Silicon Improves the Tolerance to Water-Deficit Stress Induced by Polyethylene Glycol in Wheat (*Triticum aestivum* L.) Seedlings

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Abstract Drought stress usually causes a serious yield reduction in wheat production. Silicon (Si) has been reported to be able to alleviate drought stress damage; however, the mechanism is still poorly understood. In this article, the effects of Si (as sodium silicate) on some parameters related to oxidative damage, proline, soluble sugar, and inorganic ions in the leaves of wheat under 20% (w/v) polyethylene glycol (PEG-6000) simulative drought stress are investigated. PEG stress depressed the growth of shoot and root and decreased leaf water potential and chlorophyll concentration. Addition of 1.0 mM Si could partially improve the growth of shoot (but not root) and increase the leaf chlorophyll concentrations of stressed plants. Inclusion of Si in culture solution also maintained leaf water potential of stressed plants at the same level as that of the control plants. PEG stress induced significant accumulation of leaf hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) as well as an increase in electrolyte leakage, which were all decreased by added silicon. These results suggest that stress-induced membrane lipid peroxidation could be partly alleviated by added silicon. Moreover, the results were also supported by the observation that PEG stress-induced decrease in glutathione concentration in the leaves was reversed by added silicon. The proline concentration in the leaves was markedly increased under PEG stress, whereas added silicon partially reversed this. PEG stress decreased the leaf soluble sugar concentration. There were significant negative regressions between proline concentration and both shoot dry weight and leaf chlorophyll concentrations, whereas there were positive regressions between the proline concentration and both H_2O_2 and MDA concentrations in the leaves, supporting the view that proline accumulation is a symptom of stress damage rather than stress tolerance. Addition of Si obviously increased Si accumulation in the shoot. Analyses of Na, Mg, K, and Ca showed no accumulation of these ions in the shoot (on the basis of per tissue dry weight) under water stress, and added Si even decreased their concentrations. These results suggest that under short-term PEG-induced water stress conditions (1 week), antioxidant defense, rather than osmotic adjustment, contributed to the improved wheat growth by Si.

Keywords Antioxidant defense · Drought stress · Osmotic adjustment · Silicon · Wheat (*Triticum aestivum* L.)

Introduction

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

Silicon (Si) is the second most abundant element in the earth's crust (Epstein 1999). It is in plants, especially in grasses, in amounts equivalent to those of macronutrient

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elements such as calcium, magnesium, and phosphorus (Epstein 1999). Si has been shown to be able to promote the growth and development of plants under abiotic and biotic stresses, including water stress (Epstein 1999; Gong and others 2005, 2008; Hattori and others 2005). Therefore, application of Si may be one way to improve the growth and development, and thus the yield of wheat, especially in arid regions.

Silicon taken up by plants is deposited mainly in the cell wall (Epstein 1999). The formation of Si-organic complexes was reported in rice shoots (Inanaga and Okasaka 1996). Mera and Beveridge (1993) suggested that Si can modify the cation-binding properties of cell walls. Different mechanisms for Si-mediated stress alleviation have been proposed by researchers. Si deposition in leaves was reported to be able to decrease transpiration (Matoh and others 1986), therefore alleviating salt stress. In rice (Oryza sativa L.), Si alleviated salt stress by reducing Na^+ uptake through partial blockage of the transpirational bypass flow, a major pathway of Na^+ uptake in this species (Yeo and others 1999; Gong and others 2006). The most widely reported mechanism was that Si might decrease the oxidative damage in plants subjected to environmental stresses (Saqib and others 2008). Gunes and others (2007) reported that Si alleviated sodicity and boron toxicity in spinach (Spinacia oleracea L.) and tomato (Lycopersicon esculentum Mill.) plants by reducing oxidative membrane damage. Reduced oxidative damage due to the addition of Si under saline conditions was also reported in barley (Liang 1999; Liang and others 2003, 2005) and cucumber (Zhu and others 2004).

Silicon has a positive effect on plants under drought stress. In maize, the addition of Si increased water use efficiency by reducing leaf transpiration and the water flow rate in the xylem vessel (Gao and others 2004, 2006). Hattori and others (2005, 2007) suggested that Si could facilitate water uptake and transport in sorghum [Sorghum bicolor (L.) Moench] in drought conditions. In potted wheat, Si alleviated oxidative stress by regulating the activities of antioxidant enzymes under drought (Gong and others 2005). However, the effect of Si on the concentrations of antioxidants glutathione (GSH) and ascorbic acid (AsA) has not been investigated.

