

Study of Silicon Effects on Antioxidant Enzyme Activities and Osmotic Adjustment of Wheat under Drought Stress

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Abstract: The effect of silicon (Si) was investigated on the major antioxidant enzyme activities including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), relative water content (RWC), chlorophyll and soluble protein contents, proline (Pro) and glycine betaine (GB) accumulation in three different growth stages (2nd, 4th leaf and tillering stages) of wheat (*Triticum aestivum* L.) plants under drought stress. The experiment was performed in a completely randomized design for three treatments including control, drought and Si-drought (2mM silicate sodium/kg) with three replications in a greenhouse. The results indicated that Si partially offset the negative impacts of drought stress increasing the tolerance of wheat by rising Pro and GB accumulation and soluble protein content. Compared with the plants treated with drought, applied Si significantly enhanced the activities of SOD, CAT, APX and POD. In contrast, drought stress caused a considerable decrease in RWC, chlorophyll and soluble protein contents. This Si effect was time-dependent and became stronger in the tillering stage. The results of the present experiment coincided with the conclusion that Si alleviates water deficit of wheat by preventing the oxidative membrane damage and may be associated with plant osmotic adjustment.

Keywords: drought; osmotic adjustment; oxidative stress; silicon; wheat

Drought is still a serious agronomic problem and also one of the most important factors contributing to crop yield loss. Plants have evolved a series of non-enzymatic and enzymatic antioxidant systems to cope with drought stress and to avoid photooxidative damage, either by stress avoidance or stress tolerance (JUNG 2004). Reactive oxygen species (ROS) such as superoxide anion (O_2^-), H_2O_2 , and hydroxyl (OH) are commonly generated and accumulated under abiotic stress (McCORD 2000). Excessive levels of ROS damage cellular structures and macromolecules, causing photoinhibition of the photosynthetic apparatus (SMIRNOFF 1993). However, the production and accumulation of ROS activate multiple defence

responses, thus having also a positive role. The metabolism of ROS is dependent on several functionally interrelated antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) (MITTLER 2002). SOD removes superoxide anion free radicals accompanying the formation of H_2O_2 , which is then detoxified by CAT and POD (SUDHAKAR *et al.* 2001). In the ascorbate-glutathione cycle, APX reduces H_2O_2 using ascorbate as an electron donor (ARRORA *et al.* 2002).

Although Si is the second most abundant element, it has not yet been listed among the essential elements for higher plants partly because direct evidence that Si is a part of an essential plant

constituent or metabolite is lacking (EPSTEIN 1999). Si is never found in a free form and is always combined with other elements, usually forming oxides and it is absorbed by plants in the form of uncharged silicic acid, $\text{Si}(\text{OH})_4$ (RANGANATHAN *et al.* 2006). The importance of Si has been recently recognized (EPSTEIN 1999; RICHMOND & SUSSMAN 2003; MA 2004).

Results on the beneficial effects of Si in enhancing the tolerance of plants to biotic and abiotic stresses in several crops, and their relevance to the world of agriculture have been widely described (EPSTEIN 1999; MA 2004). Si benefits to drought tolerance in wheat (GONG *et al.* 2005), maize (LI *et al.* 2007; YONG *et al.* 2007), sorghum (HATTORI *et al.* 2003, 2005) and salt tolerance of barley, tomato and cucumber (LIANG *et al.* 2003; AL-AGHABARY *et al.* 2004; ZHU *et al.* 2004) have been related to its effect on the antioxidant enzyme activity. GONG *et al.* (2005) reported that application of Si increased the activities of SOD, CAT and GR, the fatty acid unsaturation of lipids, and the contents of photosynthetic pigments and soluble proteins as well as total thiols under drought stress on wheat, whereas the content of H_2O_2 , activity of acid phospholipase (AP) and oxidative stress of proteins were decreased by applying Si compared with those of non-Si treatments under drought stress. In sorghum, Si ameliorated the decrease in dry weight under drought stress conditions, and Si-applied sorghum had a lower shoot to root (S/R) ratio, indicating the facilitation of root growth and the maintenance of the photosynthetic rate and stomatal conductance at a higher level compared with plants grown without Si application (HATTORI *et al.* 2005). Optimization of Si nutrition results in increased weight and volume of roots by 20% to 200% and enhanced drought and salt resistance of cultivated plants (MATICHENKOV *et al.* 2001).

The purpose of the present research project was to study exogenous Si and relevant ability to enhance drought stress in wheat. The hypothesis was whether Si may affect wheat to increase drought resistance via elevating the antioxidant system by the key enzymes such as CAT, SOD, APX and POD involved in the oxidative stress defence of the plant cell. Therefore, the effect of Si was determined on the forementioned traits rather than RWC, chlorophyll, Pro and GB accumulation and also total soluble protein content at different developmental stages for various stress conditions.

MATERIALS AND METHODS

Plant material

The surface of wheat seeds (*Triticum aestivum* L. VERNACK, provided by the Seed and Plant Improvement Institute of Iran) was sterilized with 1% sodium hypochlorite for 10 min and they were germinated for 24 h. The germinated seeds were sown into pots with three treatments that composed of control (C), drought (Dr) and Si-drought (Si-Dr) treatments. Drought stress was applied by gypsum block (potential -1.0 MPa). Before sowing, the soil was mingled sufficiently, divided into several parts, each of 15 kg weight, and then sodium silicate (2mM of sodium silicate/kg soil) was added to Si-Dr treatment. Each treatment was replicated three times and the experiment was carried out as a complete randomized block design. Leaf samples were collected at the 2nd, 4th leaf and tillering stages and were frozen in liquid N_2 immediately until analysis.

Measurements of RWC, chlorophyll and total soluble protein contents

The RWC of leaves was calculated by the following equation of SCHONFELD *et al.* (1988). Leaf chlorophyll was extracted in 80% acetone and the absorbance was read spectrophotometrically at 663 and 645 nm. The values of chlorophyll were evaluated using the formula proposed by ARNON (1949) to compute chlorophyll content. In order to extract total soluble protein, 1 g leaf tissue was homogenized in 3 ml of extraction buffer including 50mM phosphate buffer (pH 7.0) and 1mM sodium metabisulphate containing 100 mg insoluble PVP (polyvinylpyrrolidone). The homogenate was centrifuged (Beckman Culter, Allegra-64R) at 15 000 g for 30 min to collect the supernatant as the source of enzyme assays. All the extraction steps were carried out at 4°C. Enzyme activity was estimated spectrophotometrically in laboratory conditions at 25°C. Total soluble protein was used for determination of protein content by the method of BRADFORD (1976). Bovine serum albumin (BSA) was employed in different concentrations to draw a standard curve. Total soluble protein was also analysed by SDS-PAGE (12.5% separating and 4.5% stacking gels) at different developmental stages following the procedure described by LAEMMLI (1970). The Coomassie blue staining was used to

observe protein bands according to the method of RYBICKI and PYRUES (2003). Native-PAGE was prepared to analyse the antioxidant enzyme activity according to LAEMMLI (1970).

