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BRIEF COMMUNICATION

Effects of silicon on defense of wheat against oxidative stress under drought at different developmental stages

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Abstract

The effects of silicon application before sowing on the drought-induced oxidative stress and antioxidant defense in wheat (*Triticum aestivum* L.) were investigated. Drought stress was applied by withholding watering till sampling at booting or filling stage. Application of Si increased the water potential of drought-stressed plants at filling stage, whereas it did not at booting stage. The superoxide dismutase (SOD) activity was inhibited and peroxidase (POD) activity was enhanced by drought at booting stage, and no differences were observed due to the Si treatment. At filling stage, however, application of Si increased the SOD activity and decreased the POD activity of drought-stressed plants. The catalase (CAT) activity was slightly increased by drought only in the absence of Si and at booting stage. The activity of glutathione reductase (GR) was not greatly influenced. Application of Si did not change the contents of H_2O_2, total soluble protein and protein carbonyl of drought-stressed plants at booting stage, whereas at filling stage, it decreased the content of H_2O_2 and protein carbonyl and increased the content of total soluble protein. The content of thiobarbituric acid reactive substances (TBARS) and the activities of acid phospholipase (AP) and lipoxygenase (LOX) in drought-stressed plants were also decreased by application of Si at both stages.

Additional key words: catalase, glutathione reductase, lipoxygenase, peroxidase, phospholipase, superoxide dismutase, *Triticum aestivum*, water stress.

Although silicon has not been considered to be an essential element for higher plants (Epstein 1999), yet its beneficial effects have been demonstrated for many plants, especially when they are subjected to biotic or abiotic stresses (Ma and Yamaji 2006, Liang *et al*. 2007). Application of silicon has been reported to be able to control disease (Rodrigues *et al*. 2003, Ranganathan *et al.* 2006) and pest (Elawad *et al*. 1985, Ranganathan *et al.* 2006), alleviate toxicity of some heavy metals (Hodson and Sangster 2002), boron stress (Gunes *et al*. 2007b) and salt stress (Gong *et al*. 2006, Liang *et al*. 2006).

In rice, addition of silicon decreased the transpiration rate and membrane permeability of plants under water deficit induced by polyethylene glycerol (Agarie *et al*. 1998). In sorghum (*Sorghum bicolor* Moench), application of silicon increased relative water content and dry mass of plants (Hattori *et al*. 2001). It was suggested that the improvement of drought tolerance by added silicon in sorghum might be associated with enhancement of water uptake ability (Hattori *et al*. 2005, 2007). Addition of silicon also improved water status and increased dry mass of wheat plants in pots under drought (Gong *et al*. 2003a). Our previous results suggested that the improvement of drought tolerance of wheat by silicon was associated with the increase of antioxidant defense abilities and the alleviation of oxidative damage (Gong *et al*. 2005). All

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Abbreviations: AP - acid phospholipase; CAT - catalase; GR - glutathione reductase; LOX - lipoxygenase; POD - peroxidase; SOD - superoxide dismutase; TBARS - thiobarbituric acid reactive substances.

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these studies show that application of silicon is useful for improvement of drought tolerance of plants. However, these studies were performed with potted seedlings or PEG-stressed seedlings, in which the experimental duration was relatively short. To our knowledge, in the field, however, little work has been done. In the present work, we report the effects of silicon on the antioxidant defense and oxidative stress of wheat plants in the field under drought.