In addition to antioxidant defense, plants can also adapt to water stress by changing solute levels so that turgor and hence physiological activity are maintained at low leaf water potentials (Zhu and others 2005). It has been suggested that accumulation of solutes in the stressed leaves contributes to dehydration tolerance (Wood and others 1996; Smienoff 1998). The impact of added Si on inorganic ions and organic compound accumulation remains unknown.

In this study we investigated the effects of Si on antioxidant concentration, inorganic ions, and sugar and proline accumulation in wheat seedlings under polyethylene glycol (PEG)-induced water stress. The results could contribute to an understanding of the mechanism(s) of a silicon-induced increase in drought tolerance of wheat plants.

Materials and Methods

Plant Culture and Treatments

The experiments were conducted in a glasshouse of the College of Agriculture and Biotechnology of Zhejiang University (Hangzhou, China). Seeds of spring wheat (Triticum aestivum L. cv. Dingxi 24) were sown on a net floating in culture solution (pH 5.6) at room temperature after sterilization of the seeds' surface with 1% sodium hypochlorite for 10 min. The nutrient solution was 0.25 Hoagland solution and had the following composition: 1.5 mM KNO_3, 0.25 mM $NH_4H_2PO_4$, 0.5 mM $MgSO_4 \cdot 7H_2O$, 1.25 mM $CaCl_2 \cdot 2H_2O$, 23.125 μM H_3BO_3, 4.575 μM $MnCl_2 \cdot 4H_2O$, 0.3825 μM $ZnSO_4 \cdot 7H_2O$, 0.16 μM $CuSO_4 \cdot 5H_2O$, 0.175 μM $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and 0.0144 μM FeNaEDTA. Seven-day-old seedlings were transplanted into plastic buckets containing the same culture solution that was aerated continuously with an air pump (electromagnetic air pump AC0-007, 0.04 MPa, 90 L min^{-1}, Sunsun Industry, Zhejiang, China). The seedlings were grown under a light intensity in the range of about 200-450 μmol m^{-2} s^{-1}. The temperature was in the range of 20-30°C. The relative humidity was approximately 55%. Each treatment was replicated three times (the biological replicates were three). The solution was renewed every 5 days. From the 22nd day, the solution was changed to 0.5 Hoagland nutrient solution.

Silicon and PEG treatments were imposed simultaneously 1 month after transplant and lasted for 7 days. Silicon was introduced by the addition of Na_2SiO_4 (pH was adjusted back to 5.6 with diluted H_2SO_4, Na was supplemented in control groups by the addition of Na_2SO_4), and drought conditions were simulated by the addition of PEG-6000 of 20% (w/v) strength to achieve drought (osmotic) stress levels of approximately −0.5 MPa. The experimental design consisted of four treatments: control (CK), 20% (w/v) PEG-6000 (PEG), 1.0 mM silicon (Si), and 20% (w/v) PEG-6000 plus 1.0 mM silicon (Si + PEG). Measurements were made on the recent fully expanded leaves except where indicated, with three technical replicates.

Water Potential

The first fully expanded leaf was used to determine the water potential (WP). A piece of fresh leaf approximately

0.5–1.0 cm was cut down and used to determine WP with a vapor pressure osmometer (Wescor Inc., Logan, UT, USA).

Chlorophyll Determination

Chlorophyll was eluted from two 1-cm-diameter leaf discs per leaf by submerging the discs in 2 ml of a mixture of absolute alcohol and acetone (v:v = 5:5 v/v) in the dark for at least 72 h. Absorbance of extract solutions was read at 647 and 664 nm with a UV-Vis spectrophotometer (UV-2450, Shimadzu Corporation, Japan) and used to calculate leaf chlorophyll concentrations using equations by Inskeep and Bloom (1985).

Determination of Hydrogen Peroxide, Malondialdehyde, and Membrane Electrolyte Leakage

Hydrogen peroxide (H_2O_2) in the leaves was determined by the method described by Khalid (2004) with minor modification. Fresh leaf tissue samples were ground in cold acetone (g:ml = 1:10) and centrifuged at 3000 g for 10 min. One milliliter of the supernatant was mixed with 0.1 ml titanium reagent (20% $TiCl_4$ in concentrated HCl) and 0.2 ml of 17 M ammonia solution and then centrifuged at 3000 g for 10 min. The precipitate was washed five times with acetone by resuspension, drained, and dissolved in 3 ml of 1 M H_2SO_4. The absorbance of the solution was measured at 410 nm against blanks, which had been prepared similarly but without plant tissue.