Enzyme activity assay and isoenzyme analysis

CAT (EC 1.11.1.6) activity was determined by measuring H_2O_2 consumption at 240 nm for 3 min according to AEBI (1984) method and the enzyme activity was expressed as $\Delta_{240}/\text{mg protein/min}$. APX (EC 1.11.1.11) activity was determined following the oxidation of ascorbate to dehydroascorbate, as described by NAKANO and ASADA (1987) and the enzyme activity was shown as $\Delta_{290}/\text{mg protein/min}$. SOD (EC 1.15.1.1) activity was determined by measuring the inhibition in the photochemical reduction of nitroblue tetrazolium at 560 nm as described by BEAUCHAMP and FRIDOVICH (1971). The enzyme activity was expressed as units/mg protein. POD activity was determined by measuring peroxidation of H_2O_2 with guaiacol as an electron donor (CHANCE & MAEHLY 1955). CAT and POD activity were estimated on Native-PAGE using 6% separating and 4% stacking gels. SOD and APX activities were analysed by Native-PAGE (10% separating and 4% stacking gels). Specified staining of SOD was done according to the method of BEAUCHAMP and FRIDOVICH (1971), specified staining of APX was carried out following the procedure described by RAO *et al.* (1996), POD (EC 1.11.1.7) isoforms were detected according to the method of HART *et al.* (1971), and finally to illustrate specified staining of CAT, we used ROBERTSON *et al.* (1987) method.

Pro and GB determination

Pro was determined according to the method of BATES *et al.* (1973). Approximately 0.5 g of fresh plant material was homogenized in 10 ml of 3% aqueous sulphosalicylic acid and filtered. Two ml of filtrate was mixed with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100°C. The reaction mixture was then extracted with 4 ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was measured at 520 nm with spectrometer. Appropriate Pro standards were

included for the calculation of Pro in the sample. The amount of GB was estimated according to the method of GRIEVE and GRATTAN (1983). The dried plant material was finely ground, mechanically shaken with 20 ml deionised water for 48 h at 25°C. The samples were then filtered and filtrates were diluted 1:1 with 2M H_2SO_4 . Aliquots were kept in centrifuge tubes and cooled in ice water for 1 h. Cold KI-I_2 reagent was added and the reactants were gently stirred with a vortex mixer. The tubes were stored at 4°C for 16 h and then centrifuged at 15 000 rpm for 20 min at 0°C. The supernatant was carefully aspirated. The periodide crystals were dissolved in 9 ml of 1,2-dichloroethane. After 2 h, the absorbance was measured at 365 nm using GB as standard.

Statistical analysis

Analysis of variance was carried out on the data using software package of SPSS, version 10 and significant differences among treatment means were calculated by Duncan's multiple range test.

RESULTS

RWC of wheat leaves was significantly modified by different treatments of this study. C, Si-Dr, and Dr treatments presented 71.6%, 63.9% and 44.3% of RWC, respectively (Table 1 and Figure 1). Drought treatment significantly decreased the RWC and the Si applied plants still maintained higher RWC compared to those without application of Si under drought stress, indicating that the application of Si improved the water status of stressed wheat plants.

Drought stress significantly decreased the pigment content (Table 1 and Figure 2). Compared to the control, the contents of chlorophyll a, b and total chlorophyll were subsequently decreased to 48%, 55% and 52%, respectively, in Dr stress conditions, while Si caused an increase in these contents under drought stress and there was no significant difference between control and Si treatments. Neither were any significant changes observed between growth stages in pigment content under Dr treatment, but there were greater changes in tillering stage in C and Si-Dr treatments.

As shown in Figure 3, drought stress significantly decreased the total soluble protein content, and the level of the decrease in Si treatment

Table 1. Relative water content (RWC), pigment content (Chl), soluble protein content, catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX), proline (Pro) and glycine betaine (GB) content of the leaves of wheat plants grown under control (C), Si-drought (Si-Dr) and drought (Dr) treatments

Parameter	Treatment			F values
	C	Si-Dr	Dr	
RWC (%)	71.64 ± 2.54 ^a	63.91 ± 2.42 ^b	44.37 ± 2.01 ^c	35.1 ^{**}
Chl a (mg/g FW)	1.47 ± 0.15 ^a	1.33 ± 0.13 ^a	0.76 ± 0.09 ^b	2.49 [*]
Chl b (mg/g FW)	1.02 ± 0.13 ^a	0.927 ± 0.11 ^a	0.45 ± 0.08 ^b	1.71 [*]
Total Chl (mg/g FW)	7.78 ± 0.48 ^a	7.39 ± 0.73 ^a	3.75 ± 0.44 ^b	8.3 [*]
Soluble protein (mg/g FW)	40.1 ± 1.38 ^a	33.3 ± 1.56 ^b	25.86 ± 1.44 ^c	65.7 ^{**}
CAT (μM H ₂ O ₂ dec/min/mg protein)	0.495 ± 0.06 ^b	0.555 ± 0.05 ^a	0.501 ± 0.04 ^b	0.001 [*]
POD (μM H ₂ O ₂ dec/min/mg protein)	0.332 ± 0.03 ^c	0.573 ± 0.06 ^a	0.521 ± 0.05 ^b	0.413 ^{**}
SOD (unit/mg protein)	21.78 ± 1.15 ^c	27.98 ± 1.41 ^a	24.78 ± 1.36 ^b	17.7 ^{**}
APX (μM H ₂ O ₂ dec/min/mg protein)	0.938 ± 0.09 ^c	1.44 ± 0.05 ^a	1.28 ± 0.09 ^b	0.013 ^{**}
Pro (μM/g FW)	74.42 ± 2.83 ^c	301.67 ± 4.29 ^a	247.52 ± 3.64 ^b	318.6 ^{**}
GB (μM/g DW)	66.73 ± 1.73 ^c	219.21 ± 2.89 ^a	147.38 ± 2.28 ^b	124 ^{**}

The values are means of three replications ± SD; different letters in each row represent significant differences based on Duncan's multiple range test, * $P < 0.05$; ** $P < 0.01$

was obviously lower than that in non-Si treatment. Compared to the control, soluble protein content was decreased to 17% and 36%, respectively, by Si-Dr and Dr treatments (Table 1). These results indicate that the application of Si could reduce the decomposition of proteins in wheat plants under drought stress. In order to analyse the changes in

total protein content under drought stress, tissue extracts were subjected to SDS-PAGE (Figure 3). Si supply influenced the levels of several proteins. The result of this experiment indicated that the total soluble protein content was reduced considerably under drought stress, while only a minor decrease in total soluble protein content was observed in Si-Dr treatment.

