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Full Length Research Paper

Effects of NaCl and silicon on activities of antioxidative enzymes in roots, shoots and leaves of alfalfa

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The effects of exogenous NaCl and silicon on activities of antioxidative enzymes in the root, shoot and leaf of two alfalfa cultivars were investigated in two alfalfa (*Medicago sativa* L.) cultivars: the high salt tolerant Zhongmu No. 1 and the low salt tolerant Defor. Both cultivars were grown in a hydroponics system with control (no NaCl and no Si added), Si treatment (1 mM Si), NaCl treatment (120 mM NaCl), and Si and NaCl treatment (120 mM NaCl + 1 mM Si). After 15 days of the NaCl and Si treatments, four plants of the cultivars were removed and divided into root, shoot and leaf parts for activity of antioxidative enzyme measurements. Salinity changed the antioxidative enzyme activity to different extents in the root and shoot of both cultivars when Si was not applied. Applying Si to both cultivars under NaCl stress significantly increased ascorbate peroxidase (APX) activity in root, shoot and leaves, and catalase (CAT) activity in leaves, and peroxidase (POD) activity in shoots of both cultivars, but decreased the superoxide dismutase (SOD) activity in shoots of Zhongmu No.1 and roots of both cultivars under salt stress. It is concluded that the changes of antioxidative enzymes activity varied in different organs of alfalfa plant after salt stress, while silicon could alter the activity of antioxidative enzyme of one or several organs of plants to improve the salt tolerance.

Key words: *Medicago sativa* L., antioxidative enzymes, silicon, salt stress.

INTRODUCTION

Soil salinization is a worldwide problem that markedly reduces food production. Approximately, 20% of irrigated agricultural land is adversely affected by salinity (Viswanathan et al., 2005), which is one of the major abiotic stress that limit plant growth. Salt stress can stimulate formation of active oxygen species (AOS) such as superoxide, hydrogen peroxide and hydroxyl radicals. These activated oxygens injure the cellular components of proteins, membrane lipids and nucleic acids (Foyer et al., 1994). Malondialdehyde (MDA) is the decomposition product of polyunsaturated fatty acids of membranes and shows greater accumulation under salt stress (Gossett et al., 1994; Dionisio-Sese and Tobita 1998; Sudhakar et al., 2001). In order to avoid these oxidative injuries, plants have developed enzymatic systems for scavenging these highly active forms of AOS; superoxide is converted by superoxide dismutase (SOD) enzyme into H_2O_2, which is further scavenged by catalase (CAT) and various peroxidases. Ascorbate peroxidase (APX) and glutathione reductase (GR) also play a key role by reducing H_2O_2 to water through the Halliwell–Asada pathway (Noctor and Foyer, 1998).

Silicon (Si) which is abundant in the soil is the major constituent of many plants, but its roles in plant biology have been poorly understood (Naeem, 2010; Liang, 1999; Wang and Han, 2007). Although Si has not been listed among the generally essential elements of higher plants, there have been reports of interactions between Si supply and the responses of members of the Poaceae to biotic and abiotic stresses (Epstein, 1994; Gong et al., 2006; Kaya et al., 2006; Liang et al., 1996; Rodrigues et al., 2003). It has been reported that addition of Si to salt treated barley, significantly increases SOD activity and decreases MDA concentration in plant leaves (Liang,

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Abbreviations: AOS, Active oxygen species; **MDA,** malondialdehyde; **SOD,** superoxide dismutase; **CAT,** catalase; **APX,** ascorbate peroxidise; **GR,** glutathione reductase; **Si,** silicon; **EDTA,** ethylenediaminetetraacetic acid; **PVP,** polyvinylpyrolidone; **NBT,** nitro-blue tetrazolium.

1999), and increases SOD, peroxidase, CAT and GR activity in barley roots (Liang et al., 2003).

Alfalfa, a leguminous plant, often suffers from a significant reduction in biomass under severe salt stress and different cultivars respond differently to salt stress (Al-Khatib and Collins, 1994; Wang and Han, 2009). Thus, the present study focuses on the influence of Si addition on antioxidant enzyme activity in roots, shoots and leaves of alfalfa under salt stress. The objectives of this study are to investigate whether Si is involved in regulation of antioxidant enzymes under salt stress and therefore, to elucidate the physiological mechanism of salt stress mitigated by Si in alfalfa plants.

