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INDUCING WATER STRESS RESILIENCE IN MAIZE THROUGH REGULATION OF OSMOLYTES AND ANTIOXIDANT DEFENSE MECHANISMS VIA EXOGENOUS APPLICATION OF SILICIC ACID

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Abstract

Depletion due to intensive cropping necessitates improved nutrient management for sustainable agricultural development. Silicic acid treatment has emerged as a promising strategy in modulating the growth attributes of maize crop under water stress conditions. This study investigates how silicic acid administration affects maize plants, to cope with water stress. At 60% field capacity, a marked decrease in fresh (2.03g) and dry (0.84g) biomass was observed as compared to 100% field capacity (2.10), (0.88) in maize plants. Silicic acid foliar application led to higher chlorophyll contents and improved enzymatic activity of key antioxidants such as superoxide dismutase, ascorbate peroxidase, catalase and peroxidase. Drought stress is reflected in increased production of hydrogen peroxide and malondialdehyde contents. However, the application of silicic acid resulted in increased accumulation of various biochemical compounds, including total soluble proteins, total soluble sugars, total flavonoids, total free amino acids, and total phenolic contents. Overall, silicic acid application mitigated the adverse effects of water stress, however, 0.7 mM was the most beneficial treatment in ameliorating the negative consequences of drought stress.

It is deduced that Si fertilizer application in the form of silicic acid enhanced the development and biochemical attributes of maize plants to withstand drought effects. The heat map and principal component analysis revealed a distinct separation of attributes related to the silicic acid treatments.

Key words: Water stress; Foliar application; Silicic acid; Growth; Maize

Introduction

Global climate change and ever increased population growth have threatened food security. Water scarcity is a major factor limiting plant growth and productivity in dry and semiarid areas where drought is a frequent challenge. It reduces plant yield by 50%, making it one of the leading global causes of crop loss (Ali *et al*., 2025; Zafar *et al*., 2024). In addition, ensuring sustainable access to water is also a critical aspect of global development and human wellbeing, as highlighted in frameworks linking water rights and intellectual property for achieving SDG 6 (Kaur, 2025). The complex and interconnected responses of the plants under stress includes stunted growth (Ahmed *et al*., 2024), reduced water availability, lower chlorophyll levels, decreased transpiration, and changes in membrane permeability, which make it harder for plants to receive nutrients through their roots. The adverse effects include limited transpiration, restricted stomatal openings, reduced photosynthesis, and a disruption in electron transport required for the Calvin–Benson cycle and the activity of photosystem II (PSII) (Tahir *et al*., 2023. The imbalance may result in excessive excitation energy leading to photoinhibition of PSII oxygen evolving complex (Hasnanin *et al*., 2023), damage to the PSII oxygen-evolving complex, and inactivation of PSII reaction centers, linked to the breakdown of D1 protein (Zafar *et al*., 2024). Severe damage of the photosynthetic electron chain or manganese complex responsible for oxygen production can lead to photo induced oxidative loss (Hakala *et al*., 2005).

Plants produce excess excitation energy as a defense mechanism against the harmful effects of reactive oxygen species (ROS) produced under stress (Sachdev *et al*., 2021). Furthermore, many plants create and store certain chemicals to aid them in overcoming the challenges posed by drought. According to Zia *et al*., (2021), plants under stress regulate water channels (aquaporins), manage osmotic adjustments, minimize oxidative damage, eliminate waste, and adjust their carbon and nitrogen metabolism to protect against protein degradation or decay.

Silicon (Si), the second most common element in soils, its precise role in mitigating drought damage consequences on plants are unclear. It is a non-essential element, however, it plays a critical role in strengthening plants' resistance to a range of stresses like salt, drought, extreme temperatures, pests, heavy metals, and diseases (Zargar *et al*., 2019). For instance, adding silicon under stress can enhance water use efficiency in the xylem vessels, improve photosynthesis, increases leaf area, boosts crop quality, and delays leaf aging.

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Maize, or *Zea mays* L. is a crucial staple food that provides essential nutrients and calories to around 900 million people globally (Abbade, 2021). Unlike other cereals, maize efficiently absorbs solar energy due to its C4 plant classification and has a high photosynthetic potential, earning it the title "Queen of Cereals" (Rouf Shah *et al*., 2016). Globally, drought stress during early stages of maize crop is a significant issue, as it reduces both nitrogen and water use efficiency, leading to major losses in crop yields. To address these challenges in plant nutrition, nutrient application is employed, to provide nutrients to stressed plants in a controlled manner.

The present study was hypothesized to explore whether treating maize plants with silicic acid could help reduce the negative effects of water scarcity. The goal of the study was to determine how effectively silicic acid treatment can mitigate the adverse impacts of water shortage on maize growth, focusing on its ability to improve stress tolerance.

Materials and Methods

The research was performed to evaluate the effects of foliar spray of silicic acid on maize plants at Ayub Agricultural Research Institute Faisalabad, Pakistan. Seeds for the Malika maize variety were sown in plastic containers with a diameter of 22cm filled with clay loam soil. The pots were grouped and set aside based on 60% field capacity (FC) and 100% field capacity. Seven-day-old plants were subjected to water stress based on 60% FC. Following fifteen days of germination, foliar applications of silicic acid at various concentrations (control, 0.2, 0.4, 0.7, and 1.5 mM) were applied. The required concentrations were made using silicic acid from Sigma-Aldrich.

Estimation of morphological attributes: In order to measure the length as well as the biomass of the maize plant, one plant was removed from each container after twenty-one days of germination. The biomass was documented after the plants were dried in an oven at 65ºC for 72 hours.

Leaf photosynthetic pigments estimation: Photosynthetic pigments i.e. chlorophyll a (Chl a), b (Chl b), total chlorophyll (Chl t) and carotenoid were quantified according to Arnon (1949) and Davies' (1977) method. Fresh leaves (0.5g) were ground using a mortar and pestle in 80% acetone. The material was centrifuged at 12,000 x g. The supernatant was taken and optical density (OD) was recorded at 480 nm, 645 nm, and 663 nm. The procedure described by Kirk & Allen (1965) was used to determine the amount of carotenoids.

Evaluation of total soluble protein (TSP) and total free amino acid (TFAA): The TSP was quantified using (Bradford's, 1976) methodology at 595 nm. Leaf samples were homogenized in phosphate buffer (pH 7) and centrifuged at 14,000 rpm for 10 min. The reading was measured at 595 nm.

