black gram photosynthetic rate by reducing the production of photosynthetic characteristics under drought and salinity stress conditions (Table 2). Numerous physiological, biochemical, and molecular regulatory networks impact plant growth, according to a number of research.[48,49] Carbon fixation has been found to be an essential mechanism for plant growth and development during drought stress.[50] The photosynthetic apparatus (chloroplast degradation and chlorophyll decrease), plasma membrane stability, and enzyme degradation are all impacted by drought stress-induced oxidative stress, which eventually affects cell division and plant growth.[51] The production of photosynthetic pigments were reduced by drought stress in this study, which in turn led to a drop in the rate of photosynthesis in black gram plants. The quantities of pigments, which are necessary for the absorption of energy in plants, are significantly impacted by environmental stresses.[52] Chlorophyll is a crucial component of photosynthesis and is necessary for a plant to survive.[53]

Photosynthesis is the process by which plants convert sunlight into chemical energy for the production of food.[54] Salinity reduces photosynthesis, principally through stomatal closure.[55] When plants are exposed to salinity, their chlorophyll concentration drops. This is assumed to be an indication of oxidative stress, which is created by suppressing chlorophyll synthesis and activating the enzyme that degrades chlorophyllase.[56] Chloroplast photochemistry is disturbed by salt stressors. When the rate of light energy absorption by photosynthetic pigments exceeds the rate of light energy consumption, absorbed light energy under salt stress accelerates the process of photoinhibition and reduces photosynthetic ability.[57] Nevertheless, under drought and salinity stress circumstances, this study discovered that foliar application of silicon increased the concentrations of chlorophyll *a*, chlorophyll *b*, and chlorophyll *a* to *b* ratio, total chlorophyll contents, and carotenoids (Table 2). Under stressful circumstances, exogenous Si treatments can maintain high levels of chloroplasts, chlorophyll content, and thylakoids.[58] In summary, silicon could be involved in shielding an organelle's membrane, lessening the damage that drought stress does to chloroplasts, halting the loss of chlorophyll, and enhancing the organelles' capacity to produce more photosynthetically synthesized compounds. In the end, each of these procedures promotes plant growth.[59] Silicon's ability to improve leaf rigidity by roughening its texture.[60] stay more horizontally, which delays leaf senescence, increases leaf chlorophyll content, and activates ribulose-bisphosphate carboxylase, is another likely way how Si increases stress resistance in stressed plants.[61]

The water-related qualities in this investigation were dramatically weakened by salinity stress and drought. Drought stress reduces the water potential and RWC of leaves. It also gradually reduces stomatal conductance, which in turn reduces the rate of photosynthetic respiration, CO_2 assimilation, and the CO_2 molar percentage in chloroplasts (Figure 2). The first reaction of plants under drought stress is stomatal closure, which is usually considered to be the primary source of the drought-induced reduction in photosynthesis.[62] Increased salt concentration is thought to impede water uptake through the root when there is salt stress.[63] Salt stress inhibits the process of cell elongation by causing a drop in the RWC, which results in turgor loss. Lack of water may be causing the seedlings' entire metabolic system to collapse in saltwater environments, hindering their ability to thrive. A plant's ability to retain its water status is shown by its relative leaf water contents.[64] Consequently, it can be applied as a standard to investigate the effects of salt stress on mung bean plants that are cultivated under salinity stress. Numerous investigations verified a reduction in RWC in plants under salt stress.[30,65] The current study's reduced relative water content in leaves may be the result of reduced water intake at higher salt concentration.[66] Another theory is that the delayed sap flux flow causes a decrease in root hydraulic conductivity, which may lead to a decrease in leaf RWC.[67] Furthermore, in the current studies, plants treated with silicon demonstrated increased development of water relations and gaseous exchange properties (Table 2). A plausible explanation for the decline in photosynthetic rate could be attributed to a drop in leaf extension and stomatal conductance, which may play a crucial role in carbon fixation during stressful situations. Plant growth and development may be hampered by the loss in photosynthetic rate that follows a decrease in stomatal conductance due to a fall in leaf turgor potential, which is caused by a decrease in plant water potential. On the other hand, silicon applications could help plants maintain their water balance and control their leaf osmotic potential, which could enable them to increase gaseous exchange in stressful situations.[68] Silicon treatments have been found to affect plant water relations and stomatal conductance without any physiological implications since silicon can improve water uptake and transport to the leaves and stems by increasing hydraulic conductance.[69]

In the current experiment, there was a decrease in antioxidant activity, which was associated with an increase in ROS production in drought and salinity stressed black gram plants (Table 2).

