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Enhanced root hydraulic conductance by aquaporin regulation accounts for silicon alleviated salt-induced osmotic stress in *Sorghum bicolor* L

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ABSTRACT

It has been widely reported that silicon (Si) improves the resistance of plants to salt stress. Most of the previous studies have examined how silicon prevents Na^+ uptake, but the performance and underlying mechanism through which silicon alleviates salt-induced osmotic stress has been largely ignored. In the present study, the mechanism through which Si alleviates salt-induced osmotic stress was investigated using sorghum in a hydroponic system. Si had no effect on seedling growth under normal conditions. Under salt stress, the photosynthesis and transpiration rate were decreased, but these decreases were alleviated by Si application. In addition, the leaf water content and leaf elongation rate were maintained at higher levels with Si than without Si. The root hydraulic conductance (L_p) of the seedlings were inhibited by salt, but Si application alleviated this inhibition. Under salt stress, the transpiration rates of the seedlings both with and without Si were decreased to the same level by $HgCl_2$ treatment and partially rescued by β-mercaptoethanol treatment, suggesting that aquaporin was responsible for the alleviation of the decrease in L_p. Moreover, transcript levels of several aquaporin genes were upregulated by Si. Under salt stress, Si inhibited the increase in the root H_2O_2 levels and enhanced the activities of antioxidant enzymes. Moreover, similar to Si, pre-treatment with catalase alleviated the decrease in the transpiration rate, indicating that Si enhanced aquaporin activity by reducing H_2O_2 accumulation. These results indicate that under short-term salt stress, Si application can alleviate the decrease in L_p by mediating aquaporin activity, leading to increased water uptake and resistance to salt-induced osmotic stress.

1. Introduction

Salt stress has adverse effects on plants and causes marked decreases in crop production in 7% of arable land worldwide (Hasegawa et al., 2000; Halperin et al., 2003; Zhu 2003). A high salt concentration can result in damage to plant metabolic processes, including photosynthesis, growth, membrane integrity, and protein synthesis (Manuel Ruiz-Lozano et al., 2012). To alleviate the noxious effects of salt stress on plants, a variety of approaches have been adopted to regulate the water/osmotic homeostasis and ion balance and to prevent damage (Munns, 1993; Chen and Polle, 2010; Manuel Ruiz-Lozano et al., 2012).

Silicon (Si), the second most abundant element in the Earth's crust, has been found to alleviate salt stress in rice, barley, wheat,

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; K_{plant}, whole plant hydraulic conductance; LER, leaf elongation rate; Lp, root hydraulic conductance; PIP, plasma membrane intrinsic protein; POD, peroxidase; RWC, relative water content; SOD, superoxide dismutase.

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and cucumber (Gong et al., 2006; Liang et al., 2006; Saqib et al., 2008; Zhu et al., 2004; Epstein, 1999). It has been reported that ion accumulation is reduced by decreasing plant transpiration due to silica deposits in the leaves (Matoh et al., 1986). Gong et al. (2006) suggested that the mechanism through which Si alleviates salt stress involves a reduction in Na^+ uptake caused by deposition of Si in the root. In addition, Si application increases the antioxidant activity and membrane integrity (Zhu et al., 2004; Liang et al., 2006). In general, the salt-stress-induced reduction in plant growth occurs in two phases: a rapid response to the decrease in the external osmotic potential, which leads to difficulty in water uptake through the root, and a slow response due to the accumulation of ions in the plant, which leads to ion toxicity (Munns and Tester, 2008). Most of the previous studies have investigated the long-term responses and did not consider all of the potential underlying mechanisms. These previous experiments mainly focused on how Si alleviates ion toxicity. However, the mechanism through which Si alleviates salt-induced osmotic stress has largely been ignored.

Osmotic stress is the first stress experienced when a plant is exposed to saline soil; it has an immediate influence on the growth of the plant (Horie et al., 2011). One of the primary responses of plants to osmotic stress is a decrease in the root hydraulic conductance (L_p) (Boursiac et al., 2005). L_p represents the root water uptake capacity, and its regulation plays an important role in maintaining the water status of the entire plant (Sutka et al., 2011). The root water uptake includes three pathways: apoplastic, symplastic, and transcellular. The latter two pathways cannot be clearly distinguished, and are thus called the "cell-to-cell" pathway (Steudle, 2000). Under abiotic stress, water uptake occurs mainly through the "cell-to-cell" pathway, which is considered to be regulated by aquaporin activity (Boursiac et al., 2005; Horie et al., 2011; Sutka et al., 2011). Aquaporin activity is affected by a number of stimuli, including ABA, ethylene, Ca^{2+}, and reactive oxygen species (Parent et al., 2009; Hu et al., 2012; Azad et al., 2004; Boursiac et al., 2008a). It has been reported that under short-term salt stress, H_2O_2 can decrease the activity of aquaporin by direct oxidant gating, regulating the phosphorylation status, and inducing the relocalization of aquaporin, which leads to decreased water uptake (Boursiac et al., 2008a,b).

It has been clearly demonstrated by Romero-Aranda et al. (2006) that the application of Si improves the water status of plants under salt stress conditions. In addition, plants treated with Si maintain a high stomatal conductance and transpiration rate under salt stress (Gong et al., 2006; Soylemezoglu et al., 2009; Yin et al., 2013). Taken together, the results from previous studies suggest that Si can improve the water uptake capacity of plants under salt-induced osmotic stress, but the underlying mechanism remains unclear. Therefore, based on previous studies, we proposed the following hypothesis: Si can enhance plant salt resistance by alleviating the decrease in L_p due to the enhancement of aquaporin activity. The enhancement of aquaporin activity may be ascribed to the upregulation of the transcriptional level of the aquaporin genes and/or a reduction in the H_2O_2 content. To test this model, the L_p, aquaporin activity, H_2O_2 content, and antioxidant enzyme activities were investigated in sorghum seedlings under short-term salt treatment in a hydroponic system.

2. Materials and methods

2.1. Plant material and growth conditions

Sorghum seedlings (*Sorghum bicolor* Moench. cv. Gadambalia) were grown in a growth chamber under cycles of 14 h of light (450 μmol m^{-2} s^{-1}) at 28 °C and 10 h of darkness at 23 °C. The relative humidity was 40–50%.