The protocol reported by Hamilton & Van Slyke (1943) was used to record the TFAA. Equal amounts of ninhydrin (2%) and pyridine (10%), as well as one milliliter of the extract, were mixed together. The test tubes were placed in a water bath and heated at 95°C for half an hour and then cooled. The wavelength was measured at 570 nm.

Range of total soluble sugar contents (TSS): To regulate the amount of TSS present, 0.5 g of dried maize leaves were extracted and then incubated in an 80% ethanol solution for six hours at 60°C, following the protocol described by (Raizi *et al.,* 1985). Each test tube, containing 0.1 ml of leaf and 6 mL of anthrone reagent, heated at 95ºC for 12 min, and cooled. The readings were taken at 595 nm.

Evaluation of leaf total phenolic (TPC) and total flavonoid contents (TFC): The method described by (Julkunen, 1985) was used to calculate TPC. About 5 mL of 80% methanol solution was used to ground half a gram of fresh leaves. The material was centrifuged for 20 min at 12,000 rpm. About 1 mL of Folin-Ciocalteu Phenol Reagent (1:10), 2 mL of distilled water, and 1.0 mL of the extracted material were added to the test tubes. Two milliliters of 20% Na_2CO_3 was also added. The wavelength was measured at 750 nm.

The protocol developed by (Karadeniz *et al.,* 2005) was employed for determining the amount of TFC present in maize leaves. About 0.5 g of fresh leaves were ground in 80% methanol solution, and the resulting extract was filtered. To about 5 mL of this solution, 5% $NaNO_2$ and 3 mL of distilled water were added. To the reaction mixture, 0.6 mL of 10% aluminum chloride was introduced and the sample was kept at 25°C for 5 min and 2.0 mL of 1M NaOH was added. The absorbance was measured at 510 nm.

Evaluation of lipid peroxidation: The protocol established by Cakmak & Horst (1991) was followed to ascertain the precise differences in malondialdehyde (MDA) contents in leaves. About 1.0-gram leaf samples were grind in 3mL of a 0.1% (w/v) TCA solution. Plant extract was centrifuged for 15 minutes at 20,000 rpm. In test tube, 20% trichloroacetic acid solution 300 µL was prepared and 1mL of the supernatant was added to it. The contents were heated in a water bath at 95°C for half an hour and allowed to cool. To determine the MDA value, the spectrophotometer was calibrated to measure wavelengths between 532 and 600 nm. The difference in readings at 600 and 532 nm were used to compute malondialdehyde contents.

Using a pestle and mortar, 0.1g of fresh leaves were pulverized together with 5mL of a 10% TCA solution. The material was centrifuged for 25 minutes at 12,000 rpm, and the supernatant was recovered. The leaf extract was then mixed with 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) in separate sample tubes. This combination was mixed with 1mL of potassium iodide. The readings were measured at 390 nm, following the procedure outlined by (Velikova *et al.,* (2000).

Evaluation of enzymatic and non-enzymatic antioxidants: The superoxide dismutase enzyme activity (SOD) was evaluated using the methodology of Giannopolitis & Ries (1977). The primary method they employed was to obstruct the photochemical drop of

nitroblue tetrazolium at a wavelength of 560 nm. To measure SOD activity, 50 µM of the leaf extract was added to the solution containing 50 µM NBT, 13 mM methionine, 1.3 µM vitamin B2 (riboflavin), 50 mM phosphate buffer (pH 7.8), and 75 mM EDTA. Exposed the solution to 30 W fluorescent light in a room with inner walls covered in aluminum. The lamp was turned on to start the reaction, and after about 20minutes, it was turned off. The readings were measured at 560 nm after the reaction mixture was irradiated with white fluorescent light for 15min. A single unit of SOD was defined as the value necessary to inhibit NBT photoreduction by 50%.

For the determination of peroxidase (POD) activity, the reaction mixture contained 0.1 mL of leaf extract, 1.0 mL of guaiacol (20 mM), 0.9 mL of hydrogen peroxide (H_2O_2, 40 mM), and 1.0 mL of phosphate buffer (50 mM, pH 7.8). The change in optical density was recorded at 470 nm at 30-s intervals (Chance & Maehly, 1955).

For catalase (CAT), 50 mM phosphate buffer, 5.9 mM H_2O_2, and 0.1 mL enzyme extract were added to the test tubes. Change in optical density was taken after every 20 seconds at 240 nm. A single unit of catalase was the amount considered to decompose 0.01 units/min of H_2O_2 Chance & Maehly (1955).

Ascorbate peroxidase (APX) was quantified following the protocol of Asada (1996). A gradual drop in absorbance at 290 nm was calculated.

Statistical analysis

The experiment was conducted using a two- factor factorial arrangement in a Completely Randomized Design (CRD) with three replications. The data was computed using software 'Statistix 8.1' at $p \leq 0.05$. The mean values were recorded by employing the least significance difference (LSD) test at 5% level of significance and "R-studio (v4.0.3)" was used for recording principal component analysis.

Results

Drought stress at 60% field capacity resulted in a significant drop in the shoot length and root length of maize genotype Malika (20.85 cm, 12.16 cm) compared to 100% field capacity (22.98 cm, 15.14 cm) (Fig. 1). The damaging effects of drought stress on shoot length were significantly lowered by the foliar spray of silicic acid, however, the effects were more pronounced at 0.7 mM application (26.62 cm), in maize plants.

Fresh and dry biomass of shoot exhibited a decrease under 60% field capacity over 100% field capacity in plants of maize variety Malika (Fig. 2). Silicic acid foliar application enhanced the shoot biomass, with 0.7 mM treatment performing best in increasing biomass.

Similarly, the application of silicic acid at varying concentrations substantially alleviated the adverse effects of drought stress on root fresh and dry biomass. The maize plants exhibited improved responses to root biomass under water stress; when treated with silicic acid, the positive effects were more pronounced at 0.7 mM application.

A significant drop in chlorophyll a (1.6 mgg^{-1} f.wt.), b (0.48 mgg^{-1} f.wt.), total chlorophyll (2.18 mgg^{-1} f.wt.) and carotenoids (0.082 mgg^{-1} f.wt.) was observed under 70% FC (Fig. 3).

