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Alleviation of cadmium toxicity by silicon is related to elevated photosynthesis, antioxidant enzymes; suppressed cadmium uptake and oxidative stress in cotton

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A B S T R A C T

Biotic systems face immense environmental hazards such as accumulation of heavy metals, particularly in agricultural ecosystems that might cause deterioration of yield and quality of crops. In this study, we evaluated the role of silicon (Si) in alleviating the heavy metal (Cd) stress tolerance in cotton by analyzing the induced Physio-chemical changes. Cotton plants were grown in hydroponic culture with three different Cd levels (0, 1 and 5 μM) along with two Si treatment levels (0 and 1 mM). The data showed that Cd alone reduced the plant growth as well as the efficiency of antioxidant activity as compared to control plants. Plant growth, gas exchange characteristics (net photosynthetic rate, stomatal conductance, transpiration rate, water use efficiency) chlorophyll contents, and carotenoids as well as the performance of antioxidant enzymes were improved by the exogenous application of Si. The adverse effects of Cd on plant growth were alleviated by the exogenous application of Si. It was observed that Si effectively mitigated the adverse effects of Cd on cotton plants and markedly enhanced the growth, biomass and photosynthetic parameters while decreased the contents of malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and electrolytic leakage (EL). The antioxidant enzyme activities in cotton leaves and roots increased significantly, when Si was added to control as well as Cd stressed plants. In conclusion, Si improved the growth and photosynthesis attributes of cotton plants by mitigating the adverse effects of Cd stress through reduced EL, MDA and H_2O_2 contents and improved activities of antioxidant enzymes.

1. Introduction

Heavy metals with a density higher than 5.0 g cm^{-3} such as cadmium (Cd), lead (Pb), chromium (Cr), silver (Ag) and mercury (Hg) etc., are important environmental pollutants, mainly where there are high anthropogenic activities. Soil pollution by heavy metals is an important environmental concern for the agricultural sector VacullK et al., (2009) over the past several decades, especially in the developing countries (Tandy et al., 2006; Saifullah et al., 2009). Agricultural lands that have been polluted due to the heavy metal accumulation were proved to have highly adverse effects on soil biological activity, fertility, plant metabolism, biodiversity, and the health of humans and animals (Wagner 1993; Nawrot et al., 2006; Jarup and Akesson 2009; Ali et al., 2011a, b). Even in low concentrations can cause serious threats to all living organisms via their accumulation in soil and water. Heavy metals are well known growth inhibitors and can cause detrimental effects on plant growth, develop phototoxic responses and decrease the productivity and quality of agricultural crops (Ali et al., 2012, 2013a).

Cadmium (Cd) is one of the most toxic environmental pollutant, which is quite toxic even in minute concentration. It enters the environment principally from industrial processes, mining operations, municipal wastes, and phosphate fertilizers (Wagner, 1993; Clemens, 2006). Upon exposure to Cd stress, inhibition occurs in most of the physiological and biochemical processes of plants, such as chlorophyll synthesis, photosynthesis and nutrient uptake resulting in retardation of growth and low yield (Ali et al. 2013b; Sanita and Gabbrielli, 1999). As a non-redox metal, Cd is unable to take part in Fenton-type reactions, but can produce reactive oxygen species (ROS) and cause oxidative stress in plants (Schützendübel and Polle, 2002; Zhang et al., 2009). ROS molecules are highly toxic compounds that can oxidize most of the lipids, proteins and nucleic acids causing death of the cell due to lipid peroxidation, membrane damage, and inactivation of enzymes.

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Silicon (Si) is the second most abundant element both on the surface of the earth and in the soil (Gong et al., 2006). The unavailability of Si to plants is due to its combination with other compounds to form silicates or oxides (Richmond and Sussman, 2003). Silicon is not considered as an essential element but it has been found to minimize the various biotic and abiotic stresses (Richmond and Sussman, 2003; Ma, 2004; Ma.and.Yamaji, 2006) and has beneficial effects on growth and development of many plants, particularly of gramineous plants and some cyperaceous plants (Richmond and Sussman, 2003; Epstein 1994, 1999; Liang et al., 2007; Ma et al., 2001). For example, Si enhances plant resistance to fungi, pests as well as to the drought stresses. Si also helps in mediating the adverse effects of heavy metal stresses such as chromium (Cr) (Ali et al., 2013c) aluminum (Al) (Baylis et al., 1994; Liang et al., 2001; Hammond et al., 1995), boron (B) (Gunes et al., 2007a; Inal et al., 2009) and zinc (Zn) (Kaya et al., 2009). These beneficial effects of Si to plants are attributed to both soluble Si and Si deposited in the various plant tissues. Large amount of Si that deposited in tissues may act as a physical barrier that increases the rigidity and strength of the plant tissues (Ma, Yamaji, 2006). Soluble Si plays an active role in stimulating some defense reaction mechanisms and enhancing host resistance to plant against the diseases. (Fauteux et al., 2005).

In general, silicon has been recommended for improving the resistance of plants to abiotic stresses, including heavy metal toxicity. However, to our knowledge, the possible use of Si for improving Cd stress tolerance in cotton plants has not been tested so far. In this view, a hydroponic experiment was conducted to find out the influence of Si treatments on plant growth, photosynthesis, chlorophyll contents, oxidative stress, antioxidant enzymes, and electrolyte leakage and cd uptake under Cd stress. The objective was to investigate that whether or not the exogenous application of Si activate the protective responses in cotton plants exposed to cadmium stress.

2. Materials and Methods

2.1. Experimental site

This study was carried out in wire house of Ayub Agricultural Research Institute (AARI) and, in labs of Government College University Faisalabad and Nuclear Institute for Agriculture and Biology (NIAB) Faisalabad, Pakistan.

2.2. Growth conditions

Healthy seeds of cotton genotype MNH 886 were immersed in concentrated sulfuric acid solution for 15 min to remove the short fibers on the surface of the seeds. Seeds were then rinsed with distilled water thoroughly and sown in 2 in. layers of sterilized quartz sand trays were put in a growth chamber with a photoperiod of 16 h light/8 h dark with light intensity of 400 ± 25 μmol m^{-2} s^{-1}. The light/dark temperature was set at 30 °C/25 °C with relative humidity at 85%. After two weeks of sowing, the uniform seedlings were wrapped with foam at a root shoot junction, and transplanted in thermopole sheets having evenly spaced holes (15 in. × 17 in. in size) floating on 40 L capacity iron tubs, lined with polyethylene sheet containing modified Hoagland's solution. The basic nutrient medium had composition: ($Ca(NO_3)_2$ 2.5 mM, $MgSO_4$ 1 mM, KCl 0.5 mM, KH_2PO_4 0.5 mM, $FeCl_3$ 0.1 μM, $CuSO_4$ 0.2 μM, $ZnSO_4$1 μM, H_3BO_3 20 μM, H_2MoO_4 0.005 μM, $MnSO_4$ 2 μM). Continuous aeration was given through an air pump in the nutrient solution by making bubbles. The solution was changed every week. Thereafter two weeks of transplantation, Cd levels (control, 1 and 5 μM) were developed with $CdCl_2$ and two levels of Si as sodium silicate (Na_2SiO_3) (control and 1 mM) with three replicates were applied. Complete randomized design (CRD) was applied. By adding 1 M H_2SO_4 and NaOH, solution pH (6.0 ± 0.1) was maintained.

