

Effects of exogenous silicon on seed germination and antioxidant enzyme activities of *Momordica charantia* under salt stress

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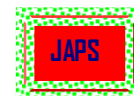
1 SUMMARY

The influences of exogenous silicon (Si) concentrations (1, 2, 3 and 5 mM) on germination rate (GR), germination index (GI), and vitality index (VI), as well as malondialdehyde (MDA) contents, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities were investigated in *Momordica charantia* under 50 and 100 mM NaCl during 5, 10 and 15-day periods. Our results indicated that GR, GI and VI were significantly decreased when treated with 50 and 100 mM NaCl, but they improve significantly by the addition of different Si concentrations compared to those of salt stress conditions. MDA contents in the leaves decreased significantly at different treated periods by addition of different Si concentrations compared to that of salt stress (50 mM NaCl). However, the activities of SOD, POD and CAT were significantly increased compared to that of NaCl stress. These results suggested that exogenous silicon may increase GR, GI and VI, which contributes to reducing MDA contents and increase the antioxidant enzyme activities under NaCl stress.

2 INTRODUCTION

Salt stress in the soil or water is the major factor especially in arid and semi-arid regions which greatly influence plant growth and yield. In plants, salt stress can lead to affects intracellular ion homeostasis and water balance. When plants are exposed to salt stress, there will be impairment of the electron transport, and then reactive oxygen species (ROS) will be produced in both chloroplasts and mitochondria (Parvaiz and Satyawati, 2008). These cytotoxic ROS can potentially disrupt normal metabolism via oxidative damage to

nuvledic acids, proteins and lipids. However, antioxidant system can protect plant cells from oxidative damage that includes antioxidant molecules as well as antioxidant enzymes. The latter includes catalase (CAT), superoxide dismutases (SOD), peroxidase (POD) and glutathione reductase (GR). (Ashraf, 2009). Various studies have suggested that SOD, POD, and CAT increased significantly in some plants when subjected to salt stress (Gao *et al.*, 2008; Azooz *et al.*, 2009). These findings suggested that an efficient antioxidant enzyme



system in these plant species plays an important role in the alleviation of oxidative damage under salt stress.

Silicon (Si) is the second most abundant element in the soil, but it is not considered an essential element. Recently, numerous studies have shown that added silicon to treated plant can significantly alleviate manganese, aluminum, salt, drought, chilling and freezing stresses, and is considered beneficial effects on plant growth and production (Liang *et al.*, 2007; Ma and Yamaji, 2008). Recently, the mitigating role of Si in salt stress has received worldwide attention. In addition, some earlier studies have shown that Si is effective in mitigating salinity in different plant species, such as barley (Liang *et al.*, 2003), cucumber (Zhu *et al.*, 2004), and maize (Moussa, 2006), tomato (Romero-Aranda *et al.*, 2006), wheat (Mukkram *et al.*, 2006; Saqib *et al.*, 2008; Tuna *et al.*, 2008). Previous results have also suggested that Si may increase superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities but reduced the malondialdehyde (MDA) concentration in barley, tomato and maize plants (Liang *et al.*, 2003; Al-Aghabary *et al.*,

2004; Moussa, 2006). These findings suggested that Si may decrease lipid peroxidation in salt-stressed plants through enhancing antioxidant enzyme activity and non-enzymatic antioxidants. As far as we know, the effects of Si on the salt tolerance of crops have been studied in many hydroponics experiments, but the protective effects of Si are still under investigation.

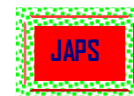
Momordica charantia L., commonly known as 'bitter melon', is a multi-purpose herb cultivated in different parts of the world for its edible fruits. In addition, bitter melon is also a traditional herb commonly used for its anti-diabetic, antioxidant, contraceptive and antibacterial properties (Liu *et al.*, 2002). All these studies show that application of silicon is useful for improvement of salt tolerance of plants, but there is currently no information available about the possible beneficial effects of Si application in *Momordica charantia* plant. Thus, the objective of the present study was to test the effects of silicon application on germination and antioxidant responses of *Momordica charantia* plants under salt stress.

