

# Silicon-mediated improvement of biomass yield and physio-biochemical attributes in heat-stressed spinach (*Spinacia oleracea*)

Quratulain Khosa<sup>A</sup>, Qamar uz Zaman<sup>A,\*</sup> , Tingting An<sup>B</sup>, Kamran Ashraf<sup>C</sup>, Asim Abbasi<sup>D</sup> , Saba Nazir<sup>A</sup>, Riffat Naz<sup>A</sup> and Yinglong Chen<sup>E,\*</sup> 

For full list of author affiliations and declarations see end of paper

**\*Correspondence to:**

Yinglong Chen  
The UWA Institute of Agriculture, and  
School of Agriculture and Environment, The  
University of Western Australia, Perth,  
WA 6009, Australia  
Email: [yinglong.chen@uwa.edu.au](mailto:yinglong.chen@uwa.edu.au);  
Qamar uz Zaman  
Department of Environmental Sciences, The  
University of Lahore, Lahore 54590, Pakistan  
Email: [qamar.zaman1@envs.uol.edu.pk](mailto:qamar.zaman1@envs.uol.edu.pk)

**Handling Editor:**

Enrico Francia

**Received:** 3 June 2022  
**Accepted:** 1 August 2022  
**Published:** 6 September 2022

**Cite this:**

Khosa Q *et al.* (2023)  
*Crop & Pasture Science*, **74**(3), 230–243.  
doi:[10.1071/CP22192](https://doi.org/10.1071/CP22192)

© 2023 The Author(s) (or their  
employer(s)). Published by  
CSIRO Publishing.  
This is an open access article distributed  
under the Creative Commons Attribution-  
NonCommercial-NoDerivatives 4.0  
International License (CC BY-NC-ND).

OPEN ACCESS

## ABSTRACT

**Context.** Defensive action of plants against biotic and abiotic stresses has been augmented by silicon (Si). Spinach (*Spinacia oleracea* L.) is a nutritious leafy vegetable that is a cold-tolerant but heat-sensitive crop. **Aims and methods.** The ability of exogenous application of Si (0, 2, 4 and 6 mmol L<sup>-1</sup> in the form of K<sub>2</sub>SiO<sub>3</sub>) to alleviate heat stress in spinach cv. Desi Palak was investigated. After an acclimatisation period, plants were grown with or without heat stress for 15 days, followed by Si treatment for 25 days. Plant growth and physiology were assessed at 65 days after sowing. **Key results.** Heat stress significantly inhibited plant growth, water status and photosynthesis, soluble sugar and protein contents, and osmolyte status in spinach leaves, but increased electrolyte leakage, activities of antioxidant enzymes, and proline content. Application of Si alleviated heat stress by enhancing water status, photosynthetic pigments, soluble proteins and essential minerals, and by reducing damage of the plasma membrane. The accumulation of osmolytes counterbalance the osmotic stress imposed by heat. **Conclusions.** Silicon plays a vital role in alleviating effects of heat stress by improving water status and photosynthetic rate, and accruing osmoprotectants other than proline. **Implications.** Exogenous application of Si is an efficient strategy to boost tolerance of spinach plants to heat stress, having significant impact on growth and productivity of spinach at higher temperatures.

**Keywords:** antioxidants, heat stress, photosynthetic pigments, physio-biochemical, plasma membrane, silicon, spinach, water relations.

## Introduction

Food security is essential to a growing global population, but it is jeopardised by climate change and global warming. Various studies have shown that crop production is endangered by increases in temperature at the regional level (Abdelrahman *et al.* 2017). By the end of the 21st Century, the average temperature will have been raised by 1.8–4.0°C (Bita and Gerats 2013; Alizadeh *et al.* 2022).

Heat stress significantly affects plant activities including seed germination, development, photosynthesis and reproduction, resulting in serious impacts on plant growth and, ultimately, yield of useful products (Hasanuzzaman *et al.* 2011). In order to survive under stressed conditions, plants have various morphological, physiological and molecular responses (Lobanov *et al.* 2008; Janská *et al.* 2010; Govindaraj *et al.* 2018). Specific field management options (selection of cultivar, date of sowing, method of sowing and irrigation scheduling) can be practiced to improve production under stressed conditions. However, heat stress severely limits the productivity of crop plants; for example, it limits wheat global productivity by >6% for each degree increase in temperature (Asseng *et al.* 2015). Despite the negative effects on overall crop production, increasing temperature has some beneficial effects on productivity in colder regions (Challinor *et al.* 2014; Zandalinas *et al.* 2021).

Spinach (*Spinacia oleracea* L.), a green nutritious leafy vegetable, is a cold-tolerant, heat-sensitive crop (Zhao *et al.* 2018; Giordano *et al.* 2021). Heat stress significantly affects its growth and development, causing a considerable decline in quality and yield. Studies have been done on the heat-shock response of spinach, both with whole plants and with detached leaf tissue (Moradpour *et al.* 2021). After being exposed to heat shock (35–50°C) for 30 min, CO<sub>2</sub> assimilation decreases and pigment proteins in thylakoid membranes aggregate, slowing the plant's ability to photosynthesise (Tang *et al.* 2007; Shabbir *et al.* 2022). The first heat-shock proteins in spinach leaf tissue are induced when the temperature reaches 28°C, and a full range of heat-shock proteins are produced at 36°C. If a spinach genotype had a high germination percentage at high temperature (e.g. 35°C), it could be tolerant to heat at the germination stage (Chitwood *et al.* 2016; Zaman *et al.* 2022).

Cultural practices such as irrigation, crop residues and mulching in combination with genetic modifications of crop plants are considered important tools for managing different abiotic stresses (Wahid 2007; Zaman *et al.* 2022). Exogenous application of mineral nutrients helps plants to combat heat stress by increasing their heat-tolerance capacity (Sarwar *et al.* 2019). In general, silicon (Si) supplementation to soil improves seed germination, root and shoot development, photosynthesis, nutrient uptake, nitrogen fixation, secondary metabolism and different yield attributes of crops (Mostofa *et al.* 2021). Furthermore, plants supplemented with Si have shown enhanced tolerance to various abiotic stresses including temperature extremes, salinity, drought, radiation and heavy metal toxicity (Khan *et al.* 2020b; Younis *et al.* 2020). Silicon applications to tomato, cucumber, rice, strawberry, date palm, barley, poinsettia and salvia effectively reduced the deleterious effects of heat stress by improving various growth-related attributes (Muneer *et al.* 2017; Khan *et al.* 2020a). In terms of plant growth, Si an essential nutrient involved in various key functions (i.e. membrane stability, cell wall stabilisation, enhancement of key enzyme activities and phytohormones interaction) under abiotic stress (Naz *et al.* 2022; Shabbir *et al.* 2022).

Use of mineral fertilisation is considered a novel approach to mitigate deleterious effects of heat stress in spinach. Moreover, balanced Si fertilisation is essential for normal physiological functioning and maintaining structural stability of plants (Waraich *et al.* 2012; Shabbir *et al.* 2022; Zaman *et al.* 2022). Therefore, understanding the response of spinach to heat stress when receiving nutrient amendments will be of value to spinach growers. In this regard, the present study was designed to assess the efficiency of exogenously applied Si at varying levels on plant growth, biomass, physio-biochemical parameters, antioxidant activity and osmolyte status under heat stress in spinach plants.

## Materials and methods

### Experimental design and treatments

A pot experiment with spinach plants was conducted in a naturally lit glasshouse at the Department of Environmental Sciences, The University of Lahore, Pakistan. A completely randomised design was used consisting of two factors with three replications per treatment: heat stress application (normal growth conditions, or heat stress); and Si application (0, 2, 4 and 6 mmol L<sup>-1</sup>). Heat stress was applied at 15 days after sowing (DAS) by placing the pots in a transparent, polyethylene-sheet tunnel, and Si was applied in the form of K<sub>2</sub>SiO<sub>3</sub>.

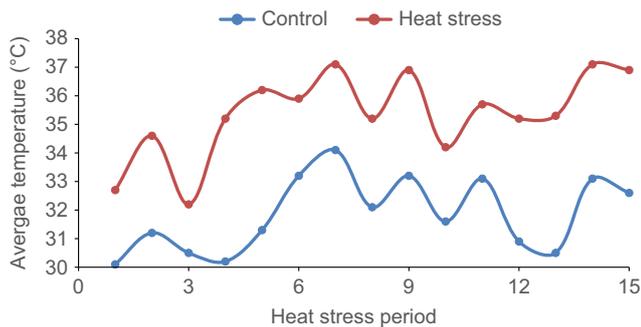
### Experiment establishment and management

Homogenous seeds of spinach cv. Desi Palak were sterilised with 0.1% (w/v) sodium dodecyl solution and then washed with deionised water. Ten seeds were sown into each plastic pot (22.5 cm top diameter, 16.5 cm base diameter, 18 cm depth) filled with 7 kg sterilised soil. At 10 DAS, five healthy plants were maintained per pot. All pots were kept in open space under normal environmental conditions until application of the stress treatments. Tap water was used as source of irrigation to pot soil capacity on daily basis. A 50% Hoagland solution of moderate strength as a source of essential nutrients was applied weekly at a rate of 1 L per pot. The optimum temperature range for spinach growth is considered to be 25–30°C (Atherton and Farooque 1983) and its growth is suppressed at 35°C (Leskovar *et al.* 1999; Katzman *et al.* 2001).

After an acclimatisation period of 15 days, heat stress was imposed. A plastic tunnel made of black-tinted transparent polyethylene sheet was constructed over the pots by using bamboo sticks. Tiny holes were made in the sheeting to minimise the humidity. The pots in control treatment were placed under normal conditions (Shahid *et al.* 2017). Temperature and humidity were recorded with a digital temperature and humidity probe (Digital Multimeter 50302; Novanna Measurement Systems, Bury St Edmunds, UK). During heat stress, the temperature of control and heat-stressed pots was recorded twice a day and averaged (Both *et al.* 2015). A considerable increase in temperature was observed in heat-stressed pots compared with control pots (Fig. 1). After 15 days of heat stress, foliar application of Si (0, 2, 4, 6 mmol L<sup>-1</sup>) in the form of potassium silicate (K<sub>2</sub>SiO<sub>3</sub>) salt in distilled water was applied, using 500 mL of the solution in each of two sprays at a 10-day interval (Naz *et al.* 2022). After 25 days of exposure to Si (65 DAS), data for morphological, biochemical and physiological attributes were recorded.

