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ORIGINAL ARTICLE

Silicon and water-deficit stress differentially modulate physiology and ultrastructure in wheat (*Triticum aestivum* L.)

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Abstract Plants combat drought stress by coordinating various metabolic enzymes, and endogenous phytohormones, such as indole acetic acid (IAA) and abscisic acid (ABA). In the present study, 37-day-old wheat seedlings were subjected to the Hoagland solution with 20% PEG for 7 days (to create the artificial osmotic stress environment) in the greenhouse, and were supplemented with an optimized concentration (1.0 mM) of silicon (Si) to alleviate the negative effects of former stress on physiological, biochemical and phytohormones contents. Exogenous Si significantly improved plant growth parameters under osmotic stress compared to PEG treatment alone (the increase was up to 6 and 9% for shoot and root fresh weight, 4 and 12% for shoot and root dry weight, respectively). Moreover, Si significantly decreased the H_2O_2, MDA contents, electrolyte leakage, antioxidant enzyme activity (POD), and mineral contents (K and Ca) under osmotic stress but markedly increased the ascorbic acid (AsA), soluble sugar and mineral (Mg and Si) contents. Interestingly, Si application under water-deficit stress differently modulated the endogenous levels of ABA, IAA and JA in wheat plants compared to PEG treatment alone. This study suggests that exogenous Si improves the plant growth by modulating the nutrient (Na, Mg and Si) uptake and phytohormone levels in wheat under water-deficit stress.

Keywords Water-deficit stress · Elements uptake · Phytohormones · Polyethylene glycol · Wheat · Ultrastructural study

Introduction

Wheat (*Triticum aestivum* L.) is an important food crop that is grown worldwide, and its yield is affected by diverse adverse environments. Among the relevant factors in wheat establishment, drought is the main cause of severe yield reductions. Increasing the drought tolerance of wheat is one way to overcome drought-related problems.

Drought stress (DS) is a major abiotic stress that can influence crop production dramatically and adversely affects plant growth by reducing leaf area, the length of roots and shoots, and photosynthesis. However, the effects of drought stress are not well recognized at the biochemical, molecular and hormonal levels due to the complex interactions between drought stress and plant physiology. To overcome these stresses, antioxidant systems and hormones play key roles in regulating several physio-biochemical processes (Ali et al. 2014; Islam et al. 2014). The role of phytohormones in drought stress is important in driving physiological mechanisms in plants under unfavorable environment (Iqbal et al. 2014). Likewise, indole acetic acid (IAA) and jasmonic acid (JA) are involved in defence-related signaling under unfavorable conditions. Moreover, ABA is involved in stomatal closure, which

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ensures that the plants do not lose a specific amount of water (Kim et al. 2014a, b).

Silicon (Si) has been used to mitigate drought stress and increase the drought resistance of crops. Increased Si concentrations in plants maintain not only water status but also improve drought resistance by regulating leaf water potential, helping in CO_2 assimilation and decreasing transpiration by adjusting the leaf area (Zhu and Gong 2014). Other ameliorating mechanisms may include decreasing water contents by transpiration, restricting the uptake of toxic ions, and modulating the osmolytes and antioxidative mechanisms. Si application affects hormone regulation during stress conditions, but the role of the Si-induced regulation of phytohormones under drought stress is not fully understood (Iqbal et al. 2014). Enhanced stress tolerance in plants is dependent upon the biosynthesis and the action of plant hormones. For example, phytohormones such as IAA and JA are involved in responses to stress conditions and may play a vital role in the cross talk between different hormones under abiotic conditions. However, little information is available about changes in these phytohormones in response to stress conditions in cereal crops (Du et al. 2013; Wang et al. 2010).

Silicon taken up by plants is deposited mainly in the cell wall (Epstein 1999). The formation of Si-organic complexes has been reported in rice shoots (Inanaga and Okasaka 1996). Different mechanisms for Si-mediated stress alleviation have been proposed by researchers (Pei et al. 2010; Mera and Beveridge 1993). Si deposition in leaves has been reported to decrease transpiration (Matoh et al. 1986), thereby alleviating salt stress. Si could alleviate the salt stress by reducing Na+ uptake in *Oryza sativa* L. (Yeo et al. 1999; Gong et al. 2006). The most widely reported mechanism is that Si might decrease oxidative damage in plants subjected to environmental stress (Saqib et al. 2008). That Si could reduce the oxidative damage induced by saline has been reported in barley (Liang 1999; Liang et al. 2003, 2005) and in cucumber (Zhu et al. 2004). Reduced oxidative damage due to the addition of Si under saline conditions has also been reported in spinach (*Spinacia oleracea* L.) and tomato (*Lycopersicon esculentum* Mill.) plants by reducing oxidative membrane damage (Gunes et al. 2007). Gong et al. (2005) also reported that Si alleviated oxidative stress by regulating the activities of antioxidant enzymes under drought in wheat. The addition of Si increases water use efficiency by reducing leaf transpiration and the water flow rate in xylem vessels of maize (Gao et al. 2004, 2006). Hattori et al. (2005, 2007) suggested that Si might facilitate water uptake and transport in sorghum (*Sorghum bicolour* L. Moench) in drought stress.

In addition to affecting antioxidant defences, plants can adapt to water stress by changing their solute levels and hence physiological activity are maintained at low leaf water potentials (Zhu et al. 2005). The accumulation of solutes in stressed leaves contributes to dehydration tolerance (Wood et al. 1996; Smienoff 1998). However, the effects of Si application on inorganic ion and organic compound accumulation remain unknown (Pei et al. 2010).

In this study, we investigated the effects of Si on plant growth, nutrient uptake, antioxidant systems, sugar and proline accumulation, phytohormone levels and ultrastructures in wheat seedlings under polyethylene glycol (PEG)-induced water stress. The results obtained might contribute to the understanding of the mechanism(s) of Si-induced increase in the drought tolerance of wheat plants.

Materials and methods

Plant materials and treatments

Healthy wheat seeds (*Triticum aestivum* L. cv. Longchun 8139) were sown on a floating net in 0.5 Hoagland solution (pH 5.6) for germination. Seven-day-old seedlings were transplanted into plastic buckets containing Hoagland solution, which was continuously aerated using an air pump. The plants were grown in a greenhouse under a light intensity of 200–450 μmol m^{-2} s^{-1}, a temperature of 20–30 °C and a relative humidity of approximately 55%. All treatments were replicated three times. The nutrient solution was changed every 5 days.

After 1 month of seedling acclimation, well-grown and uniformly sized plants were selected for the treatments. Si was applied as Na_2SiO_3, and the plants were exposed to four different treatments: control (CK), 1 mM silicon (Si), 20% (w/v) PEG-6000 (PEG), and Si plus PEG (Si + PEG). Seven days after the treatments, morphological parameters were determined; and samples for chlorophyll content assay, biochemical analysis, elemental uptake assay, transmission electron microscope and phytohormone determination were collected as described below.

Biomass determination

Ten plants were harvested for each treatment. Fresh biomass was sampled after the plants were harvested. To assay dry biomass, plants were dried in oven at 80 °C for 5 d and then weighed (Momoh and Zhou 2001).

Chlorophyll contents

Chlorophyll pigments (chlorophylls a, b and total chlorophyll) were estimated by following the method of Inskeep and Bloom (1985).

Leaf water contents and water potential

Total leaf water contents and relative leaf water contents were estimated according to the following formulas:

$$\text{TWC}\ (\%) = [(\text{FW} - \text{DW})/\text{FW}] \times 100$$

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

Here FW represents fresh weight, DW represents dry weight after drying at 75 °C for 48 h, and TW represents turgid weight after soaking in deionized water for 6 h at room temperature.

The first fully expanded leaf from the up was selected for water potential (WP) determination. Approximately, 0.5–1.0 cm fresh leaf was snipped to determine WP by the vapor pressure osmometer (Wescor Inc., Logan, UT, USA).