The activities and electrophoresis pattern of CAT, SOD, APX and POD are given in Figures 4 to 7. CAT activity in drought-stressed leaves was similar to that in leaves of well-watered control plants (Figure 4). However, plants exposed to Si showed a significant increase in the activity of CAT compared to the control. In Si treatment, CAT activity was subsequently increased to 12%. Also, this activity was increased up to 1.2% under drought stress (Table 1). With the progressing leaf age, CAT activity was also increased. Gels stained for CAT activity revealed only one isoform and Si-Dr treatment caused the increased intensity of isoform in all three stages.

POD plays a role in decreasing the accumulation of H₂O₂ content, eliminating MDA (malondialdehyde) resisting cell peroxidation of membrane lipids and maintaining the cell membrane integrity. Figure 5 shows that the enhanced activities of POD were apparent under conditions of Dr and Si-Dr treatments.

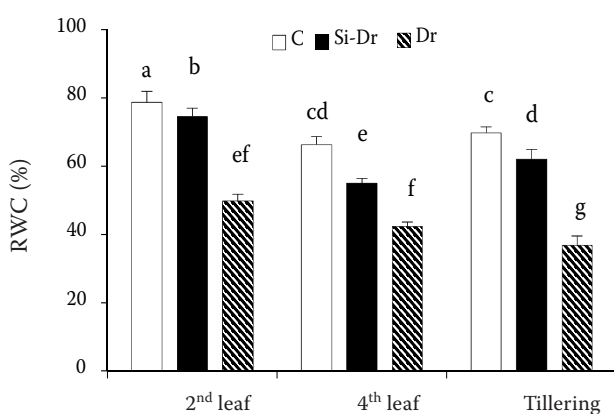


Figure 1. Effect of Si on relative water content (RWC) of wheat leaves under drought stress in three stages of growth; data are mean ± S.E. of three replications; bars with different letters are significantly different at the $P < 0.05$ level (C – control, Si-Dr – Si-drought, Dr – drought treatments)

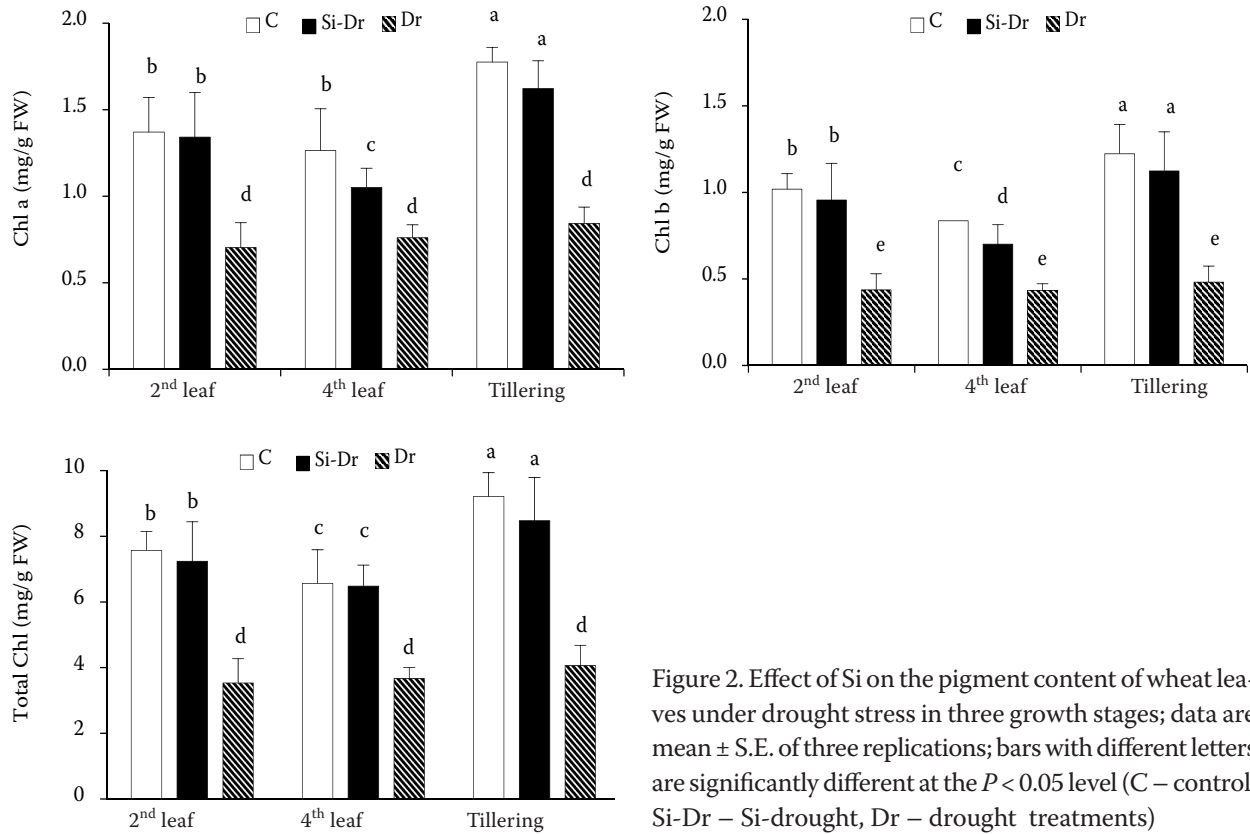


Figure 2. Effect of Si on the pigment content of wheat leaves under drought stress in three growth stages; data are mean \pm S.E. of three replications; bars with different letters are significantly different at the $P < 0.05$ level (C – control, Si-Dr – Si-drought, Dr – drought treatments)

Compared to the control, POD activity was subsequently increased to 57.5% and 72.5% under Dr and Si-Dr treatments, respectively (Table 1). There was no significant difference between growth stages in POD activity, while it was significantly increased by Si-Dr and Dr treatments in the 4th leaf and tillering stages. Based on the results from Native-PAGE, POD activities were consistent with spectrophotometric data (Figure 5). Leaves of Vynack wheat exhibited three isoforms of POD for all treatments, while Si-Dr

treatment enhanced the intensities of the existing isoforms.

