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EFFECT OF SALINITY AND SILICON APPLICATION ON OXIDATIVE DAMAGE OF SORGHUM [*SORGHUM BICOLOR* (L.) MOENCH.]

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Abstract

Application of silicon (Si) to soil is considered as an alternative approach to alleviate salinity stress in crop plants. Therefore, a field experiment was conducted to investigate the effects of Si application [control (without Si), 1.44 and 1.92 $g.kg^{-1}$soil on membrane stability index (MSI), relative water content (RWC), leaf proline, soluble sugars, antioxidant activity, total phenols and dry matter accumulation of two sorghum (*Sorghum bicolor*) cultivars under three levels of salinity of irrigation water (5.2, 10.5 and 23.1 dS m^{-1}). The results showed that leaf proline content, activities of ascorbate peroxidase (APX) and glutathione reductase (GR), Na^+ concentration significantly increased only at high level of salinity, while, RWC and dry matter accumulation were significantly decreased at all salinity levels. Soil application of 1.44 $g.kg^{-1}$ Si caused an increase in the activities of APX, catalase (CAT), superoxide dismutase (SOD), peroxidase (PRO), glutathione reductase (GR), total antioxidant and total phenol contents and 1.92 $g.kg^{-1}$soil Si caused an increase in membrane stability index, soluble sugar and total phenol contents, CAT, SOD and total antioxidant activity. Soluble sugars, total phenols, SOD and total antioxidant activity and dry matter accumulation in cv. Omidbakhsh were higher than those in cv. Sepideh. In conclusion, alleviation of salinity stress by exogenous application of Si was found to be associated partly with enhanced antioxidant activity.

Introduction

Salinity is one of the most important environmental factors limiting crop production in arid and semi-arid regions. Salinization of land and water resources is a threat for sustainability of irrigated agriculture (Qureshi *et al.*, 2007). Qureshi *et al*., (2007) reported that half of the irrigated area of Iran falls under different types of salt-affected soils and average yield losses may be as high as approximately 50%.

Following primary effects of salt stress, secondary stresses such as oxidative damage may occur. Oxidative damage occurs by accumulation of reactive oxygen species (ROS) that cause lipid and protein oxidation and eventually leads to cell death (Molassiotis *et al.*, 2006). The antioxidant defence system in the plant cell includes both enzymatic antioxidants such as APX (EC1.11.1.11), CAT (EC1.11.1.6), SOD (EC1.15.1.1), POX (EC 1.11.1.7), and GR (EC 1.6.4.2) and some non-enzymatic antioxidants such as ascorbate, glutathione and α-tocopherol (Ashraf & Harris2004; Gunes *et al.*, 2007; Ashraf, 2009).

Application of Si suggested as an alternative approach to alleviate salinity stress in crops (Liang *et al.*, 2007). However, Si content of the plant varies greatly with the plant species, ranging from 0.1 to 10.0% of dry weight (Takahashi *et al.*, 1990). Si, increases root activity, K uptake, reduction of Na uptake, improvement of membrane permeability and anti-oxidative activity (Liang *et al.*, 2007).

Sorghum is one of the most important crops of arid and semi-arid regions. It is moderately tolerant to salinity and can grow well in saline soils (Maas *et al*., 1986). However, at higher levels of salinity, considerable reduction in its growth takes place, therefore, improvement of its salinity tolerance by any means is a great challenge for plant scientists. Although a variety of strategies are currently in vogue to counteract the salinity problem, application of Si considered as one of the convenient and cost-effective approaches of overcoming the salinity menace. Thus, in the present investigation, we have assessed the ameliorative effect of exogenous application of Si on the adverse effects of salinity stress on two sorghum cultivars.

Material and Methods

Plant materials and growth conditions: A field study was conducted in 2008 at the Salinity Research Station (36°15′N, 59°28′E) of Faculty of Agriculture Ferdowsi University of Mashhad, Iran. The annual maximum and minimum temperatures were 42 and -27.8°C, respectively but during the sorghum growth temperature was always above 15°C. The source of irrigation water for low-level of salinity was the water pumped from a deep well near the site (Table 1). For the remaining two higher levels of salinity, a tanker transferred ground water from the same basin within a distance of 5 km. Chemical analysis of the water sources in terms of the three levels of salinity are shown in Table 1. Low salinity level (5.20 dS m^{-1}) played the role of control because previous experiments have shown that sorghum showed no significant yield reduction under moderate salinity (6.8 dS m^{-1}) compared to that in fresh water (1.5 dS m^{-1}) (Igartua *et al*., 1984). The soil had a loamy–silty–clay texture, with reasonable water-holding capacity. The clay, silt and sand contents of the soil were 39, 46 and 15%, respectively.