2.3. Measurements of plant growth and biomass

Plants were harvested after five weeks of growth under Cd stress. Data regarding shoot, root length, shoot, and root fresh and dry weight were recorded.

2.4. Leaf area

Leaf area was estimated with a leaf area meter (LI-2000, LI-COR, USA).

2.5. Gas exchange parameters

Five weeks after application of the treatment, photosynthetic rate (A), stomatal conductance (gs), transpiration rate (E), water use efficiency (A/E) was determined by using Infra-Red Gas Analyzer (IRGA) (Analytical Development Company, Hoddesdon, England).

2.6. Determination of chlorophyll contents

Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were determined spectrophotometrically (Metzner et al., 1965). After five weeks of Cd treatment, the topmost fully expanded fresh leaves were weighed and dipped overnight in 85% (v/v) aqueous acetone for the extraction of the chlorophyll pigments. Supernatant taken was centrifuged at 4000 rpm for 10 min and diluted with 85% aqueous acetone to the suitable concentration for spectrophotometric measurements. The disappearance was calculated at absorbance of 452.5, 644 and 663 nm alongside blank of untainted 85% liquid acetone. Chl a, b, total chlorophyll and carotenoids were analyzed using the following equations:

Chlorophyll a (μg/ml) $= 10.3{*}E_{663} - 0.98{*}E_{644}$
Chlorophyll b (μg/ml) $= 19.7{*}E_{644} - 3{:}87{*}E_{663}$
Total chlorophyll = chlorophyll a+chlorophyll b
Total carotenoids (μg/ml) $= 4.2{*}E_{452.5} - \{(0.0264{*}\text{chl a}) + (0{:}426{*}\text{chl b})\}$

Finally, these pigment fractions were calculated as mg/g fresh weight.

2.7. Estimation of electrolyte leakage

Electrolyte leakage was estimated by using the method of Dionisio-Sese, Tobita (1998). After five weeks of Cd treatment, leaves (1 g) were cut into small parts of 5 mm length and put in test tubes containing 8 ml deionized and distilled water. The tubes were placed in a water bath at 32°C for two hours. The initial electrical conductivity of the medium (EC_1) was assessed. The samples were placed in an autoclave at the 121 °C for 20 min to expel all electrolytes. Samples were cooled at 25°C and second electrical conductivity (EC_2) was measured. Total electrolyte leakage was calculated by using the following formula.

$$EL = (EC_1/EC_2) \times 100$$

2.8. Assay of antioxidant enzymes

Anti-oxidant enzymes such as superoxide dismutase (SOD), guaiacol peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) of roots and leaves were determined spectrophotometrically.

After five weeks of Cd treatment fresh samples (0.5 g) of leaves and roots were ground with the help of a motor and pestle and homogenized in 0.05 M phosphate buffer (pH 7.8) under chilled condition. The homogenized mixture was filtered through four layers of muslin cloth and centrifuged at 12,000 × g for 10 min at 4 °C.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was analyzed by the nitroblue tetrazolium (NBT) method (Beauchamp and Fridovich, 1971) at 560 nm by calculating the photoreduction of NBT. The reaction mixture (3 ml) consisted of 50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 10 μM EDTA, 2 mM riboflavin and enzyme extract (100 μl). Reaction was started by placing tubes below two 15 W fluorescent lamps for 10 min.

Guaiacol peroxidase (POD, EC 1.11.1.7) activity was assayed according to method of (Putter, 1974) with some modification. The reaction mixture (3 ml) consisted of 100 μl enzyme extract, 100 μl guaiacol (1.5%, v/v), 100 μl H_2O_2 (300 mM) and 2.7 ml 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0). Increase in the absorbance due to oxidation of guaiacol was measured spectrophotometrically at 470 nm ($\varepsilon = 26.6$ mM^{-1} cm^{-1}).

Catalase (CAT, EC 1.11.1.6) activity was determined by the method of (Aebi, 1984). The assay mixture (3.0 ml) was comprised of 100 μl enzyme extract, 100 μl H_2O_2 (300 mM) and 2.8 ml 50 mM phosphate buffer with 2 mM EDTA (pH 7.0). The CAT activity was assayed by monitoring the reduction in the absorbance at 240 nm as a consequence of H_2O_2 disappearance ($\varepsilon = 39.4$ mM^{-1} cm^{-1}).

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was assayed according to the method of (Nakano and Asada, 1981). The reaction mixture consisted of 100 μl enzyme extract, 100 μl ascorbate (7.5 mM), 100 μl H_2O_2 (300 mM) and 2.7 ml 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0). The oxidation of ascorbate was observed by the change in absorbance at 290 nm ($\varepsilon = 2.8$ mM^{-1} cm^{-1}).

2.9. Malondialdehyde (MDA) content

The level of lipid peroxidation in the leaf tissue was measured in terms of malondialdehyde (MDA, a product of lipid peroxidation) content determined by the thiobarbituric acid (TBA) reaction using the method of Heath and Packer (1968), with minor modifications as described by Dhindsa et al., (1981) and Zhang and Kirham (1994). A 0.25 g leaf sample was homogenized in 5 ml 0.1% TCA. The homogenate was centrifuged at 10,000 × g for 5 min. To 1 ml aliquot of the supernatant, 4 ml of 20% TCA containing 0.5% TBA was added. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000 × g for 10 min, the absorbance of the supernatant at 532 nm was read and the value of the nonspecific absorption at 600 nm was subtracted. The MDA content was calculated by using an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.10. Determination of soluble protein

The soluble protein content was analyzed according to Bradford (1976), using Coomassie Brilliant Blue G-250 as dye and albumin as a standard.

2.11. Hydrogen peroxide contents

H_2O_2 was extracted by homogenizing 50 mg leaf and root tissues with 3 ml of phosphate buffer (50 mM, pH 6.5) (Jana and Choudhuri, 1981). To analyze H_2O_2 content, 3 ml of extracting solution was mixed with 1 ml of 0.1% titanium sulfate in 20% (v/v) H_2SO_4 and the mixture was centrifuged at 6000 × g for 15 min. The intensity of the yellow color of the supernatant was measured at 410 nm. H_2O_2 content was computed by using the extinction coefficient of 0.28 $\mu mol^{-1}cm^{-1}$.