### Growth attributes

Following harvest at 65 DAS, plants were separated into leaves and roots for measurement of growth parameters.



**Fig. 1.** Mean daily temperature of normal and heat-stress treatments for 15 days.

Number of leaves was counted, and plant height, leaf length and width were measured (in cm) using a ruler. Leaf area ( $\text{cm}^2$ ) was measured using the LI-3100 Area Meter (LI-COR Biosciences, Lincoln, NE, USA). Plants were then washed with distilled water to remove adhered soil particles and air-dried. Roots and leaves were oven-dried at  $70^\circ\text{C}$  for 48 h for estimation of dry weights of root and leaves separately.

### Gas exchange attributes

At 65 DAS, photosynthetic rate ( $A$ ), stomatal conductance ( $g_s$ ) and transpiration rate ( $E$ ) were measured on fully expanded uppermost leaves by using a portable infrared gas analyser (ADC Bioscientific, Hoddesdon, UK) at light saturation intensity between 09:00 and 12:00 on a sunny day, as described by Emanuil *et al.* (2020).

### Biochemical attributes

#### Electrolyte leakage

Small pieces of leaves (at 65 DAS) were dipped in deionised water and electrolyte leakage (EL) level was measured. The first reading of EL was taken after incubation of the sample at  $32^\circ\text{C}$  for 2 h, and the second reading after incubation at  $121^\circ\text{C}$  for 20 min (Dionisio-Sese and Tobita 1998). The following formula was used to calculate the EL level of samples:

$$\text{EL} = (\text{EC}_1 / \text{EC}_2) \times 100$$

#### Chlorophyll content

A crushed sample of plant leaf ( $\sim 5$  g) was added to a test tube containing 85% acetone ( $v/v$ ) and placed under dark conditions for 24 h for pigment extraction. The sample was then centrifuged for 10 min at  $4000g$  and  $4^\circ\text{C}$ . A spectrophotometer (Halo DB-20/DB-20S; Dynamica Scientific, Newport Pagnell, UK) at wavelengths of 470, 647 and  $664.5$  nm was used to measure the amount of chlorophyll in the supernatant, following the methods described by Lichtenthaler (1987).

### Enzymatic antioxidants

Fresh spinach leaves (1.0 g) were extracted in 50 mM phosphate buffer ( $\text{pH} \sim 7.8$ ) and the homogenate was centrifuged at  $15000g$  for 10 min; the supernatant was used for assaying enzyme activity. The activity of peroxidase (POD) was measured according to the method described by Velikova *et al.* (2000), catalase (CAT) activity following the method presented by Aebi (1974), and superoxide dismutase (SOD) activity following the procedure presented by Beauchamp and Fridovich (1971).

### Water-related attributes

The method of Turner and Kramer (1980) was used for determination of relative water content (RWC), and the following formula was used for the calculation:

$$\text{RWC} = ((\text{FW} - \text{DW}) / (\text{TW} - \text{DW})) \times 100$$

where FW is fresh weight, TW is turgid weight, and DW is dry weight.

The water potential ( $\Psi_w$ ) obtained by fresh leaves was measured by the use of a Pressure Bomb (Soilmoisture Equipment, Santa Barbara, CA, USA). Leaf samples, which were already used for RWC, were frozen, thawed, squeezed and centrifuged ( $5000g$ ). An osmometer (Digital Osmometer; Wescor, Logan, UT, USA) was used to obtain osmotic potential.

### Determination of osmolytes

Fresh leaf (0.5 g) was taken and ground in buffer ( $\text{pH} 7.2$ ); a protease inhibitor cocktail in  $1 \mu\text{M}$  phosphate buffered saline was added to make a homogenous mixture. The saline buffer comprised deionised water,  $1.37$  mM NaCl,  $2$  mM  $\text{KH}_2\text{PO}_4$ ,  $2.7$  mM KCl and  $10$  mM  $\text{Na}_2\text{HPO}_4$ , with the pH adjusted by adding HCl. The solution was autoclaved and then centrifuged ( $12000g$ ) for  $\sim 5$  min to separate the supernatant. Total soluble proteins were detected via Bradford assay (Bradford 1976), and samples were stored. Various dilutions of bovine serum albumin ( $10, 20, 30, 40, 50, 60, 70, 80, 90$  and  $100 \mu\text{g} \mu\text{L}^{-1}$ ) were taken to construct standard curves. Deionised water ( $400 \mu\text{L}$ ) with dye stock was added and the prepared sample tubes were incubated for 30 min at room temperature and then vortexed. A UV 4000 UV-Vis spectrophotometer (Dynamica Company, London, UK) was used to read the absorbance level of the sample.

The method of Maehly and Chance (1954) was followed for proline determination. Sulfosalicylic acid (3%  $w/v$ ) was mixed with 0.5-g samples of fresh leaf and then filtered; the filtered samples were placed in test tubes and treated with glacial acetic acid and ninhydrin (2.5%). Samples were heated in test tubes in a water bath ( $100^\circ\text{C}$ ) for 60 min, after which toluene was added to the test tubes for the separation of chromophores. A UV-Vis spectrophotometer was used to record the optical density ( $520$  nm). The

method defined by Giannakoula *et al.* (2008) use to detect soluble sugar content.

### Statistical analyses

Data were analysed using Fisher's analysis of variance (ANOVA) for significance testing. Tukey's honestly significant difference test at  $P = 0.05$  was applied for comparison of means where the ANOVA indicated significant differences. Regression and correlation analyses were computed by using the Minitab-19 statistical software. All statistical computations were performed with Statistix software ver. 10 (Analytical Software, Tallahassee, FL, USA).

## Results

### Growth and biomass attributes

Heat stress, foliar-applied Si and their interaction significantly ( $P \leq 0.01$ ) affected growth and biomass attributes of spinach plants. Heat stress decreased plant height (20.4%), number of leaves (22.8%), leaf length (22.1%), leaf width (13.2%), leaf area (29.8%), root dry weight (11.1%) and leaf dry weight (25.92%) compared with the unstressed control (Fig. 2). Under normal and heat-stressed conditions, respectively, maximum plant height (27.1 and 21.4 cm), number of leaves (8.67 and 7.33), leaf length (16.8 and 13.5 cm), leaf width (3.6 and 3.1 cm), leaf area (55.5 and 42.5 cm<sup>2</sup>), root fresh weight (3.72 and 3.36 g), root dry weight (0.39 and 0.35 g), leaf fresh weight (11.93 and 10.46 g) and leaf dry weight (1.15 and 0.96 g) were observed with foliar application of 4 mmol L<sup>-1</sup> of Si solution (Fig. 2).

### Photosynthetic attributes

Heat stress, Si application and their interaction significantly ( $P \leq 0.01$ ) affected photosynthetic attributes of spinach plants. Decreases in transpiration rate (9.45%), photosynthetic rate (24.4%) and stomatal conductance (10.5%) were observed with heat treatment relative to the unstressed control. However, maximum increases in transpiration rate (58.7%), photosynthetic rate (158%) and stomatal conductance (64.1%) were observed with foliar application of 4 mmol L<sup>-1</sup> of Si solution compared with the nil-Si control under heat-stress conditions (Fig. 3).

### Enzymatic antioxidants and physio-biochemical attributes

Heat stress, foliar application of Si and their interaction showed significant ( $P \leq 0.01$ ) impact on biochemical (Fig. 4) and enzymatic (Fig. 5) attributes of spinach. Under heat stress, decreases in chlorophyll contents (21.1%) and carotenoid contents (22.1%), and increases in SOD (11.2%), POD (12.08%), CAT (18.8%) and electrolyte leakage (49.9%)

were observed compared with the unstressed control. Maximum improvements in chlorophyll and carotenoid contents and decreases in SOD (56.67%), POD (45.7%), CAT (58.4%) and electrolyte leakage (33.5%) were observed with foliar application of 4 mmol L<sup>-1</sup> of Si compared with the nil-Si control under heat-stress conditions (Figs 4 and 5).

### Water-related attributes

Foliar-applied Si, heat stress and their interaction showed significant ( $P \leq 0.01$ ) impact on water-related attributes of spinach. Under heat stress, decreases in RWC (11.81%), water potential (25.66%) and osmotic potential (21.67%) were observed compared with the unstressed control. Under normal and heat-stressed conditions, respectively, maximum RWC (91.67% and 77.22%), water potential (-1.86 and -2.25 MPa), and osmotic potential (-2.24 and -2.63 MPa) were observed with foliar application of 4 mmol L<sup>-1</sup> of Si (Fig. 6).