3 MATERIALS AND METHODS

3.1 Plant materials and chemical: Mature *Momordica charantia* seeds were collected in July, 2009 at the garden, College of Life Science, Sichuan University. Seeds were selected and stored in a gauze bag at 4 °C until to use. Nitro blue tetrazolium (NBT) and 2-thiobarbituric acid (TBA) were obtained from Sigma (St. Louis, MO, USA). Others used were of reagent grade or higher.

3.2 Seed germination: Seeds with uniform size were surface sterilized with 5% (w/v) calcium hypochlorite for 15 min, and rinsed four times thoroughly with distilled water. The seeds were transferred into 11 sterile Petri dishes (100 seeds per

dish) with quartz sand and watered using 1/2 strength Hoagland nutrient solution. A control (no NaCl and silicon added), two NaCl (50 and 100 mM) and four Si treatments (1, 2, 3 and 5) were added as K₂SiO₃. Three replicates from each treatment were prepared. Germination experiment was carried out in greenhouse, maintained at 28/20 °C day/night (12/12 h) temperature cycles, relative humidity 70 % and light intensity 250 μM m⁻² s⁻¹. The pH of the nutrient solution was adjusted at 6.2 ± 0.1. Seeds were considered to be germinated after the radical emerged through the seed coat and reached more than 5 mm in length. Seed germination and the



length of seedlings were recorded every other day. Germination rates (GR), germination index (GI) and vitality index (VI) were calculated for each treatment using the following equation: $GR = n/N \times 100 \%$, where n is the number of germination, and N represents the total number of tested seeds. $GI = \sum Gt/Dt$, where Gt is the number of germination at time t , and Dt represents the corresponding day of germination. $VI = S \cdot GI$, where S is the length of seedlings.

3.3 Seedlings growth: This experiment was designed to investigate the effects of Si application on MDA content, SOD, POD and CAT activities in *Momordica charantia* leaves under salt stress. The germination conditions were the same as above. Uniform 7-day-old seedlings with two expanded leaves were transplanted into culture devices filled with 6000 ml of aerated half-strength Hoagland solutions in a growth chamber and maintained at the same conditions of seed germination. Six treatments with three replicates were established including control (0.0 mM NaCl + 0.0 mM Si), 50 mM NaCl, 50 mM NaCl + 1mM Si, 50 mM NaCl + 2mM Si, 50 mM NaCl + 3mM Si and 50 mM NaCl + 5 mM Si. After having been treated for 0, 5, 10 and 15 days, the second leaves were harvested for determination of MDA content, SOD, POD and CAT activities.

3.4 Assay of lipid peroxidation: It was measured as MDA content determined by thiobarbituric acid (TBA) reaction (Bailly *et al.*, 1996). Fresh sample (0.5 g) was homogenized in 5 ml of 0.1 % trichloroacetic acid (TCA), and the homogenates were centrifuged at 10 000 rpm for 15 min. To 1.0 ml aliquot of the supernatant, 3.0 ml of 0.5 % thiobarbituric acid (TBA) in 5 % TCA was added. The mixture was heated at 95 °C for 30 min and then cooled immediately in an ice bath. After centrifugation at 12 000 rpm for 10 min, the absorbance of the extract was recorded at 532 nm and 600 nm in spectrophotometer. The MDA

concentration was expressed as μmol per gram fresh weight ($\mu\text{mol g}^{-1} \text{fw}$).

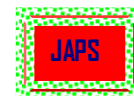
3.5 Enzyme extraction: Fresh leaves (1 g) were grounded with a pestle in an ice-cold mortar with 10 ml ice-cold 50 mM sodium phosphate buffer (pH 7.0). The homogenates were centrifuged at 12 000 rpm for 20 min at 4 °C. The supernatant was used as the crude extract for the assays of SOD, POD and CAT activities. Protein content was assayed by the Bradford method (1976) using bovine serum albumin as a standard.