2.12. Estimation of cadmium (Cd) concentration

After five weeks of treatment, cotton plants were harvested and washed thoroughly with tap water, distilled water and deionized water. Plant samples were separated into roots, stem and leaves dried at 80 °C in an oven for 48 h, and ground into powder. Each sample (0.5 g) was dry-ashed at 450 °C in muffle furnace for 10 h, and digested in 10 mL of 1 M HCl for 12 hs, and centrifuged at 3600 rpm for 15 min. Concentrations of Cd in root, stem and leaves were determined by a flame atomic absorption spectrometry (Nov Aa 400 Analytik Jena, Germany).

Table 1
Effect of different concentrations of cadmium (Cd) (0, 1 and 5 μM) and Si (0 and 1 mM) on growth characteristics of cotton plants.

Treatments	Plant height (cm)	Root length (cm)	Leaf area (cm^2)	Number of leaf $plant^{-1}$
Ck	56.69 ± 1.20a	43.49 ± 0.88a	71.74 ± 2.70a	27 ± 1a
Si_0	59.56 ± 2.40a	46.33 ± 2a	76.03 ± 2.25a	28.67 ± 1.76a
Cd1	32.61 ± 2.60c	29.29 ± 1.21c	46.66 ± 4.00c	16 ± 2.30c
Cd1+Si1	42.50 ± 1.45b	35.16 ± 2.32b	57.66 ± 1.85b	19.66 ± 1.76b
Cd5	14.71 ± 1.20e	14.00 ± 0.57e	27.00 ± 3.51e	11 ± 1.52d
Cd5+Si1	22 ± 1.15d	20.67 ± 1.45d	38.33 ± 2.02d	14.66 ± 1.70cd

Values show the means of three replicate ± SE. Means followed by same small letters are not significant different at $P \le 0.05$ by using the Tukey test.

2.13. Statistical analysis

All values reported in this experiment are mean of three replicates. Analysis of variance (ANOVA) was done by using a statistical package, SPSS version 16.0 (SPSS, Chicago, IL) followed by Tukey test between the means of treatments to determine the significant differences.

3. Results

3.1. Plant growth characteristic

The growth of plant in term of plant height, root length, number of leaves $plant^{-1}$ and leaf area was decreased in the presence of cadmium (Cd) in the nutrient medium at both Cd levels (1 and 5 μM). Cd stress caused a significant reduction in plant growth, Plant height, root length, number of leaves $plant^{-1}$ and leaf area showed significant decrease as compared to control. The adverse effects of Cd stress on the plant growth were alleviated when Si was applied (Table 1). The application of Si favored the growth of plant and got rid of the toxic effects generated by cadmium. Si application markedly decreased the inhibitory effect on plant growth that reflected as in increase of plant height, root length, number of leaves $plant^{-1}$ and leaf area. Exogenously applied Si alone also led to a slight increase in growth as compared to control.

3.2. Plant biomass

Table 2 delineates the biomass of cotton under Cd stress treated without or with Si. A significant difference was observed in plant biomass in terms of leaves shoot and root fresh and dry weight under Cd stress in comparison to the control with increasing Cd concentration. However, follow-up treatment to the stressed plants Si (1 mM) significantly countered the inhibitory effect of Cd more efficiently as compared to those which did not receive Si treatment. The response of plants to cadmium stress was avoided by Si application which markedly improved the growth of plants. Similarly, the plants treated with Cd were smaller in comparison with the plants treated with Cd+Si. The growth-promoting effect of Si was greater in plants treated with Cd.

3.3. Chlorophyll contents

Chlorophyll content expressed as chlorophyll a, chlorophyll b, total chlorophyll and carotenoids value in cotton leaves is illustrated in Table 3. Cd treatments greatly influenced the chlorophyll contents. After the treatment at both level (1 and 5 μM) chlorophyll a, chlorophyll b, total chlorophyll and carotenoids value decreased as compared with those of plants grown without Cd stress. However plants receiving Si supply imposed positive impact on chlorophyll contents than that of the control ones. The plants treated with Si in combination with Cd (1 and 5 μM)

Table 2
Effect of different concentration of cadmium (Cd) (0, 1 and 5 μM) and Si (0 and 1 mM) on biomass of cotton plants.

Treatments	Fresh weight (g)			Dry weight (g)		
	Leaf	Stem	Root	Leaf	Stem	Root
Ck	15.14 ± 0.70a	13.66 ± 1.20a	4.04 ± 0.22a	2.76 ± 0.19a	3.01 ± 0.16a	1.1 ± 0.1a
Si_0	14.88 ± 1.56a	14.20 ± 1.56a	4.08 ± 0.19a	2.79 ± 0.18a	3.02 ± 0.15a	1.14 ± 0.10a
Cd1	7.29 ± 0.53c	7.31 ± 1.45c	2.51 ± 0.19c	1.51 ± 0.03c	1.72 ± 0.26c	0.56 ± 0.05c
Cd1+Si1	9.59 ± 0.95b	9.69 ± 1.76b	3.16 ± 0.26b	1.98 ± 0.11b	2.17 ± 0.17b	0.7 ± 0.12b
Cd5	3.74 ± 0.14e	3.16 ± 0.08e	1.63 ± 0.27d	0.75 ± 0.08e	0.94 ± 0.12d	0.26 ± 0.02e
Cd5+Si1	6.11 ± 0.48d	5.36 ± 0.33d	1.98 ± 0.19cd	1.18 ± 0.01d	1.27 ± 0.18d	0.42 ± 0.08d

Values show the means of three replicate ± SE. Means followed by same small letters are not significant different at $P \le 0.05$ by using the Tukey test.

Table 3
Effect of different concentrations of cadmium (Cd) (0, 1 and 5 μM) and Si (0 and 1 mM) on chlorophyll contents of cotton plants.

Treatments	Chl a ($mg\ g^{-1}$)	Chl b ($mg\ g^{-1}$)	Total Chl ($mg\ g^{-1}$)	Carotenoids ($mg\ g^{-1}$)
Ck	0.61 ± 0.01a	0.33 ± 0.012a	0.95 ± 0.02a	0.35 ± 0.02a
Si_0	0.65 ± 0.02a	0.36 ± 0.015a	0.99 ± 0.01a	0.36 ± 0.035a
Cd1	0.37 ± 0.01c	0.18 ± 0.031c	0.51 ± 0.02bc	0.18 ± 0.029c
Cd1+Si1	0.45 ± 0.01b	0.25 ± 0.006b	0.69 ± 0.008b	0.26 ± 0.011b
Cd5	0.21 ± 0.02e	0.08 ± 0.003e	0.27 ± 0.024d	0.07 ± 0.016c
Cd5+Si1	0.30 ± 0.01d	0.13 ± 0.008d	0.43 ± 0.027c	0.12 ± 0.014d

Values show the means of three replicate ± SE. Means followed by same small letters are notsignificant different at P≤0.05 by using the Tukey test.

Table 4
Effect of different concentration of cadmium (Cd) (0, 1 and 5 μM) and Si (0 and 1 mM) on gas exchange parameters of cotton plants.