In particular, the plants of maize cultivar showed a positive response to drought stress when silicic acid was applied foliarly at 0.7 mM dose. In stressed plants, there was a more pronounced increase in chlorophyll pigment. Under drought stress, total soluble protein (TSP) in maize cultivar (2.87 mgg^{-1} f.wt.) showed a significant decrease. Furthermore, plants given different foliar applications of silicic acid showed an increase in the overall soluble protein content. Drought stress resulted in a noticeable increase in free amino acid (FAA) concentration in maize, with the variety Malika exhibiting the highest increase compared with the other varieties, reaching 1.32 mg g^{-1} fresh weight (Fig. 4).

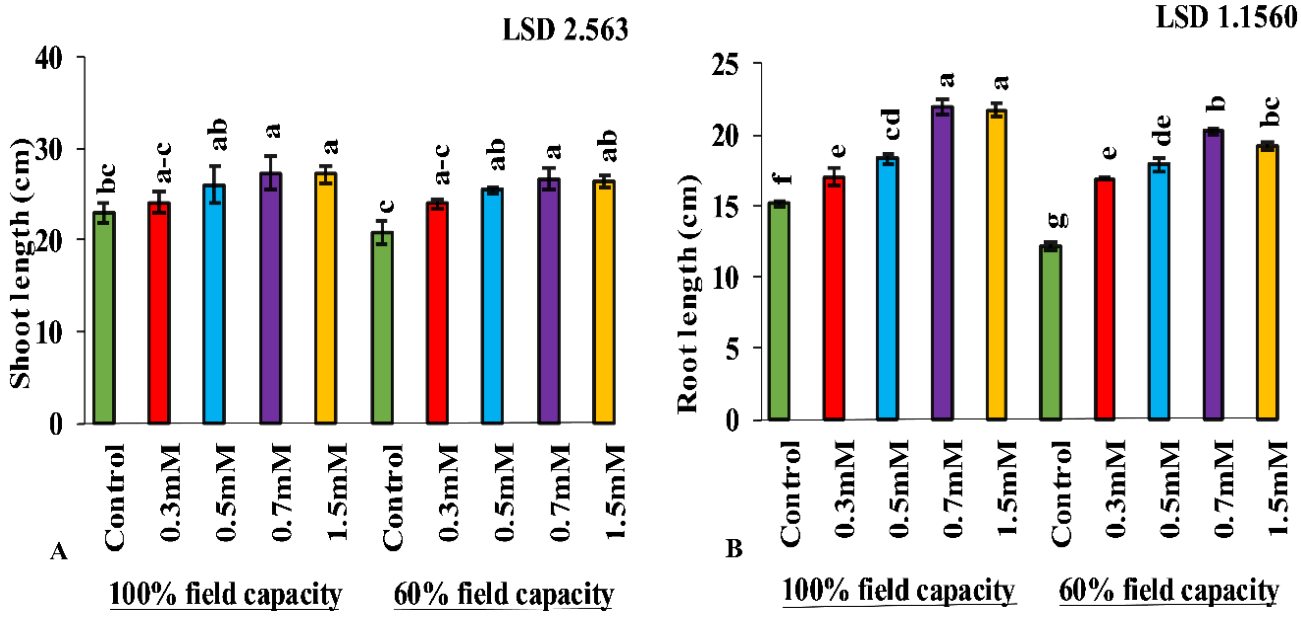

Fig. 1. Silicic acid foliar treatments on shoot and root length of maize plants under control and drought stress.

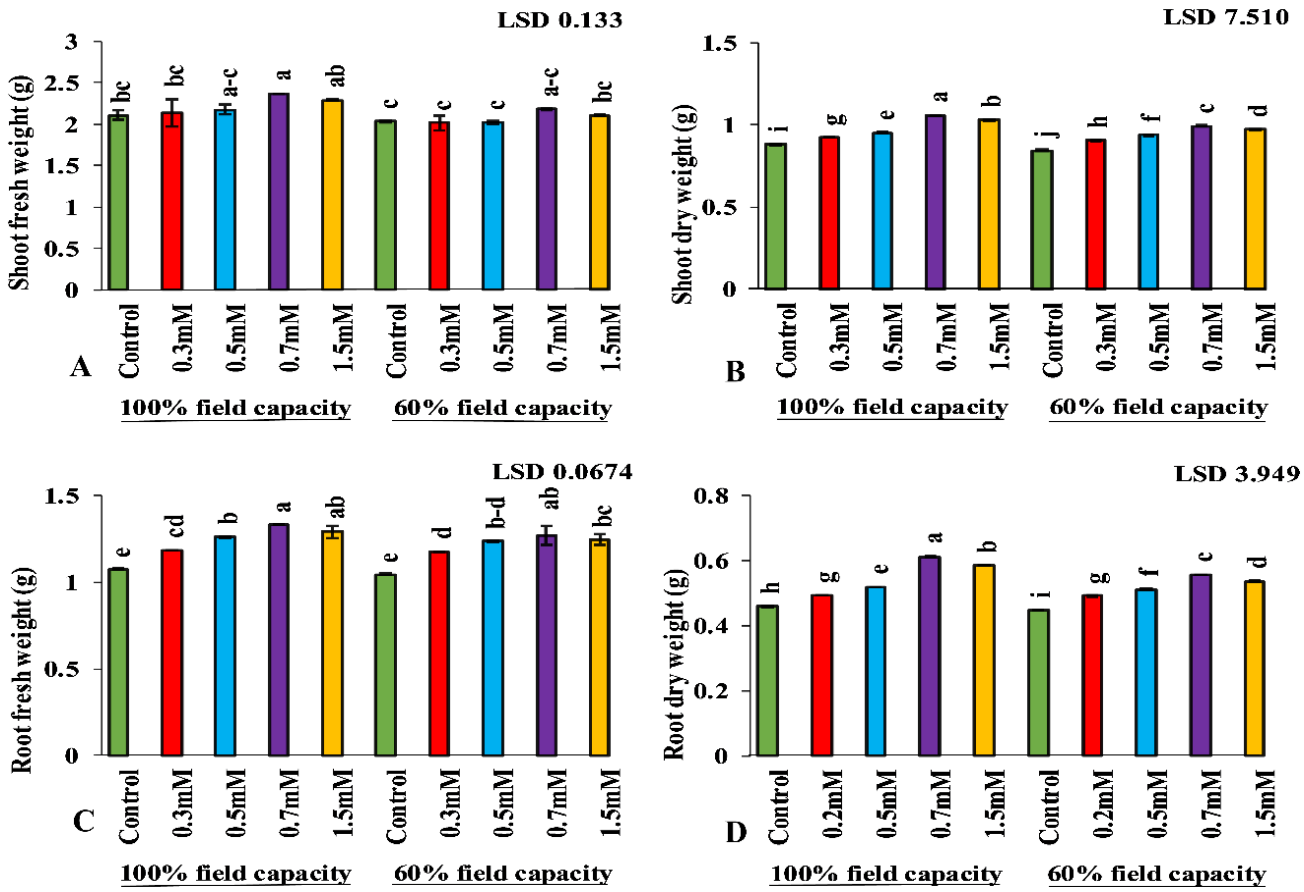

Fig. 2. Silicic acid foliar treatments on fresh and dry biomass of maize plants under control and drought stress.