Plants of spring wheat (*Triticum aestivum* L. cv. Longchun 8139) were grown in the campus of Lanzhou University. The seeds provided by the Institute of Agriculture, Dingxi County, Gansu Province, China were sown on 15th March 2003. Three treatments consisted of CK (control), DR (drought) and DSi (drought + silicon, 0.1167 g m^{-2}, as sodium silicate). Before application of silicon, the soil properties were as follows: pH 8.2, organic matter 2.9 % , total nitrogen 0.1 % , available phosphorus 73.5 mg kg^{-1}, available potassium 119.0 mg kg^{-1}, slowly available potassium 926.0 mg kg^{-1} and available silicon 281.5 mg kg^{-1}. Drought treatments were applied by withholding irrigation for 52 and 69 d till sampling at booting and filling stages, respectively, and movable rainout shelters were used to shield the plants from rain. The control was continuously maintained under optimal irrigation with tap water. The fully expanded leaves were harvested on 19th May and 5th June at about 09:00 and immediately frozen in liquid N_2. At the same time, soil samples were taken from the field at depth of 10, 30, 60 and 100 cm for moisture analysis. The soil moisture content was measured gravimetrically based on soil samples after drying at 105 - 110 °C. The leaf water potential was measured with the pressure chamber (*ZLZ*, Lanzhou University, Lanzhou, China) (Gong *et al*. 2005). For enzyme extraction, leaves were homogenized in ice-cold 0.1 M sodium phosphate buffer (pH 6.8). The homogenate was centrifuged at 12 000 *g* for 20 min, after which the supernatant was used for enzyme assays. SOD was assayed by the nitroblue tetrazolium (NBT) method (Dhindsa *et al*. 1980). One unit of SOD was defined as that being contained in the volume of extract that caused a 50 % inhibition of the NBT reduction. CAT and POD activities were determined according to the method of Cakmak *et al*. (1993) by monitoring the rates of H_2O_2 decomposition and guaiacol oxidation, respectively. GR activity was determined based on the decrease in absorbance at 340 nm due to the oxidation of NADPH to NADP according to the method of Fryer *et al*. (1998). LOX activity was assayed by monitoring the rate of linoleic acid oxidation according to the method of Egert and Tevini (2002). For extraction of acid phospholipase (AP), leaves were homogenized in 50 mM Tris-HCl (pH 7.5). The homogenate was centrifuged at 4 000 *g* for 15 min and the supernatant was used for activity determination using the method of Xu *et al*. (1994). All the above procedures were carried out at 0 - 4 °C. The H_2O_2 contents in the leaves were assayed according to the method of Velikova *et al*. (2000). Lipid peroxidation in the leaves was estimated by determining the amount of thiobarbituric acid reacting substances (TBARS) following the method of Ma *et al*. (2002). The concentration of TBARS was calculated using coefficient of absorbance of 155 mM^{-1} cm^{-1}. For the measurements of total soluble protein content and protein oxidation in the leaves, plant materials were homogenized in ice-cold extraction buffer (50 mM potassium phosphate, pH 7.4, 1 mM EDTA). The extracts were centrifuged at 15 000 *g* for 20 min, and the resulting supernatants were used for determinations. Protein contents were assayed by Bradford's method (Bradford 1976) with bovine serum albumin as a standard. Oxidative damage to protein was estimated as the content of carbonyl groups, which was determined by reaction with 2,4-dinitrophenylhydrazine according to the methods of Landry *et al*. (1995). The data were subjected to statistical analysis by one-way *ANOVA* using *Microcal Origin 6.0*.

The control field maintained soil moisture content of about 15 % at both developmental stages. At booting stage, the soil moisture contents in the drought fields decreased to about 5, 8, 10 and 13 % at depth of 10, 30, 60 and 100 cm, respectively. With drought progressing, the soil moisture contents at filling stage decreased further to about 2.5, 6.5, 7 and 9 %. There were no obvious differences in the presence or absence of Si. The water potential of wheat leaves was also decreased under drought stress (Table 1). DSi plants maintained higher water potential in comparison with DR plants at filling stage. This is in line with pot experiments in previous studies (Hattori *et al*. 2001, Gong *et al*. 2003a), suggesting that application of Si could improve the water status of drought stressed wheat plants at this stage.

