



# Silicon alleviates drought stress by up-regulating physiological and biochemical responses in two contrasting bread wheat cultivars

Waseem Ashfaq<sup>1</sup> · Muhammad Kaleem<sup>2</sup> · Graham Brodie<sup>3</sup> · Sigfredo Fuentes<sup>1,4</sup> · Alexis Pang<sup>1</sup> · Dorin Gupta<sup>1</sup>

Received: 7 November 2024 / Accepted: 3 February 2025 / Published online: 21 February 2025  
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## Abstract

Drought stress (DS) substantially reduces plant growth and productivity in a rainfed cropping environment. Silicon (Si) has been recognized for its potential to alleviate the detrimental effects of various environmental stresses. However, its precise mechanisms in mitigating early growth DS and its impact on related physiological and biochemical attributes remain less understood. The present study, conducted in a glasshouse using a complete randomized design with four replications, was aimed to explore the role of pre-sowing Si treatment on two contrasting wheat cultivars (RAC875, drought-tolerant; Kukri, drought-susceptible) in mitigating the physiological and biochemical disruptions induced by early growth DS (14 days at  $38 \pm 3\%$  pot field capacity). The results showed that Si treatment significantly increased the number of productive tillers and fresh weight in both cultivars under DS. Compared to the control (DS without Si treatment), Si treatment significantly enhanced chlorophyll content (RAC875, 8.7%; Kukri, 12.7%), relative water content (RAC875, 10.8%; Kukri, 18.1%), and chlorophyll fluorescence (RAC875, 10.1%; Kukri, 22.3%) under DS conditions. Additionally, Si treatment increased total soluble sugars, fructose content, and free amino acids while reducing proline content and lipid peroxidation concentrations in both cultivars under DS. Moreover, Si treatment significantly boosted cellular antioxidant activities, including ascorbate peroxidase (RAC875, 11.3%; Kukri, 9.2%), catalase (RAC875, 22.2%; Kukri, 25.5%), and peroxidase (RAC875, 19%; Kukri, 15.8%) under DS. Overall, these results show that Si effectively alleviates early growth DS by up-regulating antioxidants, osmoprotectants, and the photochemical process, thereby improving plant growth and productivity through enhanced photosynthesis.

**Keywords** Antioxidants · Drought stress · Oxidative stress · Silicic acid · *Triticum aestivum*

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Communicated by Gábor Kocsy.

✉ Dorin Gupta  
dorin.gupta@unimelb.edu.au

<sup>1</sup> Faculty of Science, School of Agriculture, Food and Ecosystem Sciences (SAFES), The University of Melbourne, Melbourne, Australia

<sup>2</sup> Department of Botany, University of Agriculture, Faisalabad 38040, Pakistan

<sup>3</sup> College of Science and Engineering, James Cook University, Townsville, QLD, Australia

<sup>4</sup> Faculty of Science, Digital Agriculture, Food and Wine Research Group, SAFES, The University of Melbourne, Melbourne, Australia

## Introduction

Bread wheat (*Triticum aestivum* L.) is a globally cultivated staple food crop and its growth is significantly impacted by drought stress, which disrupts physiological and biochemical processes (Barnabás et al. 2008). Depending on the growth stage, genotypic behavior, duration, and intensity of drought, yield losses in wheat can reach up to 82% (Khadka et al. 2020).

In a Mediterranean environment, drought stress poses a significant challenge and is a prevalent yield-limiting factor in dryland agriculture (Shiferaw et al. 2013). Due to changing climate, droughts are becoming more frequent across many wheat-growing regions worldwide (Leng and Hall, 2019). Although drought stress may affect wheat at any developmental stage (Pessaraki 2010), it is particularly detrimental during vegetative and stem elongation stages and before the transition to the reproductive stage when the

peduncle and the penultimate internode rapidly grow (Z30-39; Zadoks et al. 1974; Rivera-Amado et al. 2019). During these critical growth stages, drought stress disrupts source-sink relationships, potentially causing early senescence, reduced yield, and premature plant death due to desiccation (Blum 2010; Wang et al. 2015).

Grain yield in wheat is significantly influenced by stress-responsive and stress-adaptive mechanisms, which are linked with edaphic factors, the intensity and duration of water stress, and the crop's growth stage (Oliveras-Villegas et al. 2007; Blum 2010). Wheat plants exhibit complex responses to drought stress, involving significant morphophysiological and biochemical changes that directly impact plant growth and productivity (Barnabás et al. 2008; Khadka et al. 2020). Wheat cultivars respond differently to drought stress, depending mainly on their genetic potential for stomatal regulation, photosynthesis, leaf water content, antioxidant levels, and other regulatory processes that mitigate oxidative damage (Wang et al. 2019).

Drought stress during the plant life cycle disturbs antioxidant activity, leading to the excessive accumulation of reactive oxygen species (ROS), i.e., singlet oxygen ( $^1\text{O}_2$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), hydrogen superoxide ( $\text{HO}_2$ ), superoxide ( $\text{O}_2^-$ ) and hydroxyl radicals ( $\text{OH}^-$ ). These ROS damage biomolecules and interfere with normal metabolic processes (Alzahrani et al. 2018). A key mechanism for drought tolerance is osmotic adjustment through the accumulation of compatible organic solutes, including total soluble sugars (TSS), free amino acids, fructose content (FC), total soluble protein (TSP), and proline, in the protoplasm (Loutfy et al. 2012; Biju et al. 2017). These solutes and associated metabolic adjustments protect subcellular structures, maintain cell turgor and hydration, and enhance  $\text{CO}_2$  assimilation for continued photosynthesis, thereby supporting plant growth under drought stress (Blum 2010, 2017).

However, changes in physiological and biochemical factors further complicate plant responses to drought stress. Abiotic stress can downregulate chlorophyll fluorescence due to substantial photoinhibition of photosystem II (PSII) by photooxidation, which suppresses PSII repair by inhibiting PSII protein synthesis (Takahashi and Murata 2008; Murchie and Lawson 2013). The inhibition of PSII activities during drought stress is also linked with leaf senescence. However, a delayed senescence or stay-green phenotype maintains photosynthesis for longer after anthesis, a trait commonly targeted to improve crop resilience in drought-prone environments (Christopher et al. 2016).

Among five non-essential elements (Si, silicon; Se, selenium; Na, sodium; Al, aluminum; Co, cobalt), Si plays an important role in enhancing plant growth and productivity under environmental stress conditions (Pilon-Smits et al. 2009). Although Si is not readily available to plants for uptake due to its polymerized form (Walsh et al. 2018),

its application as a nutritional element to alleviate abiotic stresses has become a promising prospect in recent decades (Wang et al. 2021). In wheat, Si uptake by roots is facilitated by two key transporters: low silicon transporter 1 (Lsi1), a nodulin-26-like intrinsic protein, and Lsi2, an efflux Si transporter (Ma and Yamaji 2015). Once absorbed, Si is deposited in the cell walls and serves as a physical barrier, enhancing the tissue rigidity and structural integrity. Furthermore, Si plays a vital role in improving mineral nutrient uptake, mitigating oxidative stress, and maintaining cellular homeostasis under drought stress by improving osmotic adjustment, enhancing the activities of antioxidant enzymes, and regulating the accumulation of compatible osmolytes (Debona et al. 2017). The beneficial effects of Si have been extensively documented in several crops, i.e., rice (Agostinho et al. 2017), sorghum (Hattori et al. 2005), lentil (Biju et al. 2017; 2021; 2023), wheat (Ashfaq et al. 2022b; 2024) and tomato (Shi et al. 2014). These findings highlight the potential of Si supplementation as an effective strategy for enhancing drought tolerance and agricultural productivity in water-limited environments.