3.6 Determination of SOD activity: It was determined according to the method of Dhindsa *et al.* (1981). In brief, the assay mixture consisted of 50 μl enzyme extract, 50 mM sodium phosphate buffer (pH 7.0), 13 mM methionine, 75 μM NBT, 0.15 M NaCl and 2 μM riboflavin. The absorbance of solution was recorded at 560 nm. One unit of SOD activity was defined as the enzyme activity that reduced the photo reduction of nitroblue tetrazolium to blue formazan by 50%. Enzyme activities were expressed as enzyme units per gram fresh weight (U/g fw).

3.7 Determination of POD activity: It was performed according to Kar and Choudhuri method (1987) with slight modifications. Reaction solution contained 2.85 ml 3 % guaiacol (water solution), 0.1 ml 2 % H_2O_2 and 50 μl enzyme extract. Activity unit was calculated using the coefficient of absorbance for tetraguaiacol at 470 nm (22.6 mM^{-1}). Enzyme activities were expressed as enzyme units per gram fresh weight (U/g fw).

3.8 Determination of CAT activity: It was measured following the change in absorbance of the reaction mixture at 240 nm due to hydrogen peroxide reduction (Aebi 1984). Activity unit was calculated using the coefficient for H_2O_2 at 240nm ($40 \text{ mM}^{-1} \text{ cm}^{-1}$). Enzyme activities were expressed as enzyme units per gram fresh weight (U/g fw).

3.9 Statistical analysis: All experiments were performed at least for three replicates. Statistical



significance was evaluated with Student's t-test, and less than 0.05. considered to be significant when the *P* value was

4 RESULTS

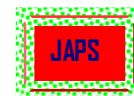
As shown in Table 1, GR, GI and VI in the addition of different Si concentrations treatments has a significant difference with control and NaCl treatment. GR was significantly depressed under 50 and 100 mM NaCl stresses, and reduced 48.9% and 71.1% compared to that of the control, respectively. This way, the progressive drop in the GR is directly affected by the increasing NaCl concentrations. However, the addition of Si concentrations of 1, 2, 3 and 5 mM significantly alleviated inhibitory effects of NaCl stress. GR at the Si applications of 1, 2, 3 and 5 mM increased by 32.6%, 47.8%, 23.9% and 13% compared to that of 50 mM NaCl treatments, respectively. Similarly, the GR increased by 17.6%, 47.1%, 29.4% and 11.8% compared to that of 100 mM NaCl treatments. GI and VI are two important parameters that reflect the seed quality. From Table 1, it may be seen that GI and VI showed a significant decrease under salt stress compared to the control, but application of Si can increase GI and VI compared to those of under salt stress. The increase of GI was to the extent of 2.08%, 42.6%, 7.01%, 4.14% compared to that of 50 mM NaCl treatments, and reaching 3.94%, 101%, 54.7% and 64% compared to that of 50 mM NaCl treatments, respectively. Exogenous application of all Si levels used also increased significantly the VI of *Momordica charantia* seeds compared to those of 50 and 100 mM NaCl treatments. Thus, the greatest values were recorded at 2 mM Si levels, and increased by 192.1% and 119.5%, respectively.

Table 1: Effects of Si application on germination rate (GR), germination index (GI) and vitality index (VI) of *Momordica charantia* seeds under salt stress. Si (0), Si (1), Si (2), Si, (3) and Si(4) are 50 mM NaCl, 50 mM NaCl+1mM Si, 50 mM NaCl+2mM Si, 50 mM NaCl+3mM Si and 50 mM NaCl+5 mM Si, respectively. Data are displayed as mean \pm SD (n=3).