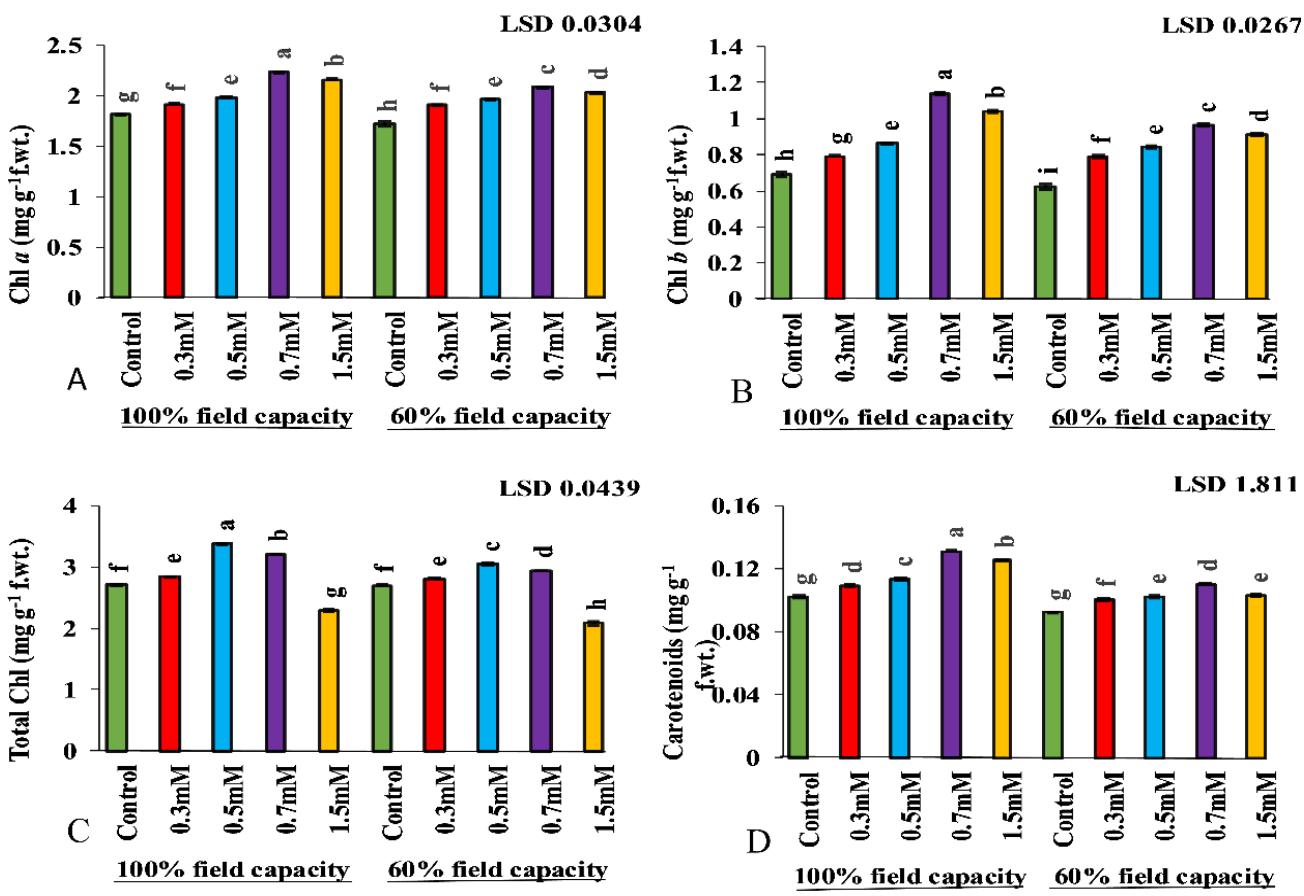

Fig. 3. Silicic acid foliar treatments on pigment contents of maize cultivars under control and drought stress.