Determinations of MDA and H_2O_2 and electrolyte leakage

The malondialdehyde (MDA) contents were measured according to the procedure described by Zhang et al. (2008) and Pei et al. (2010). Fresh samples (0.2 g) were homogenized and extracted in 10 ml of 0.5% thiobarbituric acid (TBA) made in 5% trichloroacetic acid (TCA) then extract was heated at 95 °C for 15 min and cooled on ice. The samples were centrifuged at 5,000 g for 10 min. The absorbance of the supernatant was measured at 532 nm. Correction of nonspecific turbidity was made by subtracting the absorbance value taken at 600 nm. The MDA was calculated using extinction coefficient of 155 mM cm^{-1}. Hydrogen peroxide (H_2O_2) contents were estimated according to Al-aghabary et al. (2004). Leaf samples (0.5 g) were ground with cold acetone (g:ml = 1:10) in an ice bath and centrifuged at 3000 g for 10 min. The supernatant (1 ml) was collected after the centrifugation and mixed with 0.1 ml titanium reagent ($TiCl_4$:HCl = 4:1) and 0.2 ml of 17 M ammonia solution, and then centrifuged at 3000 g for 10 min. The deposition was washed five times with cold acetone before dissolving in 3 ml of 1 M H_2SO_4. H_2O_2 content was analyzed based on the absorbance of samples at 410 nm using a standard curve as a reference. The H_2O_2 content was determined using extinction coefficient of 0.28 μM cm^{-1} and expressed as μmol g^{-1} DW. Electrolyte leakage (EL) was determined by electrical conductivity meter as described by Pei et al. (2010). The samples after being washed three times with double distilled water were placed in closed vials containing 10 ml double distilled to vacuumize for 10 min and then surged for 1 h. The initial electrical conductivity (EC1) was determined at 25 °C. Samples were then boiling for 10 min to test the final electrical conductivity (EC2). The electrolyte leakage (EL, %) was expressed following the formula: EL (%) = (EC1/EC2) × 100.

Mineral determinations

Leaf samples for each treatment were dried at 70 °C for 48 h and then pulverized before digesting in 0.5 ml H_2O_2 and 2 ml HNO_3 at 160 °C for 6 h. The digest was then cooled and made up to a final 25-ml volume with double distilled water.

Elemental (sodium, magnesium, potassium and calcium) contents were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) following the method of Zhang et al. (2008). Si analysis of the plant material was performed according to the method of Eraslan et al. (2008).

Antioxidant machinery

To measure enzyme activities, leaf samples (0.5 g) were homogenized in 50 mM potassium phosphate buffer (pH 7.8) and centrifuged at 10,000*g*. The supernatants were collected and used for the further analysis of enzyme activities. Total superoxide dismutase (SOD, EC 1.15.1.1) activity was estimated by following the protocol of Zhou et al. (1997). The reaction mixture comprised of 3-ml volume of 50 mM potassium phosphate buffer (pH 7.8), 26 mM L-methionine, 750 μM NBT, 20 μM riboflavin, 1 μM EDTA and 25 μl of enzyme extract. One unit of SOD activity was measured as the enzyme amount required to cause 50% inhibition of the NBT reduction measured at 560 nm. The method of Cakmak et al. (1993) was followed to analyze catalase (CAT, EC 1.11.1.6) activity with the use of H_2O_2 for 30 s at A_{240} in 3 ml reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 45 mM H_2O_2 and 100 μl enzyme extract. Peroxidase (POD, EC1.11.1.7) activity was analyzed according to the method of Zhang et al. (2008) with minor modifications. The activity of POD was determined as the variation in guaiacol absorbance measured at 470 nm. The reaction solution consisted of 50 mM potassium phosphate buffer (pH 7.0), 100 μl enzyme extract, 2% H_2O_2 and 0.3% guaiacol. The method of Moons et al. (1997) was followed to analyze the ascorbate peroxide (APX, EC 1.11.1.11) activity. The reaction solution contains 100 mM phosphate (pH 7.0), 0.3 mM ascorbic acid, 0.06 mM H_2O_2, 0.1 mM EDTA-Na_2 and 100 μl enzyme extract. The spectrophotometer was set at 290 nm and the absorption was taken 30 s after addition of H_2O_2.

Analysis of glutathione, proline, ascorbic acid and soluble sugar contents

Fresh leaf samples (0.5 g) were mixed with 5% (w/v) TCA (2 ml) and centrifuged at 10,000*g* for 10 min. Supernatants

were collected and used to measure the contents of glutathione (GAH) and ascorbic acid (AsA).

Reduced glutathione (GSH) was measured according to the method of Anderson (1985) with some modifications. To 0.5 ml of supernatant, 0.6 ml of 100 mM phosphate buffer (pH 7.0) and 40 µL of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) were added. The mixture was incubated at 30 °C for 5 min; then the absorbance was measured at 412 nm. A standard curve was used to calculate the total glutathione concentration. Ascorbic acid (AsA) contents were estimated following the method of Law et al. (1983). The absorbance of samples was measured at 525 nm, and the amount of AsA in the extracted leaf samples was worked out from a standard curve. The methods of Pei et al. (2010) were used to estimate proline and soluble sugar contents. Soluble sugar contents were determined by measuring absorbance at 485 nm on spectrophotometer and calculated using a standard curve.

Estimation of endogenous JA, ABA and IAA contents

Endogenous JA, ABA and IAA contents were measured using enzyme-linked immunosorbent ELISA kits (Rapidbio, USA). Samples for the study of hormones were taken at different time intervals (1, 3, 5 and 7 days) after the treatments. Approximately, 0.1 g of each plant sample was homogenized in 2 ml of extraction buffer (containing 1 mM BHT in 80% methanol) on an ice bath, and the samples were extracted for 4 h at 4 °C. The homogenate was then centrifuged at 3500×*g* for 8 min at 4 °C, saving the supernatant. To the precipitate, 1 ml of extraction buffer was added; the sample was then mixed and re-extracted for another 1 h at 4 °C and then recentrifuged again. The supernatants were combined to estimate the contents of endogenous JA, ABA and IAA.

Cell structural observations

For ultrastructural study, leaves fragments without veins (approximately 2–3 mm) were fixed in 4% (v/v) glutaraldehyde in 0.1 M sodium phosphate buffer (PBS, pH 7.0) overnight and then washed three times with PBS. The samples were post-fixed in 1% (m/v) OsO_4 for 1 h and then washed again for three times with PBS. The samples were then dehydrated in a graded ethanol series (50, 70, 80, 95, 95, 100 and 100%, v/v) for 15 min each time. After dehydration, the samples were embedded in 50% Spurr's resin for 1 h, 75% Spurr's resin for 3 h and 100% Spurr's resin overnight. After heating the specimens at 70 °C for 16 h, ultra-thin sections (80 nm) were cut and mounted on copper grids for observation under a transmission electron microscope (JEM-1200EX, JEOL, Japan) at 60.0 kV.

Statistical analysis

The data are presented as the mean values ± SE. Every treatment was replicated three times. Statistical analysis was performed using one-way ANOVA and differences between the mean values were compared using the LSD test at $P \leq 0.05$.

Results

Plant growth and chlorophyll contents

At harvest, the appearance of the wheat seedlings differed between treatments; the seedlings grown under PEG were smaller than those cultivated without PEG (Table 1). The fresh biomass of shoots was significantly reduced under water stress alone compared to the control. The addition of Si alleviated the PEG-induced stress on wheat seedlings and increased root and shoot biomass but this increase was not significant. The exogenous application of Si alone increased root and shoot fresh weight up to 14 and 44% with respect to the control, respectively (Table 1). Si increased shoot and root fresh weight by 6 and 9%, respectively, under drought stress. Similarly, Si improved root and shoot dry weight by 12 and 4%, respectively, under PEG-induced water stress compared to PEG treatment alone. Further, the data showed that PEG stress significantly improved the contents of chlorophyll pigments compared to the control, but exogenously applied Si ameliorated PEG-induced water stress in wheat seedlings insignificantly (Table 2).