Malondialdehyde (MDA) concentration in the leaves was determined according to the method of Zhang and others (2008b) with minor modification. Fresh plant leaves (0.2 g) were homogenized and extracted in 10 ml of 0.5% (w/v) thiobarbituric acid (TBA) made in 5% (w/v) trichloroacetic acid (TCA). The extract was heated at 95°C for 15 min and then quickly cooled on ice. After centrifuging at 5000 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 an extinction coefficient of 155 mM cm^{-1}.

Membrane permeability, which reflects membrane damage, was measured by an electrical conductivity method described by Yan and others (1996). Nine fresh leaf discs (diameter = 10 mm) from a recently fully expanded leaf were used to assess the electrolyte leakage percentage. Samples were washed three times with deionized water to remove surface-adhered electrolytes. Leaf discs were placed in closed vials containing 10 ml deionized water under a vacuum (via a vacuum pump) for 10 min and then surged for 1 h. Electrical conductivity of the solution (L_t) was determined at 25°C. Samples were then incubated in boiling water for 10 min and the final

electrical conductivity (L_0) was obtained after equilibration at 25°C. Electrolyte leakage was defined by the formula: electrolyte leakage percentage (%) = (L_t/L_0) $\times$ 100.

Determination of Reduced Glutathione and Ascorbic Acid

Fresh leaves (0.5 g) were homogenized in 2 ml of 5% (w:v) sulfosalicylic acid under cold conditions. The homogenate was centrifuged at 10,000 g for 10 min and the resultant supernatant was used for determination of glutathione (GSH) and ascorbic acid (AsA).

GSH was measured using the method of Anderson (1985). The supernatant (0.5 ml) was mixed with 0.6 ml of 100 mM phosphate buffer (pH 7.0) and 40 μl of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB). After 2 min, the absorbance was read at 412 nm on a UV–Vis spectrophotometer, and the GSH content was determined using a standard curve.

AsA was determined following the method of Law and others (1983). To 0.2 ml of the supernatant was added 1.4 ml of 75 mM NaH_2PO_4 (pH 7.4), 0.4 ml of 10% (w/v) trichloroacetic acid, 0.4 ml of 44% H_3PO_4, 0.4 ml of 4% (w/v) 2,2-dipyridine (dissolved with 70% ethanol), and 0.2 ml of 3% (w/v) $FeCl_3$. Then the mixture was incubated at 37°C for 1 h. The absorbance of the mixture was read at 525 nm, and the AsA content was determined using a standard curve.

Determination of Proline and Soluble Sugar

Free proline was extracted according to the method of Bates and others (1973). Dry leaf samples were extracted in 3% (w/v) aqueous sulfosalicylic acid in boiling water for 10 min. The extract was filtered and the filtrate was mixed with equal volumes of glacial acetic acid and ninhydrin reagent (1.25 g ninhydrin, 30 ml glacial acetic acid, 20 ml of 6 M H_3PO_4) and incubated for 40 min in boiling water. The reaction was stopped by placing the test tubes in cold water. The samples were rigorously mixed with 3 ml toluene. The light absorbance of the toluene phase was estimated at 520 nm on a UV-Vis spectrophotometer. The proline concentration was determined using a standard curve.

Soluble sugar was measured by the spectrophotometric method described by Zhang and others (2006). Dry samples of wheat leaves were boiled in distilled water for 30 min. The extract was filtered through two layers of cheesecloth. The filtrate (0.5 ml) was mixed with 1.5 ml distilled water and 1 ml of 9% phenol, and then 5 ml H_2SO_4. Tubes with this mixture were left at room temperature for 30 min. Color change was estimated using a UV-Vis spectrophotometer at 485 nm. The soluble sugar concentration was determined using a standard curve.

Analysis of Mineral Nutrients and Silicon

The plant samples (shoots) were dried at 70°C for 48 h and ground with a sample grinder (model Retsch MM301, Haan, Germany). The powder was digested in 2.0 ml HNO_3 and 0.5 ml H_2O_2 at 160°C for 6 h. The digestion solutions were then allowed to cool to room temperature and adjusted to a final volume of 25 ml with double deionized water.

Na, Mg, K, and Ca concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS, model 7500a, Agilent Technologies, Palo Alto, CA, USA) according to the method of Zhang and others (2008a).