Treatments	GR (%)	GI	VI
Control	90 \pm 3.96	6.83 \pm 0.31	27 \pm 1.12
50 mM NaCl	46 \pm 2.15	3.85 \pm 0.15	6.2 \pm 0.28
50 mM NaCl + 1 mM Si	61 \pm 2.85	3.93 \pm 0.16	9.52 \pm 0.43
50 mM NaCl + 2 mM Si	68 \pm 3.04	5.49 \pm 0.24	18.1 \pm 0.85
50 mM NaCl + 3 mM Si	57 \pm 2.35	4.12 \pm 0.16	10.3 \pm 0.47
50 mM NaCl + 5 mM Si	52 \pm 2.26	4.01 \pm 0.15	12.3 \pm 0.51
100 mM NaCl	17 \pm 0.69	2.03 \pm 0.09	2.57 \pm 0.11
100 mM NaCl + 1 mM Si	20 \pm 0.92	2.11 \pm 0.08	3.25 \pm 0.12
100 mM NaCl + 2 mM Si	25 \pm 1.05	4.08 \pm 0.14	5.59 \pm 0.25
100 mM NaCl + 3 mM Si	22 \pm 0.93	3.14 \pm 0.12	3.92 \pm 0.16
100 mM NaCl + 5 mM Si	19 \pm 0.86	3.33 \pm 0.13	2.98 \pm 0.13

As shown in Table 2, MDA contents in the leaves of *Momordica charantia* increased gradually as the experiments continued under the control conditions. However, MDA contents increased significantly under 50 mM NaCl stress in comparison to

non-stress conditions, and increased by 504.4%, 523.4% and 264.3% at days 5, 10 and 15, respectively. The addition of different Si concentrations significantly reduced MDA contents in *Momordica charantia* leaves in comparison to that



of 50 mM NaCl stress at day 5, and decreased by 19.9%, 8.1%, 13.9% and 32.4%, respectively. Similarly, MDA contents in the addition of different Si levels also showed significant decreases at days 10

and 15, and reduced by the greatest of 24.2% and 29.4% at the Si concentrations of 3 mM compared to that of NaCl treatment

Table 2: Effects of Si application on MDA contents of *Momordica charantia* leaves under salt stress. Data are displayed as mean \pm SD (n=3).

Treatments	0 d	5 d	10 d	15 d
Control	1.45 \pm 0.07	1.6 \pm 0.08	2.39 \pm 0.11	2.91 \pm 0.13
50mM NaCl	1.45 \pm 0.06	9.67 \pm 0.45	14.9 \pm 0.72	10.6 \pm 0.51
50mM NaCl + 1 mM Si	1.45 \pm 0.06	7.75 \pm 0.36	12.9 \pm 0.63	9.48 \pm 0.45
50mM NaCl + 2 mM Si	1.45 \pm 0.05	8.89 \pm 0.42	13.2 \pm 0.62	11 \pm 0.53
50mM NaCl + 3 mM Si	1.45 \pm 0.07	8.33 \pm 0.41	11.3 \pm 0.54	7.48 \pm 0.36
50mM NaCl + 5 mM Si	1.45 \pm 0.06	6.54 \pm 0.31	15.4 \pm 0.76	10.6 \pm 0.49

As shown in Table 3, SOD activity in *Momordica charantia* leaves increased significantly under salt stress compared to the control at 5, 10 and 15-day periods, and increased by 12.8%, 40%, and 97.1%, respectively. It is interesting to note that the addition of Si enhanced significantly SOD activity in *Momordica charantia* leaves compared to that of under salt stress. For example, the application of 3

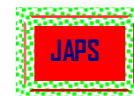
and 5 mM Si at days 5 may result in the increase of 29.2 and 31.3% compared to that of NaCl treatment, respectively. In addition, significant increases under the addition of Si conditions were also observed at days 10 and 15, and the maximum changes of 35.4% and 6.16% were recorded at the Si concentrations of 3 and 2 mM compared to that of NaCl treatment, respectively.

Table 3: Effects of Si application on SOD activities of *Momordica charantia* leaves under salt stress. Data are displayed as mean \pm standard deviation (bars) for three replications.