2.2. Seedling cultivation and Si and NaCl treatments

After sterilization, the seeds were germinated for 3 days in an incubator at 27 °C. After germination, uniform seedlings were transplanted and cultivated in one-quarter strength Hoagland's solution (Hoagland and Arnon, 1950). Six days after transplantation, half of the seedlings were supplied 1.67 mM Na_2SiO_3. The culture solution was continuously aerated, and the pH was adjusted to 6.0 every day. Nine days after transplantation, 100 mM NaCl was added at 8:00 am to induce osmotic stress.

2.3. Leaf elongation rate

The leaf elongation rate was measured in the new fully appeared leaves. The length from the bundle sheath to the leaf tip was measured using a ruler before and after 24 h of NaCl treatment. Each treatment included 12 replications.

2.4. Ion concentration

The root and shoot were sampled after 2 h of NaCl treatment and dried for 72 h at 75 °C. The dried sample was milled to powder, weighed, and then digested with nitric acid at 320 °C for 5 h. The Na^+ concentration was measured by atomic adsorption spectrophotometry (AAS) (Model iCE 3500, Thermo Scientific, USA). The ion concentration was expressed as μmol g^{-1} dry weight.

2.5. Photosynthesis rate, stomatal conductance, and transpiration rate

After 2 h of NaCl treatment, the photosynthesis rate, stomatal conductance, and transpiration rate were measured using a portable photosynthesis system (Li-6400; LI-COR Inc., Lincoln, NE, USA) between 10:00 am and 1:00 pm. A fully expanded leaf was measured at a photon flux density of 500 μmol m^{-2} s^{-1}. The flow rate through the chamber was 500 μmol s^{-1}, and the leaf temperature was 28 °C. Each treatment included six replications.

2.6. Leaf relative water content and water potential

The leaf relative water content (RWC) was measured according to Machado and Paulsen (2001). After 2 h of NaCl treatment, fully expanded leaves were cut into ten leaf discs and weighed immediately to determine the fresh weight (FW). The leaf discs were floated in distilled water for 6 h, dried with filter paper and weighed to determine the total weight (TW). The discs were then dried at 70 °C in a forced-air oven for 24 h to determine the dry weight (DW). The relative water content was calculated as follows:

$$RWC = \frac{FW - DW}{TW - DW} \times 100\%$$

After 2 h of NaCl treatment, the water potential of the fully expanded leaves was measured between 10:00 am and 1:00 pm using a pressure chamber (Model 3500, Soilmoisture Corp., Santa Barbara, CA, USA). Each treatment included six replications.

2.7. Whole plant hydraulic conductance (K_{plant})

The K_{plant} was calculated according to the following equation (Martre et al., 2002):

$$K_{plant} = \frac{\text{Transpiration rate}}{\text{Soil water potential} - \text{leaf water potential}}$$

In hydroponic culture, the soil water potential (i.e., culture potential) was −0.07 and −0.09 MPa under control conditions and with Si treatment, respectively. Under NaCl treatment, the soil water potential (culture potential) was −0.24 MPa, regardless of

whether the plants were treated with Si. Each treatment included six replications.

2.8. Root hydraulic conductance (L_p)

After 2 h of NaCl treatment, the root L_p was measured between 10:00 am and 1:00 pm according to Miyamoto et al. (2001). After the treatment, each shoot was removed at the base of the root system, leaving a 4-cm mesocotyl. The root system was inserted into the pressure chamber filled with the corresponding solution (control, Si, NaCl or NaCl + Si solution), and the root system was then sealed with silicon seals with holes adjusted to the diameter of each mesocotyl. The pressure was raised in steps of 0.1 MPa up to 0.3 MPa (P_{gas}). The exuded sap was collected with absorbent cotton and weighed. For a given pressure, the volume exuded from the root system was plotted against time. The slopes of these relationships referred to the unit root dry mass, which yields the volume flow, J_{vr} ($m^3\,g^{-1}\,s^{-1}$). The root L_p was calculated from the slopes of J_{vr} against the driving force, which consists of P_{gas} and the osmotic gradient. Under salt stress, the osmotic gradient force between the culture solution and xylem sap was too low to be considered; therefore, the osmotic gradient force was neglected. The same method was also used to calculate the L_p of the plants under salt stress, which consists solely of P_{gas} (Sutka et al., 2011; Qian et al., 2014). As a result, L_p was determined from the slopes of J_{vr} against P_{gas} according to the following equation:

$$J_{vr} = L_p \times P_{gas}$$

Each treatment included ten replications.

2.9. Transpiration rate response to aquaporin inhibitor

The root aquaporin activity was investigated by exposing the sorghum seedlings to the aquaporin inhibitor $HgCl_2$ (Knipfer et al., 2011). The transpiration rates of the seedlings were determined gravimetrically in a growth chamber between 10:00 am and 1:00 pm. After 2 h of NaCl treatment, the transpiration rates of the seedlings were measured before and after exposure to 50 μM $HgCl_2$ for 5 min. The reversibility of the effect of $HgCl_2$ on aquaporin activity was established by first treating the root with 50 μM $HgCl_2$ and then placing it for 15 min in 5 mM β-mercaptoethanol before measuring the transpiration rate.

2.10. Expression analysis of sorghum aquaporin genes

After 3 days of Si treatment, uniform plants were exposed to 0 (control) or 100 mM NaCl. After 2 h of NaCl treatment, 1-cm root tips were collected and frozen in liquid nitrogen to measure the expression of aquaporin genes. Eight sorghum plasma membrane intrinsic protein (*SbPIP*) genes were identified based on the GenBank database. The genes and the sequences of their specific primers are presented in Table 1. DNA sequence comparisons were performed to ensure that each pair of primers was specific to the corresponding *SbPIP* gene.