Silicon in shoot tissues was determined by the blue silico-molybdate procedure as described by Figen and others (2008). To the digestion solutions was then added 0.08 M H_2SO_4 and 40% HF. Color development was accomplished by adding 1.5 ml of this solution to 1.5 ml of the reagent mixture of 0.08 M H_2SO_4 and ammonium molybdate (20 g L^{-1}), then 1.5 ml of 0.25 M tartaric acid; finally, 1.5 ml of 0.2 M ascorbic acid was added. After mixing the tubes, absorbance at 811 nm was measured. The silicon concentration was determined using a standard curve.

Statistical Analysis

Statistical analysis was conducted using Genstat 10.0 and the analysis of variance (ANOVA) was followed by Fisher's protected LSD test to identify homogeneous groups within the means. Significant differences among treatments were considered at $p \leq 0.05$.

Results

Effects of PEG and Si on the Growth and Leaf Water Potential of Wheat Seedlings

One week of PEG-induced water stress significantly decreased the growth of wheat seedlings. Compared with the control, in the absence of added silicon the shoot fresh and dry weights under water stress were decreased by 63 and 51%, respectively, whereas these levels were decreased by 38 and 22%, respectively, in the presence of added silicon (Table 1). Our results showed no improvement of root growth in stressed plants by adding Si. Added silicon did not change the chlorophyll concentrations of leaves in non-stress conditions. However, water stress significantly decreased the chlorophyll levels, and these obviously improved in the presence of added silicon (Table 2).

The leaf water potential was decreased in plants treated with PEG alone. However, application of Si significantly improved leaf water potential of PEG-stressed plants and

Table 1 Effects of silicon (1.0 mM) and PEG-6000 (20%) treatments on the biomass (per seedling) of wheat seedlings

Treatment[a]	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
CK	2.404 a[b]	0.283 a	0.659 a	0.044 a
Si	2.280 a	0.267 a	0.555 a	0.036 a
PEG	0.888 b	0.140 b	0.335 b	0.031 a
PEG + Si	1.494 b	0.221 a	0.331 b	0.035 a

[a] The treatments lasted 1 week

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

Table 2 Effects of silicon (1.0 mM) and PEG-6000 (20%) treatments on the chlorophyll contents in leaves of wheat seedlings

Treatment[a]	Chlorophyll a (mg g^{-1}DW)	Chlorophyll b (mg g^{-1}DW)	Total chlorophyll (mg g^{-1}DW)
CK	9.32 a[b]	3.04 a	12.35 a
Si	8.35 a	2.75 a	11.10 a
PEG	5.63 b	1.96 b	7.59 b
PEG + Si	8.14 a	2.73 a	10.87 a

[a] The treatments lasted 1 week, and means are given ($n = 3$)

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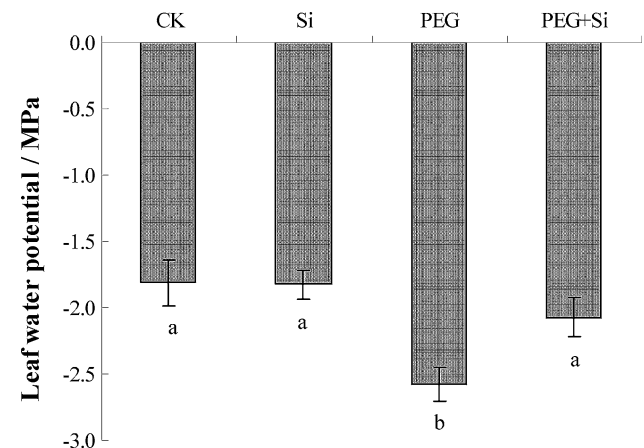

Fig. 1 Effects of silicon (1.0 mM) and PEG-6000 (20%) treatments on leaf water potential. The means followed by the same small letters are not significantly different by the LSD test at $P \leq 0.05$. Vertical bars represent SE

maintained it at the same level as that of the control plants (Fig. 1).