MATERIALS AND METHODS

Plant materials and growth conditions

Two alfalfa (*Medicago sativa* L.) cultivars of Zhongmu No. 1 (high salt tolerant) and Defor (low salt tolerant) were used in this study. The seeds were sterilized with 6% sodium hypochlorite solution for 5 min. Following germination in sand medium at 25/20°C for 8 h/16 h in a dark room, four seedlings fixed into the holes of quadrate foam were transplanted into plastic vessels (13 cm high, 28 cm wide and 35 cm long). The vessels were wrapped with aluminum foil to minimize irradiation-induced heating and suppress algae growth. Each vessel contained 4.4 L nutrient solution containing: 2.5 mM $Ca(NO_3)_2$, 2.5 mM KCl, 1 mM $MgSO_4$, 0.5 mM $(NH_4)H_2PO_4$, 2×10^{-4} mM $CuSO_4$, 1×10^{-3} mM $ZnSO_4$, 0.1 mM EDTA Fe Na, 2×10^{-2} mM H_3BO_3, 5×10^{-6} mM $(NH)_4Mo_{27}O_4$, and 1×10^{-3} mM $MnSO_4$. The plant grew in a room chamber with a 45% relative humility, alternative temperatures of day and night of 30 and 25°C, respectively, 13 h light with 450 µmol $m^{-2}s^{-1}$ by bio-sodium lamp. The plants were grown hydroponically in standard nutrient solution for 15 days. Then, four treatments with three replicates were maintained including control, 120 mmol/l NaCl, 120 mmol/l NaCl + 1.0 mmol/l Si and 1.0 mmol/l Si. The plants were exposed to salinity by adding NaCl to the growth medium in 60 mmol/l increments every 12 h, until the final concentrations of 120 mmol/l were reached. Silicon treatments were begun by adding potassium silicate (K_2SiO_3), and additional K introduced by K_2SiO_3 was subtracted from KNO_3 and the resultant nitrate loss was supplemented with dilute nitric acid. The pH of the nutrient solution was adjusted to 6.0 daily using 0.01 mol/l KOH and/or H_2SO_4. The nutrient solution was renewed every seven days and continuously aerated with an air pump. The losses of water through transpiration were daily supplemented with deionized water.

At 15 days after salt stress, 4 plants were removed from each treatment and cut into roots, shoots and leaves were stored at -20°C for biochemical parameter measurements.

Enzyme extractions and assays

For protein and enzyme extractions, 0.3 g of leaf samples were homogenized with 50 mM sodium phosphate buffer (PH 7.0) containing 1 mM ethylenediaminetetraacetic acid (EDTA).Na_2 and 2% (w/v) polyvinylpyrolidone (PVP). The whole extraction procedure was carried out at 4°C. The homogenate were centrifuged at 10,000 g for 15 min at 4°C and the supernatants were collected and used for the assays of enzyme activity. Protein concentrations in the enzyme extract were determined by the method of Bradford (1976) using bovine serum albumin as a standard.

Catalase (CAT, EC 1.11.1.6) activity was assayed by measuring the rate of H_2O_2 disappearance at 240 nm (Bergmeyer, 1970). The final volume of reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0) and 2% H_2O_2. The reaction was started by adding 100 µl leaf crude extract to this solution, and the activity was calculated as units (µmol H_2O_2 consumed per minute) per mg of protein.

Peroxidase (POD, EC 1.11.1.17) activity was determined according to Tatiana et al. (1999) with minor modifications. The reaction mixture contained 0.05 M sodium phosphate buffer (pH 5.5), 2% H_2O_2, 0.05 M guaiacol and 0.1 ml enzyme extract in a final volume of 5 ml. The reaction was started by the addition of enzyme extract. The formation of tetraguaiacol was measured at 470 nm. One unit of enzyme was defined as the amount of enzyme to decompose 1 µmol of H_2O_2 per min at 25°C.