Despite its significance, limited research has focused on wheat's response to early growth drought stress and the mitigating effects of Si supplementation, particularly in contrasting wheat cultivars. This study aims to address this gap by exploring the role of Si in alleviating drought stress at early growth stages of two wheat cultivars (drought stress tolerant and susceptible), focusing on physiological and biochemical responses and their impact on grain yield. We hypothesized that Si supplementation enhances wheat productivity under drought stress by improving water relations and increasing osmoprotectant and antioxidant levels.

## Materials and methods

### Plant materials

Two bread wheat varieties, RAC875 and Kukri, with distinct levels of drought tolerance, were selected for this study. RAC875, a drought-tolerant cultivar, can maintain grain yield under a dry environment (Izanloo et al. 2008). In contrast, Kukri, classified as susceptible to drought, experiences significant decreases in grain yield in controlled environments and the field (Fleury et al. 2010; Ashfaq et al. 2022a). Seeds of these two varieties were sourced from the Australian Grain Gene Bank, located in Horsham, Victoria, Australia.

### Experimental design and establishment

The experiment was conducted in a glasshouse at the Dookie campus of the University of Melbourne during the spring of

2019. The experiment used a complete randomized design with a three-factor factorial arrangement and four replicates, where each pot represented one replicated unit. The experimental treatments included two varieties, two Si levels (control: 0 mM Si, and Si application: 4 mM, pH 7), and two watering regimes (well-watered at  $95 \pm 5\%$  pot field capacity and drought stress at  $38 \pm 3\%$  pot field capacity).

Before sowing, a Si solution was applied and thoroughly mixed into the respective pot potting mix (comprising compost pine bark med, compost pine bark coarse, processed sawdust, and river sand in a ratio of 4:4:1:1, with a pH ~6.0). Sodium metasilicate pentahydrate ( $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$ ) was used as a source of silicic acid, and a 4 mM  $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$  concentration was chosen following the recommendation of Alzahrani et al. (2018) for drought stress studies. Based on these treatments, pots were categorized into four groups: control (C), drought stress (DS), control with Si treatment (CSi), and drought stress with pre-sowing Si treatment (DSSi). No crystals were observed in the solution at the time of Si application.

The field capacity of the potting mix was determined using a gravimetric method, as described by Pepper and Brusseau (2019). Before filling the pots, the potting mix was thoroughly mixed to ensure uniform moisture distribution. Three potting mix samples (250 gm each) were taken from the middle of randomly selected pots and saturated with tap water to determine their saturated weight. Subsequently, the samples were then oven-dried at  $70^\circ\text{C}$  for 72 h to determine their dry mass representing wilting point. The gravimetric water content (%) of the potting mix was calculated using Eq. 1.

$$\text{Gravimetric soil water content(\%)} = \left[ \frac{(\text{wet mass} - \text{dry mass})}{\text{dry mass}} \right] \times 100 \quad (1)$$

Gravimetric soil water content is the ratio of water mass to dry soil mass. The wet mass refers to the weight of the saturated potting mix (in grams) and the dry mass refers to the weight of oven-dried mass of the potting mix (in grams).

## Growth conditions

Plants were grown in perforated circular nursery pots containing potting mix with an oven-dry weight of 3.25 kg. Six seeds were planted in each pot and later thinned to four. Inside the glasshouse, a day/night temperature cycle was maintained at  $24 \pm 1/18 \pm 1^\circ\text{C}$ , respectively, with an average relative humidity of  $65 \pm 5\%$ . Temperature control within the glasshouse was automated using the Argus Control Systems. The glasshouse received 11–12 h of natural light daily during the experimental period, and no artificial lighting was used to extend the photoperiod. The ICL Peters Excel CalMag grower fertilizer (2 gm/liter per week) was applied

twice after sowing to ensure an adequate nutrient supply. To prevent excessive drainage, each pot was irrigated every alternate day, maintaining the potting mix field capacity at  $95 \pm 5\%$ .

Plant phenology was monitored using the Zadoks scale, and DS was initiated at the Z29 stage (before pseudo-stem elongation) for 14 days (Zadoks et al. 1974). A 14-day drought period was chosen as a standardized timeframe to represent medium-term droughts, which are common during critical growth stages in rainfed cropping systems. Before applying the drought, irrigation to the DS-designated group of pots was gradually reduced over five days, bringing the pot field capacity to  $38 \pm 3\%$  and facilitating acclimatization. During the growth cycle, the glasshouse relative humidity was monitored and maintained at  $65 \pm 5\%$ . Each pot was weighed daily during the DS period to measure moisture loss through evapotranspiration. Water was then added to each pot according to moisture loss measurements to maintain target field capacity. During DS, water was consistently supplied to C and CSi pots to maintain  $95 \pm 5\%$  pot field capacity.

## Physiological measurements, leaf sampling, and experiment termination

Plants were harvested 14 days after exposure to drought and 49 days after sowing. Before harvest, non-destructive measurements of chlorophyll content (measured as SPAD value) and photosystem II maximum photochemical efficiency ( $F_v/F_m$ , average of five readings) were taken using the flag leaf from each biological replicate within each pot. Chlorophyll content was calculated using the mean of nine values taken from each plant flag leaf's mid, tip, and base using a self-calibrating portable SPAD-502 Plus.

The  $F_v/F_m$  of the flag leaf was recorded by using the Mini-Palm-II system with a leaf clip holder (a portable red LED modulation fluorescence system, Heinz Walz, Germany). Minimal fluorescence ( $F_0$ ) was recorded from the dark-adapted leaves (after 30 min of darkness) by applying a low-modulated measuring beam ( $> 0.01 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). From the disk of the same leaf, maximal fluorescence ( $F_m$ ) was recorded through a beam of saturated white light ( $> 3500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The quantum yield of photosystem II for dark-adapted flag leaves was estimated using Eq. (2) (Carvalho and Amâncio 2002).

$$F_v/F_m = \frac{(F_m - F_0)}{F_m} \quad (2)$$

Following the methodology outlined by Barrs and Weatherley (1962), the relative water content (RWC) was estimated before harvest. Subsequently, five fully emerged flag leaves were harvested from each biological replicate within each pot and stored at  $-80^\circ\text{C}$  for the assay of enzymatic

antioxidants, fructose content, total soluble sugars, free amino acids, proline content, lipid peroxidation (LPX), and total soluble protein. At harvest, the fertile tillers (TLR), plant height (cm), and shoot fresh and dry weight (gm) of each biological replicate from each pot were recorded. The shoot fresh weight was recorded using a scale ( $\pm 0.01$  gm) in glasshouse conditions. Samples were then oven-dried at 70 °C for 72 h, and the shoot dry weight was measured on a scale ( $\pm 0.001$  gm) at standard laboratory conditions. After harvesting the shoots at the crown, roots from each pot were carefully retrieved, delicately washed, and preserved in 70% (v/v) alcohol at 10 °C for root crown scanning and image analysis (Ashfaq et al. 2024).