Treatments	0 d	5 d	10 d	15 d
Control	25.5 \pm 1.07	39.1 \pm 1.85	45 \pm 2.05	33 \pm 1.35
50mM NaCl	25.5 \pm 1.15	44.1 \pm 2.03	63.1 \pm 2.97	65.1 \pm 3.02
50mM NaCl + 1 mM Si	25.5 \pm 1.05	42.8 \pm 1.94	61.2 \pm 2.81	56.5 \pm 2.42
50mM NaCl + 2 mM Si	25.5 \pm 1.21	42.3 \pm 1.88	82.1 \pm 4.07	69.1 \pm 3.15
50mM NaCl + 3 mM Si	25.5 \pm 1.13	56.9 \pm 2.34	85.4 \pm 3.89	68 \pm 3.17
50mM NaCl + 5 mM Si	25.5 \pm 1.20	57.9 \pm 2.82	72.3 \pm 3.22	54.4 \pm 2.09

As shown in Table 4, POD activity in *Momordica charantia* leaves under NaCl stress increased significantly at days 5, 10 and 15 compared to that of the control, and increased by 10.3%, 258.7% and 214.3%, respectively. At treatment of days 5, no significant increase in POD activity was observed between the NaCl treatment and the addition of Si

except for the addition of Si concentration of 5 mM. However, POD activity under the Si-amended salt treatment was significantly higher at days 10 and 15 compared to the salt treatment alone. Thus, POD activity enhanced by 28% at days 10 when the addition of Si concentrations of 3 mM, and by 40.4% at days 15 when the application of Si levels



of 5 mM.

Table 4: Effects of Si application on POD activity in *Momordica charantia* leaves under salt stress. Data are displayed as mean \pm SD (n=3).

Treatments	0 d	5 d	10 d	15 d
Control	5.16 \pm 0.23	7.48 \pm 0.31	5.38 \pm 0.21	8.75 \pm 0.37
50mM NaCl	5.16 \pm 0.21	8.25 \pm 0.39	14.3 \pm 0.68	27.5 \pm 1.06
50mM NaCl + 1 mM Si	5.16 \pm 0.22	5.83 \pm 0.25	15.2 \pm 0.72	26.6 \pm 1.13
50mM NaCl + 2 mM Si	5.16 \pm 0.25	7.91 \pm 0.33	17.9 \pm 0.85	29.6 \pm 1.28
50mM NaCl + 3 mM Si	5.16 \pm 0.19	7.48 \pm 0.34	18.3 \pm 0.87	32.1 \pm 1.45
50mM NaCl + 5 mM Si	5.16 \pm 0.24	11.1 \pm 0.51	13.2 \pm 0.61	38.6 \pm 1.73

As shown in Table 5, CAT activity under the control condition increased gradually with the experiment continued up to days 10, and then decreased. However, the activity in leaves treated with 50 mM NaCl increased significantly at days 5, 10 and 15 compared to the control, and increased by 94.4%, 73.8% and 65.1%, respectively. The

addition of different Si concentrations into the salt treatment increased significantly CAT activity at day 5, 10 and 15 compared to that of the NaCl treatment, and the peak activities increased by 15.3%, 37.5% and 11.5% at the Si concentrations of 1, 2 and 5 mM, respectively

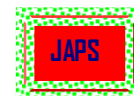
Table 5 : Effects of Si application on CAT activity in *Momordica charantia* leaves under salt stress. Data are displayed as mean \pm SD (n=3).

Treatments	0 d	5 d	10 d	15 d
Control	5.99 \pm 0.25	6.38 \pm 0.29	17.2 \pm 0.76	16.9 \pm 0.71
50 mM NaCl	5.99 \pm 0.27	12.4 \pm 0.57	29.9 \pm 1.29	27.9 \pm 1.25
50mM NaCl + 1 mM Si	5.99 \pm 0.29	14.3 \pm 0.61	35.2 \pm 1.46	26.4 \pm 1.02
50mM NaCl + 2 mM Si	5.99 \pm 0.26	13.9 \pm 0.65	41.1 \pm 1.85	29.7 \pm 1.18
50mM NaCl + 3 mM Si	5.99 \pm 0.27	13.6 \pm 0.62	35.3 \pm 1.55	26.4 \pm 1.02
50mM NaCl + 5 mM Si	5.99 \pm 0.28	11.5 \pm 0.55	29.1 \pm 1.15	31.1 \pm 1.12