### Osmolyte attributes

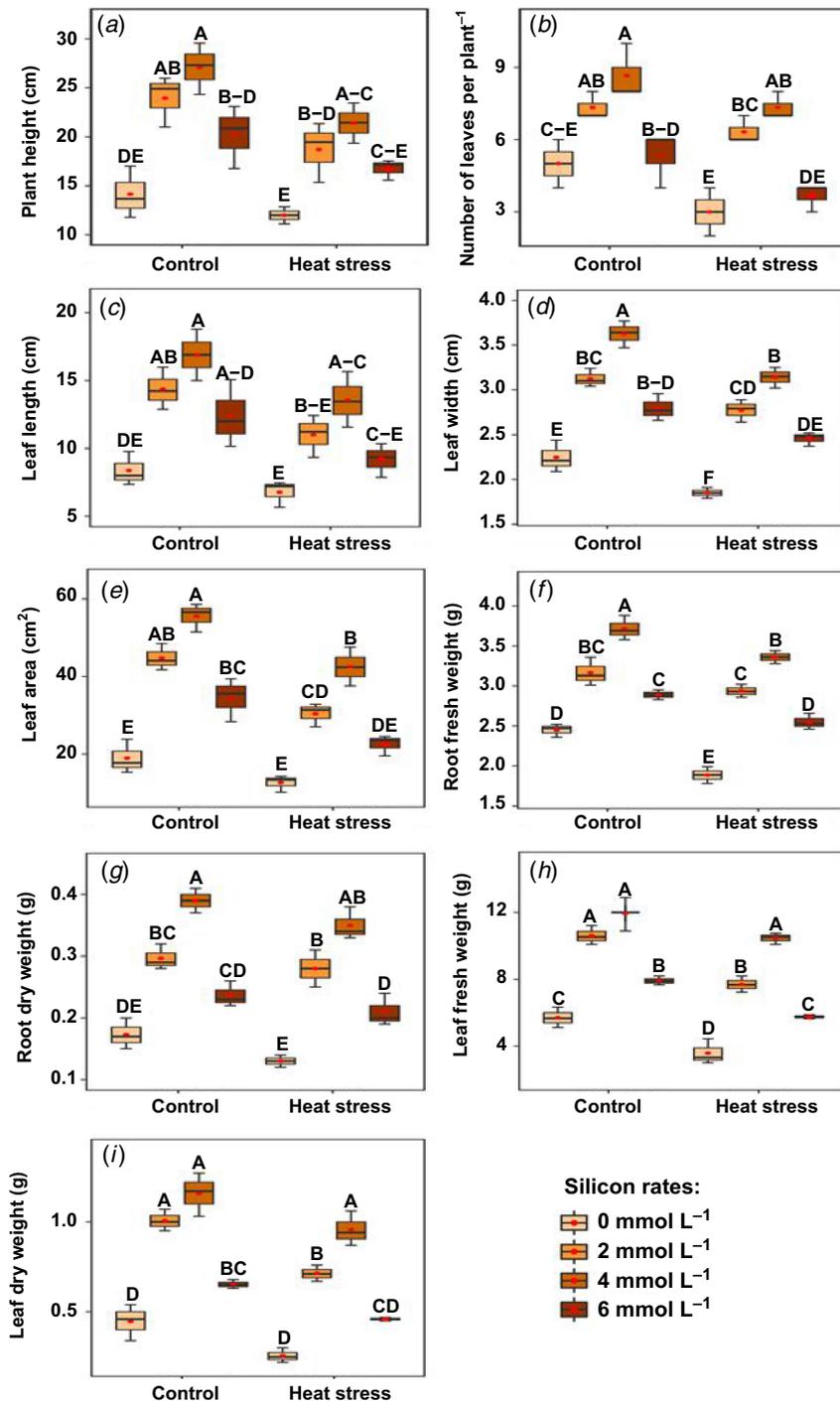
Heat stress and foliar-applied Si significantly ( $P \leq 0.01$ ) affected soluble sugar, soluble protein and proline contents of spinach plants. Heat stress significantly decreased the soluble sugar (20.92%) and soluble protein (17.71%) contents, and increased proline content (16.52%). Maximum soluble sugar and protein contents of 17.46 and 35.84 mg g<sup>-1</sup> FW under control conditions, and 14.68 and 31.05 mg g<sup>-1</sup> FW under heat-stressed conditions, were observed with foliar application of 4 mmol L<sup>-1</sup> of Si (Fig. 7).

### Correlation matrix

Growth, enzymatic, water-related and biochemical attributes were subjected to correlation analysis (Table 1). All of the enzymatic activities (SOD, POD and CAT) were negatively correlated with chlorophyll content, leaf dry weight, root dry weight and RWC. Significant positive correlations of enzymatic activities were noted with electrolyte leakage, proline content, osmotic potential and water potential, and among those four parameters. Electrolyte leakage correlated negatively with leaf dry weight, root dry weight and RWC. Chlorophyll content was positively correlated with leaf dry weight, root dry weight and RWC.

### Regression analysis

Growth, physiological, water-related and biochemical attributes were also tested using regression analysis (Fig. 8a-f). The  $R^2$  values of regressions were: leaf dry weight and RWC, 73.36% (Fig. 8a); electrolyte leakage and osmotic potential, 79% (Fig. 8b); chlorophyll content and leaf dry weight association, 94.84% (Fig. 8c); stomatal conductance and water potential, 72.35% (Fig. 8d); soluble



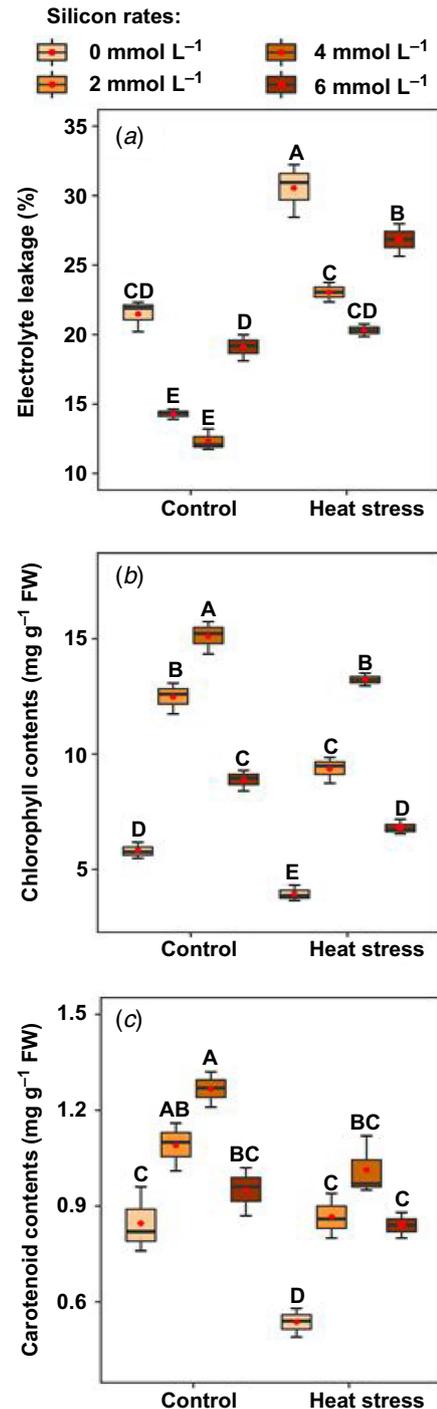
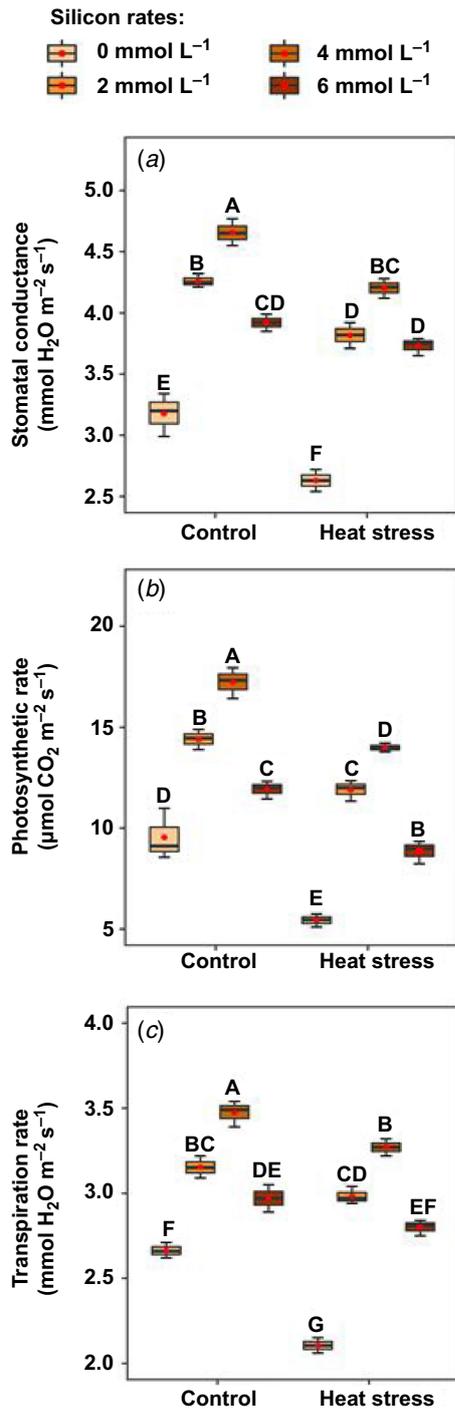
**Fig. 2.** Growth attributes of spinach cv. Desi Palak under normal and heat-stressed conditions in response to various rates of foliar-applied Si (0, 2, 4 and 6 mmol L<sup>-1</sup>): (a) plant height, (b) number of leaves per plant, (c) leaf length, (d) leaf width, (e) leaf area, (f) root fresh weight, (g) root dry weight, (h) leaf fresh weight, and (i) leaf dry weight. The line and red dot inside the box are the median and mean, respectively. For each parameter, means with the same letter are not significantly different across treatments. Capped lines denote standard deviation of three replicates.

sugar and proline contents, only 45.90% (Fig. 8e); and photosynthetic rate and leaf area, 88.38% (Fig. 8f).