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined according to the method of Nakano and Asada (1981). The reaction mixture contained 50 mM potassium phosphate (pH 7.0), 0.2 mM EDTA, 0.5 mM ascorbic acid, 2% H_2O_2 and 0.1 ml enzyme extract in a final volume of 3 ml. The decrease in absorbance at 290 nm for 1 min was recorded and the amount of ascorbate oxidized was calculated using extinction coefficient (ε = 2.8 mM^{-1} cm^{-1}). One unit of APX was defined as 1 mmol ml^{-1} ascorbate oxidized per minute at 25°C.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed spectrophotometrically as the inhibition of photochemical reduction of nitro-blue tetrazolium (NBT) at 560 nm (Beauchamp and Fridovich, 1971). The reaction mixture (3 ml) consisted of 50 mM Na-phosphate buffer (PH 7.8), 13 mM L-methionine, 75 µM NBT, 10 µM EDTA-Na_2, 2.0 µM riboflavin and 0.3 ml enzyme extract. The test tubes containing reaction mixture were weighed for 10 min under 4,000 lx at 35°C. One unit SOD activity was defined as the amount of enzyme required to cause a 50% inhibition of the rate of NBT reduction measured at 560 nm.

Glutathione reductase (GR, EC 1.6.4.2) activity was measured according to Foyer and Halliwel (1976). The assay medium contained 0.025 mM Na–phosphate buffer (pH 7.8), 0.5 mM GSSG, 0.12 mM NADPH.Na4 and 0.1 ml enzyme of extract in a final assay volume of 1 ml. NADPH oxidation was determined at 340 nm. Activity was calculated using the extinction coefficient (ε = 6.2 mM^{-1} cm^{-1}) for GSSG. One unit of GR was defined as 1 mmol ml^{-1} GSSG reduced per minute.

Statistical analysis

Date are presented as the mean ±SE for each treatment (n=4). Significant differences among treatments were analysed by analysis of variance (ANOVA). Least Significant Difference (LSD) values were calculated at the $P < 0.05$ probability level.

RESULTS AND DISCUSSION

As shown in Table 1, the shoot dry weight of the two cultivars and the weight of leaf and root of Defor were drastically reduced by NaCl stress; however, this inhibition was alleviated to various extents by Si supplement. Under salt stress, the added Si apparently increased the dry weight of shoots of both cultivars. While under non-salt stress, the added Si did not significantly increase the dry weight of the root, shoot and leaf of both cultivars.

The SOD activity was markedly higher in leaves than in roots and shoots for both alfalfa cultivars in the control treatment. The salt treatment reduced SOD activity in shoots and leaves of both cultivars without Si in the

Table 1. Dry weight (g/plant) of root, shoot and leaf of Zhongmu No. 1 and Defor grown in four treatments after 15 days of NaCl and Si treatments.

Treatment	Zhongmu No.1				Defor			
	Control	Si	NaCl	NaCl + Si	Control	Si	NaCl	NaCl + Si
Root	0.07a ± 0.00	0.07a ± 0.01	0.06ac ± 0.01	0.07a ± 0.01	0.06a ± 0.01	0.06a ± 0.01	0.03b ± 0.01	0.04bc ± 0.01
Shoot	0.08a ± 0.02	0.09a ± 0.01	0.06b ± 0.01	0.08a ± 0.01	0.07a ± 0.01	0.08a ±0.02	0.03d ± 0.00	0.04c ± 0.00
Leaf	0.13ac ± 0.01	0.14a ± 0.01	0.10bc ± 0.02	0.13ab ± 0.02	0.11bc ± 0.02	0.11bc ± 0.02	0.06d ± 0.01	0.06d ± 0.01

Data are expressed as means ± S.E. of four replicates (n = 4). Means marked with different letters in the same line are significant (P < 0.05) by L.S.D.

Table 2. SOD (unit / mg protein) and POD (unit / mg protein) activities in root, shoot and leaf of Zhongmu No. 1 and Defor grown in four treatments after 15 days of NaCl and Si treatments.