5 DISCUSSION

Salt stress is posing a severe threat to increasing demand of food in many arid and semiarid regions of the world. Germination is one of the most salt-sensitive plant growth stages and severely inhibited with increasing salinity (Parvaiz and Satyawati, 2008). Germination rate, GI and VI of *Momordica charantia* seed were inhibited by 50 and 100 mM NaCl. In addition, our results suggested that an increase of silicon concentration from 1 to 5 mM in the medium is capable of increasing of GR,

GI and VI of *Momordica charantia* seeds. Similar results in previous reports have been suggested that Si has many positive effects on the growth and yield as well as physiology and metabolism in different plant species (Liang *et al.*, 2007; Ma and Yamaji, 2008). These findings suggested that silicon may be involved directly or indirectly in both morphological changes and physiological processes in plants. Thus, our findings seemed plausible that Si shows a protective role in germination of *Momordica charantia*



seeds to prevent them from being severely affected by salt stress.

Peroxidation of membrane lipids is an indication of membrane damage at the cellular level under stress conditions. The change in MDA contents, especially in oil rich seeds, is often used as an indicator of oxidative damage (Sung, 1996). The present results suggested that MDA contents increase significantly in *Momordica charantia* leaves under salt stress compared to the control. Elevated MDA contents mediated by ROS are considered to be one of the likely explanations for lipid peroxidation. Germination inhibition in the present study is good correlation with the increase in MDA content under salt stress. However, lower MDA contents in *Momordica charantia* leaves were observed by the addition of Si compared to those of under salt stress condition. It has been reported that Si application may decrease membrane lipid peroxidation in the cells of barley plants exposed to salt stress (Liang *et al.*, 2003). These findings suggested that Si may prevent the structural and functional deterioration of cell membranes in these plants species exposed to salt stress. Thus, our results suggested that oxidative damage in *Momordica charantia* seedlings induced by salt stress might be alleviated by the addition of Si.

It is generally recognized that plants can protect themselves by inhibiting lipid peroxidation due to the effects of activated antioxidant enzymes under salt stress (Parvaiz and Satyawati, 2008). SOD, POD and CAT are the major antioxidant enzymes

associated with scavenging ROS. SOD is likely to be central in the defense against toxic ROS (Ashraf, 2009). Earlier reports have suggested that a better protection from oxidative damage caused by salt stress by increasing SOD, POD and CAT in *Jatropha curcas*, maize and other plant species (Parvaiz and Satyawati, 2008; Gao *et al.*, 2008; Azooz *et al.*, 2009). These results suggest that salt stress can led to significant changes in SOD, POD and CAT activities involved in ROS metabolism. In the present study, Si application increased SOD, POD and CAT activity in *Momordica charantia* leaves exposed to salt stress. These results suggested that they can be triggered by increased production of ROS or it might be a protective mechanism adopted by *Momordica charantia* plants against oxidative damage. Moreover, the changes of MDA content were in parallel with the changes of SOD, POD and CAT activities in *Momordica charantia* leaves. A lower lipid peroxidation resulting from elevated antioxidant enzyme activities by NaCl addition in presence of Si was also reported in barley, tomato and maize plants (Liang *et al.*, 2003; Al-Aghabary *et al.*, 2004; Moussa, 2006). These findings suggested that the oxidative damage exposed to salt stress was alleviated by Si addition by virtue of increased SOD, POD and CAT activities and decreased MDA contents. Thus, our results lend further support to those findings.

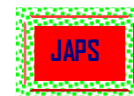
6 CONCLUSION

In conclusion, the application of Si may increase significantly GR, GI and VI of *Momordica charantia* under salt stress. The improvement of Si on salt tolerance of *Momordica charantia* plant was associated with the decreased MDA contents and increased antioxidant enzyme activities. Thus, our results suggested that Si may be involved in the defensive

mechanisms of *Momordica charantia* under salt stress.