### Discussion

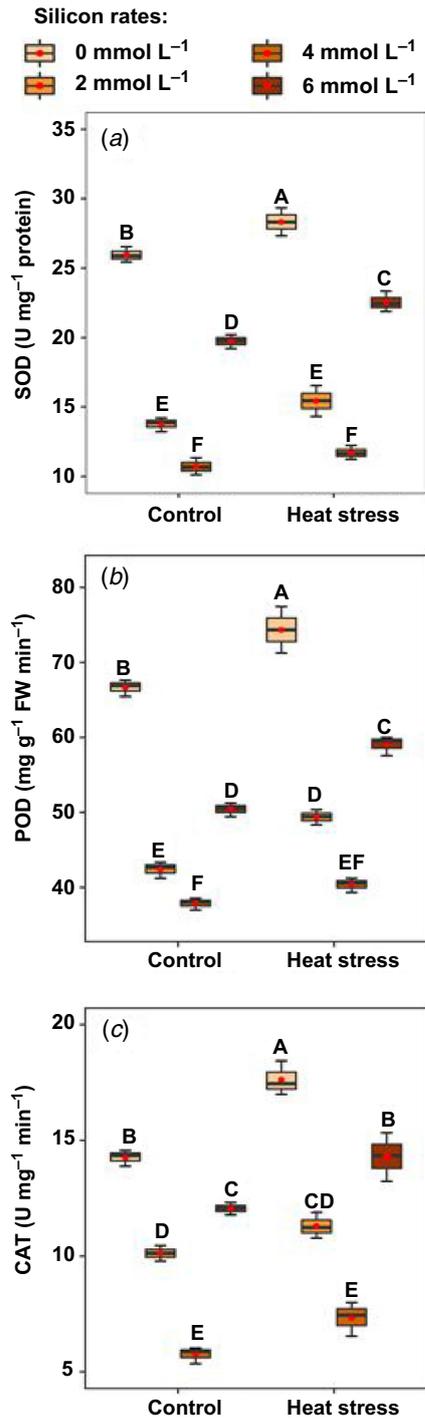
Under stressful conditions such as drought, salt and high temperature, plants may alter their morphological,

molecular, physiological and biochemical processes as adaptation strategies (Wahid 2007; Chaudhry and Sidhu 2022). The present study showed that heat stress significantly decreases the growth and biomass of spinach plants. High temperature causes loss of cell water content, ultimately reducing cell size and growth (Ali et al. 2021; Hassan et al. 2021; Okereke et al. 2021). Under extreme heat stress, plants can show programmed cell death in specific cells or

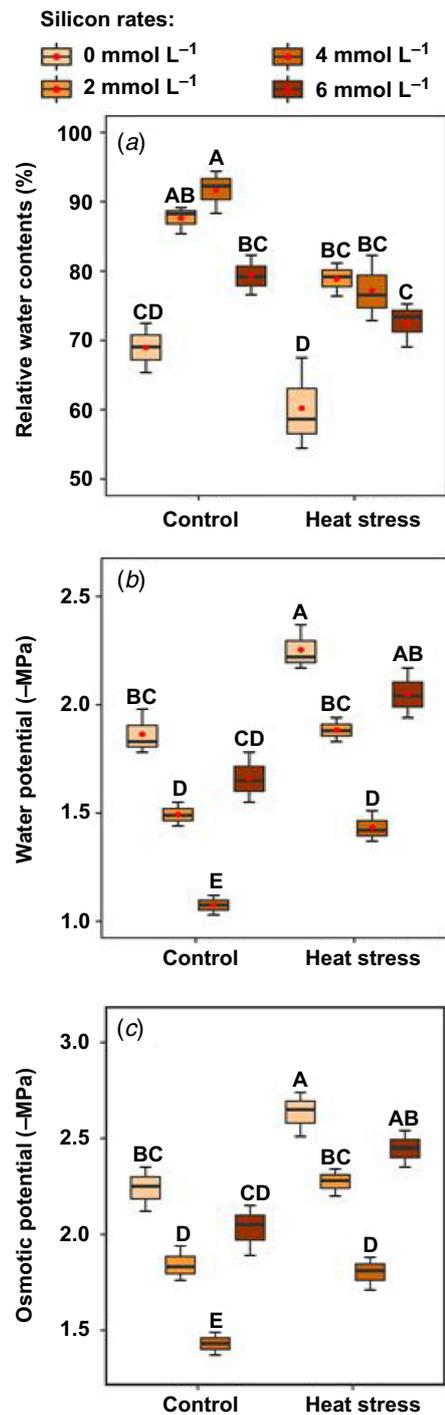


**Fig. 3.** Physiological attributes of spinach cv. Desi Palak under normal and heat-stressed conditions in response to various rates of foliar-applied Si (0, 2, 4 and 6 mmol L<sup>-1</sup>): (a) stomatal conductance, (b) photosynthetic rate, and (c) transpiration rate. The line and red dot inside the box are the median and mean, respectively. For each parameter, means with the same letter are not significantly different across treatments. Capped lines denote standard deviation of three replicates.

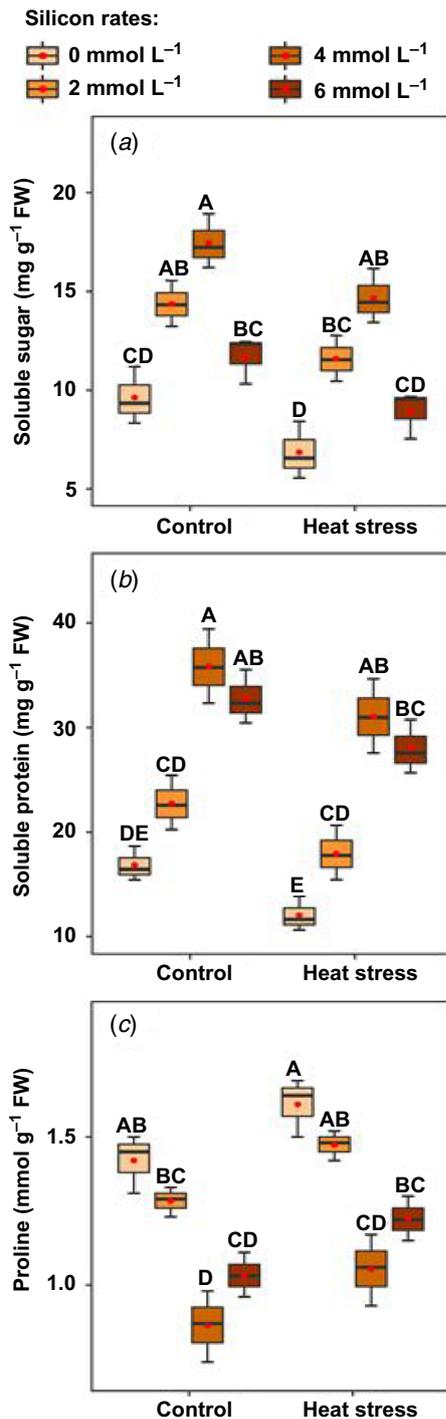
**Fig. 4.** Biochemical attributes of spinach cv. Desi Palak under normal and heat-stressed conditions in response to various rates of foliar-applied Si (0, 2, 4 and 6 mmol L<sup>-1</sup>): (a) electrolyte leakage, (b) chlorophyll content, and (c) carotenoid contents. The line and red dot inside the box are the median and mean, respectively. For each parameter, means with the same letter are not significantly different across treatments. Capped lines denote standard deviation of three replicates.



**Fig. 5.** Enzymatic attributes of spinach cv. Desi Palak under normal and heat-stressed conditions in response to various rates of foliar-applied Si (0, 2, 4 and 6 mmol L<sup>-1</sup>): (a) SOD, (b) POD, and (c) CAT. The line and red dot inside the box are the median and mean, respectively. For each parameter, means with the same letter are not significantly different across replicates. Capped lines denote standard deviation of three replicates.



**Fig. 6.** Water related attributes of spinach cv. Desi Palak under normal and heat-stressed conditions in response to various rates of foliar-applied Si (0, 2, 4 and 6 mmol L<sup>-1</sup>): (a) relative water contents; (b) water potential and (c) osmotic potential. The line and red dot inside the box are the median and mean, respectively. For each parameter, means with the same letter are not significantly different across treatments. Capped lines denote standard deviation of three replicates.



**Fig. 7.** Osmolytes status of spinach cv. Desi Palak under normal and heat-stressed conditions in response to various rates of foliar-applied Si (0, 2, 4 and 6 mmol L<sup>-1</sup>): (a) soluble sugar, (b) soluble protein, and (c) proline contents. The line and red dot inside the box are the median and mean, respectively. For each parameter, means with the same letter are not significantly different across treatments. Capped lines denote standard deviation of three replicates.

tissues; on the other hand, moderate heat stress for extended periods causes gradual death. Both types of injury or death

can lead to the shedding or shortening of leaves, reduction in fresh and dry biomass, or even death of the entire plant (Abdelrahman *et al.* 2017; Das *et al.* 2017; Fahad *et al.* 2017; Hussain *et al.* 2019). The results presented here concur with those of previous reports by Mahdiah *et al.* (2015), Abbas *et al.* (2017), and An *et al.* (2022), where the application of exogenous Si alleviated the negative effects of abiotic stress and restored plant growth. In the present investigation, we found improved leaf area, better growth, and greater fresh and dry weights in response to application of Si (Fig. 9). This suggests the involvement of Si in cell division and expansion, which might lead to internodal elongation and influence the height of the plant (Soundararajan *et al.* 2014; Hussain *et al.* 2019; Younis *et al.* 2020). The literature reveals that Si is considered a plant-growth-regulator-like compound that is necessary for cell division and expansion (Hwang *et al.* 2007; Khan *et al.* 2020a; An *et al.* 2022), and has a defensive role against a wide range of environmental stresses (Tripathi *et al.* 2013; Shalaby *et al.* 2021).

In all green plants, the most fundamental physiological process is photosynthesis, and all of its components are sensitive to stress conditions; any type of stress at any stage of life affects overall photosynthetic efficiency of green plants (Alabdallah *et al.* 2021). The present findings reveal that heat stress significantly decreases the photosynthetic attributes of spinach plants compared with control temperatures, which might be due to the decreased stomatal conductivity and transpiration rate (Mahdavi *et al.* 2021). In tomato, high stomatal conductivity and transpiration rate under heat stress improve leaf cooling, providing better protection for chlorophyll and maintaining relatively high photosynthetic rate (Haque *et al.* 2021). Under abiotic stress environments, photosynthetic pigment degradation occurs; for example, under salt stress conditions, chlorophyll pigments break down (Iqbal *et al.* 2021). The improvement of spinach physiological attributes might be the result of increased total chlorophyll content with optimum Si application under heat-stressed conditions (Fig. 9). Breakage in the walls of chloroplasts could be delayed with Si application, by which photosynthetic and transpiration rate and efficiency can be improved (Xie *et al.* 2014; Das *et al.* 2021). Effects of foliar-applied Si in improving photosynthetic potential and efficiency by opening angle of leaves, keeping the leaf erect, and decreasing self-shading have been reported in rice, barely, wheat and sugarcane (Soratto *et al.* 2012; Othmani *et al.* 2021).