The SOD activity was inhibited by drought at booting stage, and there was no significant difference between the DR and DSi (Table 1). The decrease in the activity of SOD might cause the accumulation of superoxide anion radicals. At filling stage, the activity of SOD was still decreased by drought, but in the presence of Si, the activity was not significantly different from the control. The CAT activity was slightly increased by drought at booting stage in the absence of applied Si. At both developmental stages, the activity of GR was not greatly influenced. The POD activity was obviously increased by drought at booting stage, regardless of Si application. At filling stage, this enzyme activity was still stimulated by drought only in the absence of applied Si. The increase in the activity of POD at booting and filling stage under drought in the present study might be an adaptive response (Table 1). In barley, it was found that added Si increased the SOD, POD, CAT and GR activities of salt stressed plants (Liang *et al*. 2003). Zhu *et al*. (2004) also observed that addition of Si increased the activities of antioxidant enzymes SOD, GPX, APX, DHAR and GR of salt stressed cucumber. High activities of antioxidant enzymes induced by addition of Si might protect plant tissues from membrane oxidative damage under salt stress, as suggested by Liang *et al*. (2003) and Zhu *et al*. (2004). In drought conditions, we have previously observed that application of Si increased the activities of SOD, CAT and GR of wheat plants in pots,

Table 1. Water potential [-MPa], activities of SOD [U mg^{-1}(protein)], CAT [nmol(H_2O_2) mg^{-1}(protein) min^{-1}], POD [nmol(guaiacol) mg^{-1}(protein) min^{-1}] and GR [nmol (NADPH) mg^{-1}(protein) min^{-1}], H_2O_2 content [µmol g^{-1}(d.m.)], protein content [mg g^{-1}(d.m.)] and oxidation [nmol(C=O) mg^{-1}(protein)], TBARS [nmol g^{-1}(d.m.)] and activities of LOX [nmol(linoleic acid) mg^{-1}(protein) min^{-1}] and AP [nmol(Pi) mg^{-1}(protein) min^{-1}] of wheat leaves at booting and filling stage under drought in the presence or absence of applied silicon. Means ± SE, $n = 6 - 9$ for water potential; 6 for SOD, POD, GR, AP and protein content; 8 for CAT; 3 for H_2O_2, oxidized protein and TBARS. Different letter between three treatments at each developmental stage indicates significant differences at $P < 0.05$ according to one-way *ANOVA*.

Parameters	Booting stage CK	DR	DSi	Filling stage CK	DR	DSi
Water potential	0.91 ± 0.09a	1.49 ± 0.16b	1.41 ± 0.11b	1.35 ± 0.18a	2.42 ± 0.22c	2.16 ± 0.26b
SOD	6.50 ± 0.43a	4.42 ± 0.45b	4.41 ± 0.42b	6.07 ± 0.39a	5.34 ± 0.24b	5.99 ± 0.46a
CAT	122.45 ± 6.26a	131.78 ± 6.72b	120.78 ± 9.51a	143.97 ± 6.40a	141.40 ± 11.09a	140.05 ± 10.55a
POD	422.88 ± 27.67a	511.62 ± 33.61b	505.23 ± 35.56b	556.76 ± 13.64a	618.16 ± 49.64b	560.94 ± 4.80a
GR	71.52 ± 5.89a	70.70 ± 5.94a	73.68 ± 1.42a	76.58 ± 7.44a	72.29 ± 3.93a	72.60 ± 1.67a
H_2O_2	2.53 ± 0.09b	4.24 ± 0.08a	4.02 ± 0.22a	10.15 ± 0.06b	14.61 ± 0.13a	9.08 ± 0.12c
Protein content	235.84 ± 11.58a	196.63 ± 8.94b	201.92 ± 10.49b	221.06 ± 8.25a	175.97 ± 9.92c	189.95 ± 5.53b
Oxidized protein	8.13 ± 0.98b	25.84 ± 1.56a	18.88 ± 1.84a	14.29 ± 1.91c	26.75 ± 3.86a	21.76 ± 1.24b
TBARS	57.48 ± 1.00c	80.30 ± 7.42a	70.37 ± 6.99b	109.56 ± 1.80c	131.95 ± 4.11a	115.75 ± 3.26b
LOX	349.53 ± 50.95a	419.18 ± 27.66b	343.95 ± 12.47a	316.88 ± 12.96a	363.20 ± 26.68b	321.05 ± 11.17a
AP	9.78 ± 2.65c	26.59 ± 0.90a	23.70 ± 0.68b	25.07 ± 1.15c	35.22 ± 2.28a	31.16 ± 0.65b

but there were no effect on the activities of POD and APX (Gong *et al*. 2005). The different responses of enzymatic antioxidants to drought stress and Si application between pot and field experiments might be associated with stress modes and/or stress intensity.