Water potential and water contents in wheat seedlings

Drought stress (PEG) alone significantly lowered water potential in the wheat plants. Si application considerably improved the water potential of the wheat seedlings under PEG stress, but this improvement was non-significant when compared to PEG treatment alone. Likewise, relative water content (RWC) and total water content (TWC) were significantly reduced under PEG treatment alone. Exogenously applied Si under PEG stress significantly improved RWC and TWC compared to PEG treatment alone. However, TWC was not significantly different in PEG + Si treatment from that in the control, which suggested a regulatory role of Si in maintaining RWC and TWC in stressed plants (Table 3).

PEG-induced oxidative stress

PEG induced considerable oxidative stress in the wheat plants. The H_2O_2 accumulation increased significantly in

Table 1 Effects of different silicon (1.0 mM) and PEG-6000 (20%) treatments on the biomass (per seedling) of wheat seedlings (*Triticum aestivum* L. cv. Longchun 8139)

Treatment	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
CK	4.682 ± 0.11 b[a]	0.493 ± 0.01 a	1.373 ± 0.07 a	0.082 ± 0.01 a
Si	6.722 ± 0.16 a	0.650 ± 0.06 a	1.566 ± 0.05 a	0.095 ± 0.01 a
PEG	2.983 ± 0.09 c	0.441 ± 0.06 a	1.285 ± 0.04 a	0.094 ± 0.01 a
PEG + Si	3.174 ± 0.14 bc	0.458 ± 0.01 a	1.400 ± 0.04 a	0.105 ± 0.01 a

[a] Within each column, means followed by the same small letters are not significantly different according to the LSD test at $P \leq 0.05$

Table 2 Effects of different silicon (1.0 mM) and PEG-6000 (20%) treatments on chlorophyll contents in the leaves of wheat seedlings (*Triticum aestivum* L. cv. Longchun 8139)

Treatment	Chlorophyll a (mg g^{-1} DW)	Chlorophyll b (mg g^{-1} DW)	Total Chlorophyll (mg g^{-1} DW)
CK	10.02 ± 0.15 bc[a]	2.97 ± 0.19 b	12.99 ± 0.17 c
Si	9.28 ± 0.14 c	2.70 ± 0.13 b	11.98 ± 0.07 c
PEG	13.41 ± 0.16 a	3.84 ± 0.05 a	17.25 ± 0.12 a
PEG + Si	11.84 ± 0.21 ab	3.35 ± 0.09 ab	15.20 ± 0.19 ab

[a] Within each column, means followed by the same small letters are not significantly different according to the LSD test at $P \leq 0.05$

Table 3 Effect of different silicon (1.0 mM) and PEG-6000 (20%) treatments on total water content (TWC), relative water content (RWC) and water potential (WP) in the leaves of wheat seedlings (*Triticum aestivum* L. cv. Longchun 8139)

Treatments	TWC (water Fw^{-1}, %)	RWC (%)	WP (MPa)
CK	86.08 ± 1.47 ab[a]	91.69 ± 2.47 a	−1.12 ± 0.12 a
Si	88.02 ± 0.92 a	97.72 ± 1.71 a	−1.03 ± 0.10 a
PEG	79.87 ± 1.63 c	53.72 ± 1.70 c	−2.20 ± 0.14 b
PEG + Si	84.53 ± 0.83 b	68.53 ± 2.72 b	−2.01 ± 0.19 b

[a] Within each column, means followed by the same small letters are not significantly different according to the LSD test at $P \leq 0.05$

Table 4 Effect of different silicon (1.0 mM) and PEG-6000 (20%) treatments on hydrogen peroxide and malondialdehyde contents and electrolyte leakage in the leaves of wheat seedlings (*Triticum aestivum* L. cv. Longchun 8139)

Treatment	Hydrogen peroxide (μmol g^{-1} DW)	Malondialdehyde (nmol g^{-1} DW)	Electrolyte leakage (%)
CK	391.76 ± 16.19 b[a]	100.9 ± 2.93 b	3.44 ± 0.17 c
Si	314.60 ± 14.30 c	93.0 ± 3.05 c	3.94 ± 0.18 bc
PEG	508.04 ± 18.33 a	110.2 ± 2.24 a	4.87 ± 0.12 a
PEG + Si	384.30 ± 17.23 b	105.6 ± 2.29 ab	4.23 ± 0.22 b

[a] Within each column, means followed by the same small letters are not significantly different according to the LSD test at $P \leq 0.05$

the PEG-treated wheat plants as compared to the control, while the addition of Si obviously decreased the content of H_2O_2 as compared to the PEG treatment alone (Table 4). After PEG exposure, higher MDA accumulation was observed in the wheat seedlings, while after PEG + Si treatment, a significant decline in MDA accumulation was noted compared to PEG treatment alone (Table 4). Under PEG-induced oxidative stress, membrane electrolyte leakage was increased by 42% over the control. Further, the application of Si significantly decreased electrolyte leakage in the stressed seedlings (Table 4).

Elemental contents

The effects of the various Si and PEG treatments on the contents of Si, Ca, K, Mg and Na are shown in Fig. 1. PEG treatment alone significantly decreased Si accumulation in the wheat plants. Interestingly, we found significantly higher Si content when using the Si + PEG treatment than that obtained using the PEG treatment alone. Si application showed a significant role in increasing leaf Si contents. The maximum accumulation of Si was recorded when applying Si alone (Fig. 1). In addition, PEG-induced osmotic stress

significantly reduced mineral (Mg and Na) leaf contents with respect to the control. However, the K contents were obviously increased under PEG-induced water stress, and the Ca content was only slightly lower than that in the control. The application of exogenous Si significantly improved the K content of plant shoots. Interestingly, water stress also significantly enhanced the K content of the shoots compared to the control. Moreover, the application of Si under PEG enhanced the accumulation of Mg (6%) and Na (24%) but decreased the contents of Ca (9%) and K (13%) compared to PEG treatment (Fig. 1).

Antioxidant enzyme activities

To observe the changes in antioxidant activities under different concentrations of Si and PEG, we measured changes in SOD, CAT, POD and APX activities in the wheat seedlings (Fig. 2). The results showed that CAT activities were significantly upregulated under PEG stress alone, while SOD, APX and POD activities were reduced. The addition of Si in combination with PEG significantly decreased the activity of POD (15%). However, the exogenous application of Si decreased APX activity (6%) but enhanced SOD (0.02%) and CAT (7%) activities in wheat plants under water stress (Fig. 2).

Contents of glutathione, ascorbic acid, proline and soluble sugar

The results showed that drought stress decreased leaf contents of GSH with respect to the control. However, Si application under PEG stress enhanced GSH contents, although this enhancement was non-significant compared to that in response to PEG treatment. Under PEG stress, we observed a significant decrease in AsA content in the wheat seedlings (14%) compared to the control, while exogenous Si significantly enhanced the AsA contents (42%) in the wheat plants compared to PEG treatment alone (Table 5). Moreover, the data showed that PEG induced proline accumulation of up to 22% in the wheat seedlings, while Si significantly decreased proline contents (6%) under PEG treatment. Furthermore, Si treatment improved (35%) soluble sugar contents under water stress compared to the control (Table 5).

Hormonal changes

To observe the influence of osmotic stress and Si on the contents of ABA, IAA and JA, we determined the levels of these three hormones in the wheat seedlings after different intervals under treatment (Fig. 3). The results showed that both PEG stress and Si application influenced endogenous hormones contents. Compared with the control, plants under PEG stress had higher contents of ABA, and these contents were increased as the time interval increased; the content of ABA reached the highest level, which was significantly higher than that obtained under any other treatment (Fig. 3a). However, the application of Si under PEG stress significantly enhanced ABA contents on the third day.