Chlorophyll is very sensitive to heat stress (Murkowski 2001; Song *et al.* 2018). High-temperature-induced decline in chlorophyll and carotenoid contents has been reported in field and vegetable crops (Jeon *et al.* 2006). The same trend was found in spinach plants grown under a high-temperature regime in our study. Leakage of electrolytes, which are indicators of stress sensitivity, was higher in heat-treated spinach plants than the control. The increase

**Table 1.** Correlation matrix of growth, water-related, enzymatic and biochemical attributes of spinach in response to various rates of foliar applied silicon (0, 2, 4 and 6 mmol L<sup>-1</sup>) under normal and heat-stressed conditions.

Variables	CAT	CC	EL	LDW	POD	PRO	RDW	RWC	SOD	OP
CC	-0.96**									
EL	0.81**	-0.83**								
LDW	-0.93**	0.96**	-0.85**							
POD	0.93**	-0.96**	0.80**	-0.92**						
PRO	0.73**	-0.71**	0.65**	-0.65**	0.72**					
RDW	-0.92**	0.92**	-0.79**	0.87**	-0.91**	-0.69**				
RWC	-0.78**	0.85**	-0.84**	0.84**	-0.86**	-0.67**	0.84**			
SOD	0.94**	-0.95**	0.75**	-0.92**	0.97**	0.64**	-0.92**	-0.83**		
OP	0.91**	-0.91**	0.89**	-0.88**	0.85**	0.82**	-0.88**	-0.82**	0.83**	
WP	0.91**	-0.91**	0.89**	-0.89**	0.86**	0.83**	-0.88**	-0.82**	0.83**	0.99**

\*\* $P \leq 0.01$ .

CAT, catalase activity; CC, chlorophyll content; EL, electrolyte leakage; LDW, leaf dry weight; PRO, proline content; RDW, root dry weight; RWC, relative water content; SOD, superoxide dismutase activity; OP, osmotic potential; WP, water potential.

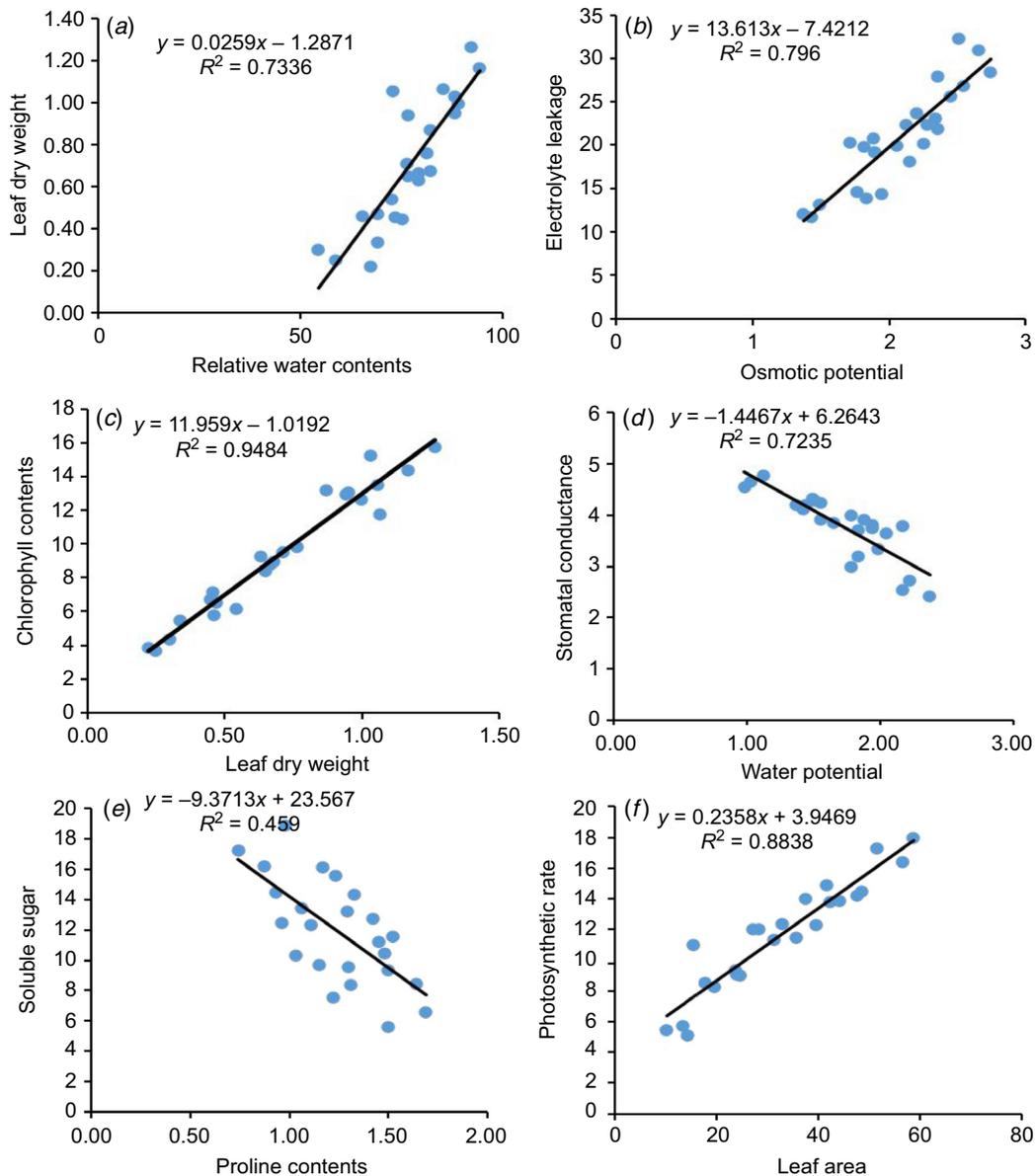
in lipid peroxidation might be a result of the overproduction and accumulation of reactive oxygen species (ROS), which then causes membrane peroxidation, protein degradation and DNA damage, severely inhibiting growth (Awasthi *et al.* 2015; Zhang *et al.* 2017). In one study, Si enhanced chlorophyll and carotenoid contents in a barley cultivar under high-temperature regimes, possibly by protecting the chlorophyll pigments from oxidative damage through strengthening of the level of carotenoids (Heile *et al.* 2021; Zaman *et al.* 2021). Exogenously applied Si improved plant defence systems to detoxify ROS induced under heat stress, which in turn helped to increased chlorophyll and carotenoid contents by limiting electrolyte leakage (Heile *et al.* 2021; Zaman *et al.* 2021).

Tolerant plants show a tendency to protection against the damaging effects of ROS through the synthesis of various enzymatic and nonenzymatic ROS-scavenging and detoxification systems (Jing *et al.* 2020). Activities also differ depending upon tolerance or susceptibility of different crop varieties, their growth stages and growing season (Li *et al.* 2020). The antioxidants CAT, SOD and POD are known for the dismutation of hydrogen peroxide to water and molecular oxygen in cells, as well as the elimination of stress-induced ROS directly or indirectly via the production of ascorbate and glutathione (Kim *et al.* 2014). In this study, we found that the activities of ROS-eliminating enzymes differed significantly in spinach plants under heat-stressed and control conditions. Plants receiving foliar-applied Si showed enhanced enzymatic activity during heat stress compared with normal temperature conditions, concurring with previous findings (Soundararajan *et al.* 2015; Heile *et al.* 2021; Zaman *et al.* 2021).

In addition to biomass, heat stress caused a significant reduction in water-related attributes of spinach plants (Sinha *et al.* 2021). Our results are in agreement with

previous reports in rice (Fahad *et al.* 2016) and wheat (Hameed *et al.* 2012). The decrease in leaf water content and water potential might affect plant metabolism and decrease plant growth and biomass (Sun *et al.* 2020). The application of Si alleviated heat stress significantly by improving the water status of spinach plants. Naz *et al.* (2022) found that, at a balanced Si concentration, RWC was almost constant under abiotic stress, which was probably due to an advanced ability for osmotic adjustment. Moreover, Si application helps in removing restrictions and improves turgor pressure in the cells to maintain water balance. Maximum RWC in the treatment with Si at 4 mmol L<sup>-1</sup> under heat stress might be due to the protective effect of Si in stressed plants by maintaining membrane integrity, because one of the primary effects of heat stress is a disruption of membrane integrity. Similar findings have been reported for wheat, sorghum and maize plants, suggesting that the positive impact of Si application under drought stress may be associated with reduced transpiration (Wang *et al.* 2021).