Treatments	Net photosynthetic rate ($\mu\ mol\ m^{-2}\ s^{-1}$)	Transpiration rate ($mmol\ m^{-2}\ s^{-1}$)	Water use efficiency	Stomatal conductance ($mmol\ m^{-2}\ s^{-1}$)
Ck	11.01 ± 0.77a	2.73 ± 0.20a	4.47 ± 0.22a	2.86 ± 0.03a
Si_0	11.65 ± 1.16a	2.78 ± 0.11a	4.84 ± 0.26a	2.91 ± 0.06a
Cd1	6.29 ± 0.36c	1.49 ± 0.06c	2.59 ± 0.15c	1.35 ± 0.03c
Cd1+ Si1	8.27 ± 0.37b	1.92 ± 0.24b	3.64 ± 0.32b	1.84 ± 0.02b
Cd5	2.15 ± 0.10e	0.61 ± 0.06e	1.47 ± 0.07d	0.67 ± 0.10e
Cd5+ Si1	4.09 ± 0.04d	1.08 ± 0.04d	2.24 ± 0.25c	1.006 ± 0.08d

Values show the means of three replicate ± SE. Means followed by same small letters are not significant different at *P*≤0.05 by using the Tukey test.

showed a significant increase in chlorophyll contents when compared with their corresponding planets under Cd stress.

3.4. Gas exchange attributes

According to the chlorophyll fluorescence parameters viz net photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (*E*) and water use efficiency (Pn/*E*), the photosynthesis processes decreased their normal activities under cadmium supply in nutrient medium, and a serious damage occurred to photosystem (Table 4). A slight decrease in the gas exchange parameters with increasing Cd stress was observed and this decrease in the activity of gas exchange attributes was more obvious at 5 μM as compared to the plants under control condition.. Application of Si significantly prohibited the stress condition and improved the activity of the gas exchange parameters in comparison to their corresponding control condition plant.

3.5. Antioxidant enzymes and protein contents

Antioxidative enzymes play a key role in oxidative stress management. Therefore, the present parameters were evaluated to find out the effects of Si application on the enzyme activities in Cd-stressed cotton. Cadmium stress led significant alteration in antioxidant defense in leaves and roots of cotton plants (Fig. 1 A–H). Decline in the activities of antioxidant enzymes (SOD, POD, CAT and APX) was observed in cotton plants due to the imposition of Cd stress than those in non-stressed plants. However, in silicon treated Cd stress plants, antioxidant enzyme (SOD, POD, CAT and APX) activities enhanced, as compared with Cd stressed plants. It means, exogenously applied Si caused an increase in antioxidant enzyme activity in Cd stressed plants.

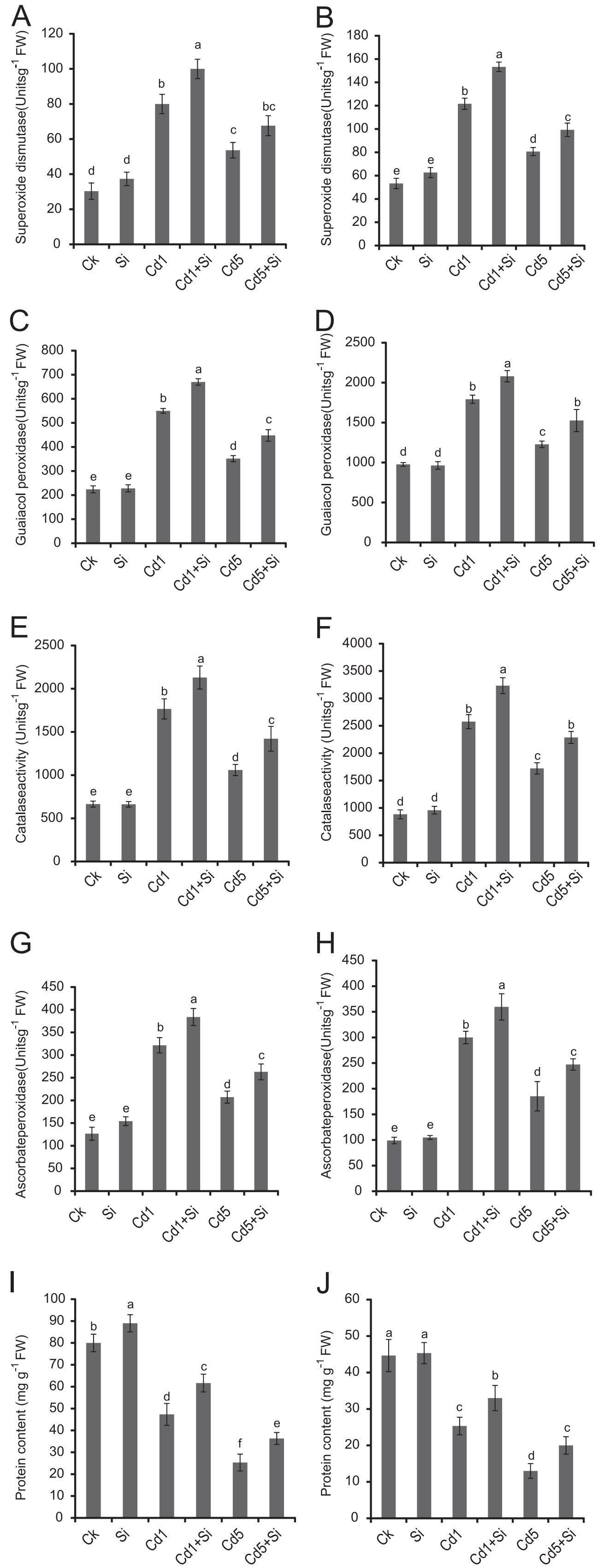

Fig. 1. Effect of different concentrations of cadmium (Cd) (0, 1 and 5 μM) and Si (0 and 1 mM) on superoxide dismutase (SOD) of leaf (A) root (B), guaiacol peroxidase of leaf (C) root (D), catalase (CAT) of leaf (E), root (F), ascorbate peroxidase (APX) of leaf (G) and root (H) and protein content of leaf (I), root (J) in cotton plants. Values show the means of three replicate ± SE. Means followed by same small letters are not significant different at *P*≤0.05 by using the Tukey test.

The evaluation of protein contents also indicated significant effects of Si treatment on Cd stressed and unstressed plants (Fig. 1 I, J). The observations on Cd stressed cotton plants revealed that protein contents decreased with rising concentrations of metal. Minimal protein contents were observed when treated with the 5 μM Cd. Application of Si showed significant increase in soluble protein contents in comparison to Cd treated plants. The treatment with 1 mM Si resulted in a significant increase in protein when compared to the control. The protein contents were also higher in Cd treated plants along with Si (1 mM) treatment than Cd alone.