In order to provide drought stress tolerance, plants scavenge ROS by activating their built-in antioxidant defense mechanism. For drought stress resistance, it may be especially crucial to strengthen the antioxidant defense system, which consists of antioxidant compounds and several antioxidative enzymes such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD).[70] On the other hand, silicon treatment in the current experiment boosted antioxidant activity, which was essential in lowering oxidative stress and allowing plants to thrive under harsh conditions (Table 2). As the initial line of defense against oxidative damage to cells, the increase in SOD activity is essential in converting $O2\bullet^-$ radicals to H_2O_2 and O_2.[71] Furthermore, CAT is necessary for plant metabolism and signal reception, and it participates in the process of turning H_2O_2 into H_2O.[72] According to Hasanuzzaman et al. (2018).[73] the application of silicon increased enzymatic activity and encouraged the ascorbate-glutathione cycle, which produces antioxidants and lowers stress-induced oxidative damage. Applying Si improved cell osmolytes, which decreased oxidative stress, and scavenged reactive oxygen species (ROS).[74] The ideal silicon concentrations increased the activities of SOD, POD, and CAT during drought stress, which may enhance plant growth and yield. As the concentration of Si rose, the enhanced range first increased and subsequently decreased.[30]

In the current study, total phenolic and total soluble protein levels dramatically enhanced under salinity and drought stress (Figure 4). Increased osmolyte accumulation under drought

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stress is one of the most effective strategies to control osmotic pressure and lessen drought stress.[75] Plant water status may go out of balance during drought stress, which impacts osmotic adjustment and ultimately results in a greater accumulation of compatible osmolytes in crops. Proline's antioxidant function lowers lipid peroxidation and aids in cell homeostasis by maintaining the redox balance.[76] Plants combat dryness by accumulating more soluble sugar and protein in their tissues, according to research. However, persistent drought-induced water shortages will harm plant structure and impede the synthesis of proteins and carbohydrates.[77] Salt stress causes an excess of ROS to be produced. According to Taibi et al. (2016),[78] avoiding ROS multiplication requires the activation of an efficient antioxidant defense system.[79,80] Under salinity stress, phenolic compounds aid in scavenging reactive oxygen species (ROS).[81,82] The formation of various phenolic compounds depends on the phenyl-propanoid biosynthesis pathway.[83]

Similar to findings under salt stress, plants displayed enhanced PAL activity following salt stress, salinity stress had a substantial impact on PAL enzyme activity.[84] Our findings concur with those of Thadani et al. (2023), who reported that one of the anabolic processes that is most adversely impacted by stress in plants is protein synthesis. An essential component of the phenylpropanoid pathway, phenylalanine ammonium lyase has been identified as a marker of several abiotic stressors in a variety of plant species. Linking primary and secondary (phenyl-propanoid) metabolism, it is a crucial enzyme.[85] Similar to findings in chamomile herb under salt stress, where plants displayed enhanced PAL activity following salt stress, salinity stress had a substantial impact on PAL enzyme activity.[86] Exogenously applied Si may improve plants' resistance to drought and salt stressors by raising osmolyte levels and modifying osmotic potentials.[87] This study also found that applying Si enhanced the amounts of GSH, AsA, and total soluble sugars. In glycophytic plants exposed to osmotic stress, the primary solutes engaged in osmotic adjustment are the accumulation of organic solutes, particularly sugars.[88]

We determined that root length and surface area increased in the combined stress of salinity and drought, respectively, and the diameter of the roots decreased under drought, which is consistent with other research (Figure 3). As a consequence of more slender roots, the results indicated that when roots were stressed by drought, root length increased and root weight decreased.[89] A deep and abundant root system is required to operate well against drought and salt stress, and it was discovered that root development was dramatically decreased under drought stress due to restricted growth in low-water conditions. This may have reduced the plant growth. However, because silicon can increase the number of lateral roots in plants, its application resulted in an increase in growth indices. Plants treated with silicon have increased the length and density of their roots in a number of crops. Through the preservation of the roots' characteristics, a higher concentration of silicon in the aerial sections was important under combined stress.[90]

This study offers scientists a solid grasp of the physiological mechanisms underlying the black gram's ability to withstand salt stress and drought by application of silicon, which will serve as a guide for future cellular research. Silicon is involved in the uptake of water under water shortage conditions and the production of antioxidants to alleviate the adverse effects of abiotic stresses.

■ CONCLUSION

Since silicon was declared to be "non-essential," plant silicon research has advanced significantly. Although most plant-growth-media formulations still largely exclude this ingredient, it is now widely acknowledged to benefit many crops that are important to agriculture. Although these advantages might be more noticeable in situations of stress, such as salinity and drought, there is mounting evidence that Si may also enhance development in stressful environments. Black gram's morphology, physiology, and biochemical characteristics are negatively impacted by salinity and drought, which lower crop production. However, in both normal and abiotic stress conditions, crop growth and development were enhanced significantly by the application of silicon at 40 mg/kg. In order to control stomatal conductance and photosynthetic efficiency, silicon treatment increased the water relations within the plant, which improved the agricultural output. From all findings, it was concluded that salinity and drought significantly reduced the morpho-physiological characteristics of black gram while Si amendment significantly improved these. Overall, this study offers scientists a solid grasp of the physiological mechanisms underlying the black gram's ability to withstand salt stress and drought by application of silicon, which will serve as a guide for future cellular research. Silicon is involved in the uptake of water under water shortage conditions and the production of antioxidants to alleviate the adverse effect of abiotic stresses.

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Notes

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ABBREVIATIONS

Si, silicon; CAT, catalase; POD, peroxidase; SOD, superoxide dismutase; H_2O_2, hydrogen peroxide; MAD, malondialdehyde

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