The total RNA from 100 mg of frozen root samples was extracted using the RNeasy® Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and then treated with Recombinant DNase I (RNase-free; Takara Bio Shiga, Japan) to remove the remaining genomic DNA. Reverse transcription (1 μg RNA) was performed using the iScript™ cDNA Synthesis Kit (Bio-Rad Hercules, CA, USA) according to the manufacturer's instructions. Then, the cDNA was diluted three-fold in water, and 1 μL of cDNA was used for semi-quantitative reverse transcription polymerase chain reaction (RT-PCR). The PCR conditions used were 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. The sorghum housekeeping gene *SbActin* was used as an endogenous control for sample normalization. The reaction was repeated for 25 cycles to obtain an appropriate amount of DNA. The obtained DNA was subjected to agarose gel electrophoresis and stained with ethidium bromide. The stained bands were photographed with a Gel Imaging System (Bio-Rad Hercules, CA, USA).

2.11. Transpiration rate responds to catalase (CAT) and salt stress

To investigate whether H_2O_2 affects water uptake, the transpiration rates of the seedlings were determined in a growth chamber between 10:00 am and 1:00 pm under salt stress. Based on Boursiac et al. (2008a), before salt application, half of the seedlings were treated with 400 U mL^{-1} CAT for 30 min. After 2 h of NaCl treatment, the transpiration rates of all of the seedlings were measured. Four treatments, namely CAT − Si−, Si+, CAT+ and CAT + Si+, were included in this experiment, and each treatment included six replications.

2.12. H_2O_2 content

The root H_2O_2 content was measured according to Yin et al. (2010). Root tips (1 cm, weighing 0.5 g) were homogenized with 2 mL of cold 0.1% (w/v) trichloroacetic acid in precooled mortars. The homogenate was centrifuged at 12,000 g and 4 °C for 30 min. After centrifugation, 0.4 mL of the supernatant was added to 0.4 mL of 10 mM potassium phosphate buffer (pH 7.0) and 0.8 mL of 1 M KI. The absorbance of the mixture was read at 390 nm. The content of H_2O_2 was calculated against the calibration curve obtained using H_2O_2 standards.

2.13. Antioxidant enzyme activities

Root tips (1 cm, 0.5 g) were sampled after 2 h of NaCl treatment and stored at −80 °C. The sample was homogenized with 50 mM sodium phosphate buffer (pH 7.8) containing 1 mM EDTA-Na_2 and 2% (w/v) polyvinylpolypyrrolidone (PVPP). For APX activity determination, 2 mM ascorbate was added to the homogenization buffer. All of the experiments were performed at 4 °C. Samples

Table 1
Primers for the *SbPIP* aquaporin genes and reference genes.

	Gene	Primer	Product size (bp)
Sb01g010030	Actin1	F 5′-TGTTCCCTGGGATTGCTG-3′R 5′-GCCGGACTCATCGTACTCA-3′	185
Sb06g025150	PIP1;3/1;4	F 5′-AATCGGGTTCGCGGTGTT-3′R 5′-CCAGGCATGGTTCTGGTTGTA-3′	115
Sb04g032430	PIP1;3/1;4 (2)	F 5′-GTGGAGCTGGAGTGGTGAA-3′R 5′-GCAAGGATAGGAACATGGGAGT-3′	199
Sb04g037800	PIP1;5	F 5′-TTTCGCCGTCTTCCTCGTC-3′R 5′-GGTCGTTCCATGCGTTGG-3′	116
Sb10g007610	PIP1;6	F 5′-TGACGGTGCTGACGGTGAT-3′R 5′-GGAGGAGCCCGAAGGTGAC-3′	168
Sb02g010760	PIP2;2	F 5′-GACTCCCACGTCCCGGTTCT-3′R 5′-CCCAGGGCTTGTCCTTGTTGT-3′	148
Sb04g026650	PIP2;3	F 5′-CCGTGACCTTCGGTTTGTTC-3′R 5′-GCACGTAGTAGGCGCTCTGG-3′	132
Sb06g022840	PIP2;5	F 5′-TCGCGGTGTTCATGGTCC-3′R 5′-TCCCAGGTCTTGTCGTTGTTGT-3′	109
Sb02g010800	PIP2;6	F 5′-CTTCCGATTGGATTCGCTGTG-3′R 5′-CGGAGGACGATCTGGTGGTA-3′	197

were centrifuged at 15,000 g and 4 °C for 20 min at, and the supernatants were used for determination of the protein content and the enzyme activity assays. The total soluble protein contents of the enzyme extracts were determined according to Bradford (1976), using bovine serum albumin as the standard. All of the spectrophotometric analyses were conducted using a Shimadzu ultraviolet spectrophotometer (UV-1600, Shimadzu Corp., Japan).

The superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed by its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm (Beauchamp and Fridovich, 1973). The catalase (CAT; EC 1.11.1.6) activity was estimated according to Hamurcu et al. (2013) by measuring the initial rate of the disappearance of H_2O_2 at 240 nm. The peroxidase (POD; EC 1.11.1.7) activity was determined using the guaiacol oxidation method (Kochba et al., 1977). The ascorbate peroxidase (APX; EC 1.11.1.11) activity was measured according to the method described by Nakano and Asada (1981), which depends on the decrease in absorbance at 290 nm as the ascorbate is oxidized.

2.14. Statistical analysis

The data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS version 8.0) software. The differences between the means were compared using the Tukey–Kramer test at $p < 0.05$. All of the experiments were repeated at least twice.

3. Results

3.1. Leaf elongation rate (LER)

As shown in Fig. 1, under control conditions, the LER was not affected by Si, whereas under NaCl treatment, the LER was significantly decreased. However, Si application alleviated the salt-induced decrease in the LER.

3.2. Ion concentration

Under normal conditions, both the shoot and root Na^+ levels were extremely low. After 2 h of NaCl treatment, the Na^+ concentration in the shoots and roots were increased. Si application had no effect on the Na^+ concentrations in the shoots and roots under either control or salt stress conditions (Fig. 2).

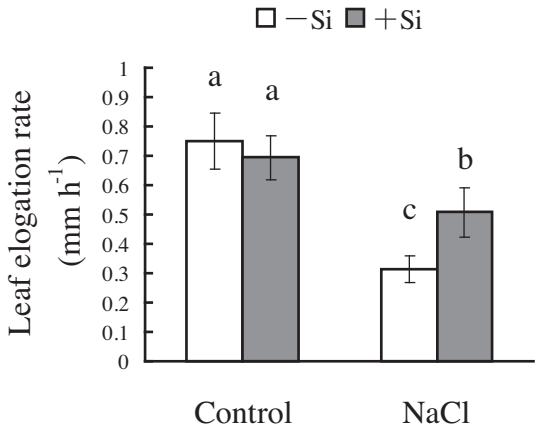

Fig. 1. Changes in the leaf elongation rate (LER) of sorghum seedlings in response to Si application under control and salt treatments. New fully appeared leaves were used for measurement of the LER after 24 h of NaCl treatment. The values are means ± standard deviation (SD) of twelve independent replicates. Different letters indicate significant differences ($p < 0.05$).