Effect of Si on Hydrogen Peroxide Concentration, Malondialdehyde Level, and Membrane Electrolyte Leakage of Leaves

Table 3 shows the effect of water stress and Si treatment on hydrogen peroxide (H_2O_2) and malondialdehyde (MDA)

Table 3 Effects of silicon (1.0 mM) and PEG-6000 (20%) treatments on electrolyte leakage, malondialdehyde, and hydrogen peroxide concentrations of wheat leaves

Treatment[a]	Hydrogen peroxide (μmol g^{-1}DW)	Malondialdehyde content (nmol g^{-1}DW)	Electrolyte leakage (%)
CK	156.0 c[b]	83.2 c	10.7 b
Si	165.9 bc	74.3 d	13.6 b
PEG	238.3 a	127.1 a	45.8 a
PEG + Si	176.1 b	103.8 b	14.6 b

[a] The treatments lasted 1 week

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

Table 4 Effects of silicon (1.0 mM) and PEG-6000 (20%) treatments on glutathione and ascorbic acid concentrations of wheat leaves

Treatment[a]	Glutathione concentration (μmol g^{-1} DW)	Ascorbic acid concentration (μmol g^{-1} DW)
CK	238.1 b[b]	18.7 bc
Si	234.2 b	19.1 ab
PEG	202.8 c	18.4 c
PEG + Si	252.1 a	19.4 a

[a] The treatments lasted 1 week, and means are given ($n = 5$ and 6 for glutathione and ascorbic acid, respectively)

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

concentrations, as well as membrane electrolyte leakage of wheat leaves. The H_2O_2 concentration of water-stressed plants was obviously higher than that of control plants. However, addition of Si significantly reduced its accumulation.

The MDA content is often used as an indicator of oxidative damage (Leul and Zhou 1999; Gunes and others 2007). The MDA level in the leaves of Si-treated plants was lower compared to that of the control in non-stress conditions (Table 3). Water stress significantly increased the MDA level. However, inclusion of Si in the culture solution decreased the MDA concentration of water-stressed plants, suggesting that addition of Si decreased lipid peroxidation of stressed plants.

Under the present water-stress conditions, membrane electrolyte leakage increased 4.3-fold compared with the control in the absence of added silicon. However, in the presence of added silicon, the electrolyte leakage was not changed by water stress (Table 3).

Glutathione and Ascorbic Acid Concentrations in the Leaves

As shown in Table 4, addition of Si did not change the glutathione (GSH) level in non-stress conditions. Water stress decreased the GSH concentration of leaves significantly. However, in the presence of added silicon, its level was significantly increased and it was even higher than the control.

Relatively, there was no obvious change for ascorbic acid (AsA) as a result of water stress. The addition of Si only slightly increased the AsA concentration in the leaves of stressed plants (Table 4).

Proline and Soluble Sugar Changes in the Leaves

Si treatment increased the proline level of leaves by 23% under control conditions (Table 5). Water stress increased

Table 5 Effects of silicon (1.0 mM) and PEG-6000 (20%) treatments on proline and soluble sugar concentrations of wheat leaves

Treatment[a]	Proline concentration (μg g^{-1} DW)	Soluble sugar concentration (mg g^{-1} DW)
CK	368.7 d[b]	42.3 c
Si	454.9 c	56.1 a
PEG	927.9 a	32.7 d
PEG + Si	634.6 b	44.8 b

[a] The treatments lasted 1 week

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

the proline level 2.5-fold in the absence of Si, corresponding to 1.4-fold in the presence of added Si compared to the Si treatment without water stress. Moreover, addition of Si reduced the proline level of water-stressed plants by 32%. Regression analyses showed that there were significantly negative correlations between proline concentration and both shoot dry weight and leaf chlorophyll concentrations (Fig. 2). There were also positive correlations between proline concentration and both H_2O_2 and MDA concentrations. The regression relationship between leaf proline concentration and electrolyte leakage was slightly weaker ($r = 0.93$, $p = 0.0701$; Fig. 2).

The soluble sugar concentration in the leaves was significantly increased by added Si in control conditions (Table 5). Water stress reduced the soluble sugar level, with Si-treated plants having higher soluble sugar concentrations compared with the plants under water stress alone.

Mineral Nutrients and Si Concentrations in the Shoots

The concentrations of Na, Mg, K, and Ca in the shoots are shown in Table 6. The Na concentration was obviously increased in Si-treated plants under non-stress conditions. Under water stress, plants treated with added Si had only