SiO_2 used as the Si source was composed of 97.59% SiO_2 and other minor elements such as $AlSO_3$ (0.37%), $FeSO_3$ (0.73%), CaO (0.26%), Na_2O (0.1%), K_2O (0.06%), MgO (0.13%) and P_2O_5 (0.11%).

The experiment was arranged as a split-split plot based on randomized complete block design with three replications. Saline waters (5.2, 10.5, and 23.1 dS m^{-1}), silicon concentration (0, 1.44 and 1.92 $g.kg^{-1}$soil Si) and two sorghum cultivars (Omidbakhsh a salt tolerant, and Sepideh, a salt sensitive cultivar) allocated as main, sub and sub-sub plots, respectively. Seeds sown at 0.75×0.2 m distances between and within row oriented in a north–south direction on 13 June of 2008. Plants grown under non-saline conditions until fourth fully expanded leaf appeared. Nitrogen fertilizer applied twice at the rate of 200 kg/ha while di-ammonium phosphate applied at the rate of 50 kg h^{-1} before sowing.

Table 1. Chemical properties of the waters and soil (0-30cm) at the study site.

	Na	Ca	Mg	K	SO_4	CO_3	HCO_3	Cl	EC
					($meq.l^{-1}$)				$dS.m^{-1}$
Water 1	32.50	8.60	9.20	0.23	15.00	0.40	2.40	34.40	5.20
Water 2	67.10	16.40	22.20	0.38	25.00	0.00	3.00	75.60	10.50
Water 3	179.80	27.00	46.80	0.31	56.10	0.00	3.20	172.40	23.10
Soil	31.10	10.60	10.20	0.75	31.30	0.00	1.80	26.80	5.80

All measurements with fresh matter were carried out during the flowering stage. Youngest fully expanded leaves sampled for membrane stability index (MSI) and RWC. Samples for biochemical determination were frozen at -80°C until determinations. At the end of the experiment, harvested plants were dried in an oven at 70°C until constant mass reached.

Leaf membrane stability was determined by recording the electrical conductivity of leaf leakages (Sairam *et al*., 2002). Leaf relative water content estimated according to Smart & Bingham (1974). Extraction and determination of leaf proline conducted according to the procedures described by Bates *et al*., (1973). Soluble sugars were determined based on the method of phenol-sulfuric acid and calculated by comparing sample absorbance with a standard glucose curve (Dubois *et al*., 1956).

For enzyme assays, leaf fresh materials (0.1 g) was powdered in liquid nitrogen and homogenized in 1 ml of 0.1 M potassium phosphate buffer of pH 7.8 containing 1 mM ethylenediaminetetraacetic acid (EDTA) by a homogenizer into microtubes. Insoluble materials removed by Beckman refrigerated centrifuge at 12000 *g* for 20 min., at 4°C and the supernatant used as the source of enzyme extraction. To measure the activities of APX, CAT, SOD, POX and GR, 100 microliters of supernatant were taken and all steps of antioxidant determination carried out at 4°C. APX (EC 1.11.1.11) activity was determined according to Yamaguchi *et al*., (1995). CAT (EC 1.11.1.6) activity was assayed by measuring the initial rate of hydrogen peroxide disappearance according to Velikova *et al*., (2000). SOD (EC 1.15.1.1) activity was assayed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium, based on the method of Yu & Rengel (1995). POX (EC 1.11.1.7) activity was estimated based on the method described by Srinivas *et al*., (1999). GR (EC 1.6.4.2) activity was measured according to Lee & Lee (2000). For determination of DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity we used by Abe *et al*., (1998) method. Total phenolic content was assessed using the Folin-Ciocalteau phenol reagent method (Singleton & Rossi, 1965).

Data collected for all parameters were subjected to an analysis of variance (ANOVA) and the differences between the means were compared by least significant difference (LSD) ($p \leq 0$ 05).