The accumulation of JA under PEG stress was increased up to the fifth day and significantly decreased thereafter until the end of the experiment (Fig. 3b). Similarly, JA accumulation was reduced in PEG + Si-treated plants, and this value was significantly lower than that for any other treatments at the end of the experiment. However, the application of Si alone significantly enhanced JA production compared to the control on the fifth and seventh days after treatment. Furthermore, IAA contents were also altered by the different combinations of PEG and Si (Fig. 3c). Under PEG treatment, a significant decrease in the IAA content was observed on the third day compared to the control; a progressive increase was noted on the fifth day, and a slight reduction in IAA content was observed again on the seventh day (Fig. 3c). However, when we applied Si under PEG stress, the maximum contents of IAA were found on the fifth day compared to other time points. These results indicate that Si application under water stress differently modulated the endogenous levels of ABA, IAA and JA in wheat plants compared to PEG stress treatment alone (Fig. 3).

Ultrastructural study of the chloroplasts and mitochondria

Ultrastructural changes in the chloroplasts and mitochondria of leaf mesophyll cells under different Si and PEG treatments are shown in Fig. 4. TEM chloroplast micrographs of wheat leaves for the control and 1.0 mM Si alone treatments showed smooth and typically shaped thylakoid membranes. All the organelles were remarkably differentiated and well developed. Well-developed chloroplasts containing starch grains could be observed under the 1.0 mM Si treatment, and the chloroplast was lens-shaped, similar to the control (Fig. 4a). However, under the treatment using PEG stress (20%) alone, the chloroplasts showed dissolved and spongy thylakoid membranes. Furthermore, chloroplast epistrophy was observed after the addition of Si to the PEG stress treatments. The chloroplasts contained well-developed and typically shaped thylakoid membranes, and clear cell membranes were seen in the micrographs of leaves under the Si + PEG treatment (Fig. 4a).

TEM micrographs of leaf mesophyll cell mitochondria under the control and 1.0 mM Si alone treatments showed well-developed ellipsoidal mitochondrial structure. The mitochondria showed the normal ultrastructure with

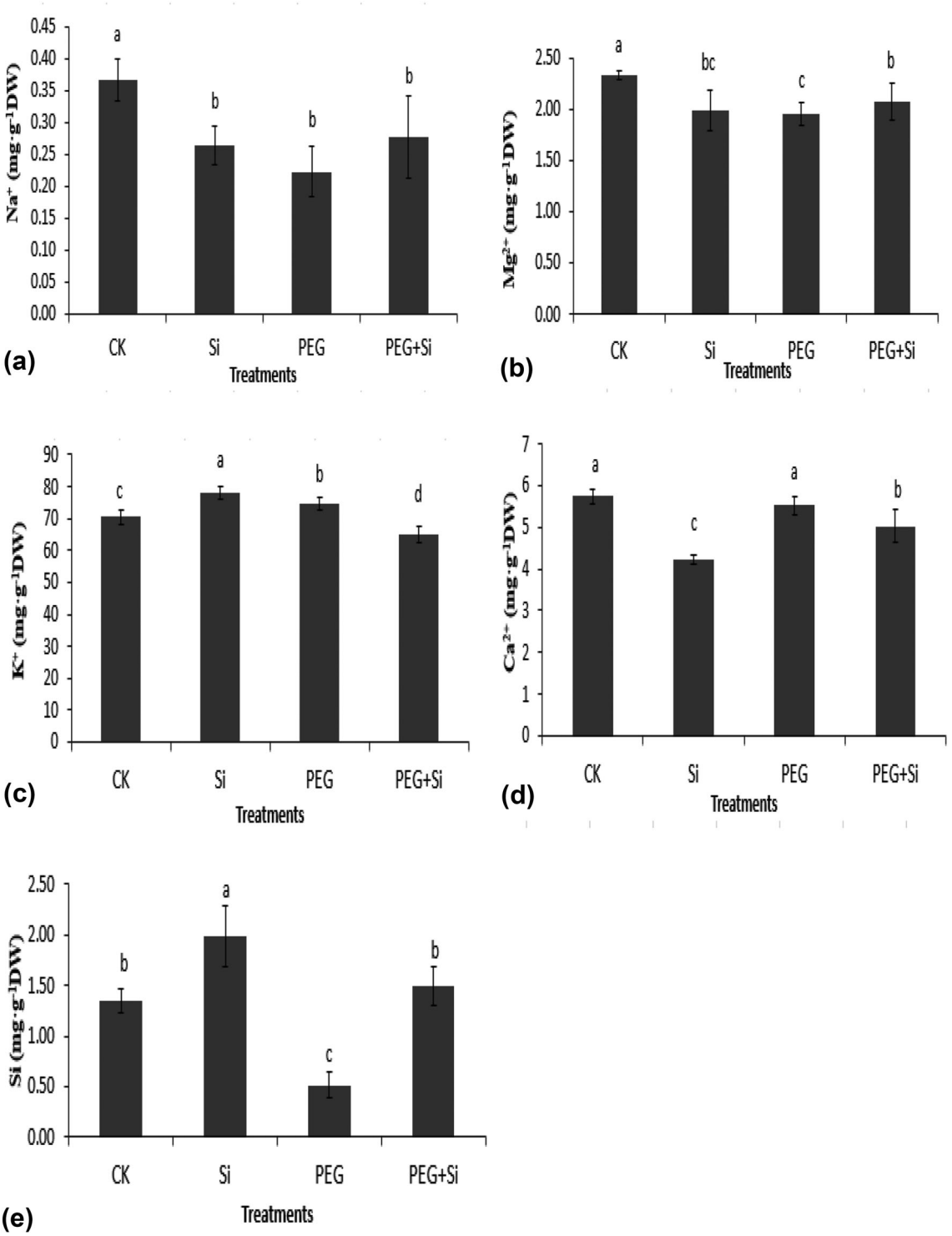

Fig. 1 Effect of different silicon (1.0 mM) and PEG-6000 (20%) treatments on Na (**a**), Mg (**b**), K (**c**), Ca (**d**) and Si (**e**) contents in wheat seedlings (*Triticum aestivum* L. cv. Longchun 8139). Values are means of three replicates ±SD. Columns marked with the same small letters are not significantly different according to the LSD test at $P \leq 0.05$

numerous cristae and dense matrix. Under PEG treatment, the mitochondria exhibited a dilated and swollen morphology with low matrix density (Fig. 4b). Moreover, mitochondria became more circular after treatment with PEG. However, the application of Si to plants under PEG stress improved mitochondrial morphology and exhibited a dense matrix; electron-transparent areas of different sizes and shapes were also observed in the micrograph (Fig. 4b).