3.6. Oxidative stress and electric conductivity

The effect of Cd and Si on oxidative stress is presented in Fig. 2 (A–D). It was observed that MDA and H_2O_2 contents of cotton plants increased in response to Cd Stress. It means, in Cd treated plants oxidative stress significantly increased in leaves and roots as compared to the control. However, Si application significantly reduced the oxidative stress under Cd-stressed in cotton plants. Membrane stability decreased highly significantly with increasing Cd stress level in cotton plants (Fig. 2E, F). An increase in the electrolyte leakage under both Cd stress levels was observed. The electrolyte leakages of roots and leaves treated with Si were significantly reduced as compared to the control showing an improvement in the membrane stability

3.7. Cadmium contents

Cd contents in roots stem and leaves are presented in Fig. 3. The degree of increase in uptake in all three plant parts viz. root, stem and leaves were dependent on dose. Cd concentrations increased with increasing Cd concentration in the nutrient medium. Cadmium concentration was significantly higher in roots irrespective of cadmium levels followed by stem and leaves. Silicon addition significantly declined Cd concentrations in all three plant parts at the two levels of Cd applied. Moreover the use of Si also restricted the translocation of Cd from roots in the aboveground parts of the plants.

4. Discussion

In the previous investigation, alleviation of metal toxicity by addition of Si has been extensively found in many plant species (Ali et al., 2013c; Gunes et al., 2007a; Wang et al., 2000) and all of those results revealed that Si application could promote plant

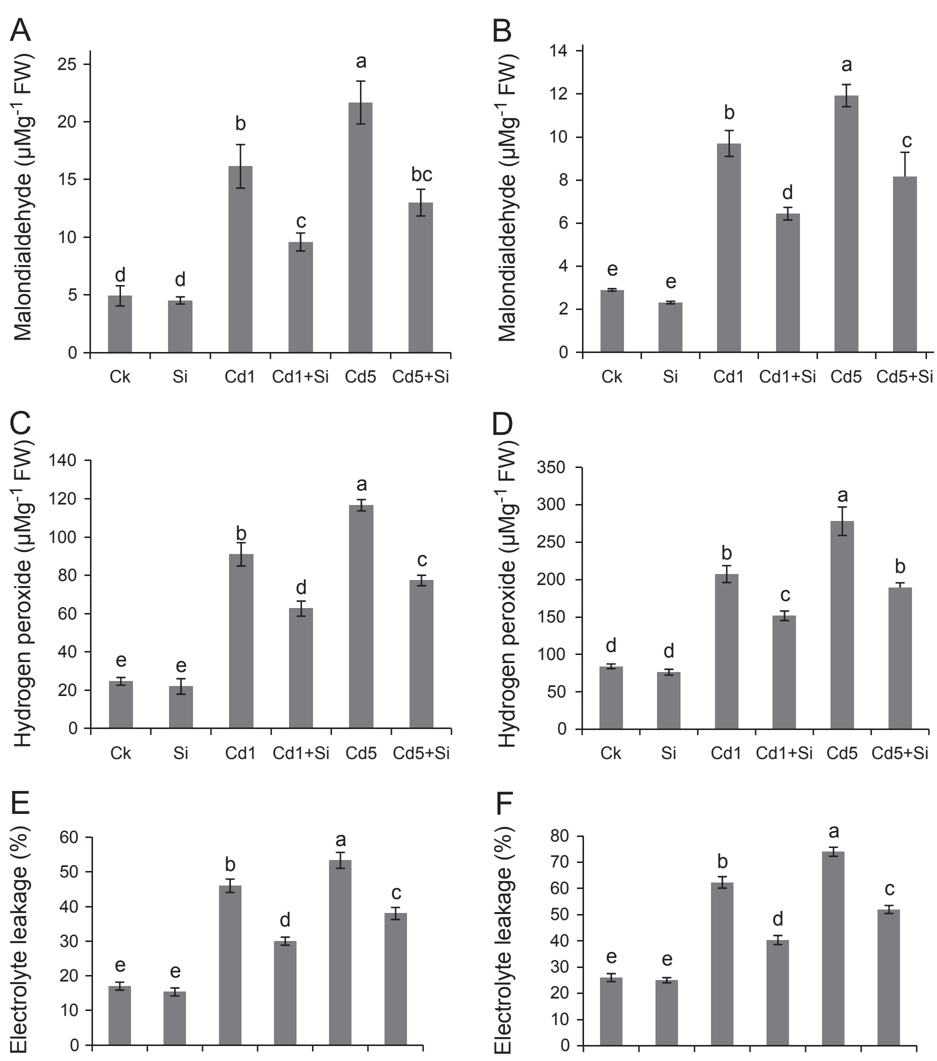

Fig. 2. Effect of different concentrations of cadmium (Cd) (0, 1 and 5 μM) and Si (0 and 1 mM) on malondialdehyde of leaf (A), root (B), hydrogen peroxide of leaf (A) root (B) and electrolyte leakage of leaf (A), root (B) in cotton plants. Values show the means of three replicate ± SE. Means followed by same small letters are not significant different at $P \leq 0.05$ by using the Tukey test.