Results

Membrane stability index (MSI): The results showed that except of cultivars and interaction of salinity levels and cultivars, other treatments showed no significant ($p \leq 0.05$) effect on MSI (Table 2). MSI in Sepideh (78.4%) was higher than that in Omidbakhsh (72.1%). In cv. Omidbakhsh, MSI decreased by increasing salinity, but in Sepideh with increasing level of salinity, MSI was also increased.

Relative water content: Increasing salinity levels significantly ($p \leq 0.01$) decreased RWC (Table 2). No significant difference in RWC was observed between 10.5 and 23.1 dS m^{-1}. The effect of Si treatments on RWC in both sorghum cultivars was non-significant. There were no significant difference in RWC between salt tolerant (Omidbakhsh), and salt sensitive (Sepideh) cultivars. However, RWC in Omidbakhsh was higher (87.2%) than that in Sepideh (86.6%).

Leaf proline and soluble sugar content: Leaf proline content was significantly ($p \leq 0.01$) increased under salinity stress (Table 2). Increase in salinity level up to 23.1 dS m^{-1}, increased proline content by 75.2% compared to that at 5.2 dS m^{-1}. There was no significant ($p \leq 0.05$) effect of Si application and cultivars on proline content (Table 2).

The soluble sugar content in leaves showed a small but non-significant ($p \leq 0.05$) increase (6.4%) in plants grown under 23.1 dS m^{-1} salinity (Table 2). The soluble sugar content differed markedly but not significantly among Si doses (Table 2). In the highest level of Si application, soluble sugar content was 4.3 and 4.9% higher than no Si application and 1.44 $g.kg^{-1}$soil Si, respectively. Soluble sugar content in the leaves of the salt tolerant cultivar was 15.36% higher than that in the salt sensitive cultivar (Table 2).

Ascorbate peroxidase: Salt stress significantly ($p \leq 0.01$) increased APX activity in sorghum leaves at the flowering stage. APX activity increased 27.48% in 23.1 dS m^{-1} compared with that at 5.2 dS m^{-1} salinity. Si doses did not show any significant effect ($P \leq 0.05$) on APX activity under salt stress (Table 2). APX activity in the salt sensitive cultivar (Sepideh) was higher (26.35%) than that in the salt tolerant cultivar Omidbakhsh (Table 2).

Catalase activity: Salinity and Si did not significantly affect CAT activity (Table 2). CAT activity showed different patterns in the two cultivars. In Sepideh, with the increase of salinity level, CAT activity increased 65.84% in comparison with the Omidbakhsh. Interaction between Si application and cultivars showed that Sepideh had significantly higher ($p \leq 0.05$) rate of CAT activity than that in Omidbakhsh at all levels of Si application (Table 2).

Superoxide dismutase: The antioxidant activity of SOD increased in sorghum cultivars under salinity stress. Among salinity treatments, 23.1 dS m^{-1} showed higher level of SOD activity (Table 2). SOD activity was higher in Si application treatments compared with Si free control (Table 2). SOD activity in the leaves of two sorghum cultivars showed that Omidbakhsh had more SOD activity than that in Sepideh (Table 2).

Table 2. Effect of different levels of salinity, silicon application on Membrane stability index (MSI) (%), relative water content (RWC), proline content, soluble sugar, ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), peroxidase (PRO), glutathione reductase (GR), total phenol content, and shoot dry matter accumulation (DM) of two sorghum cultivars.