## Quantification of organic osmoprotectants

### Total soluble sugars and fructose content

Following the methodology proposed by Dubois et al. (1951), the TSS and FC were determined spectrophotometrically at wavelengths of 620 and 420 nm, respectively, utilizing residual and soluble fractions of ethanol–water extracts. The TSS determination involved anthrone reagent, while FC determination utilized concentrated  $\text{H}_2\text{SO}_4$ , followed by incubation at 95 °C for 20 min. Absorbance readings were taken with a Halo RB-10 single-beam UV scanning spectrophotometer (Dynamica Scientific Ltd.). The amounts of TSS and FC were calculated from their respective standard curves and expressed as  $\text{mg g}^{-1}$  of fresh weight (FW).

### Proline and free amino acids

Proline and free amino acids were extracted from flag leaf tissues (0.2 gm) using a 3% aqueous sulfosalicylic acid solution. Following the protocol outlined by Bates et al. (1973), spectrophotometric measurements were performed at 520 nm for proline, and 570 nm for free amino acids, using ninhydrin reagent. The proline and free amino acid content were calculated from the calibration curves established by dissolving known concentrations (ranging from 10 to 50  $\mu\text{g/ml}$ ) of proline and leucine in 3% aqueous sulfosalicylic acid and were expressed on an FW basis.

### Total soluble protein

The TSP content was estimated using the protocols developed by Lowry et al. (1951). Leaf samples (0.2 gm) were homogenized with TissueLyser II (Qiagen, Germany) in 5 ml sodium phosphate buffer (Na-PB, pH 6.8) and centrifugation at 4000 rpm for 15 min. The resulting supernatant was pooled in 10 ml test tubes and then reacted with 5 ml diluted Bio-Rad protein assay dye reagent. After vortexing with a test tube stirrer for 10 s, the mixture was allowed to

stand for 20 min. A standard curve was generated by dissolving different concentrations of bovine serum albumin in a 5 ml phosphate buffer. The absorbance was recorded spectrophotometrically at 595 nm, and TSP content was expressed as  $\mu\text{g g}^{-1}$  FW.

### Lipid peroxidation

The lipid peroxidation was determined by assessing malondialdehyde (MDA) levels in leaf tissue. Frozen flag leaf samples (0.2 gm) were homogenized with TissueLyser II (Qiagen, Germany) in 5 ml of 0.1% trichloroacetic acid (TCA), followed by centrifugation at 10,000 rpm for 10 min. A 1 ml aliquot of the resulting supernatant was mixed with 4 ml of 0.5% thiobarbituric acid (TBA) prepared in a 20% TCA solution. After incubating at 95 °C for half an hour, the reaction was terminated in an ice bath, followed by a second centrifugation at 10,000 rpm for 10 min. The absorbance of the supernatant fraction was recorded at 532 nm after subtracting the non-specific absorbance read at 600 nm. The MDA levels were quantified using an extinction coefficient of the MDA-TBA product ( $155 \text{ mM}^{-1} \text{ cm}^{-1}$ ) and expressed as  $\mu\text{mol g}^{-1}$  FW (Heath and Packer 1968).

### Cellular antioxidants

A 300  $\mu\text{l}$  enzyme extract was prepared using a 0.2 gm frozen flag leaf sample and mixed with a reaction mixture consisting of 30%  $\text{H}_2\text{O}_2$  (v/v), 0.05 M Na-PB (pH 7.2), and 0.5 mM ascorbic acid (ASA). The activity of ascorbate peroxidase (APX, measured in  $\text{mmol ascorbate mg}^{-1} \text{ protein minute}^{-1}$ ) was assayed spectrophotometrically at 290 nm by quantifying the ASA-induced oxidation of  $\text{H}_2\text{O}_2$ , with a reduction in absorbance recorded after a two-minute interval (Chen and Asada 1989).

Catalase (CAT,  $\mu\text{mol H}_2\text{O}_2 \text{ mg}^{-1} \text{ protein minute}^{-1}$ ) activity was determined following the protocol of Chance and Maehly (1955). A 250  $\mu\text{l}$  enzyme extract was added to a reaction mixture comprising 300  $\mu\text{l}$  0.1 M  $\text{H}_2\text{O}_2$ , 1.5 ml of 50 mM Na-PB (pH 7.8), and 1 ml of distilled  $\text{H}_2\text{O}$ . Changes in absorbance were recorded spectrophotometrically at 240 nm after a minute's difference as a measure of CAT activity.

Peroxidase (POX, measured in  $\text{mmol guaiacol mg}^{-1} \text{ protein minute}^{-1}$ ) activity was assayed following the methodology developed by Chance and Maehly (1955). A 20  $\mu\text{l}$  enzyme extract was added to a reaction mixture containing 2.0 ml of 0.1 M Na-PB (pH 6.8), 50  $\mu\text{l}$  of 10 mM  $\text{H}_2\text{O}_2$ , and 0.930 ml of 20 mM guaiacol ( $\text{C}_7\text{H}_8\text{O}_2$ ). The change in absorbance at 470 nm was measured spectrophotometrically after three minutes to assess the increased oxidation of  $\text{C}_7\text{H}_8\text{O}_2$  in the presence of  $\text{H}_2\text{O}_2$ .

## Statistical analysis

The data analysis and graphical representation were performed using R software (R Core Team 2021). A two-way ANOVA ( $p < 0.05$ ) was employed to examine the effects of Si on various morpho-physiological and biochemical traits in two contrasting wheat cultivars under drought stress. Significant differences in treatment means and standard errors (SE) of treatment means were evaluated using Tukey's HSD test with the "agricolae" package in R software (Mendiburu 2021). The ggplot2 package was utilized for data visualization and plotting (Wickham 2016). Each value is presented as means ( $n = 4$ )  $\pm$  SE. Letters on error bars denote the statistical significance at  $p \leq 0.05$  between Si levels in control and drought stress within each cultivar.

## Results

### Effect of silicon on plant biomass and growth traits

No significant effect of Si was observed on plant height in both RAC875 and Kukri under control and drought stress conditions ( $p > 0.2$ ). However, compared to absolute control, Si treatment significantly increased the number of productive tillers in both the susceptible and tolerant cultivars under drought-stress Si (DSSi) treatment ( $p < 0.001$ ; Table 1). Similarly, a significant increase in fresh weight was observed with Si application ( $p < 0.001$ ) for both cultivars under control Si and DSSi treatments. Under DSSi treatment, the percentage increase in FW was 23.3% in RAC875 and 30.7% in Kukri compared to absolute drought control. However, no significant effect of Si was observed on DW for either cultivars under control or drought stress treatments (Table 1).