Plants affected by heat stress show the accumulation of compatible solutes such soluble sugars and proline (Rivero *et al.* 2014; Zhao *et al.* 2018). In the present study, enhanced proline content was observed under heat stress compared with control temperatures. Increased accumulation of proline due to heat stress has been reported by other researchers, who concluded that proline helps to stabilise membranes, subcellular structures and cellular redox potential by destroying the radicals (Hussain *et al.* 2018; Wang *et al.* 2018; Naz *et al.* 2022). Liu *et al.* (2011) observed a significant increase in starch content under heat-shock treatment >30°C. The increase in osmolyte status, soluble protein and soluble sugars and decrease in proline content in response to Si application may be due to the fact that Si has a pivotal role in binding amino acids to form specific proteins (Soundararajan *et al.* 2014; Rady *et al.* 2019;



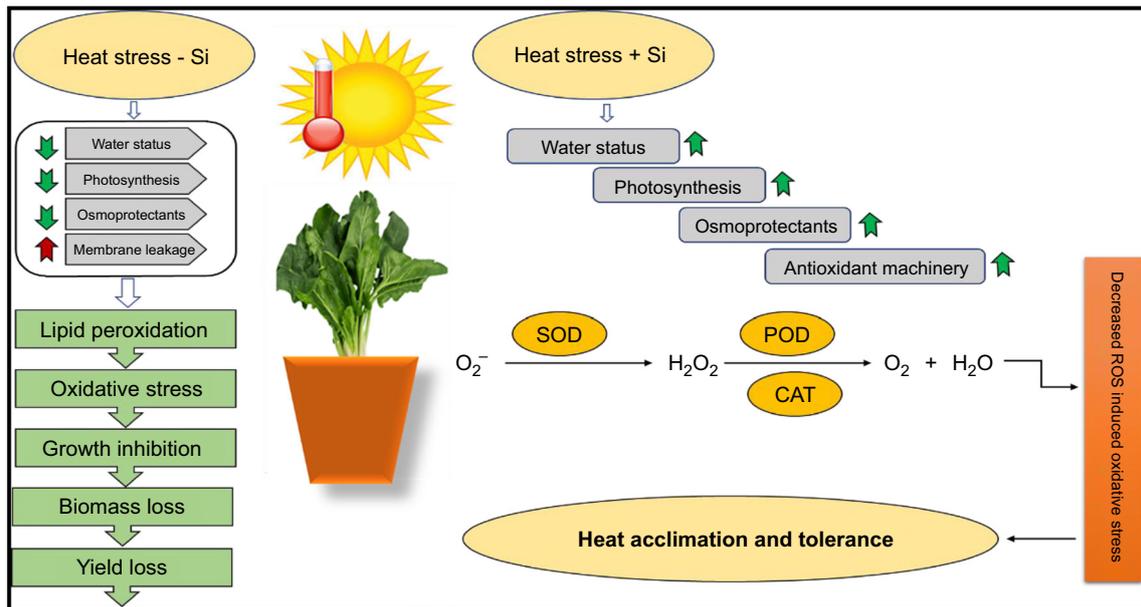
**Fig. 8.** Regressions showing significance for: (a) leaf dry weight vs relative water content, (b) electrolyte leakage vs osmotic potential, (c) chlorophyll content vs leaf dry weight, (d) stomatal conductance vs water potential, (e) soluble sugar vs proline contents, and (f) photosynthetic rate and leaf area.

Zaman *et al.* 2022), and Si is actively engaged in formation of DNA and functioning of mRNA (Abbas *et al.* 2015; AlKahtani *et al.* 2021). In addition, foliar-applied Si reduced the accumulation of proline content in the heat-stressed seedlings, which is associated with improved growth of spinach plants under heat stress (Fig. 9). This result suggests that foliar-applied Si could provide protection to cells by keeping the accumulation of proline to an optimum level, and Si probably employed other osmoprotectants for stress mitigation, such that a high level of proline accumulation was not required. The major outcomes of this research are that an appropriate level of Si not only improves the morpho-physiological parameters but

also decreases electrolyte leakage. This finding addresses issues of health and nutritional challenges and of improving farm productivity in regions facing extreme heat waves. However, further research is required to test more genotypes for thermo-tolerance and assess under field conditions.

## Conclusion

Heat stress significantly inhibited the growth, physiological, water relations and osmolyte status of spinach plants. Silicon supplementation improved plant growth, physio-biochemical,



**Fig. 9.** Pictorial representation of major damage caused by heat stress in spinach plants, and the defensive role of Si in responding. Heat stress causes oxidative, osmotic and electrolyte leakage in plants. An increase in sodium ions in soil lowers the soil water potential of plant cells. Heat stress reduces water uptake by plants, and resulting in cellular dehydration, biomass reduction, lipid peroxidation and, ultimately, yield reduction. To combat this, plants decrease ROS-induced oxidative stress pathways. These enzymatic antioxidants result in lowering of cellular water potential and lipid peroxidation and maintain a favourable gradient for water uptake. Balanced Si application alleviates osmotic stress by improving the photosynthetic process, antioxidant machinery and maintenance of osmoprotectant homeostasis. Si fertilisation reinforces the tolerance mechanism of plants to heat-induced oxidative stress.

photosynthetic, and tissue water parameters under heat stress compared with nil-Si treatment. Application of Si enhanced plant growth, most likely through decreased electrolyte leakage. Application of Si also increased enzymatic antioxidants. Application of Si at 4 mmol L<sup>-1</sup> performed best in alleviating heat stress in spinach plants. Moreover, exogenous application of Si is an environmentally friendly approach for growing spinach under heat-stressed conditions. Future research activities focusing on root architecture traits, molecular forms of Si and heat-stress interactions, economic benefits and diet diversity in addition to vital nutrients will be essential to developing agricultural strategies aiming at improving crop yield under abiotic stress.

## References

- Abbas T, Balal RM, Shahid MA, Pervez MA, Ayyub CM, Aqueel MA, Javaid MM (2015) Silicon-induced alleviation of NaCl toxicity in okra (*Abelmoschus esculentus*) is associated with enhanced photosynthesis, osmoprotectants and antioxidant metabolism. *Acta Physiologiae Plantarum* **37**, 6. doi:10.1007/s11738-014-1768-5
- Abbas T, Sattar A, Ijaz M, Aatif M, Khalid S, Sher A (2017) Exogenous silicon application alleviates salt stress in okra. *Horticulture, Environment, and Biotechnology* **58**, 342–349. doi:10.1007/s13580-017-0247-5
- Abdelrahman M, El-Sayed M, Jogaiah S, Burritt DJ, Tran L-SP (2017) The “STAY-GREEN” trait and phytohormone signaling networks in plants under heat stress. *Plant Cell Reports* **36**, 1009–1025. doi:10.1007/s00299-017-2119-y
- Aebi H (1974) Catalase. In ‘Methods of enzymatic analysis.’ pp. 673–684. (Academic Press: Cambridge, MA, USA)
- Alabdallah NM, Hasan MM, Hammami I, Alghamdi AI, Alshehri D, Alatawi HA (2021) Green synthesized metal oxide nanoparticles mediate growth regulation and physiology of crop plants under drought stress. *Plants* **10**, 1730. doi:10.3390/plants10081730
- Ali MM, Waleed Shafique M, Gull S, Afzal Naveed W, Javed T, Yousef AF, Mauro RP (2021) Alleviation of heat stress in tomato by exogenous application of sulfur. *Horticulturae* **7**, 21. doi:10.3390/horticulturae7020021
- Alizadeh MR, Abatzoglou JT, Adamowski JF, Prestemon JP, Chittoori B, Akbari Asanjan A, Sadegh M (2022) Increasing heat-stress inequality in a warming climate. *Earth's Future* **10**, e2021EF002488. doi:10.1029/2021EF002488
- AlKahtani MDF, Hafez YM, Attia K, Rashwan E, Husnain LA, AlGwaiz HIM, Abdelaal KAA (2021) Evaluation of silicon and proline application on the oxidative machinery in drought-stressed sugar beet. *Antioxidants* **10**, 398. doi:10.3390/antiox10030398
- An T, Gao Y, Kuang Q, Wu Y, Zaman Q, Zhang Y, Xu B, Chen Y (2022) Effect of silicon on morpho-physiological attributes, yield and cadmium accumulation in two maize genotypes with contrasting root system size and health risk assessment. *Plant and Soil*. doi:10.1007/s11104-022-05384-7
- Asseng S, Ewert F, Martre P, et al. (2015) Rising temperatures reduce global wheat production. *Nature Climate Change* **5**, 143–147. doi:10.1038/nclimate2470
- Atherton JG, Farooque AM (1983) High temperature and germination in spinach. I. The role of the pericarp. *Scientia Horticulturae* **19**, 25–32. doi:10.1016/0304-4238(83)90040-7
- Awasthi R, Bhandari K, Nayyar H (2015) Temperature stress and redox homeostasis in agricultural crops. *Frontiers in Environmental Science* **3**, 11. doi:10.3389/fenvs.2015.00011

- Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* **44**, 276–287. doi:10.1016/0003-2697(71)90370-8
- Bita CE, Gerats T (2013) Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Frontiers in Plant Science* **4**, 273. doi:10.3389/fpls.2013.00273
- Both AJ, Benjamin L, Franklin J, Holroyd G, Incoll LD, Lefsrud MG, Pitkin G (2015) Guidelines for measuring and reporting environmental parameters for experiments in greenhouses. *Plant Methods* **11**, 43. doi:10.1186/s13007-015-0083-5
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254. doi:10.1016/0003-2697(76)90527-3
- Challinor AJ, Watson J, Lobell DB, Howden SM, Smith DR, Chhetri N (2014) A meta-analysis of crop yield under climate change and adaptation. *Nature Climate Change* **4**, 287–291. doi:10.1038/nclimate2153
- Chaudhry S, Sidhu GPS (2022) Climate change regulated abiotic stress mechanisms in plants: a comprehensive review. *Plant Cell Reports* **41**, 1–31. doi:10.1007/s00299-021-02759-5
- Chitwood J, Shi A, Evans M, Rom C, Gbur EE, Motes D, Chen P, Hensley D (2016) Effect of temperature on seed germination in spinach (*Spinacia oleracea*). *HortScience* **51**, 1475–1478. doi:10.21273/HORTSCI11414-16
- Das A, Rushton PJ, Rohila JS (2017) Metabolomic profiling of soybeans (*Glycine max* L.) reveals the importance of sugar and nitrogen metabolism under drought and heat stress. *Plants* **6**, 21. doi:10.3390/plants6020021
- Das D, Ullah H, Tisarum R, Cha-um S, Datta A (2021) Morpho-physiological responses of tropical rice to potassium and silicon fertilization under water-deficit stress. *Journal of Soil Science and Plant Nutrition*. doi:10.1007/s42729-021-00712-9
- Dionisio-Sese ML, Tobita S (1998) Antioxidant responses of rice seedlings to salinity stress. *Plant Sciences* **135**, 1–9. doi:10.1016/S0168-9452(98)00025-9
- Emanuil N, Akram MS, Ali S, El-Esawi MA, Iqbal M, Alyemeni MN (2020) Peptide-Induced physio-biochemical modulations reduce cadmium toxicity and accumulation in spinach (*Spinacia oleracea* L.). *Plants* **9**, 1806. doi:10.3390/plants9121806
- Fahad S, Hussain S, Saud S, Hassan S, Tanveer M, Ihsan MZ, Huang J (2016) A combined application of biochar and phosphorus alleviates heat-induced adversities on physiological, agronomical and quality attributes of rice. *Plant Physiology and Biochemistry* **103**, 191–198. doi:10.1016/j.plaphy.2016.03.001
- Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Huang J (2017) Crop production under drought and heat stress: plant responses and management options. *Frontiers in Plant Science* **8**, 1147. doi:10.3389/fpls.2017.01147
- Giannakoula A, Moustakas M, Mylona P, Papadakis I, Yupsanis T (2008) Aluminum tolerance in maize is correlated with increased levels of mineral nutrients, carbohydrates and proline, and decreased levels of lipid peroxidation and Al accumulation. *Journal of Plant Physiology* **165**, 385–396. doi:10.1016/j.jplph.2007.01.014
- Giordano M, Petropoulos SA, Roupael Y (2021) Response and defence mechanisms of vegetable crops against drought, heat and salinity stress. *Agriculture* **11**, 463. doi:10.3390/agriculture11050463
- Govindaraj M, Pattanashetti SK, Patne N, Kanatti AA (2018) Breeding cultivars for heat stress tolerance in staple food crops. In 'Next generation plant breeding'. (Ed. YÖ Çiftçi) pp. 45–74. (InTechOpen: London, UK)
- Hameed A, Goher M, Iqbal N (2012) Heat stress-induced cell death, changes in antioxidants, lipid peroxidation, and protease activity in wheat leaves. *Journal of Plant Growth Regulation* **31**, 283–291. doi:10.1007/s00344-011-9238-4
- Haque MS, Husna MT, Uddin MN, Hossain MA, Sarwar AKMG, Ali OM, Abdel Latef AAH, Hossain A (2021) Heat stress at early reproductive stage differentially alters several physiological and biochemical traits of three tomato cultivars. *Horticulturae* **7**, 330. doi:10.3390/horticulturae7100330
- Hasanuzzaman M, Hossain MA, Fujita M (2011) Nitric oxide modulates antioxidant defense and the methylglyoxal detoxification system and reduces salinity-induced damage of wheat seedlings. *Plant Biotechnology Reports* **5**, 353–365. doi:10.1007/s11816-011-0189-9
- Hassan MU, Chattha MU, Khan I, Chattha MB, Barbanti L, Aamer M, Aslam MT (2021) Heat stress in cultivated plants: nature, impact, mechanisms, and mitigation strategies: a review. *Plant Biosystems* **155**, 211–234. doi:10.1080/11263504.2020.1727987
- Heile AO, Zaman Qu, Aslam Z, Hussain A, Aslam M, Saleem MH, Abualreesh MH, Alatawi A, Ali S (2021) Alleviation of cadmium phytotoxicity using silicon fertilization in wheat by altering antioxidant metabolism and osmotic adjustment. *Sustainability* **13**, 11317. doi:10.3390/su132011317
- Hussain M, Farooq S, Hasan W, Ul-Allah S, Tanveer M, Farooq M, Nawaz A (2018) Drought stress in sunflower: physiological effects and its management through breeding and agronomic alternatives. *Agricultural Water Management* **201**, 152–166. doi:10.1016/j.agwat.2018.01.028
- Hussain I, Parveen A, Rasheed R, Ashraf MA, Ibrahim M, Riaz S, Afzaal Z, Iqbal M (2019) Exogenous silicon modulates growth, physio-chemicals and antioxidants in barley (*Hordeum vulgare* L.) exposed to different temperature regimes. *Silicon* **11**, 2753–2762. doi:10.1007/s12633-019-0067-6
- Hwang S-J, Hamayun M, Kim H-Y, Na C-I, Kim K-U, Shin D-H, Lee I-J (2007) Effect of nitrogen and silicon nutrition on bioactive gibberellin and growth of rice under field conditions. *Journal of Crop Science and Biotechnology* **10**, 281–286.
- Iqbal P, Ghani MA, Ali B, Shahid M, Iqbal Q, Ziaf K, Ahmad J (2021) Exogenous application of glutamic acid promotes cucumber (*Cucumis sativus* L.) growth under salt stress conditions. *Emirates Journal of Food and Agriculture* **33**, 407–416. doi:10.9755/ejfa.2021.v33.i5.2699
- Janská A, Maršík P, Zelenková S, Ovesná J (2010) Cold stress and acclimation: what is important for metabolic adjustment?. *Plant Biology* **12**, 395–405. doi:10.1111/j.1438-8677.2009.00299.x
- Jeon M-W, Ali MB, Hahn E-J, Paek K-Y (2006) Photosynthetic pigments, morphology and leaf gas exchange during ex vitro acclimatization of micropropagated CAM *Doritaenopsis* plantlets under relative humidity and air temperature. *Environmental and Experimental Botany* **55**, 183–194. doi:10.1016/j.envexpbot.2004.10.014
- Jing J, Guo S, Li Y, Li W (2020) The alleviating effect of exogenous polyamines on heat stress susceptibility of different heat resistant wheat (*Triticum aestivum* L.) varieties. *Scientific Reports* **10**, 7467. doi:10.1038/s41598-020-64468-5
- Katzman LS, Taylor AG, Langhans RW (2001) Seed enhancements to improve spinach germination. *HortScience* **36**, 979–981. doi:10.21273/HORTSCI.36.5.979
- Khan A, Bilal S, Khan AL, Imran M, Shahzad R, Al-Harrasi A, Lee IJ (2020a) Silicon and gibberellins: synergistic function in harnessing ABA signaling and heat stress tolerance in date palm (*Phoenix dactylifera* L.). *Plants* **9**, 620. doi:10.3390/plants9050620
- Khan MN, Siddiqui MH, AlSolami MA, Alamri S, Hu Y, Ali HM, Al-Amri AA, Alsubaie QD, Al-Munqedhi BMA, Al-Ghamdi A (2020b) Crosstalk of hydrogen sulfide and nitric oxide requires calcium to mitigate impaired photosynthesis under cadmium stress by activating defense mechanisms in *Vigna radiata*. *Plant Physiology and Biochemistry* **156**, 278–290. doi:10.1016/j.plaphy.2020.09.017
- Kim YH, Khan AL, Waqas M, Shim JK, Kim DH, Lee KY, Lee IJ (2014) Silicon application to rice root zone influenced the phytohormonal and antioxidant responses under salinity stress. *Journal of Plant Growth Regulation* **33**, 137–149. doi:10.1007/s00344-013-9356-2
- Leskovar DI, Esensee V, Belefant-Miller H (1999) Pericarp, leachate, and carbohydrate involvement in thermoinhibition of germinating spinach seeds. *Journal of the American Society for Horticultural Science* **124**, 301–306. doi:10.21273/JASHS.124.3.301
- Li C, Han Y, Hao J, Qin X, Liu C, Fan S (2020) Effects of exogenous spermidine on antioxidants and glyoxalase system of lettuce seedlings under high temperature. *Plant Signaling & Behavior* **15**, 1824697. doi:10.1080/15592324.2020.1824697
- Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology* **148**, 350–382. doi:10.1016/0076-6879(87)48036-1
- Liu P, Guo W, Jiang Z, Pu H, Feng C, Zhu X, Peng Y, Kuang A, Little CR (2011) Effects of high temperature after anthesis on starch granules