Treatment	Zhongmu No.1				Defor			
	Control	Si	NaCl	NaCl + Si	Control	Si	NaCl	NaCl + Si
SOD activity								
Root	2.77b ± 0.45	3.31b ± 0.45	3.76ab ± 1.00	3.17b ± 0.97	3.21b ± 0.72	4.94a ± 0.86	4.71a ± 0.70	2.92b ± 0.88
Shoot	5.26ab ± 0.99	5.33a ± 1.10	3.90c ± 0.64	3.93c ± 0.58	4.47abc ± 0.46	4.00bc ± 1.00	3.64c ± 0.90	4.16abc ± 0.66
Leaf	2.25a ± 0.29	1.26c ± 0.16	1.17c ± 0.27	1.28c ± 0.45	1.51bc ± 0.38	1.83ab ± 0.17	1.19c ± 0.11	1.29c ± 0.27
POD activity								
Root	9.91d ± 1.15	10.54cd ± 3.58	5.85e ± 1.51	6.87de ±2.57	17.25ab ±1.56	18.39a ±3.37	10.77cd ±1.72	14.12bc ±1.72
Shoot	3.76a ± 0.61	2.03c ± 0.49	0.97d ± 0.49	2.14bc ±0.39	3.67a ±1.08	2.61bc ±0.42	1.70cd ±0.33	3.04ab ±0.73
Leaf	0.82b ± 0.16	0.91b ± 0.26	1.69a ± 0.38	1.47a ±0.20	0.85b ±0.24	0.75b ±0.09	1.45a ±0.31	1.44a ±0.19

Data are expressed as means ± S.E. of four replicates (n = 4). Means marked with different letters in the same line are significant (P < 0.05) difference by L.S.D.

nutrient solution, but increased their root SOD activity, with slight difference between them. However, adding Si had no significant effect on SOD activity in roots, shoots and leaves of both cultivars under salt stress (Table 2).

The POD activity was markedly higher in roots than in shoots and leaves for both alfalfa cultivars in the control and other treatments. Salt led to a significant decline of POD activity in roots and shoots but it increased in leaves of both cultivars. It is interesting to note that higher root and shoot POD activity was found in the low salt tolerant cultivar when compared with the high salt tolerant cultivar under salt stress without additional Si; however, lower leaf POD activity was found in the low salt tolerant cultivar when compared with the high salt tolerant cultivar under the same condition. The addition of Si markedly increased the POD activity in shoots and roots, but had no effect on POD activity in leaves in either cultivar under salt stress.

Salt led to a significant decline of APX activity in roots and shoots but increased in leaves of both cultivars. Higher root and shoot APX activity was found in the low salt tolerant cultivar compared with the high salt tolerant cultivar under salt stress without additional Si. The addition of Si markedly increased the APX activity in shoots, roots and leaves in both cultivars under salt stress (Table 3).

For Zhongmu No. 1, salt stress significantly increased CAT activity of roots, but significantly decreased CAT activity of shoots irrespective of Si treatment. It is also noted that under salt stress without additional Si, much higher CAT activity was found in the shoots of the low salt tolerant cultivar when compared with the high salt tolerant

Table 3. APX (unit / mg protein), CAT (unit / mg protein) and GR (unit / mg protein) activities in root, shoot and leaf of Zhongmu No. 1 and Defor grown in four treatments after 15 days of NaCl and Si treatments.