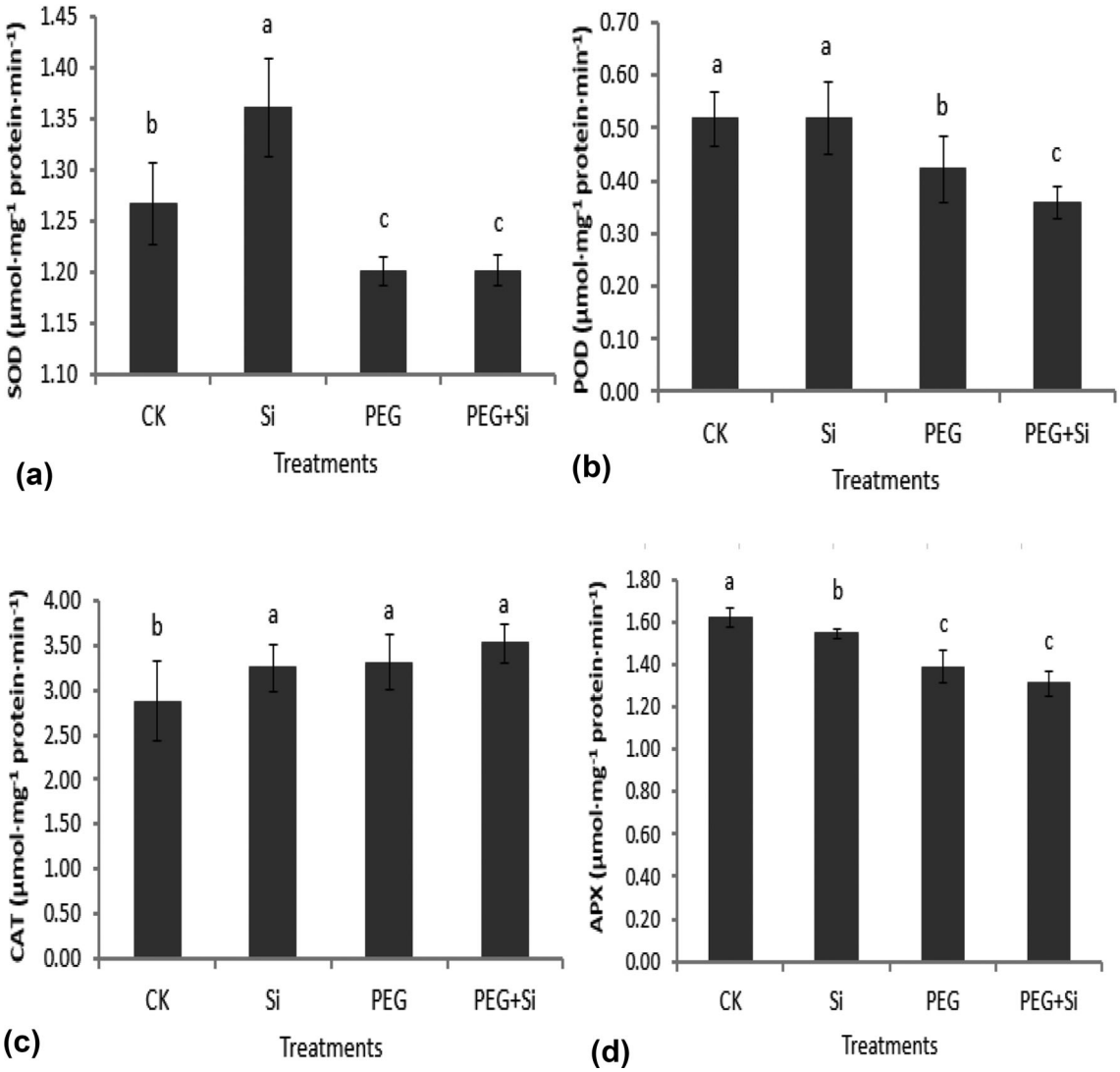

Fig. 2 Effect of different silicon (1.0 mM) and PEG-6000 (20%) treatments on biochemical changes in **a** superoxide dismutase (SOD), **b** guaiacol peroxidase (POD), (**c**) catalase(CAT), **d** ascorbate peroxidase (APX) in the leaves of wheat seedlings (*Triticum aestivum* L. cv. Longchun 8139). Values are mean ± SD ($n = 3$). Means followed by same small letters are not significantly different according to the LSD test at $P \leq 0.05$

Table 5 Effect of different silicon (1.0 mM) and PEG-6000 (20%) treatments on glutathione, ascorbic acid, proline and soluble sugar contents in the leaves of wheat seedlings (*Triticum aestivum* L. cv. Longchun 8139)

Treatment	Glutathione (μmol g^{-1} DW)	Ascorbic acid (μmol g^{-1} DW)	Proline (μg g^{-1} DW)	Soluble sugar (mg g^{-1} DW)
CK	906.3 ± 13.24 a[a]	28.9 ± 1.2 a	677.6 ± 25.10 b	62.5 ± 2.12 c
Si	903.3 ± 11.45 a	21.9 ± 1.5 b	635.5 ± 29.20 b	69.1 ± 2.08 c
PEG	720.2 ± 5.34 b	13.9 ± 1.76 c	827.3 ± 30.36 a	93.8 ± 3.10 b
PEG + Si	722.0 ± 8.45 b	19.7 ± 1.56 b	773.9 ± 25.84 a	127.0 ± 7.69 a

[a] Within each column, means followed by the same small letters are not significantly different according to the LSD test at $P \leq 0.05$

Discussion

Drought stress is a major abiotic stress factor that negatively affects plant metabolism, growth and yield (Shafiq et al. 2014). In this study, PEG-induced water stress significantly decreased shoot fresh biomass in wheat plants (Table 1). Similarly, a number of studies have shown the adverse effects of drought stress on plant fresh and dry biomass in many crop plants, including wheat (Pei et al. 2010), canola (Shafiq et al. 2014), and soybean (Shen et al.

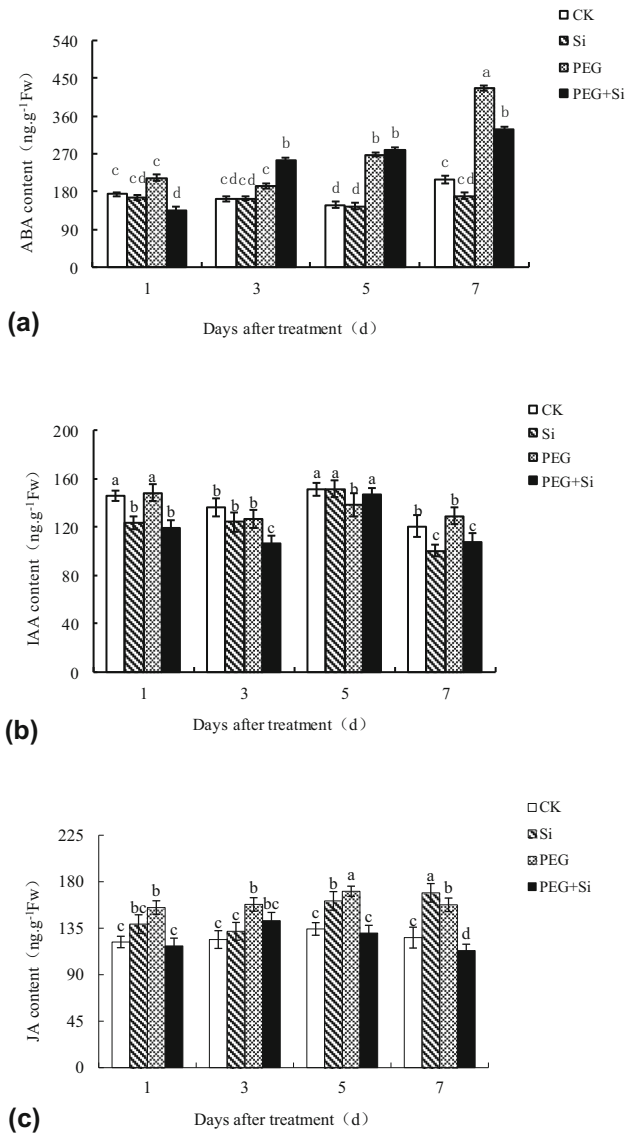

Fig. 3 Effect of different silicon (1.0 mM) and PEG-6000 (20%) treatments on the endogenous hormonal changes in **a** abscisic acid (ABA), **b** indoleacetic acidJA (IAA), **c** jasmonic acid (JA), **d** ascorbate peroxidase (APX) in the leaves of wheat seedlings (*Triticum aestivum* L. cv. Longchun 8139). Values are mean ± SD ($n = 3$). Means followed by same small letters are not significantly different according to the LSD test at $P \leq 0.05$

2010) due to alterations in various physio-chemical processes. However, the dry and fresh biomasses of wheat plants were increased by Si application under water stress (Table 1). The results also showed that drought stress enhanced chlorophyll content in the wheat leaves compared to the control plants. Furthermore, the application of exogenous Si decreased the chlorophyll contents under water stress (Table 2). Drought stress limits nutrient uptake from the soil and its transport to shoots, resulting in less nutrient availability (Farooq et al. 2009). Moreover, the application of Si under drought stress helps to balance mineral uptake and distribution in plants (Zhu and Gong 2014). The increased K and Ca levels seen under the combined treatment of Si and PEG can play an important role in osmotic adjustment (Ashraf et al. 2002), which might enhance PEG-induced drought stress tolerance in wheat plants.