7 ACKNOWLEDGEMENTS

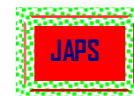
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8 REFERENCES

- Aebi M: 1984. Catalase *in vitro*. *Methods in Enzymology* 105: 121–126.
- Ashraf M: 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnology Advances* 27: 84–93.
- Al-Aghabary K, Zhu Z. and 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* 12: 2101–2115.
- Azooz MM, Ismail AM. and Abou-Elhamd MF: 2009. Growth, lipid peroxidation and antioxidant enzyme activities as a selection criterion for the salt tolerance of three maize cultivars grown under salinity stress. *International Journal of Agriculture and Biology* 11: 21–26.
- Bailly C, Benamar A, Corbineau F. and Dome D: 1996. Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seed as related to deterioration during accelerated aging. *Physiologia Plantarum* 97: 104–110.
- Bradford 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.
- Dhindsa RS, Dhindsa PP. and Thorpe TA: 1981. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *The Journal of Experimental Botany* 32: 93–101.
- Gao S, Ouyang C, Wang S, Xu Y, Tang L. and Chen F: 2008. Effects of salt stress on growth, antioxidant enzyme and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. seedlings. *Plant Soil and Environment* 54: 374–381.
- Kar PK. and Choudhuri MA: 1987. Possible mechanisms of light induced chlorophyll eradication in senescencing leaves of hydrila veticillata. *Physiologia Plantarum* 70: 729–734.
- Liang YC, Chen Q, Liu Q, Zhang W. and Ding R: 2003. Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). *Journal of Plant Physiology* 160: 1157–1164.
- Liang YC, Sun W, Zhu YG. and Christie P: 2007. Mechanisms of silicon mediated alleviation of abiotic stress in higher plants: a review. *Environmental Pollution* 147: 422–428.
- Liu X, Li S, Feng C. and Yan D: 2002. Advances in the study of *Momordica charantia* L. *Zhong Yao Cai* 25: 211–213.
- Ma JF. and Yamaji N: 2008. Functions and transport of silicon in plants. *Cellular and Molecular Life Sciences* 65: 3049–3057.
- Moussa HR: 2006. Influence of exogenous application of silicon on physiological response of salt-stressed maize (*Zea mays* L.). *International Journal of Agriculture and Biology* 8: 293–297.
- Mukkram AT, Rahmatullah, Tariq A, Ashraf M, Shamsa K. and Maqsood MA: 2006. Beneficial effects of silicon in wheat (*Triticum aestivum* L.) under salinity stress. *Pakistan Journal of Botany* 38: 1715–1722.



- Parvaiz A. and Satyawati S: 2008. Salt stress and phyto-biochemical responses of plants – a review. *Plant Soil and Environment* 54: 89–99.
- Romero-Aranda MR, Jurado O. and Cuartero J: 2006. Silicon alleviates the deleterious salt effect on tomato plant growth by improving plant water status. *Journal of Plant Physiology* 163: 847–855.
- Saqib M, Zörb C. and Schubert S: 2008. Silicon-mediated improvement in the salt resistance of wheat (*Triticum aestivum*) results from increased sodium exclusion and resistance to oxidative stress. *Functional Plant Biology* 35: 633–639.
- Sung JM: 1996. Lipid peroxidation and peroxide-scavenging in soybean seeds during aging. *Physiologia Plantarum* 97: 85–89.
- Tuna AL, Kaya C, Higgs D, Murillo-Amador B, Aydemir S. and Girgin AR: 2008. Silicon improves salinity tolerance in wheat plants. *Environmental and Experimental Botany* 62: 10–16.
- Zhu ZJ, Wei GQ, Li T, Qian QQ. and Yu JQ: 2004. Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Curcumas sativa* L.). *Plant Science* 167: 527–533.