Salinity ($dS\ m^{-1}$)	Silicon ($g.kg^{-1}soil$)	Cultivar	MSI (%)	RWC (%)	Proline ($mg.g^{-1}dw$)	Soluble sugar ($mg.g^{-1}dw$)	APX ($unit.g^{-1}dw$)	CAT ($unit.g^{-1}dw$)	SOD ($unit.g^{-1}dw$)	PRO ($unit.g^{-1}dw$)	GR ($unit.g^{-1}dw$)	Total antioxidant (%)	Total phenol ($mg.g^{-1}dw$)	Shoot DM ($ton.h^{-1}$)
5.2	0	Omidbakhsh	77.1	89.6	1.7	147.1	43.7	202.1	287.7	2882.8	80.1	50.5	9.2	11.0
	1.44	Omidbakhsh	75.4	91.1	1.4	123.2	35.2	45.5	340.2	1886.4	66.2	40.0	20.5	11.4
	1.92	Omidbakhsh	75.8	86.1	1.4	143.2	61.8	120.3	281.6	3111.4	64.2	69.8	21.2	9.3
10.5	0	Omidbakhsh	70.2	86.4	4.5	128.1	59.0	72.1	272.7	2982.2	40.7	61.3	11.1	7.0
	1.44	Omidbakhsh	73.8	86.0	4.4	143.3	101.8	157.0	357.6	3311.7	90.6	60.4	12.3	5.2
	1.92	Omidbakhsh	81.0	88.4	4.1	127.4	54.9	187.4	396.1	4181.4	68.2	56.5	10.9	6.9
23.1	0	Omidbakhsh	70.0	85.8	5.8	129.3	104.0	149.6	322.0	2306.3	140.9	67.3	12.2	8.6
	1.44	Omidbakhsh	60.1	87.9	4.8	150.9	78.1	57.1	350.0	2902.5	80.0	69.7	13.0	3.4
	1.92	Omidbakhsh	65.5	83.2	5.5	135.4	78.0	132.1	303.9	1764.7	46.4	48.8	9.2	7.4
5.2	0	Sepideh	66.9	88.4	0.9	104.2	94.8	245.8	187.3	3364.1	163.6	46.5	9.0	10.8
	1.44	Sepideh	62.1	88.8	2.3	90.1	95.4	381.0	323.5	5234.7	162.2	38.1	11.6	9.9
	1.92	Sepideh	84.2	91.2	1.0	124.6	67.0	390.4	296.7	2000.5	54.4	43.0	9.0	9.5
10.5	0	Sepideh	83.7	84.5	4.6	125.6	66.3	233.8	247.4	2551.5	116.2	43.3	17.9	4.5
	1.44	Sepideh	79.3	84.5	2.4	112.7	101.9	350.6	245.9	3689.4	240.4	52.2	9.6	5.2
	1.92	Sepideh	78.0	82.8	2.7	117.9	115.8	321.0	255.2	2344.3	160.2	61.0	10.5	6.8
23.1	0	Sepideh	83.9	87.0	6.7	111.9	99.4	327.0	350.6	4823.4	237.7	43.1	7.8	5.2
	1.44	Sepideh	84.9	85.4	7.3	121.1	107.4	494.4	266.4	3633.3	305.3	60.9	10.4	4.2
	1.92	Sepideh	82.3	86.9	4.6	130.9	89.3	230.7	344.1	4125.3	221.8	41.6	8.3	5.3
LSD† 0.05		Salinity	13.8	3.6	1.4	17.2	26.5	104.9	175.4	1755	41.7	34.1	8.3	2.59
		Silicon	5.7	3.4	1.3	21.7	19.5	87.4	70	1005	27.6	14.5	4.2	1.71
		Cultivar	6.1	1.9	1.2	13.1	14.1	57.8	42.6	807	23.1	10.9	4.1	2.5
		Salinity×Silicon	9.9	5.8	2.3	37.7	47.8	151.3	121.2	1741	47.8	25.1	7.3	2.97
		Salinity×Cultivar	10.5	3.3	2.1	22.6	24.4	242.8	73.8	1398	40	19	7.1	1.45
		Silicon×Cultivar	10.5	3.3	2.1	22.6	24.4	242.8	73.8	1398	40	19	7.7	1.45

†Least significant difference

Peroxidase activity: Salinity and Si did not significantly affect ($p \leq 0.05$) peroxidase activity based on unit.g^{-1}dry weight (Table 2). Interaction between salinity and cultivars showed that they had significant effect ($p \leq 0.05$) on peroxidase activity (Table 2). Peroxidase activity in Sepideh was remarkably but not significantly ($p \leq 0.05$) higher (20.26%) than in Omidbakhsh (Table 2).

Glutathione reductase activity: The GR activity in the leaves of sorghum at 23.1 dS m^{-1} was 42.76 and 30.60% higher than 5.2 and 10.5 dS m^{-1}salinity treatments, respectively (Table 2). The rate of increase in GR activity was slightly higher in 1.44 g.kg^{-1}soil Si application than in the 1.92 g.kg^{-1}soil and no Si application (Table 2). Combination of salinity and Si application had shown that the highest levels of GR activity were obtained at 1.44 g.kg^{-1} soil Si application (Table 2). The leaf GR activity in Sepideh was higher (59.25%) than that in cv. Omidbakhsh (Table 2). Interaction between salinity and cultivar showed that with increasing levels of salinity GR activity in cv. Sepideh was more than that in cv. Omidbakhsh (Table 2). Cultivar Sepideh showed more GR activity than that in cv. Omidbakhsh in all Si applications and GR activity in the 1.44 g.kg^{-1}soil Si application was more than that at 1.92 g.kg^{-1}soil Si (Table 2).