Water management under drought stress is an important index for measuring plant growth and development. In the present study, leaf water status, as measured by the total water content and relative water content, was reduced under drought stress (Table 3). The application of Si under PEG alleviated drought stress significantly by improving the water status of wheat plants (Table 3). 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 (Gong et al. 2005; Pei et al. 2010). Other studies also demonstrated that Si application enhances leaf transpiration in wheat and sorghum plants under water stress (Gong et al. 2005). Therefore, one may conclude that the enhanced water potential of the wheat plant under PEG-induced drought stress may be due to the stimulation of water uptake and transportation (Pei et al. 2010).

Endogenous Si accumulation is involved in plant growth promotion and development through the regulation of complex and enigmatic phytohormones signaling networks that interact under different environmental stresses (Khan et al. 2011). The effect of AsA accumulation on the amelioration of oxidative stress under salt and heavy metal stresses has been examined by various researchers (Islam et al. 2014; Ali et al. 2014). Earlier studies showed that AsA can mitigate the adverse effects of drought in maize plants (Dolatabadian et al. 2009), similar to our results (Table 5). Thus, the silicon-mediated improvement of soluble sugar and AsA contents might play a role in protecting wheat plants under drought stress (Table 5).

In the present study, the contents of H_2O_2 and MDA as well as electrolyte leakage were much lower in Si-treated plants under PEG stress (Table 4). This decrease in oxidative stress may be due to the spontaneous dismutation of O_2^- into H_2O_2, causing the production and quenching of ROS by stimulating the antioxidant defence system. These results are consistent with findings that Si decreases oxidative stress in soybeans under drought stress (Shen et al. 2010). In plants, excess ROS is scavenged by enzymatic and non-enzymatic antioxidants (Sharma et al. 2012). In this experiment, a reduction of POD activity under Si application was correlated with a decrease in H_2O_2 production under water stress; the enhanced activity of POD and the malfunction of CAT and SOD contribute to a greater accumulation of H_2O_2 in wheat plants. These results are consistent with the findings of previous studies that Si application reduces H_2O_2 contents in wheat under

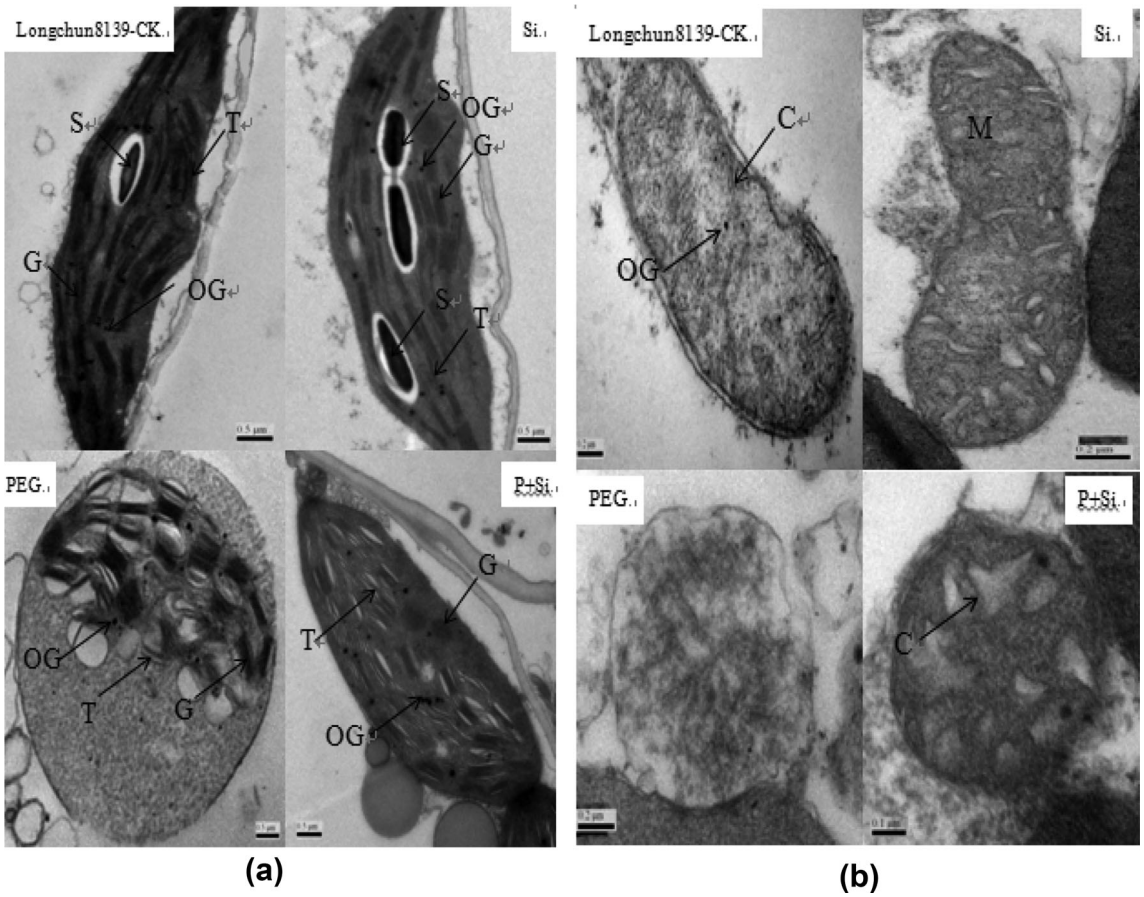

Fig. 4 Effect of different silicon (1.0 mM) and PEG-6000 (20%) treatments on the ultrastructural changes in **a** chloroplast and **b** mitochondrion of leaf mesophyll cells in wheat seedlings (*Triticum aestivum* L. cv. Longchun 8139). **a** Plate legend: *G* grana, *OG* osmophilic granule, *S* starch, and *T* stroma thylakoid. *Bar* 0.5 μm. **b** Plate legend: *C* cristae, *M* matrix, *OG* osmophilic granule. *Bar* 0.2 μm

stress conditions (Gunes et al. 2007; Kim et al. 2014a; Islam et al. 2014). Our results showed that the application of Si slightly increased the GSH content under drought stress (Table 5). A similar trend in the levels of GSH was found in sunflower and wheat plants under saline stress (Ali et al. 2013). The increase of GSH under Si application might reflect an increase in the activity of glutathione reductase in drought-stressed plants (Pei et al. 2010).

Similar to our results (Fig. 3a), Mahouachi et al. (2007) also found enhanced accumulation of ABA under drought stress. The increase in ABA content seen under stress reduces stomatal conductance, thereby affecting photosynthesis and chlorophyll synthesis (Kim et al. 2014b). The application of Si under PEG treatment decreased the ABA content compared to drought treatment alone, possibly due to the alleviation of drought stress in the plants as the result of improved RWC (Table 3) and the control of stomatal movement (Mahouachi et al. 2013; Kim et al. 2014c). Similarly, other researchers have demonstrated that the exogenous application of ABA promotes drought tolerance in various plants (Wang et al. 2003).