Fig. 2 Regression between leaf proline concentration and shoot dry weight, leaf chlorophyll concentration, H_2O_2 concentration, MDA concentration, and electrolyte leakage in wheat seedlings after treated with silicon (1.0 mM) and PEG-6000 (20%). Chl a, chlorophyll a; Chl b, chlorophyll b
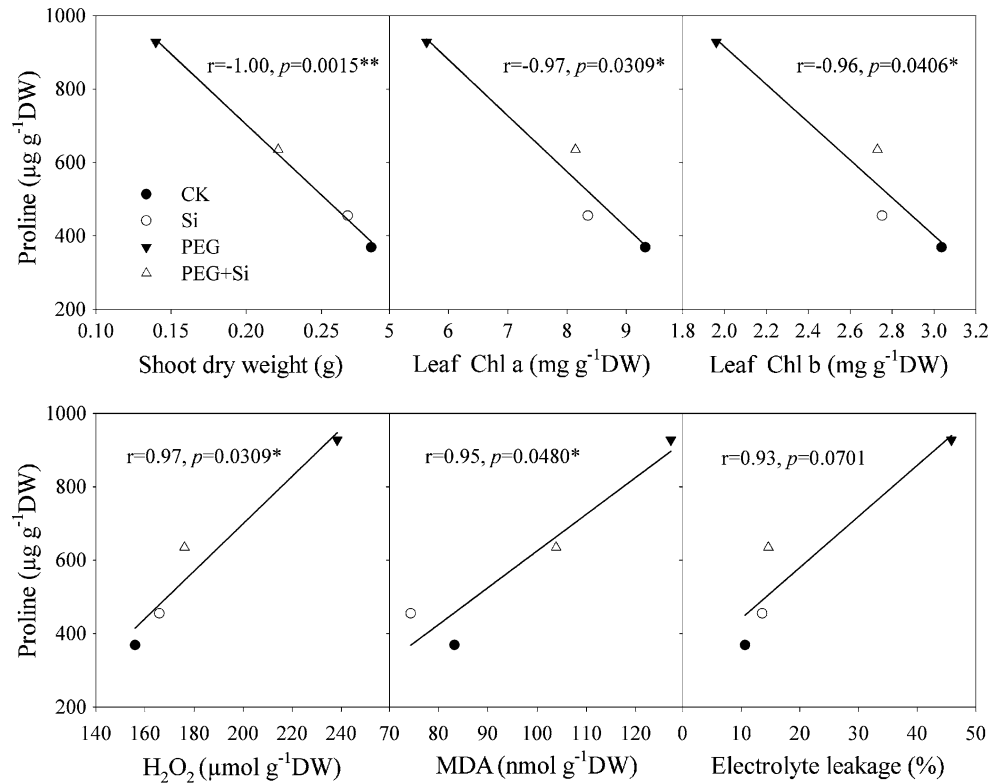
slightly higher Na compared with those without added Si. Addition of Si increased the Mg concentration in the shoots. Water stress decreased Mg concentration, especially in the plants treated with added Si. Both Si and water stress decreased K concentrations in the shoots. Under water stress, plants treated with additional Si obviously had lower Ca concentrations in the shoots. The addition of Si also significantly increased the Si concentration in the shoots, whereas water stress decreased its uptake.

Discussion

The ameliorative effects of Si on water stress observed in this study are consistent with the results of pot experiments under drought conditions (Gong and others 2005). Similar results were also obtained in sorghum (Hattori and others

2005). In field conditions the Si effect on wheat drought tolerance was related to developmental stages and stress intensity (Gong and others 2008). In maize, Li and others (2007) observed that the application of Si to soil improved plant growth under different drought conditions, whereas inclusion of Si in the nutrient solution did not improve growth under 20 or 30% PEG-6000-induced water stress conditions (Gao and others 2004). In *Brachiaria* grasses, application of Si to the soil did not change tolerance to water deficit or affect dry matter yield (de Melo and others 2003), and it was suggested that the 60% field capacity treatment used in that study was not sufficient to express the role Si plays on soil water deficit tolerance. Therefore, the effects of Si on plant growth may relate to the species used and the stress modes and intensity.

In this study, application of Si improved leaf water potential of PEG-stressed plants, which was consistent with

Table 6 Effects of silicon (1.0 mM) and PEG-6000 (20%) treatments on Na, Mg, K, Ca, and Si concentrations of wheat shoots

Treatment[a]	Na (mg g^{-1}DW)	Mg (mg g^{-1}DW)	K (mg g^{-1}DW)	Ca (mg g^{-1}DW)	Si (mg g^{-1}DW)
CK	0.67 d[b]	3.22 b	60.28 a	10.44 a	0.76 c
Si	1.60 a	3.46 a	54.43 b	10.75 a	1.65 a
PEG	0.70 c	3.11 b	49.06 c	9.92 b	0.54 c
PEG + Si	0.77 b	2.74 c	43.82 d	8.48 c	1.11 b