DPPH-radical scavenging activity: The DPPH-radical scavenging activity increased ($p \leq 0.05$) by 14% in 10.5 dS m^{-1} and 13% in 23.1 dS m^{-1} compared with 5.2 dS m^{-1} treatment (Table 2). Silicon treated plants showed higher DPPH-activity than no Si application but the differences was not significant ($p \leq 0.05$) (Table 2). For the salt-tolerant cv. Omidbakhsh, DPPH-radical scavenging activity was significantly ($p \leq 0.05$) higher than salt-sensitive cv. Sepideh (Table 2). None of interactions between treatments was significant ($p \leq 0.05$).

Total phenol: The results showed that with increasing levels of salinity, phenol content decreased but it was not significant ($p \leq 0.05$) (Table 2). Supply of 1.44 g.kg^{-1}soil Si increased total phenol content remarkably but not significantly compared with control and 1.92 g.kg^{-1}soil Si application (13% and 10% respectively). The total phenol content in salt tolerant cultivar was higher (21%), but not significant ($p \leq 0.05$), than the salt sensitive sorghum cultivar (Table 2). Interaction effect of salinity and Si application on total phenol content was also non-significant ($p \leq 0.05$). However, only in high levels of salinity, 23.1 dS m^{-1} phenol content decreased, but among Si treatments, 1.44 kg^{-1}soil Si application showed more phenol content (Table 2). Interaction between salinity and cultivars showed that under 5.2 dS m^{-1} salinity and in the salt tolerance cultivar, total phenol content was higher than in the other treatments (Table 2). Total phenol content in Si application treatments and salt tolerance cultivar was higher than Si free and salt sensitive cultivar (Table 2).

Dry matter accumulation: As shown in Table 2, the shoot dry weight of both cultivars was significantly ($p \leq 0.01$) reduced by NaCl stress. Under salt stress, Si significantly ($p \leq 0.05$) decreased the shoot dry weight of both cultivars up to 1.44 g.kg^{-1} soil Si but increased at 1.92 g.kg^{-1} soil Si.

In the present study, the activities of APX, SOD, POX, GR and DPPH in plants grown under high levels of salinity were obviously higher than control (5.2 dS m^{-1}). Effect of salinity on activity of CAT was not considerable and a decline in total phenol content measured with increased salinity (Table 2). The high antioxidant enzymes activities have a role in imparting tolerance to these cultivars against salinity stress. In the present result, increased antioxidants activity was consistent with the previous findings and maximum antioxidants activity observed at 1.44g.kg^{-1}soil Si (Table 2).

Discussion

Mechanisms of plant adaptation under salt stress are complex. As the initial sites of the cell injury by any environmental stress firstly appears in cell membranes (Ashraf & Ali, 2008). Farooq & Azam (2006) reported that cellular injury increased with increasing salinity levels in wheat varieties. Previous findings showed that added Si increased the MSI of leaf cells (Ashraf & Ali, 2008; Liang *et al*., 2003; Liang, 1999; Liang *et al*., 1996). The results of our study showed that there were no significant differences in MSI among salinity and Si treatments. However, supply of 1.92 g.kg^{-1}soil Si, increased MSI of sorghum (3.2%) leaf cells. This supports the previous findings that supplement of Si under salt stress increased the MSI of leaf cells (Liang *et al*., 1996; Liang, 1999). Electrolyte leakage affects dry matter accumulation mainly through osmotic imbalance and entrance of toxic ions.

Decrease in leaf water potential under salinity conceivably reduces expansive growth (Munns & Tester, 2008). RWC at 5.2 dS m^{-1} was higher than 10.5 and 23.1 dS m^{-1} salinity. In the present study, a maximum increase in RWC was observed at 1.44 g.kg^{-1} soil Si application (Table 2). Leaf RWC declined significantly (0.61%) with higher external Si concentrations (Table 2). Mali & Aery (2008) observed that RWC of wheat leaves was adversely affected at higher levels (800 ppm) of Si. Decreased transpiration and improved light interception characteristics by keeping the leaf blade erect by Si application have been reported in some previous studies (Epstein, 1999; Mali & Aery, 2008).