3.3. Photosynthesis rate, stomatal conductance, and transpiration rate

Under control conditions, the photosynthetic rate, stomatal conductance, and transpiration rate were not affected by Si application. The exposure of the plants to culture solution with of NaCl resulted in decrease in all of these parameters. However, the application of Si significantly alleviated the decreases in these parameters. These results show that Si may be beneficial to the maintenance of the photosynthetic rate, stomatal conductance, and transpiration rate under salt stress in sorghum seedlings (Fig. 3).

3.4. Leaf relative water content (RWC) and water potential

The RWC was not affected by Si under control conditions. Under salt stress, the RWC of leaves was significantly higher in the Si-treated plants than the non-Si-treated plants, indicating that the application of Si was beneficial to the maintenance of the water status of the plants (Fig. 4A).

Under control conditions, the leaf water potential was approximately −0.47 MPa. The exposure of the sorghum seedlings to salt stress decreased the leaf water potential to −0.91 MPa, but this parameter was maintained at −0.74 MPa in the presence of Si (Fig. 4B).

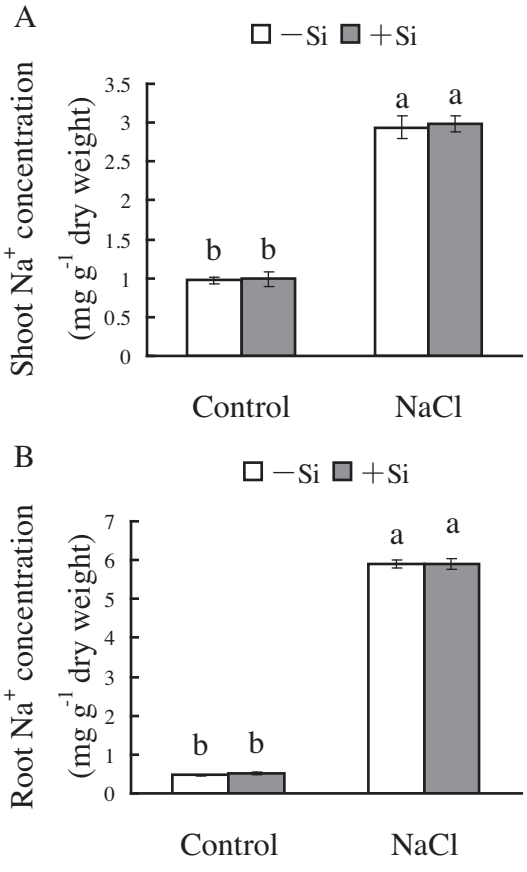

Fig. 2. Changes in the shoot and root Na^+ concentrations of sorghum seedlings in response to Si application under control and salt treatments. The shoots and roots were sampled after 2 h of NaCl treatment. The Na^+ concentration was measured by atomic adsorption spectrophotometry (AAS) (Model iCE 3500, Thermo Scientific, America). The values are means ± SD of six independent replicates. Different letters indicate significant differences ($p < 0.05$).

3.5. Whole plant hydraulic conductance (K_{plant})

Under control conditions, the K_{plant} was not affected by Si application, and after 2 h of NaCl treatment, the K_{plant} was markedly decreased. However, the salt-induced decrease in K_{plant} was significantly alleviated by Si application: the K_{plant} obtained with Si application was 34% higher than that obtained without Si (Fig. 5).

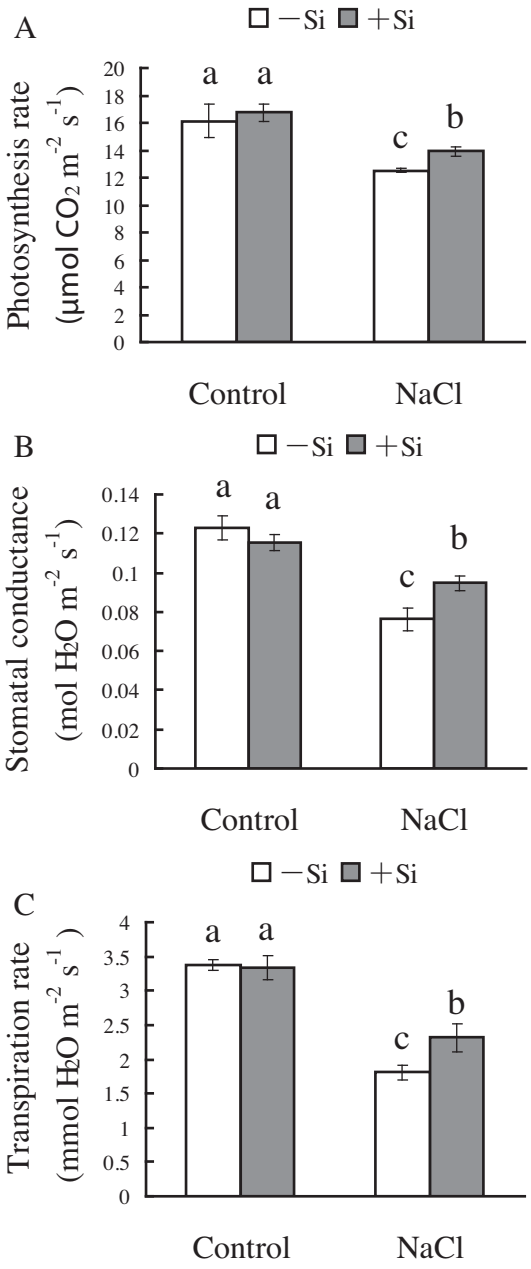

Fig. 3. Changes in the photosynthesis rate (A), stomatal conductance (B), and transpiration rate (C) of sorghum seedlings in response to Si application under control and salt treatments. After 2 h of NaCl treatment, fully expanded leaves were used for measurement in a portable photosynthesis system (Li-6400). The values are means ± SD of six replicates. Different letters indicate significant differences ($p < 0.05$).