Environmental stresses increase the formation of reactive oxygen species, which can induce oxidative damage to functional molecules in the plant cells (Egert and Tevini 2002). In this study, drought stress significantly increased the H_2O_2 content at both developmental stages in the absence of applied Si (Table 1). Application of Si did not affect the H_2O_2 content at booting stage, whereas it decreased the content obviously at filling stage (Table 1). The induction in the SOD activity decreased the toxicity of superoxide, but could also cause the accumulation of H_2O_2 if not eliminated promptly. However, no increased activities of H_2O_2-scavenging enzymes by application of Si were observed at filling stage, and the activity of POD was even decreased at this stage (Table 1). We speculated that this might be associated with the decrease in the H_2O_2 content by application of Si, and there was no demand to increase the activities of H_2O_2-scavering enzymes in the Si-applied plants at this stage. This is consistent with previous study in wheat in well watering conditions (Gong *et al*. 2003b).

The content of total soluble protein was significantly decreased by drought stress. Application of Si did not affect the protein content of drought-stressed plants at booting stage, but it increased the protein content of stressed plants at filling stage (Table 1). Drought stress increased the content of carbonyl groups in the leaves. There was no significant difference between DR and DSi at booting stage. But at filling stage, DSi plants had lower content of carbonyl group than DR (Table 1). This indicates that application of Si could alleviate oxidative damage of proteins under drought.

The TBARS content is often used as an indicator of oxidative damage (Gunes *et al*. 2007b). In the present study, the TBARS content in the leaves was increased by drought, and there was less increase in DSi than in DR at both developmental stages (Table 1), suggesting that application of Si decreased lipid peroxidation. Similar result was also observed in previous pot experiment (Gong *et al*. 2005), in which Si increased fatty acid unsaturation of drought stressed wheat.

LOXs catalyze the oxidation of polyunsaturated fatty acid to hydroperoxides, in which singlet oxygen and superoxide anions can be formed (Lynch and Thompson 1994). Drought stress increased the LOX activity at both developmental stages and Si application decreased the LOX activity of drought stressed plants (Table 1). The activity in DSi did not show any significant difference from that in CT. The effect of Si on LOX activity has been investigated by Gunes *et al*. (2007a,c). He found that the increase of LOX activity induced by boron in spinach was partially inhibited by addition of Si (Gunes *et al*. 2007a), but the LOX activities in spinach and tomato grown in combination of NaCl and B were increased by added Si (Gunes *et al*. 2007c). Reduction in LOX activity under stress conditions can be considered as beneficial to the plant (Egert and Tevini 2002, and references therein). Therefore, the decrease of LOX activity by applied Si partially reduced the production of radicals and ROS, which might contribute to the alleviation of oxidative damage to proteins, lipids and other cellular components.

The AP activity was increased by drought stress at both stages and Si treatment decreased the activity in drought stressed plants (Table 1). This is in accordance to the results observed previously in pot experiment (Gong *et al.* 2005), indicating that application of Si decreased lipid deesterification.

In summary, these results indicate that application of Si could alleviate the oxidative stress of wheat and regulate activities of antioxidant enzymes, which contributed to improvement of growth of plants in field under drought. The ameliorative effects were more obvious at filling stage. At early developmental stage, there might be sufficient Si in the field for the growth of plants so that the effect of Si application was not obvious. It can also be reasonable to speculate that the Si effect was related to drought stress intensity, *i.e.* drought stress was more severe at filling stage than booting stage.

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