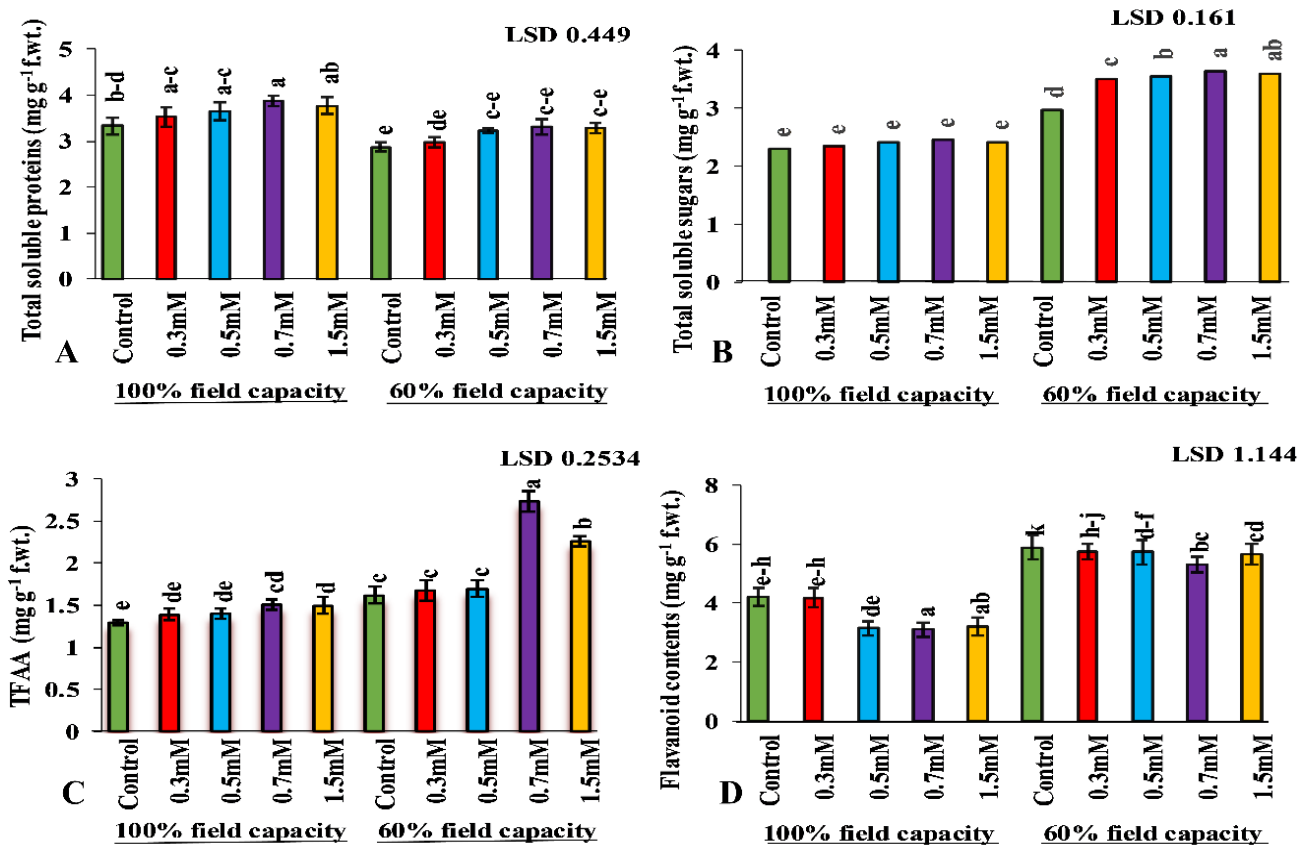

Fig. 4. Silicic acid foliar treatments on total soluble proteins (TSP), total soluble sugars (TSS), total freeamino acids (TFAA) and total flavonoid contents (TFC) of maize plants under control and drought stress.

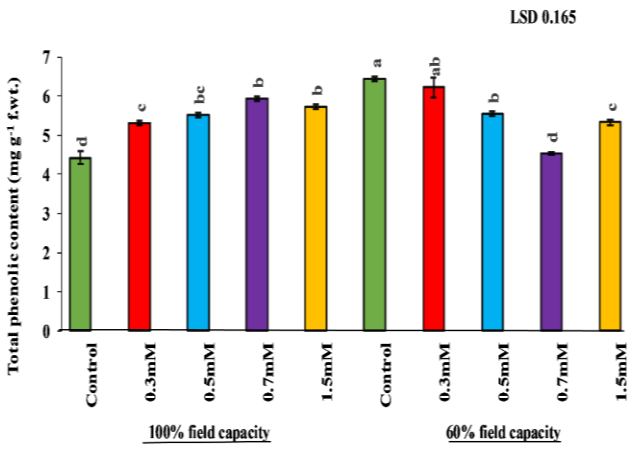

Fig. 5. Silicic acid foliar treatments on total phenolic contents of maize cultivar under control and drought stress.

Total soluble sugars (TSS) were significantly increase in maize plants (2.47 mg g^{-1} f.wt.) experiencing drought stress. On the other hand, when silicic acid was applied, the concentration of total soluble sugars further increased noticeably (Fig. 4).

The maize plants exhibited a notable decrease in total phenolic compound (TPC) contents (4.34 mg g^{-1} f.wt.) under drought stress (Fig. 5). On the other hand, plants of maize cultivars with silicic acid treatment showed a progressive rise in TPC. The total flavonoid compound (TFC) level was decreased under stress. When compared to other silicic acid treatment levels, plants treated with 0.7 mM showed higher accumulation of flavonoid contents as compared to plants given 1.5 mM treatment.

A non significant change in MDA content was observed when plants of maize cultivar were grown under drought stress conditions. The foliar application of silicic acid greatly decreased MDA production in both stressed and non-stressed conditions (Fig. 6). The plants of maize cultivar showed elevated H_2O_2 levels under drought stress, although the response differed significantly under various silicic acid levels. Notably, under drought stress, foliar application of silicic acid at 0.7 mM was found to reduce the accumulation of both MDA and H_2O_2 compared to 1mM. It was discovered that foliar application of silicic acid at different doses was beneficial in augmenting the activity of antioxidant enzyme was examined (Fig. 6). Foliar applications of 0.7 mM silicic acid resulted in maintaining the antioxidant enzyme activity in both stressed and unstressed plants (Fig. 7).

Heat map and principal component analysis

Heat map: Histogram analysis was performed to illustrate the relationship amongst morphological and biochemical parameters of maize plants by foliar application of 0.2 mM, 0.4 mM, 0.7 mM, 1.5 mM silicic acid under drought stress conditions (100% FC, 60% FC) (Fig. 8). Remarkable distinctions were observed in plant growth, photosynthetic attributes, and antioxidant defense system under various treatments. Significant results were also observed with other attributes under drought stress with silicic acid foliar application (Fig. 8). Grey color exhibited the non-significant differences in treatments, while other colors showed the significant differences. Overall, histogram analysis demonstrated strong associations among growth traits, chlorophyll contents and biochemical attributes of maize plants in response to silicic acid application under various moisture regimes.