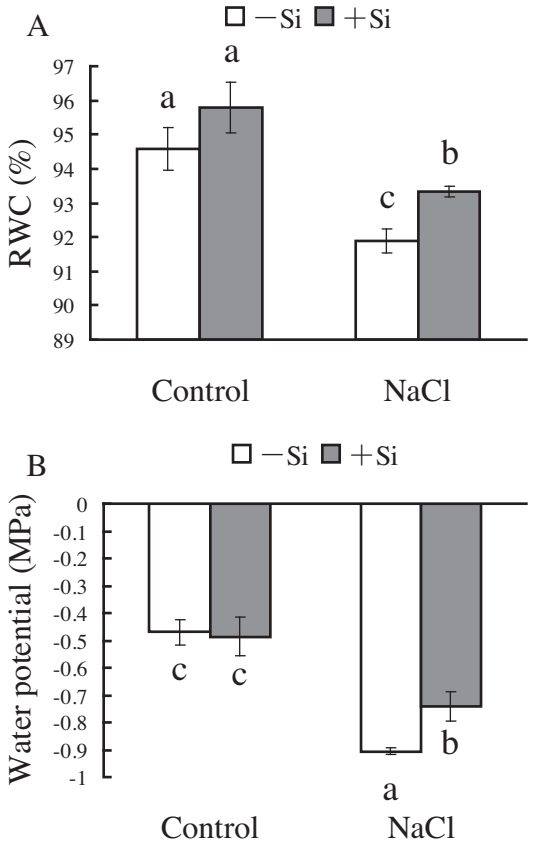

Fig. 4. Changes in the leaf relative water content (RWC) (A) and water potential (B) of sorghum seedlings in response to Si application under control and salt treatments. The RWC and leaf water potential were measured after 2 h of NaCl treatment. Ten discs were cut from the leaves to investigate the RWC. The water potential of new fully expanded leaves was measured in a pressure chamber (Model 3500, Soilmoisture Corp., Santa Barbara, CA, USA). The values are means ± SD of six replicates. Different letters indicate significant differences ($p < 0.05$).

3.6. Root hydraulic conductance (L_p)

As shown in Fig. 6, the L_p was not affected by Si application under control conditions (average $53.6 \times 10^{-9}\ m^3\ g^{-1}\ s^{-1}\ MPa^{-1}$). The exposure of the seedlings to salt stress significantly decreased the L_p ($43.40 \times 10^{-9}\ m^3\ g^{-1}\ s^{-1}\ MPa^{-1}$). However, Si application not only alleviated this decrease but also maintained the L_p at a level similar to that observed under control conditions ($51.74 \times 10^{-9}\ m^3\ g^{-1}\ s^{-1}\ MPa^{-1}$).

3.7. Transpiration rate responds to aquaporin inhibitor

To ascertain the function of aquaporin, the widely used aquaporin inhibitor $HgCl_2$ was employed in this study. Under salt stress, the transpiration rates of the seedlings treated with Si were markedly higher than of those obtained without Si. The transpiration rates of the seedlings with and without Si application decreased to the same level when exposed to $HgCl_2$. After the seedlings were successively treated with β-mercaptoethanol, the transpiration rates of seedlings treated with Si were much higher than those obtained without Si application (Fig. 7).

3.8. Expression analysis of sorghum aquaporin genes

To investigate the effect of Si on the transcription of aquaporin genes, eight *SbPIP* genes were selected from GenBank. As shown in Fig. 8, under control conditions, none of the *SbPIP* genes were

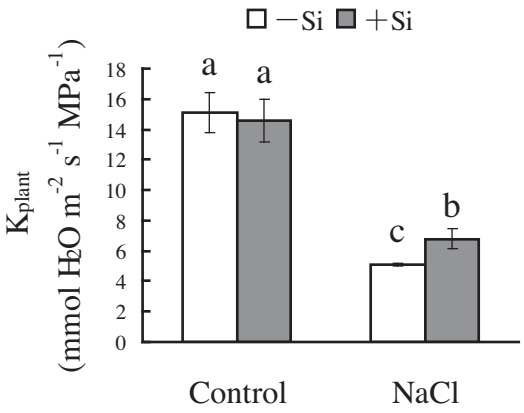

Fig. 5. Changes in the whole plant hydraulic conductance (K_{plant}) of sorghum seedlings in response to Si application under control and salt treatments. After 2 h of NaCl treatment, the K_{plant} was calculated as the transpiration rate divided by the difference between the soil and leaf water potentials. The values are means ± SD of six replicates. Different letters indicate significant differences ($p < 0.05$).

affected by Si. After 2 h of NaCl treatment, some of the *SbPIP* genes were upregulated. Moreover, Si could further increase the expression of *SbPIP1;6*,*SbPIP2;2* and *SbPIP2;6*.

3.9. Transpiration rate responds to catalase (CAT), salt stress

After 2 h of NaCl treatment, the transpiration rate was significantly lower in the plants without Si treatment than in the plants treated with Si. Pretreatment with CAT alleviated the NaCl-induced reduction in the transpiration rate, and the transpiration rate was similar to that obtained with the Si+ and the Si + CAT + treatments (Fig. 9).

3.10. H_2O_2 content

After 2 h of NaCl treatment without Si or CAT, the H_2O_2 content was significantly increased. However, the pre-treatment of the seedlings with CAT inhibited the enhancement in the H_2O_2 content. Si application also significantly reduced the H_2O_2 accumulation under salt stress (Fig. 10).