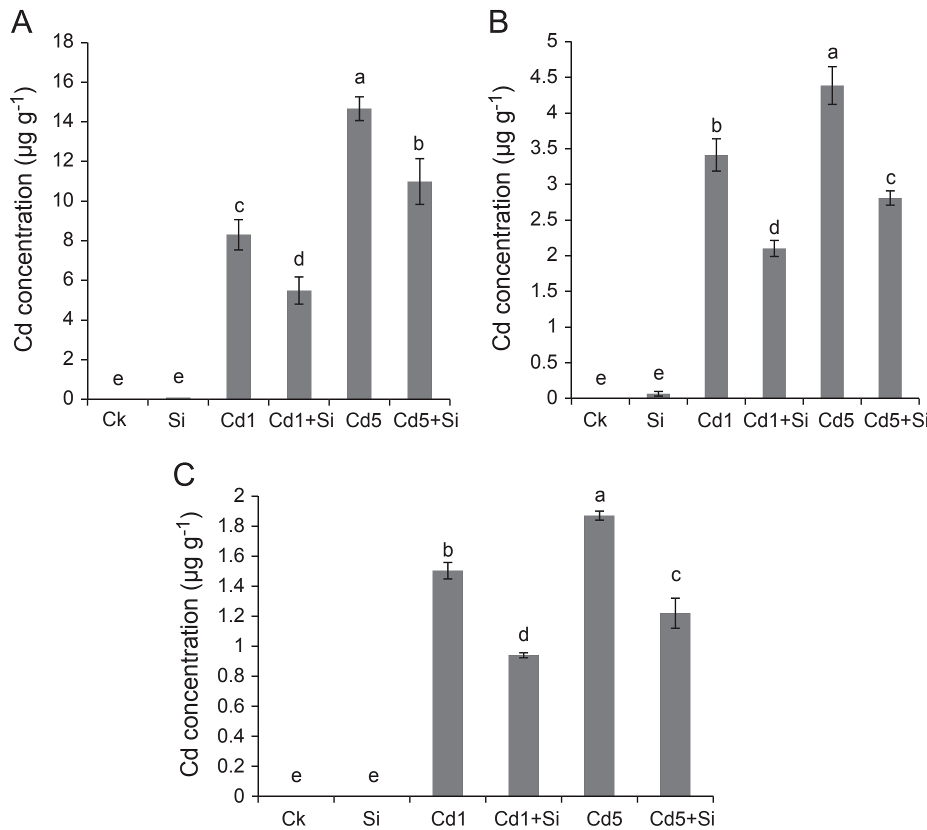

Fig. 3. Effect of different concentrations of cadmium (Cd) (0, 1 and 5 μM) and Si (0 and 1 mM) 575 on concentrations of cadmium (Cd) in roots (A), stem (B) and leaves (C) in cotton plants. Values 576 show the means of three replicate ± SE. Means followed by same small letters are not 577 significant different at $P \leq 0.05$by using the Tukey test.

growth under metal toxicity, the present study also supported the conclusions.

Our present study showed that Cd treatments significantly decreased the plant growth and development in cotton plants. Cd inhibited gas exchange parameters (net photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (E), water use efficiency (Pn/E)), chlorophyll contents (chlorophyll a, b and total chlorophyll) and carotenoids. Some such results were also reported by many researchers that Cd inhibited yield, photosynthesis, and pigments in plants (Nwugol and Huerta, 2008; Wahid et al., 2008). The result obtained showed that Cd toxicity disturbed the biochemical and metabolic processes in cotton plants (Shi et al., 2010; Nwugol and Huerta, 2008; Zhang et al., 2008; VacullK et al., 2009; Song et al., 2009). One of the main processes underlying Cd induced stress to plants is increased formation of ROS, even though Cd does not generate ROS directly, but produce oxidative burst by interfering the antioxidant defense system (Foyer and Noctor, 2005b that promotes the MDA contents due to lipid peroxidation (Krantev et al. 2008).

The present study showed that plant growth, biomass, chlorophyll contents and photosynthetic parameters of cotton plants were enhanced by the silicon addition under non-Cd stress conditions, proving the positive and beneficial effects of Si on cotton growth. The reason for this beneficial effect of Si is still unclear. Similarly, many researchers reported beneficial effects of silicon in many crops such as wheat, rice and sugarcane (Li et al., 1989, Hammond et al., 1995, Epstein, 1994,1999; Liang, 1999, 2007; Ma et al., 2001; Liang et al., 2005). Such beneficial impacts could be indirect (for instance improved disease resistance) or direct (improved nutrition). As silicon induced promotion in growth by increasing cell wall extensibility in rice plants (Hossain et al., 2002).

The results of our present study showed that silicon markedly alleviated Cd- induced reduction in growth, biomass and photosynthetic parameters in cotton plants. The alleviating effects of silicon on stresses like high temperature, chilling, diseases, drought, salinity, heavy metals etc. have been intensively studied (Hashemi et al., 2010; Jiao-Jing et al., 2009). Cadmium accumulation among the three parts of the plant (root, shoot, and leaf) increased by increasing the level of Cd concentrations in the nutrient medium. We calculated that accumulation of cadmium was greater in the root, and less was observed in the shoot of the cotton plant. Mostly with highest resistance, the plants take up less proportion of the total solution metal so have lower metal contents in the upper ground parts of the plants (Yang et al., 1998). It was reported by many scientists that silicon supplement would enhance tolerance to toxic metals by reducing the uptake and translocation of metals, including Cd, Mn and Zn (Shi et al., 2010; Kaya et al., 2009; Song et al., 2009; Nwugol and Huerta, 2008; Zhang et al., 2008).

Our present results also showed that Cd decreased the soluble protein in both roots and leaves of cotton plants. It may be due to more oxidative damage that inhibited the protein contents (Gupta et al., 2009). In the present investigation, increased reactive oxygen species (ROS) was found under metal stress as indicated by electrolyte leakage, MDA and H_2O_2 contents. Plants generally faced the oxidative damage when exposed to Cd and other metals (Dixit et al. 2001; Erdei et al. 2002; Fargasova, 1994; Macfarlane, 2003). To meet and check the oxidative damage plants developed complex antioxidant system. In the present study anti-oxidant enzymes such as SOD, POD, CAT and APX in roots and leaves decreased at both Cd levels (1 and 5 μM). The reduction in antioxidant enzymes at higher Cd concentrations might be due to the severe stress of oxidative damage to antioxidant enzymes, hence, the activity of enzymes was diminished (Mishra et al., 2006). Alleviation of metals toxicity by Si was also related with protection against oxidative damage caused due to oxidative stress. Si alleviated B (Boron) stress in spinach, barley and tomato by preventing oxidative damage (Gunes et al., 2007b). Similarly, Song et al. (2009) reported that the alleviation of cadmium toxicity by Si in pakochi plants was related to a marked and significant increase in enzymatic and non-enzymatic antioxidants and decrease in formation of lipid peroxidation (MDA). According to Shi et al. (2005), tolerance against Mn toxicity in cucumber plants was diminished by Si application. In the present investigation, the exogenous addition of silicon in solution culture showed marked beneficial effects on the reduction of producing MDA, H_2O_2 and electrolyte leakage, and the enhancement of anti-oxidant activities of SOD, APX, POD and CAT under Cd Stress. From our results, it might be suggested that silicon addition could markedly enhance the capacity of defense against oxidative damage induced by Cd toxicity in cotton seedlings.

In this investigation, Cd was less translocated from roots to shoots in cotton plants. Mostly, the plants with highest resistance took up a less proportion of the total solution metal and had the lowest upper ground metal contents (Liu et al., 2004). But, exogenous application of Si decreased the uptake of Cd in all three parts of plants such as roots, stem and leaves and translocation of Cd in Cd stressed plants as compared to Cd alone. The possible mechanisms for inhibition of metal transport in plants by Si are mainly involved in the two aspects: (1) Si deposits lignin in cell walls and induces metal ions bound to cell walls, thus reduce translocation of metals from roots to shoots (Ma.and.Yamaji, 2006). It was reported that Mn stress tolerance enhancement in *Cucumis sativus* by Si was a result of stronger binding of manganese to cell walls and a reduction of Manganese contents within symplast (Rogalla, Römheld, 2002). Similarly, it was observed that other metals such as Cd and Al behaved in the same manner (Wang et al. 2000, Liang et al. 2001) (2) the complex formation or co-precipitation of toxic metal ions with Si. Hodson and Sangster (1993) reported that silicon could detoxify Al toxicity in *Sorghum bicolor* by forming a complex with Al in the medium and/or roots and ultimately inhibiting Al translocarion into the root cortex. Similarly, Inal et al. (2009) reported that formation of B–Si (boron-silicate) complexes in the soil led to lower boron availability and consequently lowered tissue boron concentration. In the present study, Si-induced inhibition of Cd uptake and translocation in cotton plants were also observed. Therefore, it can be supposed that Si might also have the ability to form complex with Cd or induce Cd to deposit in the cell walls, thus lowering Cd toxicity in cotton plants.