**Table 1** The effect of pre-sowing silicon on two contrasting wheat cultivars under control and drought stress. Each value is presented as the mean of four values with a range provided in parenthesis

| Traits   | 0 mM Si         | 4 mM Si          | ANOVA<br>Si |
|----------|-----------------|------------------|-------------|
|          | Mean            | Mean             |             |
| PHT (cm) | 53.3 (48–59.7)  | 53.7 (48–59.3)   | ns          |
| TLR      | 3.5 (2.7–4.7)   | 4.3 (3.3–5.3)    | <0.001***   |
| FW (gm)  | 16.3 (9.6–21.2) | 20.4 (14.1–26.9) | <0.01***    |
| DW (gm)  | 4.6 (3–6.6)     | 4.9 (3.5–6.8)    | ns          |

The abbreviations are PHT, plant height; TLR, productive tillers; FW, fresh weight; and DW, dry weight

$P < 0.001 = ***$ ,  $p > 0.05 = ns$  (non-significant)

### Effect of silicon on various physiological traits

The results showed that both cultivars had the same level of RWC ( $\sim 83\%$ , expressed as a net increase or decrease in RWC) under absolute control. Compared to their respective absolute controls, drought stress significantly lowered the RWC in RAC875 and Kukri by 24.1% and 31.6%, respectively. However, Si treatment showed a significant ( $p < 0.01$ ) effect on RWC, enhancing the leaf water status of both cultivars under CSi and DSSi treatments. Notably, under DSSi, a proportionally higher increase in RWC was observed in Kukri (18.1%) compared to RAC875 (10.8%), relative to their respective absolute controls (Fig. 1A).

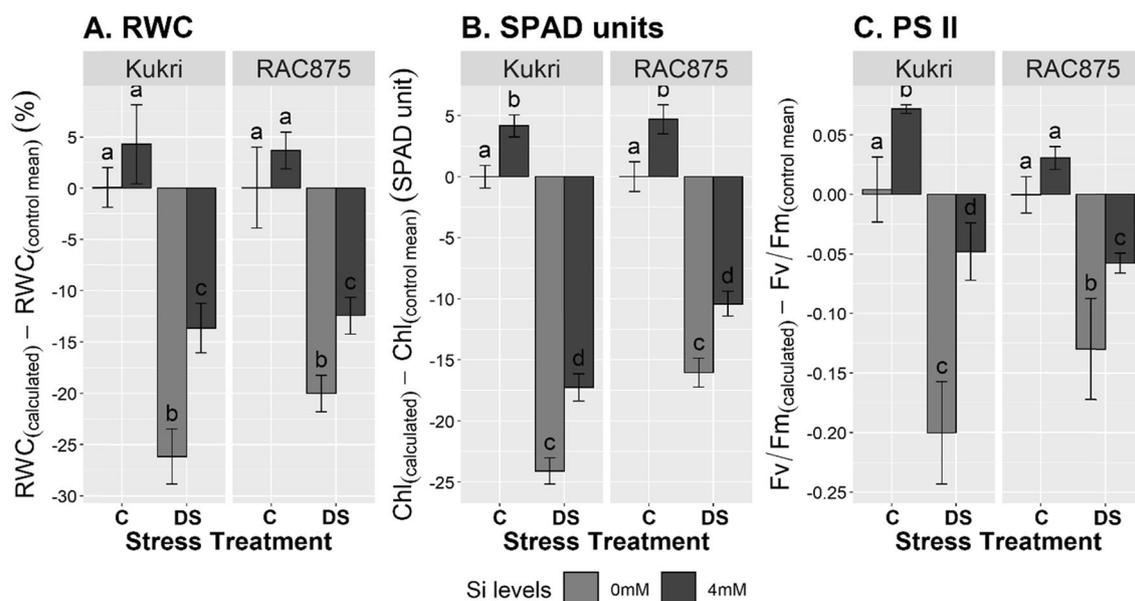
Flag leaf chlorophyll content (calculated as SPAD readings) decreased in both cultivars under drought stress, and Kukri (drought-susceptible) exhibited premature flag leaf senescence compared to RAC875 (drought-tolerant). At the end of drought stress treatment, a significant decline in flag leaf chlorophyll content was observed in both cultivars (RAC875, 21.4% & Kukri, 33.9% SPAD units). However, under DSSi, Si treatment significantly reduced the flag leaf senescence ( $p < 0.001$ ), and Kukri showed a proportionally higher stay-green phenotype (12.7%) than RAC875 (8.7%) under DSSi (Fig. 1B).

The Si treatment also showed a significant effect ( $p < 0.001$ ) on enhancing  $F_v/F_m$  in both cultivars under drought stress (Fig. 1C). In the absolute control treatment, the average  $F_v/F_m$  values for RAC875 and Kukri were 0.77 and 0.73, respectively. Following Si treatment (CSi), these values increased by 3.9% and 8.4% for RAC875 and Kukri, respectively. However, DS treatment reduced  $F_v/F_m$  by 16.8% in RAC875 and 27.8% in Kukri compared to their respective absolute controls. Silicon treatment positively influenced  $F_v/F_m$  under DSSi, resulting in a 10.1% increase in RAC875 and 22.3% in Kukri compared to their respective drought stress controls (no Si) (Fig. 1C).

### Effect of silicon on biochemical traits

#### Organic osmolytes and LPX

Under the absolute control conditions, both RAC875 and Kukri exhibited maximum TSS levels ( $99.8 \pm 3.5$  and  $92.3 \pm 3.3$  mg  $g^{-1}$  FW, respectively) and did not show significant differences upon Si treatment (CSi) ( $101 \pm 4.7$  and  $96 \pm 4.3$  mg  $g^{-1}$  FW, respectively). The imposition of drought stress significantly reduced TSS levels by 41% for RAC875 and 28.3% for Kukri compared to their respective absolute controls. However, under DSSi, the main effect of Si treatment was significant ( $p < 0.05$ ). It increased TSS levels in both cultivars (RAC875, 19.3% and Kukri, 11.7%, respectively) compared to their respective drought stress controls (Fig. 2A).



**Fig. 1** Effect of silicon application on the **A** relative water contents (RWC), **B** chlorophyll content (SPAD values), and **C** photosystem II maximum photochemical efficiency (Fv/Fm) in two contrasting wheat

cultivars, RAC875 and Kukri, in response to 14 days drought stress at stem elongation stage