One of the biochemical strategies to improve salt tolerance in plants is synthesis of compatible solutes. Munns & Tester (2008) reported that if Na^+ and Cl^- are sequestered in the vacuole of a cell, organic solutes that are compatible with metabolic activity even at high concentrations mainly accumulate in the cytosol and organelles to balance the osmotic pressure of the ions in the vacuole. Proline and sucrose are the most commonly solutes that accumulate to high concentrations in certain species at saline condition (Munns & Tester, 2008). In our experiment, under salinity stress both proline and soluble sugars concentrations were not significantly different with and without supplemental Si (Table 2). However, proline concentrations were lower in supplemental Si plants than in Si-untreated plants, and soluble sugar concentration was slightly increased with Si application than in the case where Si was absent. These results are consistent with the findings of Gunes *et al*., (2007), who showed that in the spinach and tomato plants under sodic and boron toxic soil, proline concentrations significantly decreased by Si

treatments. Tuna *et al*., (2008) also reported decrease in proline in wheat cultivars grown under salinity and silicon and they suggested that there is a definite decreasing effect of Si on proline biosynthesis/accumulation. However, proline is a plant osmoregulator under stress condition and may cause decrease of growth and dry matter accumulation in salt stress (Yoshiba *et al.*, 1997).

One effect of free oxygen radical accumulation in plant cells under stress is membrane damage and electrolyte leakage (Marschner, 1995). Although, high levels of antioxidants activity observed at 1.44 $g.kg^{-1}$soil Si addition but MSI of sorghum leaf cells increased (6.7%) by supplying of 1.92 $g.kg^{-1}$soil Si. Liang (1999) suggested that Si-grown plants operate metabolic pathways that scavenge ROS. In the present work, increased antioxidant activity could not alleviate salinity damage completely because dry matter production was not according to high levels of antioxidants activity. The results of this study are in line with previous findings, which showed that added Si enhanced leaf SOD activity under salt stress (Liang *et al.*, 1996; Liang, 1999; Liang *et al.*, 2003).

Conclusion

The results show that Si may alleviate salt stress in sorghum by increasing antioxidants activity. Supplied 1.44 $g.kg^{-1}$soil Si, caused increased activity of APX, CAT, SOD, PRO, GR, total antioxidant and total phenol concentration, while, 1.92 $g.kg^{-1}$soil Si application caused increase in MSI, soluble sugars and total phenol concentration, CAT, SOD and total antioxidant activity. It seems that Si increased antioxidant activity and inhibited the lipid peroxidation of cell plasma membranes to maintain integrity in high levels of Na^+ concentration in the cytoplasm but osmotic stress occurred in plant cells. Despite increased antioxidant activity at 1.44 $g.kg^{-1}$soil Si, growth and dry matter accumulation of salt-stressed sorghum plants was not improved by this amount of Si application.

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References

Abe, N., T. Murata and A. Hirota. 1998. Novel 1,1-diphenyl-2-picryhy- drazyl- radical scavengers, bisorbicillin and demethyltrichodimerol, from a fungus. *Biosci. Biotechnol. Biochem*., 62: 661-662.

Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnol. Adv*., 27: 84-93.

Ashraf, M. and P.J.C. Harris. 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.,* 166: 3-16.

Ashraf, M. and Q. Ali. 2008. Relative membrane permeability and activities of some antioxidant enzymes as the key determinants of salt tolerance in canola (*Brassica napus* L.). *Environ. Exp. Bot.,* 63: 266-273.

Bates, L.S., R.P. Waldran and I.D. Teare. 1973. Rapid determination of free proline for water studies. *Plant Soil*, 39: 205-208.

DuBois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anol. Chem*., 28(3): 350-356.

Epstein, E. 1999. Silicon. Ann. Rev. Plant Physiol. *Plant Mol. Biol*., 50: 641-664.

Farooq, S. and F. Azam. 2006. The use of cell membrane stability (CMS) technique to screen for salt tolerant wheat varieties*. J. Plant Physiol*., 163: 629-637.

Gunes, A., A. Inal, E.G. Bagci and D.J. Pilbeam. 2007. Silicon-mediated changes of some physiological and enzymatic parameters symptomatic for oxidative stress in spinach and tomato grown in sodic- B toxic soil. *Plant Soil*, 290: 103-114.