Treatment	Zhongmu No.1				Defor			
	Control	Si	NaCl	NaCl + Si	Control	Si	NaCl	NaCl + Si
APX activity								
Root	0.59c ± 0.12	0.58cd ± 0.05	0.38e ± 0.10	0.43de ± 0.07	0.82a ± 0.08	0.92a ± 0.18	0.61bc ± 0.05	0.76ab ± 0.07
Shoot	0.57a ± 0.06	0.47ab ± 0.11	0.19d ± 0.03	0.27cd ± 0.07	0.45ab ± 0.06	0.39bc ± 0.04	0.31c ± 0.09	0.56a ± 0.11
Leaf	0.11d ± 0.01	0.12d ± 0.06	0.26c ± 0.04	0.40a ± 0.07	0.06d ± 0.01	0.12d ± 0.06	0.29bc ± 0.01	0.36ab ± 0.06
CAT activity								
Root	0.87c ± 0.09	1.48b ± 0.31	2.07a ± 0.12	2.34a ± 0.30	1.88ab ± 0.14	2.07a ± 0.44	1.90ab ± 0.16	1.89ab ± 0.58
Shoot	2.71ab ± 0.38	2.60ab ± 0.21	1.53c ± 0.30	2.47abc ± 0.54	2.02bc ± 0.87	2.97a ± 0.78	2.93ab ± 0.21	2.86ab ± 0.63
Leaf	7.48ab ± 1.44	8.18a ± 0.09	5.75c ± 1.24	8.81a ± 0.45	5.84bc ± 1.56	7.43ab ± 0.32	6.24bc ± 0.53	8.40a ± 0.91
GR activity								
Root	0.66c ± 0.13	0.69bc ± 0.05	0.63c ± 0.09	0.58c ± 0.10	0.71abc ± 0.07	0.83a ± 0.06	0.82ab ± 0.06	0.80ab ± 0.1
Shoot	1.24ab ± 0.29	1.10bc ± 0.08	0.76d ± 0.19	0.50e ± 0.05	1.44a ± 0.19	0.91cd ± 0.11	0.84d ± 0.10	0.74de ± 0.11
Leaf	0.39a ± 0.05	0.20bc ± 0.03	0.24bc ± 0.06	0.21bc ± 0.02	0.36a ± 0.02	0.25b ± 0.03	0.24bc ± 0.03	0.19c ± 0.04

Data are expressed as means ± S.E. of four replicates (n = 4). Means marked with different letters in the same line are significantly different ($P < 0.05$) by L.S.D.

cultivar. The addition of Si markedly increased the CAT activity in leaves in both cultivar and in shoots in Zhongmu No. 1 under salt stress.

The salt treatment reduced GR activity in the shoots and leaves of both cultivars without Si in the nutrient solution. The addition of Si markedly decreased the GR activity in shoots in Zhongmu No. 1 under salt stress.

Salinity is the major environmental factor that limits plant growth and crop productivity (Asish et al., 2005). Improvement of salt tolerance by the addition of Si has been reported in several plants (Pei et al., 2010; Matoh et al., 1986; Bradbury and Ahmad, 1990; Ahmad et al., 1992; Liang et al., 1996; Al-Aghabary et al., 2004). In the present study, the growth of salt-stressed alfalfa plants is improved by the addition of Si. It has been reported that the alleviation of salt toxicity by Si addition results from the effect on enzyme activity in the plants of barley (Liang et al., 2003), tomato (Al-Aghabary et al., 2004), cucumber (Zhu et al., 2004) and wheat (Gong et al., 2008).

Salt stress can cause plant membrane damage and stimulate formation of AOS such as super-oxide, hydrogen peroxide and hydroxyl radicals. Among the AOS, superoxide is converted by SOD enzyme into H_2O_2, which is further scavenged by CAT and various peroxidases. APOX and GR also play a key role by reducing H_2O_2 to water through the Halliwell-Asada pathway (Noctor and Foyer, 1998). Salinity induced an increase in enzyme activities in cotton, cucumber, soybean and the shoots of rice and wheat, but a decrease in the roots of wheat (Zhu et al., 2004; Wang et al., 2009). In the present study, salinity increased the SOD activity in the roots of low salt tolerant cultivar, CAT activity in the roots of high salt tolerant cultivar, POD and APX activity in leaves of both alfalfa cultivars, but decreased the SOD activity in the leaves of high salt tolerant cultivar, POD and APX activity in the roots and shoots of both alfalfa cultivars and CAT and GR activity in the shoots and leaves of both alfalfa cultivars. Under salt stress, the diverse responses in enzyme activity in roots, shoots and leaves between the two cultivars show that significantl difference in the sca-venging mechanisms of AOS may exist in different organs of the two cultivars.

The effect of Si on the antioxidant enzymes activity under salt stress has been reported by Liang et al. (2003) who described an increase in SOD activity in salt-stressed barley leaves and increases in SOD, GPX, CAT and GR activity in salt-stressed barley roots. In the present study, applying Si to both alfalfa cultivars under NaCl stress significantly increased APX activity in root, shoot and leaves, CAT activity in leaves and POD

activity in shoots of both cultivars, but decreased the SOD activity in shoots of Zhongmu No.1 and roots of both cultivars under salt stress. The changes of antioxidative enzyme activity vary in different organs of alfalfa plant after salt stress, while silicon could alter the activity of antioxidative enzyme activity of one and/or several organs of plants to improve the salt tolerance.