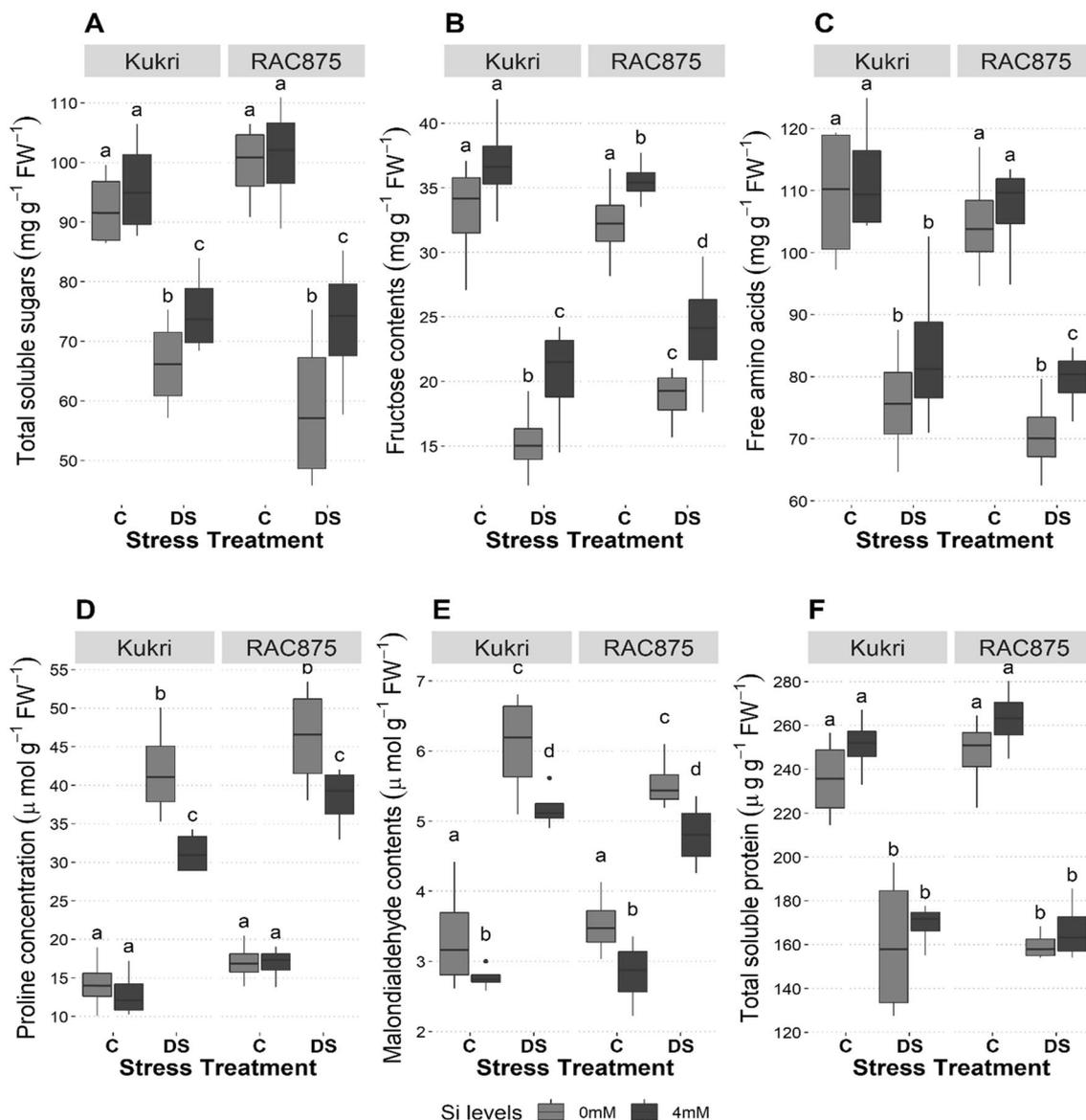
Similarly, following the imposition of drought stress, a significant decrease in FC occurred in RAC875 (41.7%) and Kukri (53.8%). In contrast, Si treatment significantly increased ( $p < 0.01$ ) FC in RAC875 and Kukri under CSi and DSSi. Under DSSi treatment, a higher FC was observed in a susceptible cultivar, Kukri (25.1%), than in a tolerant cultivar, RAC875 (21.2%) (Fig. 2B). Regarding free amino acids, the main effect of Si was non-significant ( $p = 0.11$ ). However, only RAC875 (tolerant cultivar) showed an increase in free amino acids under DSSi (12.8%) (Fig. 2C).

Significant Si effects ( $p < 0.001$ ) were observed in reducing the proline content and MDA in response to DS treatment. Drought stress significantly elevated the proline in RAC875 and Kukri, and the increase was 2.7–3.0 folds higher than their respective controls. However, Si treatment under DS led to a significant decrease in proline content in RAC875 (16.9%) and Kukri (25.3%) compared to their respective DS controls (Fig. 2D).

Silicon treatment significantly maintained cell membrane integrity by reducing the levels of MDA in RAC875 and Kukri (13.2% and 14.6%, respectively). Compared to their respective DS control, a proportionally higher reduction was observed in the susceptible cultivar, Kukri, than in the tolerant cultivar, RAC875, under DSSi (Fig. 2E). For TSP, a non-significant ( $p = 0.07$ ) effect of Si was observed in both RAC875 and Kukri under the C and DS treatments (Fig. 2F).

#### Cellular antioxidants

The Si effect on cellular antioxidant enzyme activities under DS is presented in Table 2. Activities of all antioxidant enzymes were affected by drought and Si treatments. The APX activity was significantly enhanced in RAC875 (54.6%) and Kukri (50.4%) under DS treatment compared to C. However, adding Si mitigated DS effects by further elevating the APX activity ( $p < 0.001$ ) by 11.3% in RAC875 and 9.2% in Kukri compared to the respective DS control. Similarly, the POX activity was accelerated by 18.8% in RAC875 and 14.9% in Kukri under DS compared with C. The Si under DS treatment led to a further increase ( $p < 0.01$ ) in POX activity by 19% in RAC875 and 15.8% in Kukri compared to their respective DS treatment plants. Catalase activity was also significantly enhanced by DS, with an increase of 48.5% in RAC875 and 49.7% in Kukri compared to their respective C. Silicon treatment further amplified CAT activity under both CSi and DSSi treatments. Under CSi, higher CAT activity was observed in Kukri (29.1%) compared to RAC875 (18.5%). Similarly, under DSSi, Kukri had a 25.5% increase in CAT activity than RAC875 (22.2%) compared to their respective DS controls (Table 2). Overall, Si treatment under drought stress significantly enhanced the activity of antioxidant enzymes, APX, POX, and CAT, in both drought-tolerant (RAC875) and drought-susceptible (Kukri) wheat cultivars.



**Fig. 2** Effect of silicon application on **A** total soluble sugars ( $\text{mg g}^{-1}$  FW), **B** fructose content ( $\text{mg g}^{-1}$  FW), **C** proline content ( $\mu\text{mol g}^{-1}$  FW), **D** free amino acid ( $\text{mg g}^{-1}$  FW), **E** malondialdehyde content ( $\mu\text{mol g}^{-1}$  FW) and **F** total soluble protein ( $\mu\text{g g}^{-1}$  FW) in two contrasting wheat cultivars, RAC875 and Kukri, in response to 14 days drought stress at stem elongation stage

**Table 2** Effect of Si on the activity of enzymatic antioxidants in two contrasting wheat cultivars under drought stress

| Enzymatic antioxidant | Cultivar | Treatment     |               |               |               |
|-----------------------|----------|---------------|---------------|---------------|---------------|
|                       |          | C             | CSi           | DS            | DSSi          |
| APX                   | RAC875   | 0.23 ± 0.01a  | 0.28 ± 0.01b  | 0.49 ± 0.02a  | 0.57 ± 0.02b  |
|                       | Kukri    | 0.21 ± 0.01a  | 0.26 ± 0.02b  | 0.42 ± 0.01a  | 0.49 ± 0.02b  |
| POX                   | RAC875   | 0.17 ± 0.00a  | 0.18 ± 0.00a  | 0.21 ± 1.01a  | 0.25 ± 0.00b  |
|                       | Kukri    | 0.16 ± 0.00a  | 0.17 ± 0.00a  | 0.19 ± 0.01a  | 0.22 ± 0.00b  |
| CAT                   | RAC875   | 0.036 ± 0.00a | 0.044 ± 0.00b | 0.072 ± 0.00a | 0.092 ± 0.00b |
|                       | Kukri    | 0.033 ± 0.00a | 0.047 ± 0.00b | 0.065 ± 0.00a | 0.087 ± 0.00b |