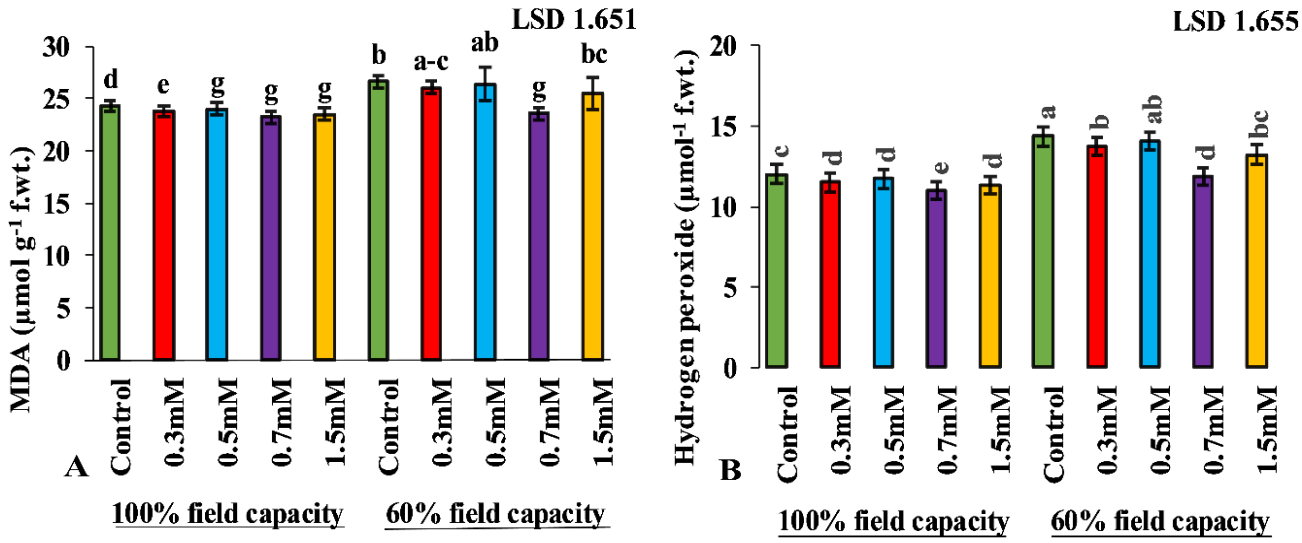

Fig. 6. Silicic acid foliar treatments on Lipid peroxidation (hydrogen peroxide, malondialdehyde contents) and antioxidant enzymes activity (Superoxide dismutase, catalase, peroxidase, ascorbate peroxidase) of maize plants under control and drought stress.

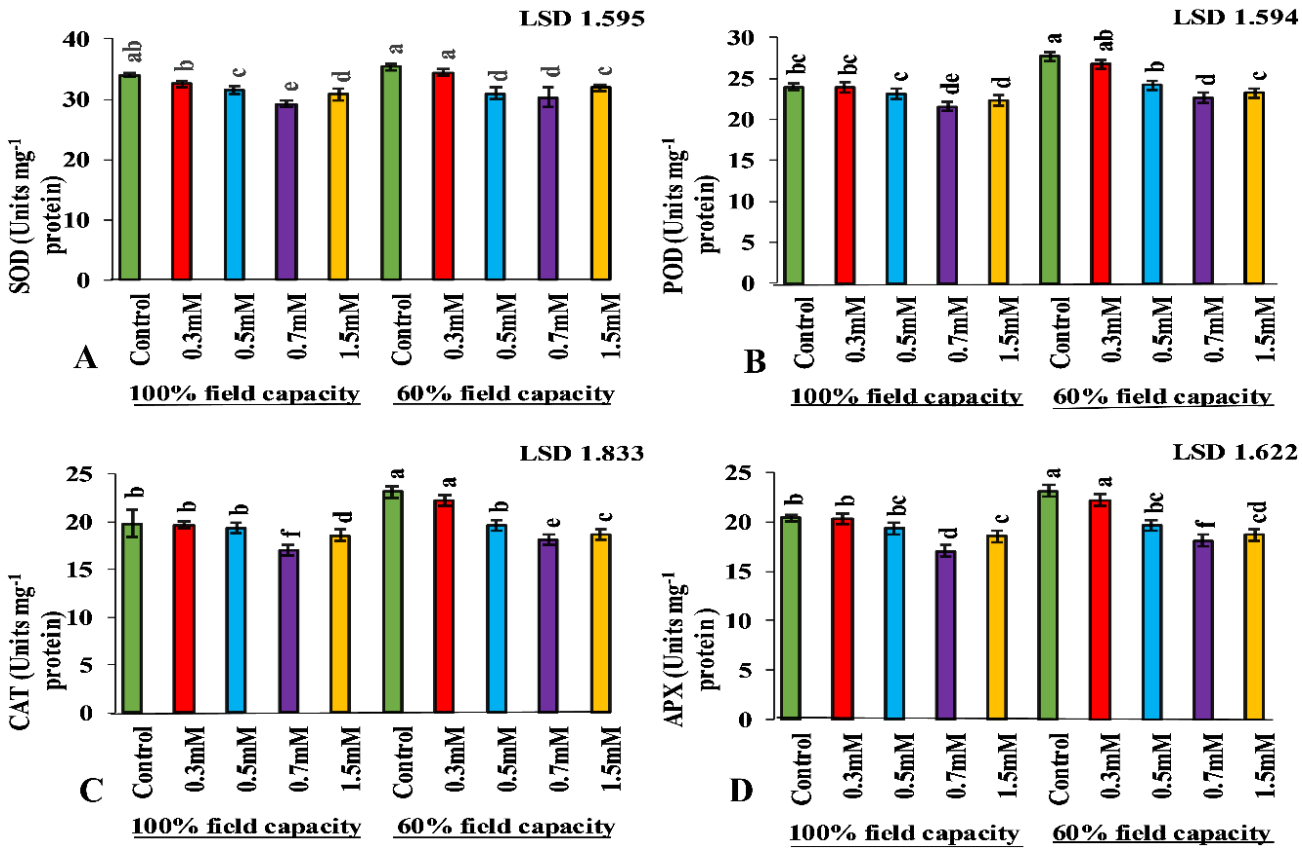

Fig. 7. Silicic acid foliar treatments on antioxidant enzymes activity (Superoxide dismutase, catalase, peroxidase, ascorbate peroxidase) of maize plants under control and drought stress.

Principal component analysis: The plots of PCA showed the outcome of water stress (100% field capacity and 60% field capacity) by foliar treatments of silicic acid under water stress conditions in maize plants. A clear separation of various parameters in response to shortage of water was presented by Dim1 and Dim2. In fig 9, Dim 1 and Dim 2 illustrated the extreme influence and occupy more than 76.0% of whole databases, with Dim 1 shows 51.3% and Dim 2 shows 24.7%. In Fig. 9A (a clear distinction of attributes with respect to 100% and 60% field capacity (9B) a clear distinction of attributes by silicic acid treatments.