Although drought stress caused an increase in the activity of SOD, it was higher in Si-Dr treatment than in the other treatments (Figure 6). Compared to C treatment, SOD activity was significantly elevated up to 14% under drought, while the application of Si caused an increase in such activity to twofold values (28%). By the application of Si, SOD activity was significantly increased to 28.45% and 18.34%

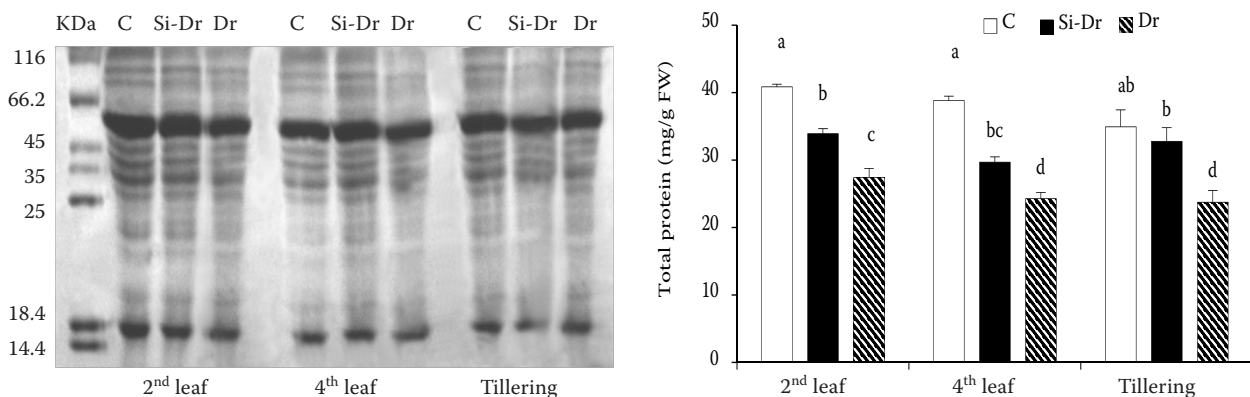


Figure 3. Effect of Si on a change in total soluble protein content and electrophoresis pattern (20 μ l per well) that was separated by SDS-PAGE under drought stress in three growth stages; bars with different letters are significantly different at the $P < 0.05$ level (C – control, Si-Dr – Si-drought, Dr – drought treatments)

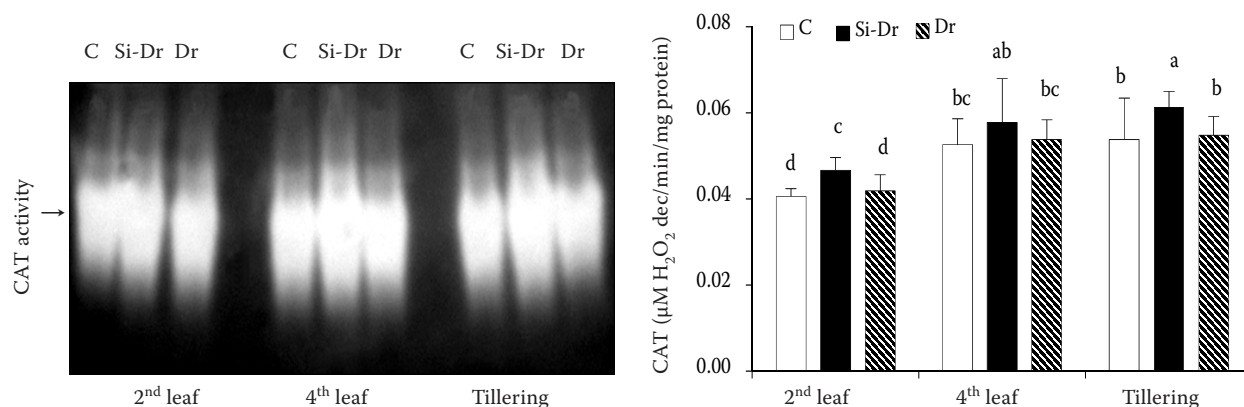


Figure 4. Effect of Si on catalase (CAT) activity and isoenzymes (20 μ g protein per well) that were subjected to Native-PAGE under drought stress in three growth stages; data are mean \pm S.E. of three replications; bars with different letters are significantly different at the $P < 0.05$ level (C – control, Si-Dr – Si-drought, Dr – drought treatments)

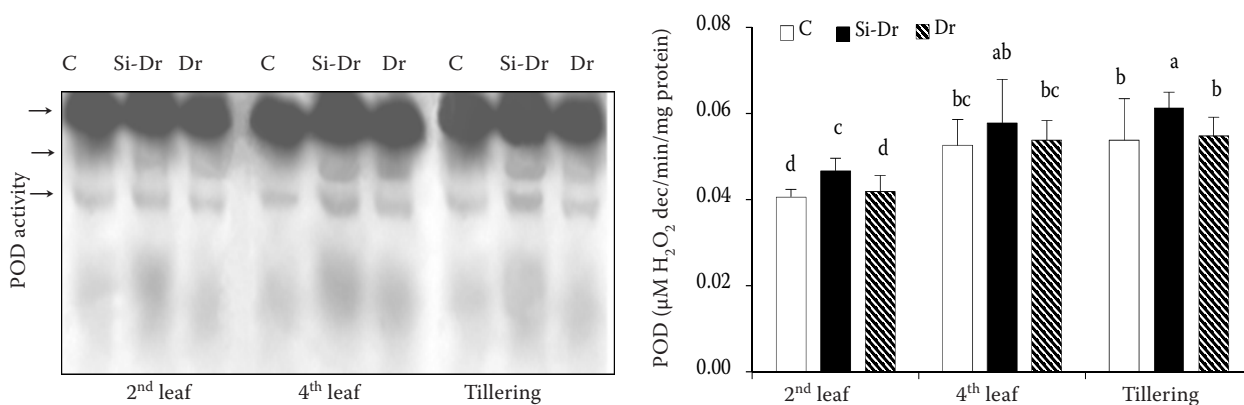


Figure 5. Effect of Si on peroxidase (POD) activity and isoenzymes (30 μ g protein per well) that were subjected to Native-PAGE under drought stress in three growth stages; data are mean \pm S.E. of three replications; bars with different letters are significantly different at the $P < 0.05$ level (C – control, Si-Dr – Si-drought, Dr – drought treatments)