## Multivariate analysis

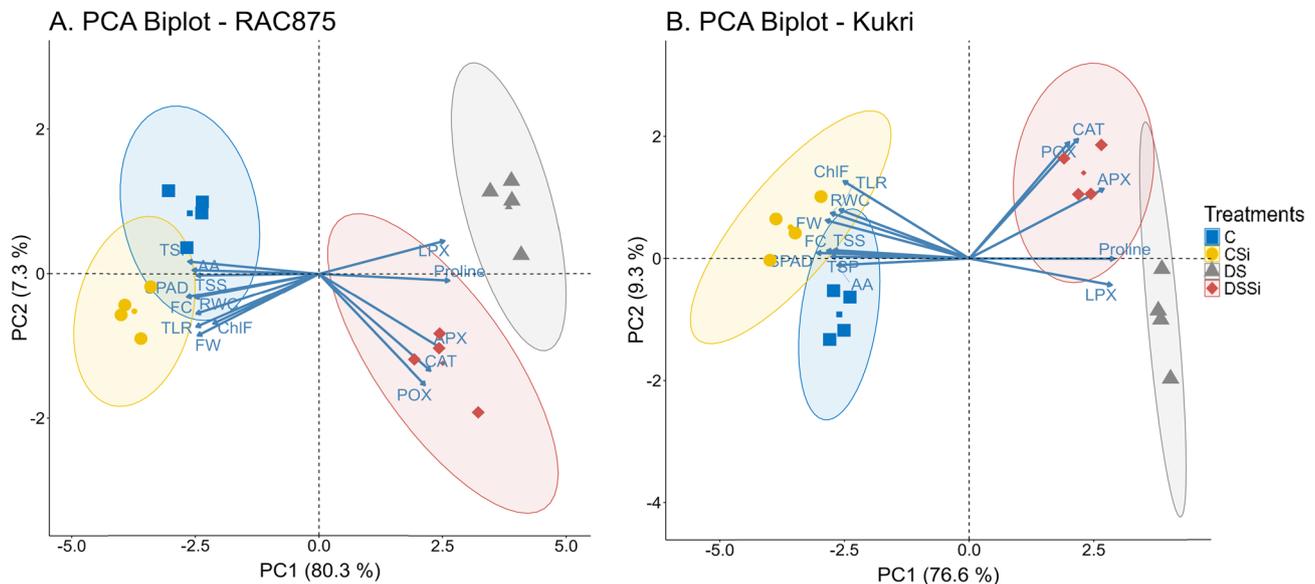
The principal component analysis (PCA) revealed important insights into the relationships between key traits and their influence on the overall variability under different treatments. The PCA explained a combined cumulative variance of 87.6% in RAC875 (PC1 = 80.3% and PC2 = 7.3), and 85.9% in Kukri (PC1 = 76.6% and 9.3%) (Fig. 3). A distinct separation of traits into four groups was observed, highlighting differential responses across treatments. The first principal component (PC1) accounted for most of the variance and was strongly correlated with eight variables, including TLR, TSS, FC, AA, FW, RWC, SPAD, and chlorophyll fluorescence (ChlF). These traits tended to increase together and were indicative of improved physiological and biochemical performance under the experimental conditions. In contrast, the PC1 exhibited a strong negative correlation with proline content and LPX concentration, inversely related to the overall variability captured by PC1. This negative association indicates that reduction in proline and LPX were aligned with increased drought tolerance and enhanced physiological and biochemical traits with Si treatment under drought stress.

The second principal component (PC2), which explained less variance compared to PC1, captured additional cultivar-specific variability (Table 3). For instance, in RAC875 and Kukri, PC2 was influenced by varying

**Table 3** Rotational PC matrix for significantly contributing top ten traits for RAC875 and Kukri

| Traits                             | RAC875 |       | Kukri |       |
|------------------------------------|--------|-------|-------|-------|
|                                    | PC-1   | PC-2  | PC-1  | PC-2  |
| TLR                                | 0.90   | 0.27  | 0.85  | 0.26  |
| TSS                                | 0.90   | 0.01  | 0.88  | 0.04  |
| FC                                 | 0.90   | 0.20  | 0.93  | 0.04  |
| Proline                            | -0.96  | 0.03  | -0.95 | 0.00  |
| AA                                 | 0.93   | -0.02 | 0.86  | 0.04  |
| FW                                 | 0.89   | 0.31  | 0.94  | 0.20  |
| RWC                                | 0.91   | 0.12  | 0.90  | 0.24  |
| SPAD                               | 0.97   | 0.12  | 0.99  | 0.03  |
| ChlF                               | 0.78   | 0.25  | 0.82  | 0.41  |
| LPX                                | -0.92  | -0.17 | -0.93 | 0.14  |
| Proportion of total variance (%)   | 80.31  | 7.33  | 76.64 | 9.30  |
| Cumulative percent of variance (%) | 80.31  | 87.64 | 76.64 | 85.90 |

levels of traits like TSS, FC, RWC, and FW, indicating cultivar-specific metabolic and growth responses under Si and drought stress treatments. This specifically underscores the differential adaptation mechanisms between the drought-tolerant (RAC875) and drought-susceptible (Kukri) wheat cultivars.



**Fig. 3** The principal component biplots showing interrelationships of traits in RAC875 and Kukri across experimental treatments. Abbreviations are SPAD (chlorophyll content), LPX (lipid peroxidation), APX (ascorbate peroxidase), CAT (catalase), and POX (peroxidase),

TSP (total soluble protein), AA (free amino acids), TSS (total soluble sugars), RWC (relative water content), ChlF (chlorophyll fluorescence), FC (fructose content), FW (fresh weight), TLR (productive tillers).