Igartua, E., M.P. Gracia and J.M. Lasa. 1995. Field responses of grain sorghum to a salinity gradient. *Field Crops Res*., 42: 15-25.

Lee, D.H. and C.B. Lee. 2000. Chilling stress-induced changes of antioxidant enzymes in the leaves of cucumber: in gel enzyme activity assays. *Plant Sci*., 159: 75-85.

Liang, Y., W. Sun, Y.G. Zhu and P. Christie. 2007. Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. *Environ. Pollut*., 147: 422-428.

Liang, Y.C. 1999. Effects of silicon on enzyme activity and sodium, potassium and calcium concentration in barley under salt stress. *Plant Soil*, 209: 217-224.

Liang, Y.C., Q. Chen, Q. Liu, W.H. Zhang and R.X. Ding. 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, Y.C., Q.R. Shen, Z.G. Shen and T.S. Ma 1996. Effects of silicon on salinity tolerance of two barley cultivars. *J. Plant Nutr*., 19: 173-183.

Maas, E.V., J.A. Poss and G.J. Hoffman. 1986. Salinity sensitivity on sorghum at three growth stages. *Irrig. Sci*., 7: 1-11.

Mali, M. and N.C. Aery. 2008. Influence of silicon on growth, relative water contents and uptake of silicon, calcium and potassium in wheat grown in nutrient solution. *J. Plant Nutr*., 31: 1867-1876.

Marschner, H. 1995. Part I. Nutritional physiology. In: *Mineral Nutrition of Higher Plants*. (Ed.): H. Marschner. Academic Press, London. Seconded, pp. 18-30, 313-363.

Molassiotis, A.N., T. Sotiropoulos, G.Tanou, G.Kofidis, G. Diamantidis and I. Therios. 2006. Antioxidant and anatomical responses in shoot culture of the apple rootstock MM 106 treated with NaCl, KCl, mannitol or sorbitol. *Biol. Plant*., 50: 61-68.

Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol*., 59: 651-81.

Qureshi, A.S., M.Qadir, N. Heydari, H. Turral and A. Javadi. 2007. A review of management strategies for salt-prone land and water resources in Iran. Colombo, Sri Lanka: International Water Management Institute. 30p. (IWMI Working Paper 125).

Sairam, R.K., K.V. Rao and G.C. Srivastava. 2002. Differential response of wheat genotypes to long-term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Sci.,* 163: 1037-1046.

Singleton, U.L. and J. Rossi. 1965. Colorimetry of total phenolics with phosphomolybdic-posphotungustic acid reagent. *Am. J. Enol. Vit*., 16: 144.

Smart, R.E. and G.E. Bingham. 1974. Rapid estimates of relative water content. *Plant Physiol*., 53: 258-260.

Srinivas, N.D., K.R. Rashmi and K.S.M.S. Raghavarao. 1999. Extraction and purification of a plant peroxidase by aqueous two-phase extraction coupled with gel filtration. *Process Biochem.,* 35: 43-48.

Takahashi, E., J.F. Ma and Y. Miyake. 1990. The possibility of silicon as an essential element for higher plants. Comm. *Agric. Food Chem*., 2: 99-122.

Tuna, A.L., C. Kaya, D. Higgs, B. Murillo-Amador, S. Aydemir and A.R. Girgin. 2008. Silicon improves salinity tolerance in wheat plants. *Environ. Exper. Bot*., 62: 10-16.

Velikova, V., I. Yordanov and A. Edreva. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. *Plant Sci.,* 151: 59-66.

Yamaguchi, K., H. Mori and M. Nishimura. 1995. A novel isoenzyme of ascorbate peroxidase localized on glyoxysomal and leaf peroxisomal membranes in pumpkin. *Plant Cell Physiol*., 36: 1157-1162.

Yoshiba, Y., T. Kyiosue, K. Nakashima, K. Yamaguchi-Shinozaki and K. Shinozaki. 1997. Regulation of levels of proline as an osmolyte in plants under water stress. *Plant Cell Physiol*. 38: 1095-1102.

Yu, Q. and Z. Rengel. 1995. Drought and salinity differentially influence activities of superoxide dismutase in narrow-leafed lupins. *Plant Sci*., 142: 1-11.

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