[a] The treatments lasted 1 week

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

that observed in potted plants (Gong and others 2005), indicating that added Si could improve the water status of water-stressed wheat. The beneficial effects of Si on plant growth have been linked to decreased transpiration. In an earlier study of rice (*Oryza sativa* L.), Si-caused physical blocking of cuticular transpiration was suggested to be the cause of Si-induced reduction in transpiration (Yoshida 1965). Recent studies of rice and maize (*Zea mays* L.) showed that Si-induced reduction in transpiration was associated mainly with decreased stomatal aperture/conductance (Agarie and others 1992, 1998; Gao and others 2006), suggesting the involvement of Si in stomata movement. In addition, Liang and others (2008) proposed that water molecules may escape less easily from leaf surfaces because the accumulation of polar monosilicic acid and/or polymerized silicic acid in the epidermal cell walls may form H bonds between H_2O and $SiO_2 \cdot nH_2O$. However, in a previous study we observed that application of Si increased leaf transpiration and stomatal conductance of drought-stressed wheat in pots (Gong and others 2005). Similar phenomena were also observed in sorghum under drought stress (Hattori and others 2005). It seems, therefore, that the increase in water potential by added Si was not mainly through these mechanisms. In sorghum [*Sorghum bicolor* (L.) Moench], Si facilitated water uptake and transportation under drought conditions (Hattori and others 2005, 2007). Therefore, the increase of water potential in the leaves of water-stressed wheat by added Si might have been due to stimulated water uptake and transport. However, this requires further investigation.

Environmental stresses break the balance between production of reactive oxygen species (ROS) and antioxidant defense, causing accumulation of ROS which induce oxidative damage to functional molecules in the plant cells (Egert and Tevini 2002). The antioxidant defense system in the plant cell includes both enzymatic [for example superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR)] and nonenzymatic constituents (for example reduced glutathione, ascorbic acid). SOD can dismutate $O_2^{\cdot-}$ to H_2O_2, which is detoxified by CAT and POD. In the ascorbate–glutathione cycle, APX reduces H_2O_2 using ascorbate as an electron donor. Oxidized ascorbate is then reduced by GSH generated from GSSG catalyzed by GR at the expense of NADPH (Lin and Kao 2000). We have observed that the activities of SOD, CAT, POD, and APX in the leaves were increased under water stress (Gong and others 2005). Inclusion of Si further increased POD activity (data not shown). Therefore, the Si-induced increase in antioxidant enzymes might contribute to the elimination of overproduced ROS under stress, as shown in Table 3. The improvement in the ability to scavenge ROS reduced the risk of oxidation of photosynthetic pigments, membrane

lipids, proteins, and nucleic acid (Egert and Tevini 2002; Yordanov and others 2000) and thus maintained normal growth of wheat plants.

To our knowledge, the effect of Si on nonenzymatic antioxidants under drought has not been investigated before. In this study, increased GSH concentration due to added Si under water stress was observed (Table 4). It was also found that added Si increased the glutathione concentration in the roots and leaves of salt-stressed barley plants (Liang and others 2003, 2006). The increase in the GSH level by added Si might be partly due to increased activity of glutathione reductase (GR), as observed in drought-stressed wheat (Gong and others 2005) and salt-stressed barley (Liang and others 2003). GSH can protect the −SH group in enzymes and structural proteins against oxidation either by acting as a scavenger for oxidizing substances or by repairing the –SH groups via the GSH-disulfide exchange reaction (Christine and others 1997; Liang and others 2006). Therefore, a Si-induced increase in the GSH level in the leaves of wheat was involved in the antioxidant defense against water stress. These results suggest that water stress induced accumulation of ROS, which caused membrane lipid peroxidation. As a result, the leaf membrane was damaged and caused electrolyte leakage. Inclusion of Si in the nutrient solution reduced the production of H_2O_2 and therefore alleviated membrane damage. The added Si and water stress did not have much effect on the ascorbic acid concentration in this study (Table 4). In leaves of salt-stressed wheat, Saqib and others (2008) observed that the ascorbic acid concentration was increased. However, added Si did not change its concentration.