### Comparative analysis of correlation matrices

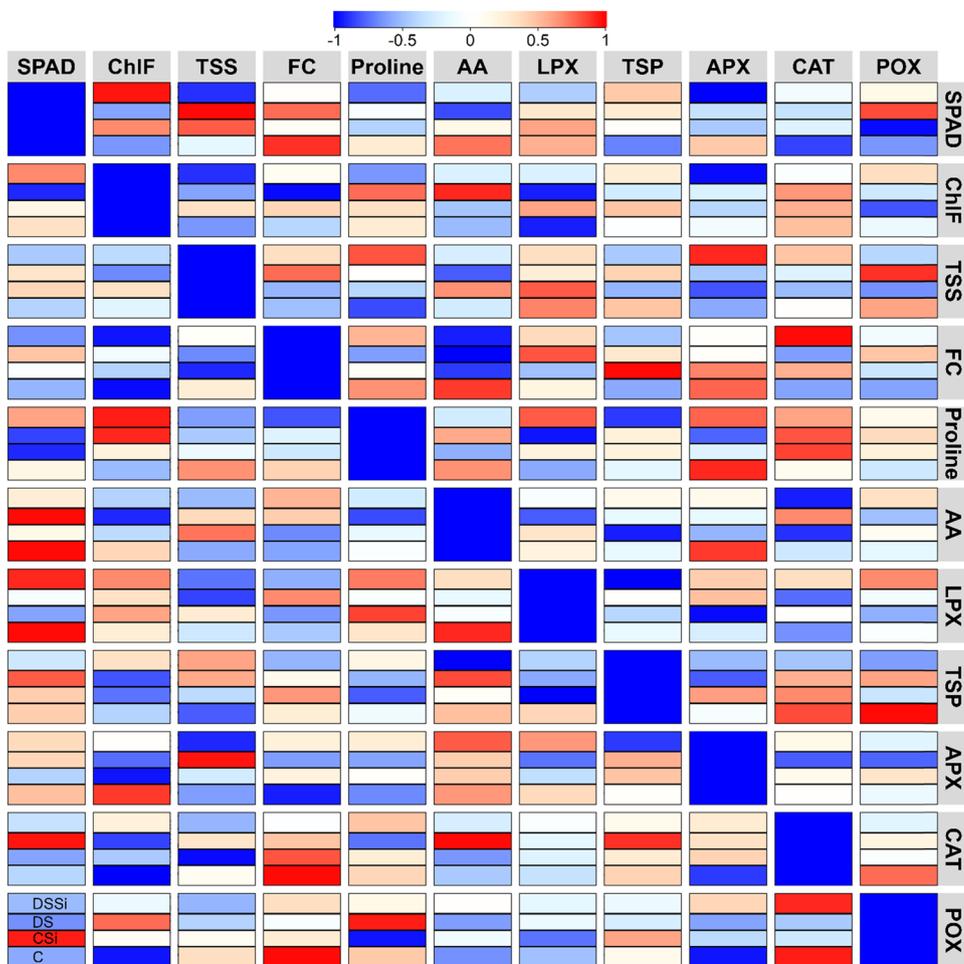
The heatmap illustrates correlation analyses between various physiological and biochemical parameters in two wheat cultivars: Kukri (top panel) and RAC875 (bottom panel) (Fig. 4). A key for each sub-box treatment in the correlation matrix is provided in the lower left corner of the bottom panel in Fig. 4.

The results indicate that Kukri exhibits stronger correlations between proline and enzymatic antioxidants under DSSi, suggesting a more pronounced Si-induced stress response than drought-tolerant RAC875. Additionally, increased Si-induced antioxidant enzyme activity is associated with LPX in both cultivars, especially under DSSi compared to DS. In addition, Chlorophyll content and chlorophyll fluorescence also show strong positive correlations under DSSi for both cultivars, highlighting the beneficial effects of Si in mitigating drought stress and maintaining photosynthetic efficiency.

### Discussion

Several studies have highlighted the beneficial impact of silicon on various growth parameters across crops, such as rice (Dorairaj et al. 2020), wheat (Ashfaq et al. 2022b; 2024), and lentils (Biju et al. 2017; 2021; 2023). In this study, a non-significant effect of Si treatment was observed on the plant height of both cultivars, which might be due to the vigorous early-stage growth of both cultivars. Generally, an increase in plant height is undesirable in wheat cultivation as taller wheat is more susceptible to lodging. These results agree with the findings by Walsh et al. (2018), who reported a non-significant effect of Si on the plant height in irrigated wheat grown under non-stressed environments. However, results showed that the productive tillers increased with Si treatment. Grain yield is closely linked with more productive tillers due to the high spike number per unit area. This observation agrees with Cuong et al. (2017), who reported that Si had a non-significant effect on plant height but significantly increased the productive tillers in field-grown rice. Current study results showed that Si substantially increased the FW

**Fig. 4** Correlation matrices illustrating interrelationships among traits in Kukri and RAC875 across different experimental treatments. The upper panel represents Kukri, and the lower panel represents RAC875. Each correlation cell contains four sub-boxes: the top sub-box represents DSSi, the second sub-box represents DS, the third sub-box represents CSi, and the fourth sub-box represents C, as indicated by the key in the lower-left corner of the figure. The parameters indicated on both axes are SPAD (chlorophyll content), ChIF (chlorophyll fluorescence), TSS (total soluble sugars), FC (fructose content), Proline, AA (amino acids), LPX (lipid peroxidation), TSP (total soluble protein), APX (ascorbate peroxidase), CAT (catalase), and POX (peroxidase)



in both cultivars under DS; similar results were reported by Liang et al. (2015) and Cuong et al. (2017). They found that exogenous Si application minimizes mineral imbalance and enhances moisture and nutrient uptake by improving the root system under abiotic stresses, which could lead to vigorous plant growth.

Among various physiological indicators of drought severity, RWC is a reliable plant trait that can quantify the plant's water status under drought. Improved plant water status maintains leaf water potential and helps in optimizing photosynthetic activity under drought stress. The current study showed that Si treatment significantly enhanced the RWC in both cultivars under drought stress (Fig. 1A). The latter might be due to (i) enhanced root growth with Si, which favors higher moisture uptake, which helps to maintain the leaf water potential under drought stress (Ashfaq et al. 2024), (ii) deposition of silica in the leaf epidermal cells to regulate the extra moisture loss through transpiration (Debona et al. 2017). In addition, elevated  $H_2O_2$  is responsible for suberin lamellae formation in roots, which forms a hydrophobic barrier for low water permeability (Razem and Bernards 2002; Shi et al. 2016). Hence, there is a possibility that Si-induced increased CAT activity eliminates  $H_2O_2$  (Table 2) and develops less suberin for higher moisture uptake under drought stress.

Chlorophyll is the primary pigment for photosynthesis in leaves, converting light energy into chemical energy. Reduction in chlorophyll content due to drought stress is mainly attributed to the deterioration of photosynthetic pigments due to the destruction of the thylakoid membrane, which is directly linked with reduced photosynthetic activity (Fang and Xiong 2015). The results of the present study revealed that Si treatment reduced the impact of drought stress on the leaf chlorophyll content in tolerant and susceptible cultivars, which might have also enhanced the photochemical efficiency in both cultivars (Fig. 1C). The higher SPAD value is an index of the leaf's photosynthetically active light transmittance characteristic, which depends on the leaf's chlorophyll density (chlorophyll content per unit of leaf area) (Songsri et al. 2009). Silicon deposition in the leaf epidermis enhances the erectness of leaves and may also assist in improving photosynthesis by maintaining or improving light interception under drought stress (Ma and Takahashi 2002).

Chlorophyll fluorescence assessment is one of the most reliable techniques for rapid and non-destructive evaluation of photosystem II maximum photochemical efficiency ( $F_v/F_m$ ) under drought (Baker 2008; Murchie and Lawson 2013). It is well known that PSII reaction centers are damaged by various abiotic stresses such as drought (Baker 2008). In a well-watered condition,  $F_v/F_m$  is consistent and directly correlates with the maximum quantum yield of photosynthesis. Drought stress results in low  $F_v/F_m$  (rise in  $F_o$ ), which indicates substantial photoinhibition and

down-regulation of PSII (Baker 2008; Murchie and Lawson 2013). The conclusion was confirmed from the current study that pronounced differentiation was observed between control and drought-stressed plants in PSII of dark-adapted plant leaves, indicating that drought treatment induces a significant decrease in the  $F_v/F_m$  of PSII electron transport. Although Si treatment enhanced  $F_v/F_m$ , the underlying mechanisms of Si enhancing PSII stability in a drought-stress environment are unclear. The possible reasons could be (i) increased accumulation of some soluble sugar compounds in the water-stressed leaves of Si-treated plants (Fig. 2A). The hypothesis agrees with the findings of Lu and Zhang (1999) that the thermostability of thylakoid improves when chloroplast is incubated with some soluble compounds, i.e., proteins and sugars. (ii) Si-induced increased quenching activities of various antioxidants under drought control the overproduction of ROS (Table 2) (Debona et al. 2017). Murata et al. (2007) and Takahashi and Murata (2008) reported that overproduction of ROS under drought inhibits the repair process of photodamaged PSII by suppressing the translation factors and thus downregulating the synthesis of D1 protein in the chloroplast. Current study results also suggested that the decline in  $F_v/F_m$  due to drought was genotype-specific, as damage to the photosynthetic apparatus of Kukri (drought-susceptible) was more severe than RAC875 (drought-tolerant). These insights have implications in agricultural research under climate change and may be used in various crop management strategies.