5. Conclusion

During the current investigation, we observed that the toxicity of Cd could decrease the growth, biomass, pigments, photosynthetic characteristics and protein contents due to increase in Cd uptake, also by the formation of ROS and lowering the antioxidant capacities. However, the application of Si improved the plant growth and photosynthetic characteristics by lowering the reactive oxygen species (ROS) damage through increased antioxidant enzyme activities and by lowering uptake and accumulation of Cd. From these observations, it was concluded that Si application can improve the Cd stress tolerance in the cotton.

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References

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121–126.
Ali, S., Bai, P., Zeng, F., Cai, S., Qiu, B., Wu, F., Zhang, G.P., 2011a. Ecotoxicological and interactive effects of chromium and aluminum on growth, oxidative damage and antioxidant enzymes of the two barley cultivars differing in Al tolerance. Environ. Exp. Bot. 70, 185–191.
Ali S., Farooq M.A., Hussain S., Yasmeen T., G.H. Abbasi A.N.D. Zhang GP 2013a. Alleviation of chromium toxicity by hydrogen sulfide in barley. Environ. Toxicol. Chem. 10.1002/etc.2309.
Ali S., Farooq M.A., Jahangir M.M., Abbas F., Bharwana S.A., Zhang G.P. 2013b. Effect of chromium toxicity on photosynthesis and anti-oxidative system in barley as affected by nitrogen form. Bio. Plant. 10.1007/s10535-013-0336-y.
Ali, S., Cai, S., Zeng, F., Qiu, B., Zhang, G.P., 2012. The effect of salinity and chromium stresses on uptake and accumulation of mineral elements in barley genotypes differing in salt tolerance. J. Plant Nutr 35, 827–839.
Ali, S., Farooq, M.A., Yasmeen, T., Hussain, S., Arif, M.S., Abbas, F., Bharwana, S.A., Zhang, G.P., 2013c. The influence of silicon on barley growth, photosynthesis and ultra-structure under chromium stress. Ecotoxicol. Environ. Saf. 89, 66–72.
Ali, S., Zeng, F., Cai, S., Qiu, B., Zhang, G.P., 2011b. The interaction of salinity and chromium in the influence of barley growth and oxidative stress. Plant Soil Environ. 57, 153–159.
Baylis, A.D., Gragopoulou, C., Davidson, K.J., Birchall, J.D., 1994. Effects of silicon on the toxicity of aluminum to soybean. Commun. Soil Sci. Plant Anal 25, 537–546.
Beauchamp, C., Fridovich, I., 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44, 276–287.
Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem 72, 248–254.
Clemens, S., 2006. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie 88, 1707–1719.
Dhindsa, R.S., Dhindsa, P.P., Thorpe, T.A., 1981. Leaf senescence: correlated with increased level of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Exp. Bot 32, 93–101.
Dionisio-Sese, M.L., Tobita, S., 1998. Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 135, 1–9.
Dixit, V., Pandey, V., SHYAM, R., 2001. Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum*). J. Exp. Biol. 52, 1101–1109.
Epstein, E., 1994. The anomaly of silicon in plant biology. In: Proceedings of the National Academy of Sciences USA. vol. 91, pp. 11–17.
Epstein, E., 1999. Silicon. Plant Physiol. 50, 641–664.
Erdei, S., Hegedus, A., Hauptmann, G., Szalai, J., Horvath, G., 2002. Heavy metal induced physiological changes in the antioxidative response system, In:Proceedings of the Seventh Hungarian Congress on Plant Physiology. pp. 89–90.
Fargasova, A., 1994. Effect of Pb, Cd, Hg, As and Cr on germination and root growth of Sinalpis alba seeds. Bull. Environ Contam. Toxicol 52, 452–456.
Fauteux, F., Re´mus-borel, W., Menzies, J.G., Be´Langer, R.R., 2005. Silicon and plant disease resistance against pathogenic fungi. Micro Lett. 249, 1–6.
Foyer, C.H., Noctor, G., 2005b. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 17, 1866–1875.
Gong, H.J., Randall, D.P., Flowers, T.J., 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.
Gunes, A., Inal, E.G., Bagci, Coban, S., Sahin, O., 2007a. Silicon increases boron toleranceand reduces oxidative damage of wheat grown in soil with excess boron. Biol.Plan 51, 571–574.
Gunes, A., Inal, A., Bagci, E.G., Coban, S., 2007b. Silicon-mediated changes on some physiological and enzymatic parameters symptomatic of oxidative stress in barley grown in sodic-B toxic soil. J. Plant Physiol 164, 807–811.
Gupta, D.K., Nicoloso, F.T., Schetinger, M.R., Rossato, L.V., Pereira, L.B., Castro, G.Y., Srivastava, S., Tripathi, R.D., 2009. Antioxidant defense mechanism in hydroponically grown Zea mays seedlings under moderate lead stress. J. Hazard. Mater. 172, 479–484.
Hammond, K.E., Evans, D.E., Hodson, M.J., 1995. Aluminium/silicon interactions in barley (*Hordeum vulgare* L.) seedlings. Plant Soil. 173, 89–95.
Hashemi, A., Abdolzadeh, A., Sadeghipour, H.M., 2010. Beneficial effects of silicon nutrition in alleviating salinity stress in hydroponically grown canola, *Brassica napus* L., plants. Soil Sci. Plant Nutr. 56, 244–253.
Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometryof fatty acid peroxidation. Arch. Biochem. Biophys. 125, 189–198.
Hodson, M.J., Sangster, A.G., 1993. The interaction between silicon and aluminum in Sorghum bicolor L. Moench: growth analysis and X-ray microanalysis. Ann. Bot. 72, 389–400.
Hossain, M.T., Ryuji, M., Soga, K., Wakabayashi, K., Kamisaka, S., Fuji, S., Yamamoto, R., Hoson, T., 2002. Growth promotion and increase in cell wall extensibility by silicon in rice and some Poaceae seedlings. J. Plant Res. 115, 23–27.
Inal, A., Pilbeam, D.J., Gunes, A., 2009. Silicon increases tolerance to boron toxicity and reduces oxidative damage in barley. J. Plant Nutr. 32, 112–128.
Jarup, L., Akesson, A., 2009. Current status of cadmium as an environmental health problem. Toxicol. Appl. Pharm 238, 201–208.