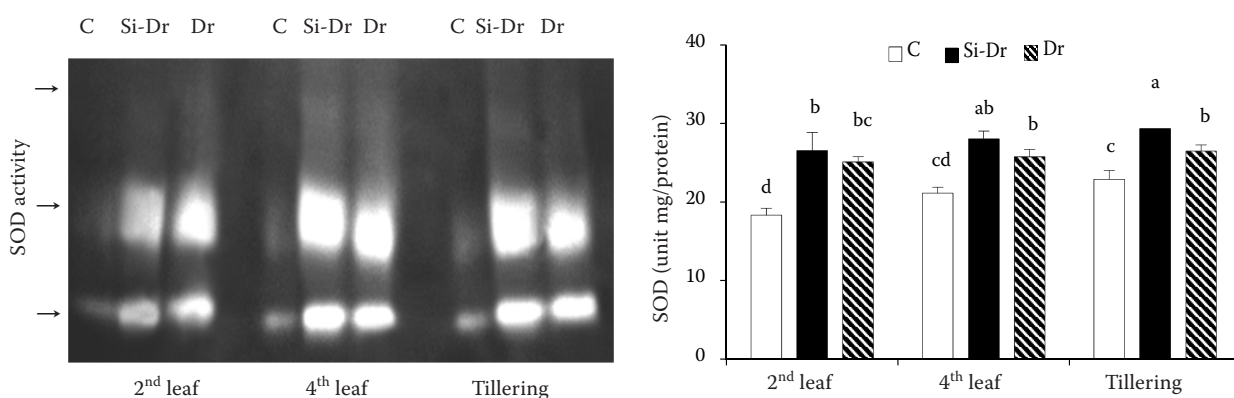


Figure 6. Effect of Si on superoxide dismutase (SOD) activity and isoenzymes (40 μ g protein per well) that were subjected to Native-PAGE under drought stress in three growth stages; data are mean \pm S.E. of three replications; bars with different letters are significantly different at the $P < 0.05$ level (C – control, Si-Dr – Si-drought, Dr – drought treatments)

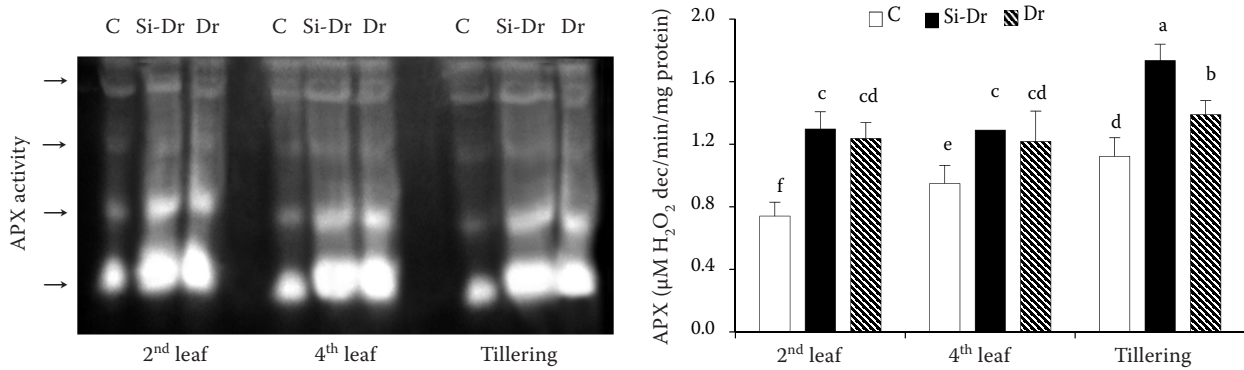


Figure 7. Effect of Si on ascorbate peroxidase (APX) activity and isoenzymes (30 μ g protein per well) that were subjected to Native-PAGE under drought stress in three growth stages; data are mean \pm S.E. of three replications; bars with different letters are significantly different at the $P < 0.05$ level (C – control, Si-Dr – Si-drought, Dr – drought treatments)

for C and Dr treatments, respectively (Table 1). The SOD activity was slightly increased in control and Si-Dr treatments in all three stages. There was no marked change in SOD activity between the 4th leaf stage and tillering stage in Dr treatment. When foliar extracts were subjected to Native-PAGE and monitored for SOD activity, three different SOD isoforms were observed in plants. According to the results of the present study, Si supplement caused a 53% increase in APX activity compared to the control. However, such activity was elevated to 36% under Dr stress (Table 1). APX activity was higher in Si-Dr and Dr treatments in tillering stage than in the other stages as presented in Figure 7. Four different isoforms were detected on the isoenzyme profiles of this enzyme. The increase in APX activity as a result

of Si-Dr treatment was due to the intensity of the isoform bands compared to C and Dr treatments.

The concentrations of Pro and GB are shown in Figure 8. With respect to this result, both Pro and GB contents were significantly increased in Si-Dr and Dr treatments compared to the control. However, drought stress frequently caused an increase in osmolyte content, so remarkably higher Pro and GB concentrations were observed in Si-Dr treatment than in other treatments. The concentration of Pro was increased from 74.42 (control) to 247.52 and 301.67 μ M/g FW, and GB content was increased from 66.73 (control) to 147.38 and 219.21 μ M/g DW, respectively, in Dr and Si-Dr treatments. Pro and GB contents were increased at the 4th leaf and tillering stages compared to the 2nd leaf stage and