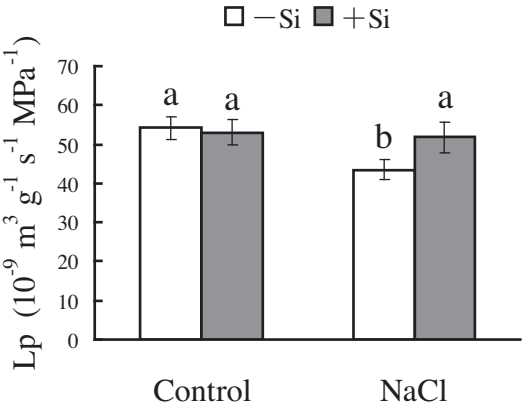

Fig. 6. Changes in the root hydraulic conductance (L_p) of sorghum seedlings in response to Si application under control and salt treatments. After 2 h of NaCl treatment, the entire root system of each nine-day-old seedling was cut off near the root base, leaving a 4-cm mesocotyl, and the root system was inserted into a pressure chamber. For a driving force, the volume exuded from the root system was plotted as a function of time. The slope of these relationship in reference to the unit root dry mass was denoted as the L_p. The values are means ± SD of ten replicates.

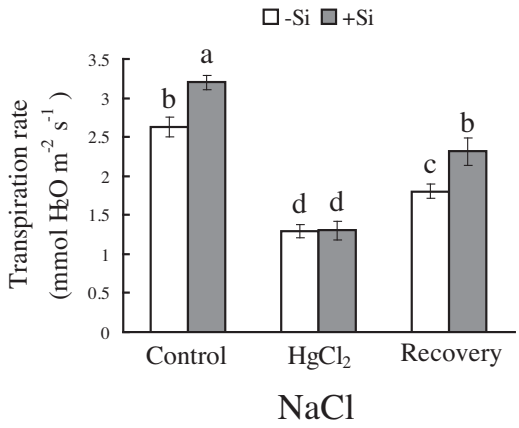

Fig. 7. Effects of the aquaporin inhibitor ($HgCl_2$) and anti-inhibitor (β-mercaptoethanol) on the transpiration rates of seedlings with and without Si application under salt stress. The transpiration rate was measured under salt stress before and after exposure to 50 μM $HgCl_2$ (5 min). The recovery of aquaporin-mediated root water uptake by β-mercaptoethanol was also indicated by the transpiration rate. The values are means ± SD of six replicates. Different letters indicate significant differences ($p < 0.05$).

3.11. Antioxidant enzyme activities

The activities of antioxidant enzymes are shown in Fig. 11. Under control conditions, the SOD and CAT activities were not affected by Si application, although these were significantly increased by Si under salt stress. The POD activity was decreased by Si application under both control and salt conditions. The APX activity was increased by salt treatment, but no change was observed upon Si treatment.

4. Discussion

Although numerous experiments have been conducted to investigate the mechanism through which Si alleviates salt stress, most have focused on how Si relieves Na^+ toxicity after long-term salt stress (Gong et al., 2006; Matoh et al., 1986; Saqib et al., 2008). Therefore, the present study investigated the effect of Si on osmotic stress under short-term exposure to salt stress. Salt stress affects plant growth in two ways: osmotic stress and ion toxicity. The early stress induced by salt is mainly caused by osmotic stress (Boursiac et al., 2005; Munns and Tester, 2008). In this study, after 2 h of NaCl treatment, Si application did not affect the Na^+ concentration in either the shoots or roots (Fig. 2). This finding is different from the results of previous studies, which showed that the Na^+ concentration is decreased by Si application. This discrepancy may be ascribed to the far shorter NaCl treatment time used in the present study compared with that used in the previous reports; furthermore, the Na^+ concentration used in the present study did not reach the toxic level. Therefore, the role of Si in decreasing the Na^+ concentration was not obvious in this study.

Under short-term salt stress, the osmotic stress induced by high salt concentration plays a dominant role in disturbing plant growth (Boursiac et al., 2005; Munns and Tester, 2008). Osmotic stress decreases the water potential of the root medium, which leads to a decrease in the root water uptake, thus limiting the transport of water from the root to the leaf. Ultimately, osmotic stress causes water loss in leaf cells and leads to a decreased leaf water potential (Munns and Tester, 2008). A reduction in the water potential can result in reduced cell elongation and division, which would ultimately lead to decreased leaf growth (Meyer and Boyer, 1972). In the current study, Si application significantly increased the LER, RWC, and leaf water potential of seedlings compared with those

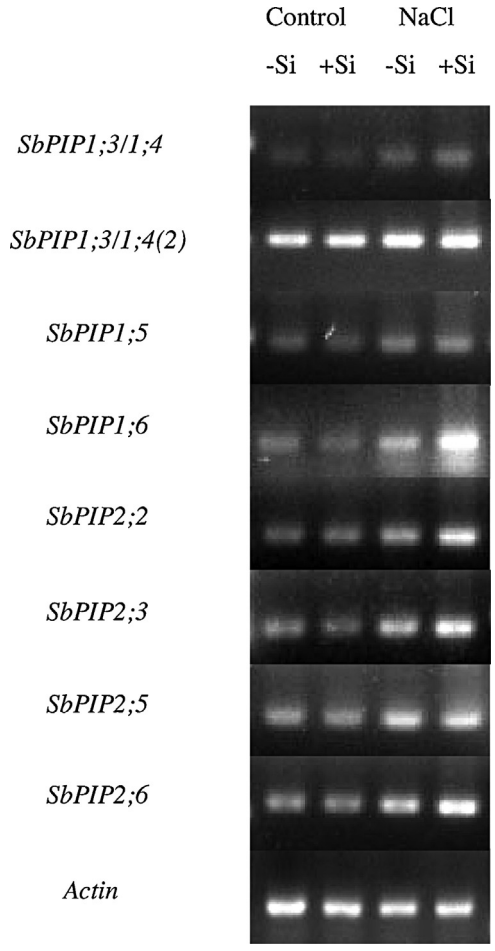

Fig. 8. Expression of *SbPIP* genes in the roots of sorghum seedlings in response to Si application under control and salt treatments. Eight *SbPIP* genes were identified based on GenBank. The roots were sampled after 2 h of NaCl treatment. The relative expression was determined by semi-quantitative PCR.

observed in seedlings not exposed to Si under salt stress, indicating that Si alleviates salt-induced osmotic stress but not ion toxicity during the early stage of salt stress.