Jiao-Jing, L., Shao-hang, L., Pei-lei, X., Xiu-juan, W., Ji-gang, B., 2009. Effects of exogenous silicon on the activities of antioxidant enzymes and lipid peroxidation in chilling-stressed cucumber leaves. Agric. Sci. Chin. 8, 1075–1086.
Krantev, A., Yordanova, R., Janda, T., Szalai, G., Popova, L., 2008. Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. J Plant Physiol. 165, 920–931.
Kaya, C., Tuna, A.L., Sonmez, O., Ince, F., Higgs, D., 2009. Mitigation effects of silicon on maize plants grown at high zinc. J. Plant Nutr. 32, 1788–1798.
Li, Y.C., Alva, A.K., Sumner, M.E., 1989. Response of cotton to aluminum in solutions with varying silicon concentrations. J.Plant Nutr. 12, 881–892.
Liang, Y., 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., Sun, W.C., Zhu, Y.G., Christie, P, 2007. Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. Environ Pollu 147, 422–428.
Liang, Y.C., Wong, J.W.C., Wei, L., 2005. Silicon-mediated enhancement of cadmium tolerance in maize (*Zea mays* L.) grown in cadmium contaminated soil. Chemosphere 58, 475–483.
Liang, Y.C., Yang, C.G., Shi, H.H., 2001. Effects of silicon on growth and mineral composition of barley grown under toxic levels of aluminum. J. Plant. Nutr. 24, 229–243.
Liu, J., Li, K., Xu, J., Zhang, Z., Ma, T., Lu, X., Yang, J., Zhu, Q., 2004. Lead toxicity, uptake and translocation in different rice cultivars. Plant Sci. 165, 793–802.
Ma, J.F., Yamaji, N., 2006. Silicon uptake and accumulation in higher plants. Trends Plant Sci. 11, 392–397.
Ma, J.F., 2004. Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. Soil Sci 50, 11–18.
Ma, J.F., Miyake, Y., Takahashi, E., 2001. Silicon as a beneficial element for crop plants. In: Datnoff, L., Snyder, G., Korndorfer, G. (Eds.), Silicon in Agriculture. Elsevier Science, New York, pp. 17–39.
Macfarlane, G.R., 2003. Chlorophyll a fluorescence as a potential biomarker of zinc stress in the grey mangrove, Avicennia marina. Bull. Environ. Contam. Toxicol 70, 90–96.
Metzner, H., Rau, H., Senger, H., 1965. untersuchungen zur synchronisierbakeit einzelner pigmentmangel-mutation von chlorella. Planta 65, 186–194.
Mishra, S., Srivastava, S., Tripathi, R.D., Govindarajan, R., Kuriakose, S.C., Prasad, M. N.V., 2006. Phytochelatin synthesis and response of antioxidants during cadmium stress in Bacopa monnieri L. Plant Physiol. Biochem 44 (1), 25–37.
Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate- specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22, 867–880.
Nawrot, T., Plusquin, M., Hogervorst, J., Roels, H.A., Celis, H., Thijs, L., Vangronsveld, J., Van Hecke, E., Staessen, J.A., 2006. Environmental exposure to cadmium and risk of cancer: a prospective population-based study. Lancet Oncol. 7, 119–126.
Nwugol, C.C., Huerta, A.J., 2008. Silicon-induced cadmium resistance in rice (*Oryza sativa*). J. Plant Nutr. Soil Sci. 171, 841–848.
Richmond, K.E., Sussman, M., 2003. Got silicon? The non-essential beneficial plant nutrient. Curr. Opinion Plant Biol 6, 268–272.
Rogalla, H., Römheld, V., 2002. Role of leaf apoplast in silicon-mediated manganese tolerance of *Cucumis sativus* L. Plant Cell Environ 25, 549–555.
Saifullah, E., Meers, Qadir, M., De. Caritat, P., Tack, F.M.G., Laing, G., Zia, M.H., 2009. EDTA-assisted Pb phytoextraction 74, 127–129Chemosphere 74, 127–129.
Sanita, D.T., Gabbrielli, R., 1999. Response to cadmium in higher plants. J. Exp. Bot. 41, 105–130.
Schützendübel, A., Polle, A., 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J. Exp. Bot. 53, 1351–1365.
Shi, QH., Bao, Z.Y., Zhu, Z.J., He, Y., Qian, Q.Q., Yu, J.Q., 2005. Silicon-mediated alleviation of Mn toxicity in Cucumis sativus in relation to activities of superoxide dismutase and ascorbate peroxidase. Phytochemistry 66, 1551–1559.
Shi, G.R., Cai, Q.S., Liu, C.F., Wu, L., 2010. Silicon alleviates cadmium toxicity in peanut plants in relation to cadmium distribution and stimulation of antioxidative enzymes. Plant Growth Regul 61, 45–52.
Song, A.L., li, Z.J., Zhang, J., Xue, G.F., Fan, F.L., A Liang, Y.C., 2009. Silicon-enhanced resistance to cadmium toxicity in *Brassica chinensis* L. is attributed to Si-suppressed cadmium uptake and transport and Si-enhanced antioxidant defense capacity. J. Hazard. Mater., 172; , pp. 74–83.
Tandy, S., Schulin, R., Nowack, B., 2006. The influence of EDDS on the uptake of heavy metals in hydroponically grown sunflowers. Chemosphere 62, 1454–1463.
VacullK, M., Lux, A., Luxovac, M., Tanimoto, E., Lichtscheidl, I., 2009. Silicon mitigates cadmium inhibitory effects in young maize plants. Ecotoxicol. Environ. Saf. 67, 52–58.
Wagner, G.J., 1993. Accumulation of cadmium in crop plants and its consequences to human health. Adv. Agro 51, 173–212.
Wahid, A., Ghani, A.F., Javed, 2008. Effect of cadmium on photosynthesis, nutrition and growth of mungbean. Agron. Sustain. Dev. 28, 273–280.
Wang, L., Wang, W., Chen, Q., Cao, W., Li, M., Zhang, F., 2000. Silicon-induced cadmium tolerance of rice seedlings. J. Plant Nutr. 23, 1397–1406.
Yang, M.G., Lin, X.Y., Yang, X.E., 1998. Impact of Cd on growth and nutrient accumulation of different plant species. Chin. J. Appl. Ecol. 19, 89–94.
Zhang, J.X., Kirham, M.B., 1994. Drought stress-induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. Plant Cell Physiol 35, 785–791.
Zhang, F.Q., Zhang, H.X., Wang, G.P., Xu, L.L., Shen, Z.G., 2009. Cadmium-induced accumulation of hydrogen peroxide in the leaf apoplast of *Phaseolus aureus* and *Vicia sativa* and the roles of different antioxidant enzymes. J. Hazard. Mater. 168, 76–84.
Zhang, H., Li, Y.H., Hu, L.Y., Wang, S.H., Zhang, F.Q., Hu, K.D., 2008. Effects of exogenous nitric oxide donor on antioxidant metabolism in wheat leaves under aluminum stress.Russ. J. Plant Physiol. 55, 469–474.