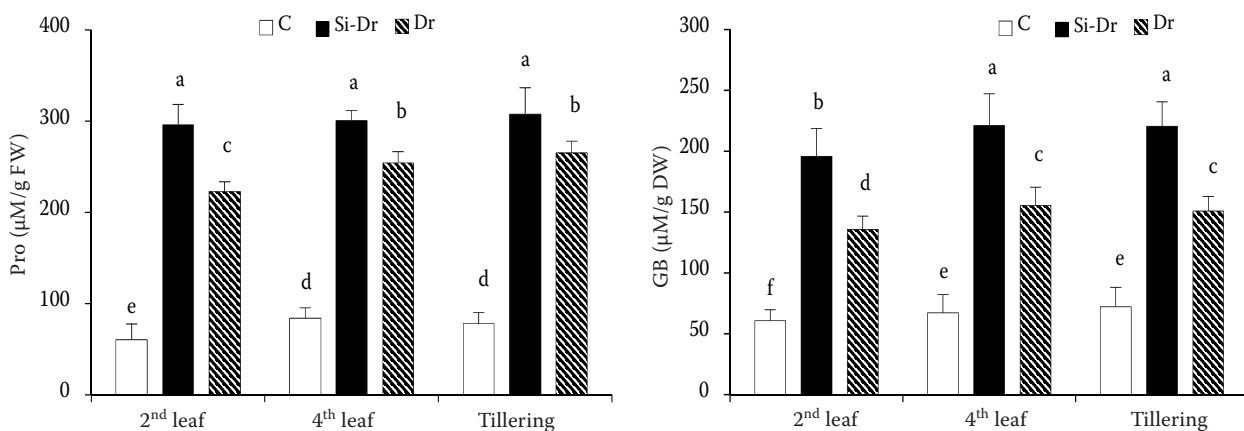


Figure 8. Effect of Si on proline (Pro) and glycine betaine (GB) content of wheat leaves under drought stress in three growth stages; data are mean \pm S.E. of three replications; bars with different letters are significantly different at the $P < 0.05$ level (C – control, Si-Dr – Si-drought, Dr – drought treatments)

no significant changes were observed between the 4th leaf stage and tillering stage.

DISCUSSION

Drought stress leads to the closure of stomata and subsequent decrease in the photosynthetic rate. The RWC of leaves, as indicated by the extent of dehydration, was used to assess cellular damage. In this study, drought stress caused a decrease in RWC content, but Si application can alleviate water stress by decreasing the transpiration. This result is in agreement with those of LOBATO *et al.* (2009), who showed that an increase in the Si concentration promoted an increase in the water retention of pepper leaf tissue. HATTORI *et al.* (2005) reported that the diurnal determination of the transpiration rate indicated that the Si-treated sorghum could extract a larger amount of water from a drier soil and maintain a higher stomatal conductance. The improved ability to retain water by plants treated with Si may result from a lowered transpiration rate and a higher value of water use efficiency (GAO *et al.* 2006). Leaf transpiration occurs mainly through the stomata and partly through the cuticle. It is obvious that most effects of Si were expressed through Si deposition on the leaves, stems, and hulls (MA & TAKAHASHI 2002). Si in the hull is also deposited between the epidermal cell wall and the cuticle, forming a cuticle-Si double layer like in the leaf blades. However, in contrast to the leaves, transpiration occurs only through the cuticle because the hull does not have any stomata (MA *et al.* 2001). Si is effective in decreasing the hull transpiration. Therefore, Si plays an important role in keeping a high moisture condition within the hull by decreasing the transpiration rate from the hull (MA & TAKAHASHI 2002). MA *et al.* (2001) reported that the rate of water loss from Si-free spikelets was about 20% higher than that from spikelets containing Si (7% Si) at both the milky and maturity stages and Si can reduce the transpiration rate by 30% in rice, which has a thin cuticle. Also the level of polysaccharides in the cell wall was higher in the leaves containing Si than in those lacking Si. These results suggest that Si in rice leaves is involved in the water relations of cells, such as mechanical properties and water permeability. MAITI *et al.* (1984) and LUX *et al.* (1999) suggested that depositions of Si on the endodermal cells might protect vascular tissues

against the invasion of parasites and the effects of drying soil via the mechanical hardening of root endodermal cells. In this experiment, the effect of Si on RWC content was higher in the tillering stage than in the 4th leaf stage (Figure 1). These results suggest that Si application may be useful to improve the drought tolerance of wheat via the enhancement of water uptake ability synchronized with time progress.

The content of photosynthetic pigments was significantly decreased by drought stress, the result of this study indicating that the application of Si could decrease the decomposition of photosynthetic pigments (Figure 2). These results are in accordance with those of GONG *et al.* (2005) in wheat under drought stress and AL-AGHABARY *et al.* (2004) in salt-stressed barley. GONG *et al.* (2005) reported that Si could increase the photosynthesis of wheat plants under drought and this might be associated with the enhancement in activities of photosynthetic enzymes, ribulose-bisphosphate carboxylase and NADP⁺ dependent glyceroldehyde-3-phosphate dehydrogenase, as well as chlorophyll content in stress conditions. Other studies showed the significant Si-induced enhancement of photosynthesis and chlorophyll fluorescence parameters in drought-stressed maize plants (LI *et al.* 2007), drought-stressed sorghum (HATTORI *et al.* 2005), salt-stressed barley (LIANG 1998) and tomato (AL-AGHABARY *et al.* 2004). KAUFMAN *et al.* (1979) proposed a “window hypothesis” for Si by suggesting that Si in the form of silica bodies deposited in leaf epidermal cells could act as a “window” that enhances the light use efficiency by facilitating the transmission of light to the photosynthetic mesophyll tissue. Consequently, the Si-induced enhancement of chlorophyll fluorescence parameters was recently demonstrated in drought-stressed maize plants (LI *et al.* 2007) and salt-stressed tomato (AL-AGHABARY *et al.* 2004).

Adaptation to drought may depend on different mechanisms, including the capacity to maintain high levels of antioxidants and/or through the induction of antioxidant enzymes. In the present study, the activity of CAT, POD, SOD and APX in wheat was increased in the leaves under drought stress, while such an increase was more significant and consistent in Si treatment than in other treatments. These results are in good agreement with the results of GONG *et al.* (2005), who found out that under drought stress the addition of Si

increased the antioxidant activity in wheat. In metabolic processes plants produce H_2O_2 which causes damage to the cell oxidation function, while CAT can eliminate H_2O_2 and play a key role in the elimination of O_2 . In this experiment, no significant change in the activity of CAT was observed in plants subjected to drought stress, when compared to the control. However, CAT activity was significantly elevated by Si treatment in the same conditions (Figure 4). These results indicated that, as compared with Dr treatment, higher constitutive levels of CAT suggest the more effective H_2O_2 dismutation capacity outside the plant chloroplasts under Si-Dr treatment. POD, as well as CAT, plays an essential role in scavenging the H_2O_2 toxicity, which is a major product produced by SOD. The combined action of CAT and SOD converts the toxic superoxide radical (O_2) and H_2O_2 to water and molecular oxygen (O_2), thus averting the cellular damage under unfavourable conditions like water stress (NOCTOR *et al.* 2000). APX plays the most important role in removing H_2O_2 , dehydroascorbate reductase and glutathione reductase, can provide substrate for APX by a catalyzing reaction. In the present study, the activities of APX were increased in plants when exposed to drought stress. Similar results were obtained by ZHU *et al.* (2004), who also observed increases in SOD and APX activities under salt stress by Si addition in barley and cucumber. The increase in the enzyme activity under Si treatment coincided with a variable increase in the individual isoform expression.