In conclusion, salinity reduced plant growth in both cultivars; however, the added Si apparently increased the shoot dry weight of both cultivars under salt stress. Summing up the results of the study, it is possible to conclude that physiologically, salt tolerance of alfalfa is associated with changes of antioxidative enzyme activity in different organs of alfalfa plant after salt stress, while silicon could only alter the activity of antioxidative enzyme of one and/or several organs of plants to improve the salt tolerance.

REFERENCES

Ahmad R, Zaheer S, Ismail S (1992). Role of silicon in salt tolerance of wheat (*Tritium aestivum* L.). Plant Sci. 85: 43-50.

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

Al-Khatib MM, Collins JC (1994). Between and within culture variability in salt tolerance in lucerne. Genet. Resour. Crop Eviron. 41: 159-164.

Bradbury M, Ahmad R (1990). The effect of silicon on the growth of *Prosopis juliflora* growing in saline soil. Plant Soil, 125: 71-74.

Dionisio-Sese ML, Tobita S (1998). Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 135: 1-9.

Epstein E (1994). The anomaly of silicon in plant biology. Proc. Natl Acad. Sci. USA. 91: 11-17.

Foyer CH, Halliwell B (1976). The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta, 133: 21-25.

Foyer CH, Maud L, Kunert KJ (1994). Photooxidative stress in plants. Physiol. Plant, 92: 696-717.

Gong HJ, Chen KM, Zhao ZG, Chen GC, Zhou WJ (2008). Effects of silicon on defense of wheat against oxidative stress under drought at different developmental stages. Biologia Plantarum, 52: 592-596.

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

Gossett DR, Millhollon EP, Lucas MC (1994). Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop Sci. 34: 706-714.

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

Liang YC, Chen Q, Liu Q, Zhang WH, Ding RX (2003). Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). J. Plant Physiol. 160: 1157-1164.

Liang YC, Shen QR, Shen ZG, Ma TS (1996). Effects of silicon on salinity tolerance of two barely cultivars. J. Plant Nutr. 19: 173-183.

Matoh T, Kairusmee P, Takahashi E (1986). Slat-induced damage to rice plants and alleviation effect of silicate. Soil Sci. Plant Nutr. 32: 259-304.

Naeem MS, Jin L, Wan GL, Liu D, Liu HB (2010). Yoneyama & W.J. Zhou. 5-Aminolevulinic acid improves photosynthetic gas exchange capacity and ion uptake under salinity stress in oilseed rape (*Brassica napus* L.). Plant Soil, 332: 405-415.

Noctor G, Foyer CH (1998). Ascorbate and glutathione: Keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49: 249-279.

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

Rodrigues FA, Vale FXR, Korndorfer GH, Prabhu AS, Datnoff LE, Oliveira AMA (2003). Influence of silicon on sheath blight of rice in Brazil. Crop Prot. 22: 23-29.

Sudhakar C, Lakshmi A, Giridarakumar S (2001). Changes in the antioxidant enzyme activities in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. Plant Sci. 161: 613-619.

Viswanathan C, Andréand J, Zhu JK (2005). Understanding and improving salt tolerance in plants. Crop Sci. 45: 437-448.

Wang XS, Han JG (2007). Effects of NaCl and silicon on ion distribution in the roots, shoots and leaves of two alfalfa cultivars with different salt tolerance. Soil Sci. Plant Nutr. 53: 278-285.

Wang XS Han JG (2009). Changes of Proline Content, Activity, and Active Isoforms of Antioxidative Enzymes in Two Alfalfa Cultivars under Salt Stress. Agric. Sci. China. 8: 101-105

Wang XS, Zhao GQ, Gu HR (2009). Physiological and antioxidant responses of three leguminous species to saline environment during seed germination stage. Afr. J. Biotechnol. 8: 5773-5779.

Zhu ZJ, Wei GQ, Li T, Qian QQ, Yu JQ (2004). Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). Plant Sci. 167: 527-533.