The first adaptive response of plants to various abiotic stresses is the excessive production of multiple osmolytes (Kim et al. 2017). A higher concentration of osmoprotectants safeguards plants from cellular dehydration. It may involve various osmotic adjustments, including the stability of cell membranes and stabilizing different enzymatic antioxidants for abiotic stress tolerance (Blum 2017). In the current study, Si increased the total soluble sugars, fructose content, and free amino acids under drought. The increase in these osmolytes with Si treatment was almost the same in susceptible and tolerant wheat cultivars under drought stress. A similar trend was found by Sonobe et al. (2010), who reported that the higher accumulation of total soluble sugars and free amino acids in sorghum roots could regulate the root osmotic potential, which ultimately helps in moisture uptake under drought stress. The results also suggest that Si-induced defense mechanisms in drought-susceptible and tolerant wheat cultivars can help farming communities sustain grain yield under changing climatic events.

Malondialdehyde is a stable metabolite of LPX, and its higher concentration has been considered an indicator of oxidative stress in plants (Shi et al. 2014). The increased LPX and proline content concentration in plants is a typical oxidative stress response (Shi et al. 2014; Biju et al. 2017). The current study confirms that Si treatment decreased

proline and LPX in both cultivars, suggesting that added Si performs a protective role that could reduce the LPX in wheat plants under drought stress to prevent oxidative damage. Previously, it was reported that decreased LPX concentration is attributed to increased antioxidant activity for scavenging excessive ROS (Shi et al. 2014). Moreover, Si-induced reduced proline content is a sign of stress relief and alleviation of various oxidative damages, i.e., reduced peroxidative damages to the cell membrane due to an added tolerance effect. However, the magnitude of reduction in proline and LPX with Si treatment was different regarding contrasting drought tolerance, and it was higher in Kukri (drought-susceptible cultivar) than RAC875 (Fig. 2D and E). These results show that Si application can also sustain productivity in susceptible wheat cultivars under drought-stress conditions.

Exposure to drought stress during the plant cycle disturbs the quenching ability of various antioxidants, leading to the overproduction of several ROS (Alzahrani et al. 2018). Plants have an efficient antioxidant system for suppressing the toxic level of ROS and maintaining homeostasis across cells through detoxifying these molecules (Kim et al. 2017). The present study showed that POX, APX, and CAT activities were triggered with Si treatment under drought stress. The latter is an elevated adaptive response of a plant to drought stress, which facilitates the scavenging of excessive ROS and contributes to drought stress tolerance (Table 2). Similar research findings that exogenous Si application significantly enhanced the activities of various antioxidants to scavenge excessive ROS under drought stress and protect cells from oxidative damage were also reported previously in lentils (Biju et al. 2017), wheat (Ashfaq et al. 2022b) and tomato (Shi et al. 2014).

Silicon-induced higher APX and CAT activities led to the  $H_2O_2$  decomposition into oxygen and water molecules under both control and drought stress. Previous studies have reported that Si treatment enhances APX and CAT activity to prevent potential  $H_2O_2$ -derived cellular damage (Kim et al. 2017; Alzahrani et al. 2018; Ashfaq et al. 2022b). Typically, APX is involved in scavenging  $^1O_2$ ,  $O_2^-$ , and  $OH^-$ , which can damage cell biomolecules (Kim et al. 2017). This study showed that POX activity was non-significant in both the cultivars under C and CSi. The latter result might be associated with increased CAT activity under both Si treatment and absolute control, leading to lower  $H_2O_2$  levels. Therefore, there was less demand for enhanced POX activity for scavenging  $H_2O_2$  (Shi et al. 2014).

The PCA results for RAC875 (Fig. 3A), and Kukri (Fig. 3B) align with the criteria set by Sneath and Sokal (1973), which recommend that PC1 and PC2 should together explain at least 70% of the total variance to avoid overfitting. The PCA results highlight the interplay of physiological and biochemical traits in mitigating drought stress in wheat. The

length of vectors on the factor map approximates the relative influence of each variable in response to experimental treatments. Antioxidants such as APX, CAT, and POX contributed strongly to mitigate drought stress through Si treatment. This suggests plant's antioxidant levels improve when treated with Si under drought stress, and these alleviated antioxidant levels helped both wheat cultivars mitigate oxidative damage and improve their stress tolerance. This finding aligns with previous studies (e.g., Biju et al. 2017; Ashfaq et al. 2022b) that highlighted Si-induced activation of the antioxidant system to mitigate oxidative damage under drought conditions.

While this study utilized a controlled glasshouse environment, the findings should be validated in the field trials to account for more realistic environmental variability. Additionally, exploring the genetic and physiological factors underlying genotype-specific responses to Si treatment would enhance our understanding of Si-mediated stress tolerance mechanisms.

## Conclusion

The present study highlights the potential of Si to enhance physiological and metabolic activities while mitigating oxidative damage during early growth stage drought stress in contrasting wheat cultivars. The Si-induced tolerance for drought stress was associated with increased antioxidant activities, a higher concentration of various osmoprotectants, and the maintenance of different physiological processes under drought stress. The findings also suggest that Si application can effectively alleviate early growth drought stress in wheat, irrespective of the cultivar's tolerance level to drought stress. Overall, the study concludes that Si inclusion as a nutritional element could be beneficial as a new management strategy to enhance growth and productivity, particularly for drought-susceptible wheat cultivars. Furthermore, Si application shows promise in improving the performance of drought-tolerant wheat cultivars under drought-stress conditions.

**Acknowledgements** The authors are grateful to The University of Melbourne for awarding the Melbourne Research Scholarship to WA for PhD research and providing funding support for this study. The authors also extend their thanks to Dr Ravneet Kaur Jhaji (Dookie Campus lab manager) for her assistance and support during the biochemical analysis.

**Authors' contributions** WA: Study conception, design, methodology, and execution of the experiment, biochemical analysis, data collection, and data analysis, prepared the first draft, reviewed, edited the draft, and approved the final version. MK: Study conception, methodology, biochemical analysis, reviewed the draft, and approved the final version. GB, SF, AP: Study conception and experiment design, reviewed the draft, and approved the final version. DG: Study conception,

supervision, experiment design, reviewed the draft, and approved the final version.

**Funding** Open Access funding enabled and organized by CAUL and its Member Institutions.

**Data availability** The data are contained within the article.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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