Similarly, Xie et al. (2003) also found a decrease in IAA contents in wheat leaves under water stress. The decrease in IAA contents observed in the present study (Fig. 3c) indicates that the biosynthesis of auxin may be suppressed by drought stress, helping plant adaptation to adverse environments (Du et al. 2013). ABA production and IAA inhibition in response to Si application might be related to the fact that these two hormones signaling pathways are closely linked. In the present study, higher levels of JA were observed under drought stress (Fig. 3b). Previous studies also demonstrated increased levels of JA under saline and drought stresses (Du et al. 2013; Moons et al.1997). Interestingly, under the combined treatment of Si and PEG, a remarkable reduction in JA content was found. This decline in JA might have occurred because the plants were less affected by drought stress or because the wheat plants utilized less α-linolenic acid for JA synthesis. α-linolenic acid is considered a substrate for lipoxygenase and the biosynthesis of JA and is produced by stress-activated lipases from plant membrane lipids. Similar JA behavior has been observed in rice plants (Kim et al.

2014b). The data obtained indicate that the biosyntheses of ABA, JA and IAA were differentially regulated under the different treatment combinations, and the balance between these hormones is critical for plant development under drought stress (Fig. 3). Moreover, the investigation of molecular mechanisms as a means to study the signaling pathways of endogenous hormones and environmental factors is required for a better understanding of the hormone balance and adaptive capacity of plants under stress conditions (Liang et al. 2013; Yang et al. 2012; Xia et al. 2015, 2016).

Previous studies demonstrated that Si application was obviously able to alleviate the damage caused to chloroplasts under stress in maize (Vaculik et al. 2015) and tomato leaves (Cao et al. 2015). The results were consistent with our findings revealed by the TEM micrographs (Fig. 4). In our study, the swelling of chloroplasts, an increased number of osmophilic granules, and dissolved and spongy thylakoid membranes were seen under PEG treatment only. The chloroplast ultrastructure recovered showing well-developed and typically shaped thylakoid membranes when the plants were treated with Si and PEG, indicating that Si might play a role in alleviating PEG-induced osmotic stress. In contrast, under PEG treatment, the mitochondria exhibited dilated and swollen morphology with low matrix density, and Si application under PEG stress improved the morphology of all mitochondria, which showed a dense matrix. Therefore, Si alleviated the ultrastructural damages as reflected by the improved chloroplast and mitochondria structures.

In conclusion, the Si-mediated changes in the morphological and physiological attributes were closely related to the alterations in hormone concentration. The plants to which Si was applied exhibited decreased IAA and JA levels and increased ABA contents, RWC and antioxidant enzyme activities (POD and APX) under water stress conditions. These Si-mediated changes resulted in a new balance of endogenous hormones and physiology, thereby enhancing the tolerance of the wheat plants against drought stress. This new balance of endogenous hormones in the plants to which Si had been applied and the enhanced growth suggests that endogenous levels of ABA, IAA and JA are differentially regulated by drought and Si in wheat, thus showing diverse roles of these hormones under different stress conditions.

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Compliance with ethical standards

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

References

Al-aghabary K, Zhu ZJ, Shi QH (2004) Influence of silicon supply on chlorophyll content, chlorophyll fluorescence, and antioxidative enzyme activities in tomato plants under salt stress. J Plant Nutr 27:2101–2115

Ali B, Tao QJ, Zhou YF, Gill RA, Ali S, Rafiq MT, Xu L, Zhou WJ (2013) 5-Aminolevolinic acid mitigates the cadmium-induced changes in *Brassica napus* as revealed by the biochemical and ultra-structural evaluation of roots. Ecotoxicol Environ Safe 92:271–280

Ali B, Xu X, Gill RA, Yang S, Ali S, Tahir M, Zhou WJ (2014) Promotive role of 5-aminolevulinic acid on mineral nutrients and antioxidative defense system under lead toxicity in *Brassica napus*. Ind Crops Prod 52:617–626

Anderson ME (1985) Determination of glutathione and glutathione disulfides in biological samples. Meth Enzymol 113:548–570

Ashraf M, Karim F, Rasul E (2002) Interactive effects of gibberellic acid (GA3) and salt stress on growth, ion accumulation and photosynthetic capacity of two spring wheat (*Triticum aestivum* L.) cultivars differing in salt tolerance. Plant Growth Regul 36:49–59

Cakmak I, Strboe D, Marschner H (1993) Activity of hydrogen peroxide scavenging enzymes in germinating wheat seeds. J Exp Bot 44:127–132

Cao BL, Ma Q, Zhao Q, Wang L, Xu K (2015) Effects of silicon on absorbed light allocation, antioxidant enzymes and ultrastructure of chloroplasts in tomato leaves under simulated drought stress. Sci Hortic 194:53–62

Dolatabadian A, Modarres Sanavy SAM, Sharifi M (2009) Alleviation of water deficit stress effects by foliar application of ascorbic acid on *Zea mays* L. J Agron Crop Sci 195:347–355

Du H, Wu N, Chang Y, Li X, Xiao J, Xiong L (2013) Carotenoid deficiency impairs ABA and IAA biosynthesis and differentially affects drought and cold tolerance in rice. Plant Mol Biol 83(4–5):475–488

Epstein E (1999) Silicon. Annu Rev Plant Physiol Plant Mol Biol 50:641–664

Eraslan F, Inal A, Pilbeam DJ, Gunes A (2008) Interactive effects of salicylic acid and silicon on oxidative damage and antioxidant activity in spinach (*Spinacia oleracea* L. cv. Matador) grown under boron toxicity and salinity. Plant Growth Regul 55:207–219

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. Agron Sustain Dev 29:185–212

Gao X, Zou C, Wang L, Zhang F (2004) Silicon improves water use efficiency in maize plants. J Plant Nutr 27(8):1457–1470

Gao X, Zou C, Wang L, Zhang F (2006) Silicon decreases transpiration rate and conductance from stomata of maize plants. J Plant Nutr 29:1637–1647

Gong HJ, Zhu XY, Chen KM, Wang SM, Zhang CL (2005) Silicon alleviates oxidative damage of wheat plants in pots under drought. Plant Sci 169:313–321

Gong HJ, Randall DP, Flowers TJ (2006) Silicon deposition in the root reduces sodium uptake in rice (*Oryza sativa* L.) seedlings by reducing bypass flow. Plant Cell Environ 29:1970–1979

Gunes A, Inal A, Bagci EG, Pilbeam DJ (2007) Silicon-mediated changes of some physiological and enzymatic parameters symptomatic for oxidative stress in spinach and tomato grown in sodic-B toxic soil. Plant Soil 290:103–114

Hattori T, Inanaga S, Araki H, An P, Morita S, Luxova M, Lux A (2005) Application of silicon enhanced drought tolerance in *Sorghum bicolour*. Physiol Plant 123:459–466

Hattori T, Sonobe K, Inanaga S, An P, Tsuji W, Araki H, Eneji AE, Morita S (2007) Short term stomatal responses to light intensity changes and osmotic stress in sorghum seedlings raised with and without silicon. Environ Exp Bot 60:177–182

Inanaga S, Okasaka A (1996) Calcium and silicon binding compounds in cell walls of rice shoots. Soil Sci Plant Nutr 41:103–110

Inskeep WP, Bloom PB (1985) Extinction coefficients of chlorophyll a and chlorophyll b in *N*, *N*-dimethyl formamide and 80% acetone. Plant Physiol 77:482–483

Iqbal N, Umar S, Khan NA, Khan MIR (2014) A new perspective of phytohormones in salinity tolerance: regulation of proline metabolism. Environ Exp Bot 100:34–42

Islam F, Yasmeen T, Ali Q, Ali S, Arif MS, Hussain S, Rizvi H (2014) Influence of *Pseudomonas aeruginosa* as PGPR on oxidative stress tolerance in wheat under Zn stress. Ecotoxic Environ Safe 104:285–293

Khan TA, Mazid M, Mohammad F (2011) A review of ascorbic acid potentialities against oxidative stress induced in plants. J Agrobiol 28(2):97–111