Many plant species naturally accumulate Pro and GB as major organic osmolytes when subjected to different abiotic stresses. These compounds are thought to play adaptive roles in mediating osmotic adjustment and protecting subcellular structures in stressed plants. GB is abundant mainly in chloroplasts where it plays a vital role in adjustment and protection of thylakoid membrane, thereby maintaining photosynthetic efficiency (GENARD *et al.* 1991). We found out a higher Pro and GB accumulation in Si-Dr treatment than in Dr treatment. A direct consequence of the higher Pro concentration in Si-Dr is the relatively higher water-retaining capacity as reflected by RWC (Figure 1) and also the more efficient antioxidant enzyme activity. CARLOS *et al.* (2009) observed that concentrations of Pro in potato are increased under lower water availability and higher Si availability in the soil, which indicates that Si may be associated with plant osmotic adjustment. The result of this experiment showed that the effect of Si was considerably increased in the tillering stage

of plant. These results are consistent with findings of ZHU *et al.* (2004). They reported that Si effect was time-dependent and became stronger as the experiments continued. The level of polysaccharides in the cell wall was higher in the leaves containing Si than in those lacking Si. These results suggest that Si in rice leaves is involved in the water relations of cells, such as mechanical properties and water permeability (MA 2004).

Mechanisms which are important for plant resistance to drought stress are as follows: improvement of the plant water status by reduced water loss through transpiration, maintenance of the membrane stability, regulation of osmotic adjustment, elimination of ROS and prevention of oxidative stress by increasing the antioxidant enzymes. The addition of Si significantly increased RWC in drought-stressed wheat leaves that corroborated the increase in (osmotic adjustment) Pro and GB concentration. Si may act to alleviate drought stress by improvement of the plasma membrane and tonoplast structure, integrity and functions, increasing CAT, SOD, APX and POD activity and reduction of lipid peroxidation, decreased pigments and soluble protein content. It was reported that Si enhanced the stability of lipids in cell membranes of wheat plants exposed to drought stress, suggesting that Si prevented the structural and functional deterioration of cell membranes when wheat plants were exposed to environmental stress (GONG *et al.* 2005).

In the case of leaves (HOSSAIN *et al.* 2002), silicon application to rice promoted the elongation of leaf blades with a concomitant increase in cell-wall extensibility in its basal elongating zone. Silicon plays two separate functions in root cell walls, strengthening the endodermal cell walls in the mature basal region and keeping the young expanding cell walls extensible in the apical region of the roots (HATTORI *et al.* 2003). The application of silicon seems to be quite beneficial to plants grown under drought conditions by encouraging the development of a big root system and providing protection to roots against soil drying. SACALA (2009) reported that a possible role of Si in plant resistance mechanisms to water stress may be considered at different levels (molecular, cellular, and whole-plant). In future studies, further research, including other species, is needed to confirm present results. Moreover, some researchers should focus on the mode of Si action, how it affects the abiotic stress response in higher plants. It is also suggested that detection and analysis of

induced proteins/enzymes in stressed Si tolerant plants might help to increase our understanding of the mechanism of Si tolerance in plants.

References

- AEBI H.E. (1984): Catalase *in vitro*. Methods in Enzymology, **105**: 121–126.
- AL-AGHABARY K., ZHU Z., SHI Q. (2004): Influence of silicon supply on chlorophyll content, chlorophyll fluorescence and antioxidative enzyme activities in tomato plants under salt stress. Journal of Plant Nutrition, **27**: 2101–2115.
- ARNON D.I. (1949): Copper enzymes in isolated chloroplast. Poly-phenoloxidase in *Beta vulgaris*. Journal of Plant Physiology, **24**: 1–15.
- ARRORA A., SAIRAM R.K., SRIVASTAVA G.C. (2002): Oxidative stress and antioxidant system in plants. Journal of Plant Physiology, **82**: 1227–1237.
- BATES L.S., WALDREN R.P., TEARE I.D. (1973): Rapid determination of free Pro for water stress studies. Journal of Plant Soil, **39**: 205–217.
- BEAUCHAMP C., FRIDOVICH F. (1971): Superoxide dismutase: cadmium assay and an assay applicable to acryl amide gels. Analytical Biochemistry, **44**: 276–287.
- BRADFORD M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, **72**: 248–254.
- CARLOS A.C.C., ADRIANO L.P., LEANDRO B.L., ROGERIO P.S., GIUSEPPINA P.P.L. (2009): Effects of silicon and drought stress on tuber yield and leaf biochemical characteristics in potato. Crop Science, **49**: 949–954.
- CHANCE B., MAEHLY A. (1955): Assay of catalase and peroxidase. Methods in Enzymology, **2**: 764–817.
- EPSTEIN E. (1999): Silicon. Plant Physiology, **50**: 641–664.
- GAO X., ZOU G.H., WANG L., ZHANG F. (2006): Silicon decrease transpiration rate and conductance from stomata of maize plants. Plant Nutrition, **29**: 1637–1647.
- GENARD H., SAOS J.L.E., HILLARD J., TREMOLIERES A., BOUCAUD J. (1991): Effect of salinity on lipid composition, glycine betaine content and photosynthetic activity in chloroplasts of *Suaeda maritima*. Plant Physiology and Biochemistry, **29**: 421–427.
- GONG H.Z., CHEN K., WANG S., ZHANG C. (2005): Silicon alleviates oxidative damage of wheat plants in pots under drought. Plant Science, **169**: 313–321.
- GRIEVE C.M., GRATAN S.R. (1983): Rapid assay for determination of water soluble quaternary ammonium compounds. Plant and Soil, **70**: 303–307.
- HART M.A., TYSON H., BLOOMBERG B. (1971): Measurement of activity of peroxidase isoenzymes in flax (*Linum usitatissimum*). Canadian Journal of Botany, **49**: 2129–2137.
- HATTORI T., INANAGA S., TANIMOTO E., LUX A., LUXOVA M., SUGIMOTO Y. (2003): Silicon-induced changes in viscoelastic properties of sorghum root cell walls. Plant Cell Physiology, **44**: 743–749.
- HATTORI T., INANAGA S., ARAKI H., AN P., MORITA S., LUXOVA M., LUX A. (2005): Application of silicon enhanced drought tolerance in sorghum bicolor. Physiologia Plantarum, **123**: 459–466.
- HOSSAIN M.T., MORI R., SOGA K., WAKABAYASHI K., KAMISAK S., FUJII S., YAMAMOTO R., HOSON T. (2002): Growth promotion and an increase in cell wall extensibility by silicon in rice and some other *Poaceae* seedlings. Journal of Plant Research, **115**: 23–27.
- JUNG S. (2004): Variation in antioxidant metabolism of young and mature leaves of *Arabidopsis thaliana* subjected to drought. Plant Science, **166**: 459–466.
- KAUFMAN P.B., TAKEOKA Y., CARLSON T.J., BIGELOW W.C., JONES ONES J.D., MOORE P.H., GHOSHEH N.S. (1979): Studies on silica deposition in sugarcane (*Saccharum* spp.) using scanning electron microscopy, energy dispersive X-ray analysis, neutron activation analysis and light microscopy. Phytomorphology, **29**: 185–193.
- LAEMMLI U.K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T₄. Nature, **227**: 680–685.
- LI Q.F., MA C.C., SHANG Q.L. (2007): Effects of silicon on photosynthesis and antioxidative enzymes of maize under drought stress. Chinese Journal of Applied Ecology, **18**: 531–536.
- LIANG Y.C. (1998): Effects of silicon on leaf ultrastructure, chlorophyll content and photosynthetic activity in barley under salt stress. Pedosphere, **8**: 289–296.
- LIANG Y., CHEN Q., ZHANG W., DING R. (2003): Exogenous silicon increases antioxidant enzyme activity and reduces lipid peroxidation in root of salt-stressed barley (*Hordeum vulgare* L.). Plant Physiology, **160**: 1157–1167.
- LOBATO A.K.S., COIMBRA G.K., NETO M.A.M., COSTA R.C.L., SANTOS F.B.G., OLIVEIRA C.F., LUZ L.M., BARRETO A.G.T., PEREIRA B.W.F., ALVES G.A.R., MONTEIRO B.S., MAROCHIO C.A. (2009): Protective action of silicon on water relation and photosynthetic pigments in pepper plants induced to water deficit. Research Journal of Biological Sciences, **4**: 617–623.
- LUX A., LUXOVA M., MORITA S., ABE J., INANAGA S. (1999): Endodermal silicification in developing seminal roots of lowland and upland cultivars of rice (*Oryza sativa* L.). Canadian Journal of Botany, **77**: 955–960.