Discussion

Water shortage adversely affects photosynthesis and modifies the morphological, physiological, and biochemical processes of plants (Ali *et al.,* 2024). The oxidative stress indicators immediately jeopardize crop growth and yield (Tahir *et al.,* 2024). The current study found that water shortage stunted growth, yield, and chlorophyll content in the plants while increasing oxidative stress indicators, antioxidant rank, and solute accumulation. Consistent with the present outcomes, (Zafar *et al.,* 2024) reported a

significant decrease in crop development, yield, and physiological indicators due to water shortage stress. Water shortage situations affect the growth and physiological functions of almost all plants, including grain crops (Seleiman *et al.,* 2021). However, a number of studies have already shown that silica enhances plants resistance to adverse environments (Ma *et al.,* 2001; Ogedengbe *et al*., 2026). When challenged with water constraint, a number of species, including soybeans (Anda *et al.,* 2020), rice (Xu *et al.,* 2020), and wheat (Zhao *et al.,* 2020), have demonstrated advances in growth administered by silicon.

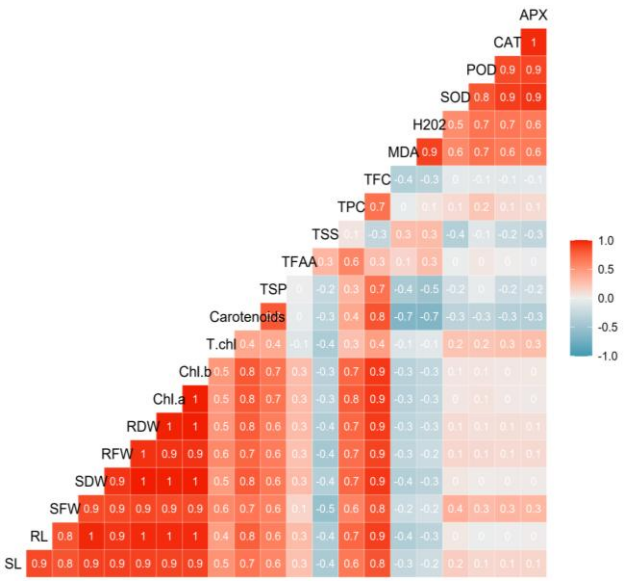

Fig. 8. Heat map histogram showing correlation of various attributes of maize variety Malika. Shoot length (SL); Root length (RL); Shoot fresh weight (SFW); Shoot dry weight (SDW); Root fresh weight (RFW); Root dry weight (RDW); Chlorophyll a (Chl.a); Chlorophyll b (Chl.b); Total Chlorophyll (T.Chl); Total soluble proteins (TSP); Total free amino acids (TFAA); Total soluble sugars (TSS); Total phenolic contents (TPC); Total flavonoid contents (TFC); Malondialdehyde contents (MDA); hydrogen peroxide (H2O2); Superoxide dismutase (SOD); Peroxidase (POD); Catalase (cat); Ascorbate peroxidase (APX).

The current investigation found that the drought significantly reduced the biomass and growth of maize plants. All of these metrics were, however, improved under water stress by foliar application of silicic acid. Particularly, in terms of morphological criteria, the plants that received a 0.7mM silicic acid foliar spray demonstrated superior growth. Even though they are essential for ion and water absorption, roots are also essential for the absorption, synthesis, and movement of several solutes. According to Wang & Grimm (2021), Fathi (2022), chlorophyll is essential for photosynthetic biomass production of photosynthesis. This study found that in maize plants, drought stress decreased the amounts of photosynthetic pigments such as carotenoids, total chlorophylls, chlorophyll a and b. A previous study (Eid *et al.,* 2022) discovered that low water conditions led to a decrease in the levels of chlorophyll a, chlorophyll b, and total chlorophylls in cotton plant leaves; this was caused by increased ROS generation (Jin *et al.,* 2020; Sharma *et al.,* 2020). On the other hand, silicic acid applied topically resulted in an increase in the amount of chlorophyll pigments. The plants treated with 0.7 mM of silicic acid exhibited a noteworthy rise in photosynthetic pigment accumulation; similarly, plants treated with 1.5 mM of silicic acid demonstrated favorable outcomes. Similar to recent findings, exogenous silicon treatment was reported to increase the levels of chlorophyll a and b in tomato and mungbean plants (Li *et al.,* 2020; Abbas *et al*., 2026)). Si treatment increased tomato chlorophyll a and b levels in drought-stressed environments (Dou *et al.,* 2023; Ning *et al*., 2026). The role of silicon in enhancing photosynthesis and the dispersion of light energy in chloroplasts is well documented (Li *et al.,* 2020; Murtaza *et al*., 2026). Under drought stress, total soluble protein concentration increase in the current investigation. In order to control osmotic pressure and to reduce water loss, plants under water stress increased the concentrations of total soluble proteins. It is possible that rise in the overall quantity of soluble proteins is in charge of maintaining cellular pH, osmotic adjustment, and shielding macromolecules within cells (Hasnain *et al.,* 2023). It is reported that water-starved gerbera plants were helped to keep their water level by the application of foliar silicon, which significantly increased TFA accumulation. It is also reported that Si stimulates amylase activity to increase starch breakdown under drought stress. Our findings are in consistent with the research on maize (Xu *et al.,* 2022) and peach (Gao *et al.,* 2022), which indicated that exposure to drought stress triggers the biosynthesis and gathering of amino acids that actively participate in the regulation of osmotic potential in drought-stressed environment. The current study indicated that under drought stress, both kinds of maize cultivars exhibited a considerably higher percentage of total soluble sugar contents. In comparison to controls plants under drought stress, foliar application of silicic acid further enhanced the total soluble sugar content. These outcomes agreed with the findings of (Bukhari *et al.,* 2021., Aurangzaib *et al.,* 2022) on wheat. According to (Ali *et al.,* 2025), secondary metabolites, total phenolic and flavonoid compounds, are produced in the cytoplasm and endoplasmic reticulum of plants and play a crucial role as signal molecules in antioxidant protection systems in drought-stressed maize plants. The beneficial effects of Si on nutritional content have been noted in certain plants; these effects may be attributed to the enhancement of total phenolic contents and the control of important enzyme activity in the phenyl propanoid pathway (Ebeed *et al.,* 2023). Drought stress causes ROS generation, which in turn triggers an oxidative explosion. In order to scavenge ROS and ultimately improve plant stress tolerance, oxidative stress activates a variety of antioxidant enzymes. Studies by (Loutfy *et al.,* 2020) showed that ascorbate peroxidase, catalase, superoxide dismutase, and peroxidase enzymes are activated during drought stress. The findings showed that supplementing plants with silicon, experiencing water stress triggered the antioxidant enzymes. Significant reductions in H_2O_2 and MDA levels in maize plants demonstrated the scavenging of ROS generation by elevated levels of CAT, POD,