Kim YH, Khan AL, Waqas M, Shim JK, Kim DH, Lee KY, Lee IJ (2014a) Silicon application to rice root zone influenced the phytohormonal and antioxidant responses under salinity stress. J Plant Growth Regul 33(2):137–149

Kim YH, Khan AL, Kim DH, Lee SY, Kim KM, Waqas M, Jung HY, Shin JH, Kim JG, Lee IJ (2014b) Silicon mitigates heavy metal stress by regulating P-type heavy metal ATPases, *Oryza sativa* low silicon genes, and endogenous phytohormones. BMC Plant Biol 14(1):13

Kim JG, Back K, Lee HY, Lee HJ, Phung TH, Grimm B, Jung S (2014c) Increased expression of Fe-chelatase leads to increased metabolic flux into heme and confers protection against photodynamically induced oxidative stress. Plant Mol Biol 86(3):271–287

Law MY, Charles SA, Halliwell B (1983) Glutathione and ascorbic acid in spinach (*Spinacea oleracea*) chloroplasts. The effect of hydrogen peroxide and of paraquat. Biochem J 210:899–903

Liang Y (1999) Effects of silicon on enzyme activity and sodium, potassium and calcium concentration in barley under salt stress. Plant Soil 209:217–224

Liang Y, Chen Q, Liu Q, Zhang W, Ding R (2003) Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). J Plant Physiol 160:157–1164

Liang Y, Zhang W, Chen Q, Ding R (2005) Effects of silicon on H^+-ATPase and H^+-PPase activity, fatty acid composition and fluidity of tonoplast vesicles from roots of salt-stressed barley (*Hordeum vulgare* L.). Environ Exp Bot 53:29–37

Liang ZS, Ma YN, Xu T, Cui BM, Liu Y, Guo ZX, Yang DF (2013) Effects of abscisic acid, gibberellin, ethylene and their interactions on production of phenolic acids in *Salvia miltiorrhiza* Bunge hairy roots. PLoS One 8(9):e72806

Mahouachi J, Arbona V, Gomez-Cadenas A (2007) Hormonal changes in papaya seedlings subjected to progressive water stress and re-watering. Plant Growth Regul 53(1):43–51

Mahouachi J, Fernandez-Galvan D, Gomez-Cadenas A (2013) Abscisic acid, indole-3-acetic acid and mineral—nutrient changes induced by drought and salinity in longan (*Dimocarpus longan* Lour.) plants. Acta Physiol Plant 35(11):3137–3146

Matoh T, Kairusmee P, Takahashi E (1986) Salt-induced damage to rice plants and alleviation effect of silicate. Soil Sci Plant Nutr 32:295–304

Mera MU, Beveridge TJ (1993) Mechanism of silicate binding to the bacterial cell wall in *Bacillus subtilis*. J Bacteriol 175:1936–1945

Momoh EJJ, Zhou WJ (2001) Growth and yield responses to plant density and stage of transplanting in winter oilseed rape (*Brassica napus* L.). J Agron Crop Sci 186:253–259

Moons A, Prinsen E, Bauw G, Van Montagu M (1997) Antagonistic effects of abscisic acid and jasmonates on salt stress- inducible transcripts in rice roots. Plant Cell 9:2243–2259

Pei ZF, Ming DF, Liu D, Wan GL, Geng XX, Gong HJ, Zhou WJ (2010) Silicon improves the tolerance to water-deficit stress induced by polyethylene glycol in wheat (*Triticum aestivum* L.) seedlings. J Plant Growth Regul 29(1):106–115

Saqib M, Zorb C, Schubert S (2008) Silicon-mediated improvement in the salt resistance of wheat (*Triticum aestivum*) results from increased sodium exclusion and resistance to oxidative stress. Funct Plant Biol 35:633–639

Shafiq S, Akram NA, Ashraf M, Arshad A (2014) Synergistic effects of drought and ascorbic acid on growth, mineral nutrients and oxidative defense system in canola (*Brassica napus* L.) plants. Acta Physiol Plant 36(6):1539–1553

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot. doi:10.1155/2012/217037

Shen XF, Zhou YY, Duan LS, Li ZH, Eneji AE, Li JM (2010) Silicon effects on photosynthesis and antioxidant parameters of soybean seedlings under drought and ultraviolet-B radiation. J Plant Physiol 167(15):1248–1252

Smienoff N (1998) Plant resistance to environmental stress. Curr Opin Biotechnol 9:214–219

Vaculik M, Pavlovic A, Lux A (2015) Silicon alleviates cadmium toxicity by enhanced photosynthetic rate and modified bundle sheath's cell chloroplasts ultrastructure in maize. Ecotoxicol Environ Safe 120:66–73

Wang ZL, Huang BR, Xu QZ (2003) Effects of abscisic acid on drought responses of Kentucky bluegrass. J Am Soc Hortic Sci 128:36–41

Wang SK, Bai YH, Shen CJ, Wu YR, Zhang SN, Jiang DA, Guilfoyle TJ, Chen M, Qi YH (2010) Auxin-related gene families in abiotic stress response in *Sorghum bicolor*. Funct Integr Genom 10(4):533–546

Wood AJ, Saneoka H, Rhodes D, Joly RJ, Gildsbrough PB (1996) Betaine aldehyde dehydrogenase in sorghum. Molecular cloning and expression of two related genes. Plant Physiol 110:1301–1308

Xia PG, Li JZ, Wang RL, Zhang Y, Guo HB, Yan XJ, Liu Y, Liang ZS (2015) Comparative study on volatile oils of four *Panax, genus* species in Southeast Asia by gas chromatography–mass spectrometry. Ind Crop Prod 74:478–484

Xia PG, Guo HB, Zhao HG, Jie J, Deyholos MK, Yan XJ, Yan L, Liang ZS (2016) Optimal fertilizer application for *Panax notoginseng* and effect of soil water on root rot disease and saponin contents. J Ginseng Res 40(1):38–46

Xie ZJ, Jiang D, Cao WX, Dai TB, Jing Q (2003) Relationships of endogenous plant hormones to accumulation of grain protein and starch in winter wheat under different post-anthesis soil water statuses. Plant Growth Regul 41(2):117–127

Yang DF, Ma PD, Liang X, Liang ZS, Zhang MX, Shen S, Liu HY, Liu Y (2012) Metabolic profiles and cDNA-AFLP analysis of *Salvia miltiorrhiza* and *Salvia castanea* Diel f. *tomentosa* Stib. PLoS One 7(1):e29678

Yeo AR, Flowers SA, Rao G, Welfare K, Senanayake N, Flowers TJ (1999) Silicon reduces sodium uptake in rice (*Oryza sativa* L.) in saline conditions and this is accounted for by a reduction in the transpirational bypass flow. Plant Cell Environ 22:559–565

Zhang WF, Zhang F, Raziuddin R, Gong HJ, Yang ZM, Lu L, Ye QF, Zhou WJ (2008) Effects of 5-aminolevulinic acid on oilseed rape seedling growth under herbicide toxicity stress. J Plant Growth Regul 27:159–169

Zhou W, Zhao D, Lin X (1997) Effects of waterlogging on nitrogen accumulation and alleviation of waterlogging damage by application of nitrogen fertilizer and mixtalol in winter rape (*Brassica napus* L.). J Plant Growth Regul 16:47–53

Zhu YX, Gong HJ (2014) Beneficial effects of silicon on salt and drought tolerance in plants. Agron Sustain Dev 34(2):455–472

Zhu Z, Wei G, Li J, Qian Q, Yu J (2004) Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). Plant Sci 167:527–533

Zhu X, Gong H, Chen G, Wang S, Zhang C (2005) Different solute levels in two spring wheat cultivars induced by progressive field water stress at different developmental stages. J Arid Environ 62:1–14