One may argue whether the decreased oxidative damage in Si-amended wheat plants was just the result of improved water status or was caused by the increased abilities of the antioxidant defense system. We suggest that neither aspect can be excluded. Si-induced improvement in the water status of leaves resulted in plants with less cellular dehydration and therefore less oxidative stress by overproduction of ROS. On the other hand, Si might have been actively involved in the antioxidant defense. In salt-stressed barley and cucumber, addition of Si increased the antioxidant defense activity and decreased oxidative damage (Liang and others 2003; Zhu and others 2004). A similar phenomenon has been observed in freeze-stressed wheat plants (Liang and others 2008). In cucumber, Si applied to the roots alleviated oxidative stress and enhanced defense resistance in response to infection by powdery mildew (Liang and others 2005); this was further supported by a complete transcriptome analysis of powdery mildew-stressed *Arabidopsis* (Fauteux and others 2006; Liang and others 2008). Liang and others (2008) suggested that Si-enhanced antioxidant defense activity might be a

universal mechanism for Si-enhanced tolerance to various abiotic and biotic stresses in plants. However, transcriptome analysis needs to be conducted to explore the role of Si in the metabolism of ROS under water-deficit stress. Further investigations are also needed to elucidate how Si might trigger the antioxidant defense.

A common response of plants to environmental stresses is overproduction of different types of compatible organic solutes, which are of low molecular weight, highly soluble, and usually nontoxic at high cellular concentrations (Ashraf and Foolad 2007). Proline and soluble sugars are such organic solutes. They can protect plants from stress through different mechanisms, including cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of proteins/enzymes (Ashraf and Foolad 2007). In this study, an increased proline concentration in wheat leaves was observed under water stress (Table 5). The accumulation of proline in the leaves might be involved in one or more of the above processes and contribute to drought tolerance. However, the actual role of proline in osmotolerance remains controversial. In some studies, accumulation of proline under stress has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants (Madan and others 1995; Nayyar and Walia 2003). However, other researchers suggested that the accumulation of proline was a symptom of stress injury rather than an indication of stress tolerance (Lutts and others 1999; de-Lacerda and others 2003). In this study, addition of Si decreased proline accumulation in the leaves under water stress (Table 5). There are significantly negative linear correlations between the proline concentration and both shoot dry weight and chlorophylls. Positive linear correlations are seen between the proline concentrations and H_2O_2 concentration, MDA concentration, and electrolyte leakage (Fig. 2). Our results seem to support the view that proline accumulation under stress is an injury symptom. A Si-induced decrease in proline accumulation was a sign of stress injury alleviation.

Water stress decreased the soluble sugar concentration in the leaves, irrespective of Si addition (Table 5). Compared with the stressed plants without additional Si, plants with added Si had a significantly higher soluble sugar concentration. This suggests that in the short-term water stress conditions used in this study, the catabolism of soluble sugar was enhanced and that adding Si decreased the catabolism under water stress. This observation was contrary to a previous study in which increased soluble sugar levels were found in the field (Zhu and others 2005). This may be related to the difference in stress modes and durations. Further studies using different stress modes and duration in wheat cultivars need to be done to confirm this.

The addition of Si to the nutrient solution increased the Si concentration in wheat shoots (Table 6), which was expected and consistent with other studies in *Cucumis sativus* L. and wheat plants (Rogalla and Römheld 2002; Tuna and others 2008).

Accumulation of inorganic ions is another way for plants to cope with environmental stresses, as observed in previous studies (Zhu and others 2005). However, in the experimental conditions of our study, no obvious accumulation of inorganic ions was observed in the shoots under PEG stress, except that the Na concentration was slightly increased (Table 6). The results potentially suggest that these ions did not contribute to osmotic adjustment under the water stress used in this study. Although compared to the plants without added Si, stressed plants with added Si had lower Mg, K, and Ca concentrations in the shoots, yet, considering the improvement of shoot dry matter by Si, their total contents in the shoots were actually increased. This indicates higher uptake of these ions in the roots. One possible explanation for the increased K uptake could be the stimulating effect of Si on the plasma membrane H^+-ATPase in the roots, as found in barley under salt stress (Liang and others 1999). Calcium is transported via the xylem and its transport is dependent on transpiration (Arndt and others 2000). Therefore, increased Ca transport in Si-treated plants under water stress was possibly due to increased transpiration by Si under stress, as observed in previous studies (Gong and others 2005; Hattori and others 2005). However, the mechanism for the increased uptake of these ions by added Si under water stress remains to be further investigated.

In conclusion, addition of Si could alleviate PEG-induced water stress in wheat. The alleviative effect was attributed to an enhanced antioxidant defense ability and leaf water potential. According to the organic solutes and inorganic ions investigated, osmotic adjustment seemed to show little contribution to the Si-induced tolerance to the short-term water stress of this study.

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