Water homeostasis plays a crucial role not only in plant growth but also in adaptation to osmotic stress induced by salt and drought (Horie et al., 2011). When plants are exposed to osmotic stress, the first response is to decrease water loss by closing the stomata. The photosynthesis rate is simultaneously decreased because the CO_2 uptake is reduced as a result of stomata closure (Cornic, 2000). A previous study indicated that stomata aperture is a direct response to the leaf water status (Comstock, 2002); in other words, if the water content in leaf is high, the stomata will remain open, which will increase the CO_2 uptake and further increases the photosynthesis rate. In the current study, the application of Si significantly alleviated the decrease in the photosynthesis rate, and this phenomenon was due to the enhancement in stomatal conductance originating from an improvement of the leaf water content (leaf water potential and RWC). It has been reported that changes in the K_{plant} affect stomatal conductance (Hubbard et al., 2001). In the present study, the changing tendency of K_{plant} was similar to the changing tendency of stomatal conductance.

K_{plant}, which represents the soil-to-leaf water transport capacity, consists of the leaf, stem and root hydraulic conductance (Martre et al., 2002). Because the sorghum seedlings used in the present study did not form stems, the K_{plant} was only affected by the root and leaf hydraulic conductance. Under control conditions, the L_p was not affected by Si. Salt stress significantly decreased the L_p, but Si application maintained the L_p at the same level as that observed under control conditions (Fig. 6). It should be noted that salt stress decreased the K_{plant} to a larger extent than the L_p in this study. This result may be due to the decrease in K_{leaf}, which was not measured in this study because of technical limitations. The decrease in K_{leaf} indicated that the water transport resistance in leaf was enhanced (Sack and Holbrook, 2006). In this study, salt stress decreased the K_{leaf}, but Si application did not eliminate this decrease. This may explain the fact that Si did not enhance the leaf water potential to the level observed under control conditions.

It is worth noting that Si enhanced the transpiration rate by 28% compared with that obtained without Si under salt stress, and a similar tendency was also observed for the leaf water potential and L_p, which was increased by 22% and 20%, respectively. The similar extent of the changes in those indices suggested that the Si-increased water uptake (transpiration rate) could be ascribed mostly to an improvement in the L_p. In addition, under osmotic stress, water is mainly transported through the symplastic pathway (Javot and Maurel, 2002). However, Na^+ in root is mainly transported through the apoplastic pathway (Munns and Tester, 2008). In the present study, Si application enhanced the L_p and

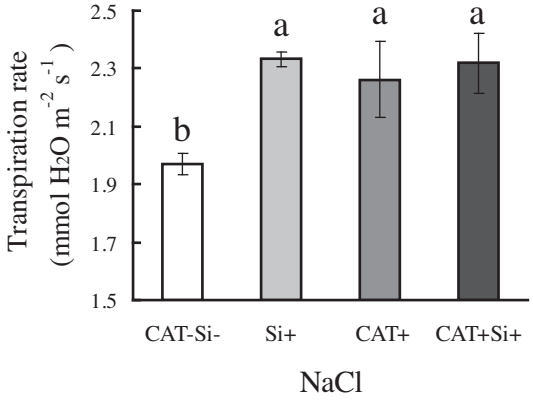

Fig. 9. Effects of catalase (CAT) and Si on the transpiration rate under salt stress. Before NaCl treatment, half of the seedlings were pre-treated with 400 U mL^{-1} CAT for 30 min. After 2 h of NaCl treatment, the transpiration rate was determined. The values are means ± SD of six replicates. Different letters indicate significant differences ($p < 0.05$).

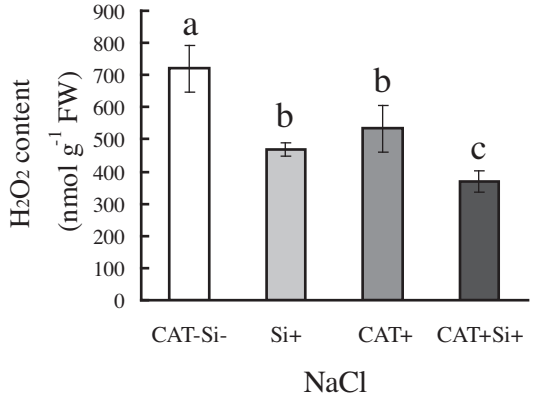

Fig. 10. Effects of catalase (CAT) and Si on H_2O_2 content under salt stress. Before NaCl treatment, half of the seedlings were pre-treated with 400 U mL^{-1} CAT for 30 min. After 2 h of NaCl treatment, H_2O_2 content was measured in root tips (1 cm). The values are means ± SD of six replicates. Different letters indicate significant differences ($p < 0.05$).