- in grains of wheat (*Triticum aestivum* L.). *The Journal of Agricultural Science* **149**, 159–169. doi:10.1017/S0021859610001024
- Lobanov AV, Hatfield DL, Gladyshev VN (2008) Reduced reliance on the trace element selenium during evolution of mammals. *Genome Biology* **9**, R62. doi:10.1186/gb-2008-9-3-r62
- Maehly AC, Chance B (1954) The assay of catalases and peroxidases. *Methods of Biochemical Analysis* **1**, 357–424. doi:10.1002/9780470110171.ch14
- Mahdavi S, Arzani A, Maibody SAMM, Mehrabi AA (2021) Photosynthetic and yield performance of wheat (*Triticum aestivum* L.) under sowing in hot environment. *Acta Physiologiae Plantarum* **43**, 106. doi:10.1007/s11738-021-03278-2
- Mahdieh M, Habibollahi N, Amirjani MR, Abnosi MH, Ghorbanpour M (2015) Exogenous silicon nutrition ameliorates salt-induced stress by improving growth and efficiency of PSII in *Oryza sativa* L. cultivars. *Journal of Soil Science and Plant Nutrition* **15**, 1050–1060. doi:10.4067/S0718-95162015005000073
- Moradpour M, Abdullah SNA, Namasivayam P (2021) The impact of heat stress on morpho-physiological response and expression of specific genes in the heat stress-responsive transcriptional regulatory network in *Brassica oleracea*. *Plants* **10**, 1064. doi:10.3390/plants10061064
- Mostofa MG, Rahman MM, Ansary MMU, Keya SS, Abdelrahman M, Miah MG, Phan Tran L-S (2021) Silicon in mitigation of abiotic stress-induced oxidative damage in plants. *Critical Reviews in Biotechnology* **41**, 918–934. doi:10.1080/07388551.2021.1892582
- Muneer S, Park YG, Kim S, Jeong BR (2017) Foliar or subirrigation silicon supply mitigates high temperature stress in strawberry by maintaining photosynthetic and stress-responsive proteins. *Journal of Plant Growth Regulation* **36**, 836–845. doi:10.1007/s00344-017-9687-5
- Murkowski A (2001) Heat stress and spermidine: effect on chlorophyll fluorescence in tomato plants. *Biologia Plantarum* **44**, 53–57. doi:10.1023/A:1017966203859
- Naz R, Zaman Qu, Nazir S, Komal N, Chen Y, Ashraf K, Al-Huqail AA, Alfagham A, Siddiqui MH, Ali HM, Khan F, Sultan K, Khosa Q (2022) Silicon fertilization counteracts salinity-induced damages associated with changes in physio-biochemical modulations in spinach. *PLoS ONE* **17**, e0267939. doi:10.1371/journal.pone.0267939
- Okereke CN, Liu B, Kaurilind E, Niinemets Ü (2021) Heat stress resistance drives coordination of emissions of suites of volatiles after severe heat stress and during recovery in five tropical crops. *Environmental and Experimental Botany* **184**, 104375. doi:10.1016/j.envexpbot.2021.104375
- Othmani A, Ayed S, Bezzin O, Farooq M, Ayed-Slama O, Slim-Amara H, Ben Younes M (2021) Effect of silicon supply methods on durum wheat (*Triticum durum* Desf.) response to drought stress. *Silicon* **13**, 3047–3057. doi:10.1007/s12633-020-00639-3
- Rady MM, Elrys AS, Abo El-Maati MF, Desoky E-SM (2019) Interplaying roles of silicon and proline effectively improve salt and cadmium stress tolerance in *Phaseolus vulgaris* plant. *Plant Physiology and Biochemistry* **139**, 558–568. doi:10.1016/j.plaphy.2019.04.025
- Rivero RM, Mestre TC, Mittler R, Rubio F, Garcia-Sanchez F, Martinez V (2014) The combined effect of salinity and heat reveals a specific physiological, biochemical and molecular response in tomato plants. *Plant, Cell & Environment* **37**, 1059–1073. doi:10.1111/pce.12199
- Sarwar M, Saleem MF, Ullah N, Ali S, Rizwan M, Shahid MR, Alyemeni MN, Alamri SA, Ahmad P (2019) Role of mineral nutrition in alleviation of heat stress in cotton plants grown in glasshouse and field conditions. *Scientific Reports* **9**, 13022. doi:10.1038/s41598-019-53297-w
- Shabbir R, Javed T, Hussain S, Ahmar S, Naz M, Zafar H, Pandey S, Chauhan J, Siddiqui MH, Pinghua C (2022) Calcium homeostasis and potential roles to combat environmental stresses in plants. *South African Journal of Botany* **148**, 683–693. doi:10.1016/j.sajb.2022.05.038
- Shahid M, Saleem MF, Anjum SA, Shahid M, Afzal I (2017) Effect of terminal heat stress on proline, secondary metabolites and yield components of wheat (*Triticum aestivum* L.) genotypes. *The Philippine Agricultural Scientist* **100**, 278–286.
- Shalaby TA, Abd-Elkarim E, El-Aidy F, Hamed E-S, Sharaf-Eldin M, Taha N, El-Ramady H, Bayoumi Y, dos Reis AR (2021) Nano-selenium, silicon and H<sub>2</sub>O<sub>2</sub> boost growth and productivity of cucumber under combined salinity and heat stress. *Ecotoxicology and Environmental Safety* **212**, 111962. doi:10.1016/j.ecoenv.2021.111962
- Sinha T, Kumar S, Singh AK (2021) Physio-biochemical and yield response of chickpea genotypes under salinity and high temperature stress. *International Journal of Environment and Climate Change* **11**, 136–147. doi:10.9734/ijecce/2021/v11i330384
- Song L, Guanter L, Guan K, You L, Huete A, Ju W, Zhang Y (2018) Satellite sun-induced chlorophyll fluorescence detects early response of winter wheat to heat stress in the Indian Indo-Gangetic Plains. *Global Change Biology* **24**, 4023–4037. doi:10.1111/gcb.14302
- Soratto RP, Crusciol CAC, Castro GSA, Costa CHMd, Ferrari Neto J (2012) Leaf application of silicic acid to white oat and wheat. *Revista Brasileira de Ciência do Solo* **36**, 1538–1544. doi:10.1590/S0100-06832012000500018
- Soundararajan P, Sivanesan I, Jana S, Jeong BR (2014) Influence of silicon supplementation on the growth and tolerance to high temperature in *Salvia splendens*. *Horticulture, Environment, and Biotechnology* **55**, 271–279. doi:10.1007/s13580-014-0023-8
- Soundararajan P, Manivannan A, Park YG, Muneer S, Jeong BR (2015) Silicon alleviates salt stress by modulating antioxidant enzyme activities in *Dianthus caryophyllus* 'Tula'. *Horticulture, Environment, and Biotechnology* **56**, 233–239. doi:10.1007/s13580-015-0111-4
- Sun YL, Wang YH, Deng LF, Shi X, Bai XF (2020) Moderate soil salinity alleviates the impacts of drought on growth and water status of plants. *Russian Journal of Plant Physiology* **67**, 153–161. doi:10.1134/S1021443720010215
- Tang Y, Wen X, Lu Q, Yang Z, Cheng Z, Lu C (2007) Heat stress induces an aggregation of the light-harvesting complex of photosystem II in spinach plants. *Plant Physiology* **143**, 629–638. doi:10.1104/pp.106.090712
- Tripathi P, Tripathi RD, Singh RP, Dwivedi S, Goutam D, Shri M, Trivedi PK, Chakrabarty D (2013) Silicon mediates arsenic tolerance in rice (*Oryza sativa* L.) through lowering of arsenic uptake and improved antioxidant defence system. *Ecological Engineering* **52**, 96–103. doi:10.1016/j.ecoleng.2012.12.057
- Turner NC, Kramer PJ (1980) 'Adaptation of plants to water and high temperature stress.' (Wiley Publishers: Hoboken, NJ, USA)
- Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Sciences* **151**, 59–66. doi:10.1016/S0168-9452(99)00197-1
- Wahid A (2007) Physiological implications of metabolite biosynthesis for net assimilation and heat-stress tolerance of sugarcane (*Saccharum officinarum*) sprouts. *Journal of Plant Research* **120**, 219–228. doi:10.1007/s10265-006-0040-5
- Wang J, Yuan B, Xu Y, Huang B (2018) Differential responses of amino acids and soluble proteins to heat stress associated with genetic variations in heat tolerance for hard fescue. *Journal of the American Society for Horticultural Science* **143**, 45–55. doi:10.21273/JASHS04246-17
- Wang M, Wang R, Mur LAJ, Ruan J, Shen Q, Guo S (2021) Functions of silicon in plant drought stress responses. *Horticulture Research* **8**, 254. doi:10.1038/s41438-021-00681-1
- Waraich EA, Ahmad R, Halim A, Aziz T (2012) Alleviation of temperature stress by nutrient management in crop plants: a review. *Journal of Soil Science and Plant Nutrition* **12**, 221–244. doi:10.4067/S0718-95162012000200003
- Xie Z, Song F, Xu H, Shao H, Song R (2014) Effects of silicon on photosynthetic characteristics of maize (*Zea mays* L.) on alluvial soil. *The Scientific World Journal* **2014**, 718716. doi:10.1155/2014/718716
- Younis AA, Khattab H, Emam MM (2020) Impacts of silicon and silicon nanoparticles on leaf ultrastructure and *TaPIP1* and *TaNIP2* gene expressions in heat stressed wheat seedlings. *Biologia Plantarum* **64**, 343–352. doi:10.32615/bp.2020.030
- Zaman Qu, Rashid M, Nawaz R, Hussain A, Ashraf K, Latif M, Heile AO, Chen Y (2021) Silicon fertilization: a step towards cadmium-free fragrant rice. *Plants* **10**, 2440. doi:10.3390/plants10112440
- Zaman Qu, Abbasi A, Tabassum S, Ashraf K, Ahmad Z, Siddiqui MH, Alamri S, Maqsood S, Sultan K (2022) Calcium induced growth, physio-biochemical, antioxidants, osmolytes adjustments and

- phytoconstituents status in spinach under heat stress. *South African Journal of Botany* **149**, 701–711. doi:[10.1016/j.sajb.2022.06.065](https://doi.org/10.1016/j.sajb.2022.06.065)
- Zandalinas SI, Fritschi FB, Mittler R (2021) Global warming, climate change, and environmental pollution: recipe for a multifactorial stress combination disaster. *Trends in Plant Sciences* **26**, 588–599. doi:[10.1016/j.tplants.2021.02.011](https://doi.org/10.1016/j.tplants.2021.02.011)
- Zhang B, Wijesundara NM, Abbey L, Rupasinghe HPV (2017) Growing medium amendments effect on growth, secondary metabolites and anti-streptococcal activity of two species of *Plectranthus*. *Journal of Applied Research on Medicinal and Aromatic Plants* **5**, 53–59. doi:[10.1016/j.jarmap.2016.11.001](https://doi.org/10.1016/j.jarmap.2016.11.001)
- Zhao Q, Chen W, Bian J, Xie H, Li Y, Xu C, Ma J, Guo S, Chen J, Cai X, Wang X, Wang Q, She Y, Chen S, Zhou Z, Dai S (2018) Proteomics and phosphoproteomics of heat stress-responsive mechanisms in spinach. *Frontiers in Plant Science* **9**, 800. doi:[10.3389/fpls.2018.00800](https://doi.org/10.3389/fpls.2018.00800)

**Data availability.** The dataset of this article can be requested from the corresponding author upon suitable request.

**Conflicts of interest.** The authors declare no conflicts of interest.

**Declaration of funding.** This research received no external funding.

#### Author affiliations

<sup>A</sup>Department of Environmental Sciences, The University of Lahore, Lahore 54590, Pakistan.

<sup>B</sup>Institute of Soil and Water Conservation, Chinese Academy of Sciences, and Northwest A&F University, Yangling 712100, China.

<sup>C</sup>Department of Food Sciences, Government College University Faisalabad, Sahiwal Campus, Sahiwal 57000, Pakistan.

<sup>D</sup>Department of Environmental Sciences, Kohsar University, Murree, Pakistan.

<sup>E</sup>The UWA Institute of Agriculture, and School of Agriculture and Environment, The University of Western Australia, Perth, WA 6009, Australia.