SOD, and APX activities. Drought stress considerably increased MDA levels, a marker of lipid peroxidation (Choudhury *et al.,* 2022). An increase in the production of H_2O_2 was also observed. The variety Malika exhibited less accumulation in MDA and H_2O_2. The findings demonstrated that the treated plants had increased resistance to oxidative stress and were less susceptible to drought. When stressed plants received treatment, their MDA levels were lowered significantly (Hnilickova *et al.,* 2021). According to Ebeed *et al.,* (2023), application of silicon under drought stress may function as the most effective supplement to endure the generation of the reactive oxygen species (ROS) by sustained oxidative damage and antioxidant defense system upregulation. Bukhari *et al.,* (2021) described that the accumulation of Si raised the antioxidant enzyme activities in plants, assisting the plants in mitigating the damage caused by reactive oxygen species (ROS), to withstand development and growth of plants under drought stress.

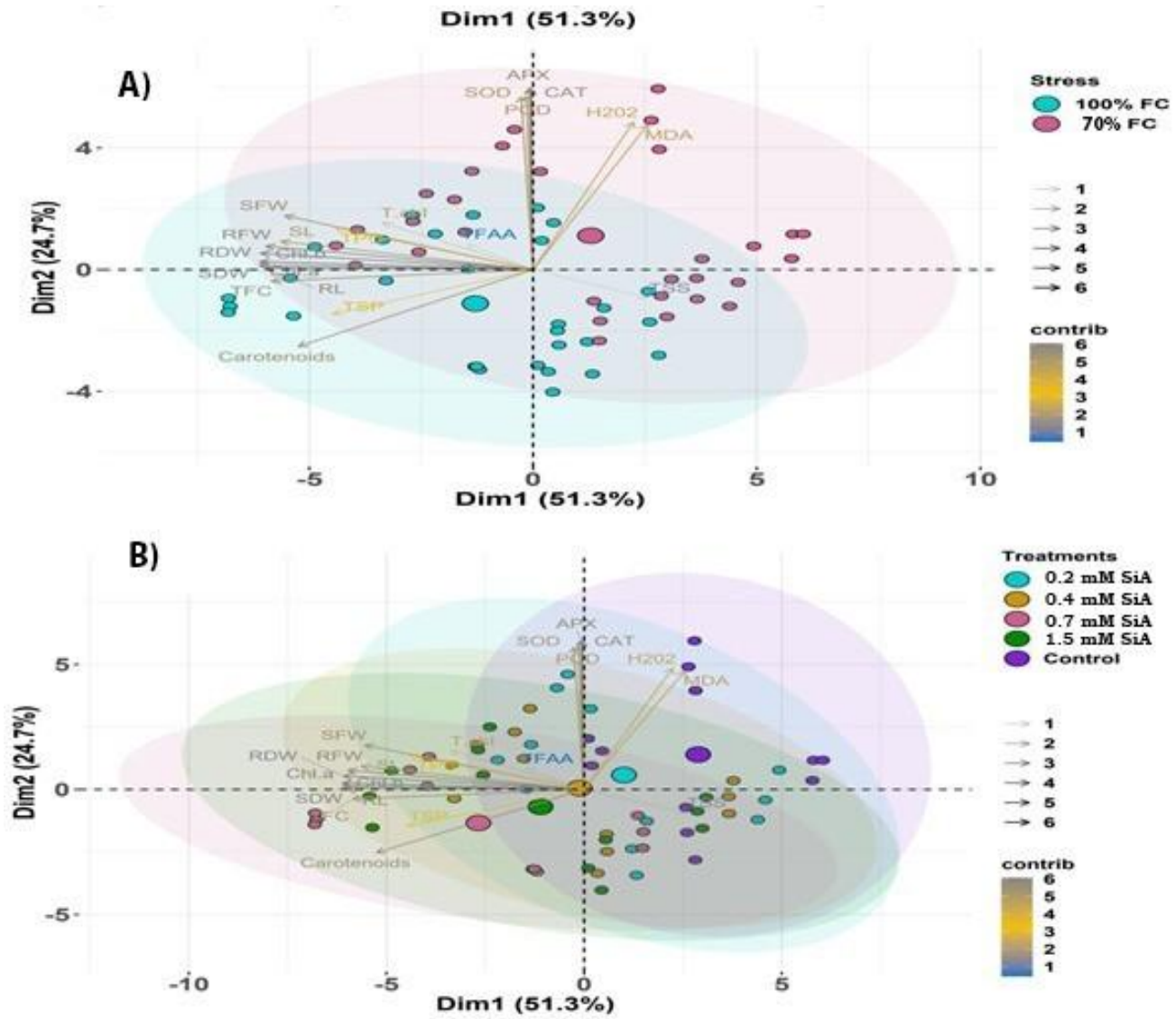

Fig. 9. Principal component analysis exhibiting relationship between (A) drought stress (100%, 60% field capacity), (B) silicic acid treatments for growth and biochemical attributes of maize plants.

Conclusion

The current study suggested that silicic acid is the best silicon source when it comes to foliar applications, which can help maize growth more productively in arid zones. Intense cultivation has depleted Si from nutrient status of agricultural soils. In the future, silicic acid may be used as a facilitator molecule to improve soil health and protection of plants against stresses, to decipher the molecular mechanisms underlying drought tolerance in cereals, particularly maize.

Future Prospects: The significance of using silicic acid as a fertilizer for improving drought tolerance in maize, helps in enhancing growth, physiological, and antioxidant mechanisms in arid environments. Soil silicon level is depleted due to the intensification of cultivation, silicic acid might play a significant role for improving soil and drought stress tolerance. In the future, it is expected that using such traditional approaches in combination with novel molecular tools might play an important role in developing climate-resilient cultivars (Maqbool *et al.,* 2026).

Author's Contribution: AA: Conducted the experiment and refined the manuscript; **SZ:** Supervised the experiment; **MA:** Planned the experiment; **NK:** Assisted with statistical analysis; **MMA:** Assisted in manuscript writing; **MS:** Provided financial support.

Declarations

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Conflict of Interest: The authors declare no conflict of interest.

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