- MA J.F. (2004): Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Science and Plant Nutrition*, **50**: 11–18.
- MA J.F., TAKAHASHI E. (2002): *Soil, Fertilizer and Plant Silicon Research in Japan*. Elsevier Science, Amsterdam.
- MA J.F., MIYAKE Y., TAKAHASHI E. (2001): Silicon as a beneficial element for crop plants. In: DATNOFF L.E., SNYDER G.H., KORNDORFER G.H. (eds): *Silicon in Agriculture*. Studies in Plant Science No. 8, Elsevier Science, Amsterdam, 17–39.
- MAITI R.K., RAMAIAH K.V., BISEN S.S., V.L. (1984): A comparative study of the haustorial development of *Striga asiatica* (L.) Kuntze on sorghum cultivars. *Annals of Botany*, **54**: 447–457.
- MATICHENKOV V.V., BOCHARNIKOVA E.A., AMMOSSOVA J.M. (2001): The influence of silicon fertilizers on the plants and soils. *Agrochemistry*, **12**: 30–37.
- MCCORD J.M. (2000): The evolution of free radicals and oxidative stress. *American Journal of Medicine*, **108**: 652–659.
- MITTLER R. (2002): Oxidative stress antioxidants and stress tolerance. *Trends in Plant Science*, **7**: 405–410.
- NAKANO Y., ASADA K. (1987): Purification of ascorbate peroxidase in spinach chloroplast: inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. *Plant Cell Physiology*, **28**: 131–140.
- NOCTOR G., VELJVOIC-JOVANOVIC S., FOYER C.H. (2000): Peroxide processing in photosynthesis: antioxidant coupling and redox signalling. *Philosophical Transactions of the Royal Society of London*, **355**: 1465–1475.
- RANGANATHAN S., SUVARCHALA V., RAJESH Y.B.R.D., PRASAD M.S., PADMAKUMARI A.P., VOLETI S.R. (2006): Effect of silicon sources on its deposition, chlorophyll content, and disease and pest resistance in rice. *Biologia Plantarum*, **50**: 713–716.
- RAO M.V., PALIYATH G., ORMROD D.P. (1996): Ultra-violet-B and ozone-induced of protein biochemical change in antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiology*, **110**: 125–136.
- RICHMOND K.E., SUSSMAN M. (2003): Got silicon? The non-essential beneficial plant nutrient. *Current Opinion in Plant Biology*, **6**: 268–272.
- ROBERTSON E.F., DANNELLY H.K., MALLOY P.J., REEVES H.C. (1987): Rapid isoelectric focusing in a vertical polyacrylamide minigel system. *Analytical Biochemistry*, **167**: 290–294.
- RYBICKI E., PURVES M. (2003): SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE). Department of Microbiology, University of Cape Town.
- SACALA E. (2009): Role of silicon in plant resistance to water stress. *Journal of Elementology*, **14**: 619–630.
- SCHONFELD M.A., JOHNSON R.C., CARVER B.F., MORNHINWEG D.W. (1988): Water relations in winter wheat as drought resistance indicator. *Crop Science*, **28**: 526–531.
- SMIRNOFF N. (1993): The role of active oxygen in the responses to water deficit and desiccation. *New Phytologist*, **125**: 27–58.
- SUDHAKAR A., LAKSHMI S., GIRIDARAKUMAR A. (2001): Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. *Plant Science*, **161**: 613–619.
- YONG Y., TAI S., BAO X. (2007): Effects of silicon on photosynthesis and antioxidative enzymes of maize under drought stress. *Plant Science*, **18**: 531–536.
- ZHU Z., WEI G., LI J., QIAN Q., YU J. (2004): Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). *Plant Science*, **167**: 527–533.

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