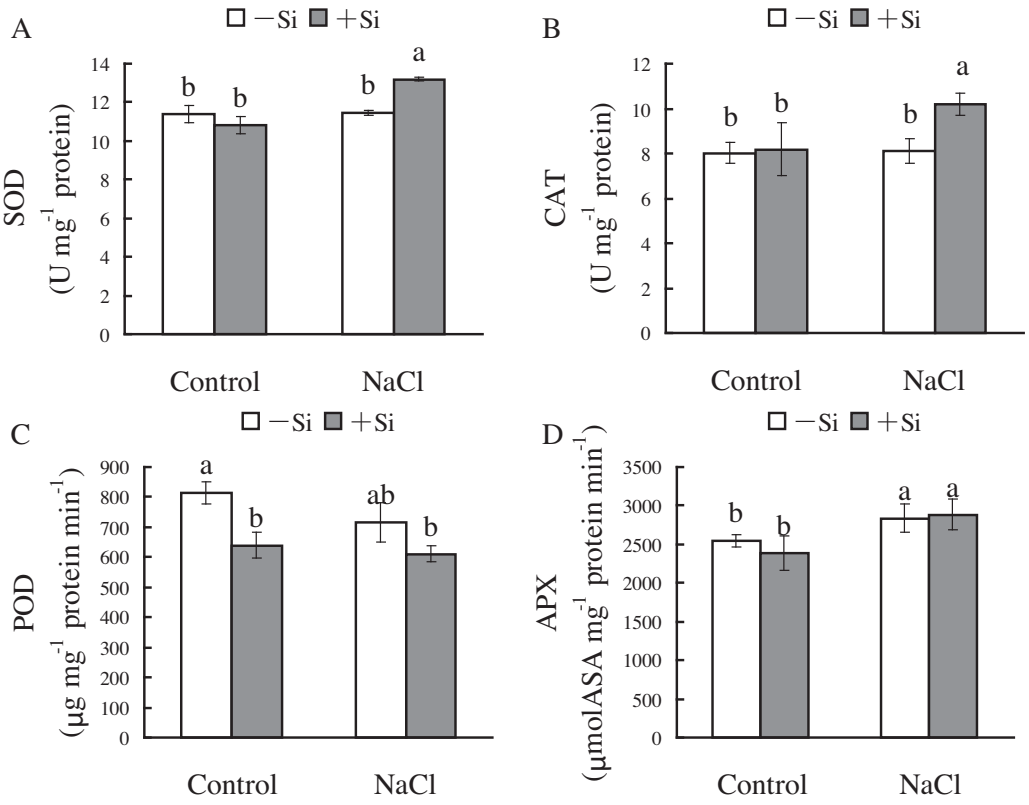

Fig. 11. Changes in antioxidant enzyme activities in the roots of sorghum seedlings in response to Si application under control and salt treatments. Root tips (1 cm) were sampled after 2 h of NaCl treatment to measure the activities of anti-oxidant enzymes. The values are means ± SD of six replicates. Different letters indicate significant differences ($p < 0.05$).

transpiration rate by regulating the symplastic pathway but not the apoplastic pathway. Therefore, Na^+ uptake in plants cannot be affected by the Si-induced changes in the L_p and transpiration.

It is now well known that the uptake of water by the roots in most plant species is largely regulated by aquaporin, which facilitates radial water transport across cell membranes, particularly under stress conditions (Boursiac et al., 2005). In the present study, the well-known aquaporin inhibitor $HgCl_2$ was used to investigate whether aquaporin activity was regulated by Si (Agre et al., 1998; Knipfer et al., 2011). Under salt stress, the transpiration rates of the seedlings treated with Si were significantly higher than those obtained without Si. However, after $HgCl_2$ treatment, the transpiration rates decreased to the same level. After recovery was induced by β-mercaptoethanol, the transpiration rates of the seedlings exposed to Si were higher than those measured in the seedlings without Si. These results indicated that aquaporin activity was enhanced by Si under salt stress, resulting in alleviating the salt-induced decrease in the L_p.

Based on this result, we investigated whether the transcriptional levels of aquaporin were affected by Si. In particular, we investigated the plasma membrane intrinsic protein (PIP), the most abundant aquaporin in the root plasma membrane, which plays a central role in regulating "cell-to-cell" water transport (Steudle and Peterson, 1998). As shown in Fig. 8, under short-term salt stress, the transcriptional levels of *SbPIP1;6*, *SbPIP2;2*, and *SbPIP2;6* were upregulated by Si. Previous studies have shown either decreases or increases in aquaporin activity under stress conditions in different plants (Hachez et al., 2012; Vandeleur et al., 2009; Rodriguez-Gamir et al., 2011). Our result was consistent with previous studies showing that the upregulation of aquaporin aids salt resistance (Qian et al., 2014). In addition, it has been reported that the overexpression of aquaporin genes results in rapid water uptake, which dilutes the Na^+ concentration (Gao et al., 2010). Sutka et al. (2011) concluded that enhancing the expression of aquaporin in the roots may provide an approach to compensate for the reduced soil water uptake under water-limiting conditions. In this study, the upregulation of aquaporin by Si was at least partly responsible for the recovery of the L_p under salt stress.

In addition to transcriptional regulation, aquaporin activity may also be regulated by translation, translocation, stability, or post-translational modification of aquaporin (Suga et al., 2002; Boursiac et al., 2008a,b). Aquaporin activity is affected by a number of factors, including abiotic stresses, phytohormones, and H_2O_2 (Henzler et al., 2004; Ruiz-Lozano et al., 2009; Hachez et al., 2012). There is a growing body of evidence showing that H_2O_2 plays a negative role in regulating the activity of aquaporin (Henzler et al., 2004; Boursiac et al., 2008a,b). In maize and chara, an oxidative gating of aquaporin was observed in the roots in the presence of H_2O_2, leading to the inactivation of aquaporin (Henzler et al., 2004; Ye and Steudle, 2006). In *Arabidopsis*, under salt stress, H_2O_2 decreased the L_p through the internalization of *AtPIPs* (Boursiac et al., 2008a). The investigation of aquaporin function in chilling–tolerant and chilling–sensitive maize genotypes showed that the chilling–tolerant cultivar exhibits higher aquaporin activity than the chilling-sensitive cultivar; this tolerance was due to lesser damage to the plasma membrane by H_2O_2 (Aroca et al., 2005). In addition, a previous study showed that H_2O_2 is involved in the gating of aquaporin through phosphorylation/dephosphorylation (Boursiac et al., 2008b). In the present study, to determine whether H_2O_2 is involved in the regulation of aquaporin activity, the seedlings were pre-treated with CAT before salt treatment. The results showed that, when pre-treated with CAT,

the transpiration rates of seedlings were similar to those with Si application under salt stress but higher than those without CAT or Si-treated plants (Fig. 9). In contrast, the root H_2O_2 content was quite low in the plants treated with both CAT and Si (Fig. 10). These results suggest that maintenance of aquaporin activity may be partly due to the decreased H_2O_2 levels caused by Si treatment. Moreover, the decreased H_2O_2 levels could be ascribed to the Si-induced enhancement of antioxidant enzyme activities (SOD, CAT, APX) under salt stress (Fig. 11), as has been widely reported in previous studies (Liang et al., 2003, 2006; Zhu et al., 2004).

5. Conclusions

At the early stage of salt stress (i.e., osmotic stress), the suppression of plant growth was mainly caused by a low water status in the leaf, which not only decreases cell division and elongation but also reduces photosynthesis. Under salt stress, Si application decreased H_2O_2 accumulation and upregulated the transcription of *SbPIP* genes, thereby enhancing aquaporin activity. The high aquaporin activity alleviated the salt-induced decrease in the L_p, which is beneficial to maintaining a high water content, stomatal conductance, and photosynthesis rate and enhanced the plant salt resistance. This research findings support the conclusion that the alleviation of salt-induced osmotic stress, as well as the mitigation of ion toxicity, is crucial to fully clarifying the mechanism through which Si alleviates salt stress.

Acknowledgments

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