Silicon Improves Growth and Alleviates Toxicity of Cadmium in Maize Seedlings

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ABSTRACT

The protective effect of silicon against cadmium (Cd) toxicity in maize seedlings was investigated. Cd is a strong environmental pollutant that negatively affects plant growth. The seedlings were treated with Cd at 100 μM for 9 days. The application of 50, 100 and 150 mM silicon was able to alleviate Cd toxicity and improve growth parameters through some mechanisms. For example, shoot and root fresh weight increased significantly from 2.47 and 1.05 g per pot to 3.11 and 1.61 g per pot, respectively. The alleviation showed by silicon adding to Cd treatment seedlings through declining in lipid peroxidation, proline and Cd uptake and rising in chlorophyll, carotenoid, shoot and root fresh and dry weight and relative water content compared to Cd-stressed seedlings, significantly.

INTRODUCTION

Cadmium (Cd) is a non-essential element that severely inhibits plant growth. Its uptake and accumulation in plants poses a serious health threat to humans and living cells via the food chain (Shah and Dubey 1998; Stohs *et al*. 2000). Harmful effects of Cd might be explained by its ability to inactivate enzymes possibly through reaction with the SH-groups of proteins (Gouia *et al*. 2000). This highly toxic heavy metal is associated with industrial processes such as metal plating and the production of nickel-cadmium batteries, pigments, plastics, and other synthetics.

Cd treatments inhibited the net photosynthetic rate of peanut (*Arachis hypogaea*) plants due to a reduction in stomatal conductance and photosynthetic pigment content, as well as alterations in leaf structure (Shi and Cai 2008). Cd toxicity in cells disrupts the photosynthesis electron chain in PSII, decreasing enzymatic and non-enzymatic antioxidants (Kalaji and Loboda 2007). In addition, it alters enzyme structure by interaction with sulphydryl groups or by replacement of metals in metaloproteins, lipid peroxidation and membrane damage, inhibiting ATPase activity, disruption of channels and transporters, induction of reactive oxygen species (ROS), all the effects of Cd toxicity (Asada and Takashi 1987; Mohsenzadeh *et al*. 2011). Plants exposed to Cd stress show significantly variation in electron transport in both chloroplasts and mitochondria (Prasad *et al*. 2001; Shah *et al*. 2001; Zhang *et al*. 2005). The uptake of Cd ions seems to be in competition for the same transmembrane carriers with cation nutrients (Korshunova *et al*. 1999; Connolly *et al*. 2002; Bernard *et al*. 2004).

Silicon (Si) is the second most abundant element on the surface of the earth and the attempts to associate Si with metabolic or physiological activities have been inconclusive (Epstein 1994). Although Si has not been classified as an essential element, it has been shown to be beneficial for the plant growth and in alleviation of toxicity stress (Liang *et al*. 1994; Epstein 1999; Oliva *et al*. 2011; Shi *et al*. 2011).

The main objective of the present study was to investigate the effect of Cd in relation to the influence of Si on growth, some biochemical responses and uptake of maize seedlings.

MATERIALS AND METHODS

Plant materials and treatments

Seeds of *Zea mays* (var KSC.704), which is cultivated in Iran and has good growth and harvest, were obtained from the agricultural research center and surface sterilized by using 20-min incubation in 5% (w/v) sodium hypochlorite. After three washes with distilled water, seeds were germinated for 48 h at 24°C and then transferred to pots containing a mixture of sand and perlite (1/1, v/v) and irrigated with nutrient solution (as mgl^{-1}; KNO_3, 1000; $Ca(H_2PO_4)_2$, 250; $MgSO_4 \cdot 7H_2O$, 250; H_3BO_3, 2.3; $MnCl_2 \cdot 4H_2O$, 1.8; $ZnSO_4 \cdot 7H_2O$, 0.22; $CuSO_4 \cdot 5H_2O$, 0.08; H_2MoO_4, 0.02; FeEDTA, 6.92). The seedlings were grown for 6 days in a greenhouse in which growth conditions were 16 h light (maximum intensity of full sunlight was about 2000 μmol m^{-2} s^{-1}) and 8 h dark, an average minimum temperature of 18°C and an average maximum temperature of 28°C, the mean humidity of 60%. Then heavy metal treatment was carrying out during 9 days. Cadmium (CdN_2O_6) was applied in solution at concentration of 100 μM. Silicon were applied at 50, 100, 150 mM concentrations as sodium metasilicate ($Na_2SiO_3 \cdot 9H_2O$). The fully developed leaf from 14 days old seedlings was used as a source material for biochemical analysis and uptake measurement.

Lipid peroxydation measurement

Lipid peroxidation was assayed using 0.3 g of leaf tissue after homogenization in 10 ml of 0.1% (w/v) trichloroacetic acid (TCA). Then the mixture was centrifuged at 10,000 × *g* for 15 min and vortexed 1 ml of the supernatant with 4 ml of 20% (w/v) TCA containing 0.5% (w/v) 2-thiobarbituric acid (TBA). The solution was heated for 30 min at 95°C. The samples were cooled on ice for 5 min and then recentrifuged for 10 min at 10,000 × *g*. The absorbance of the samples was measured at 535 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm (Heath and Packer 1968). The level of lipid peroxidation products in roots and shoots was expressed as μmol of malondialdehyde (MDA) per g^{-1} FW. The MDA concentration was calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} (Lozano-Rodríguez 1997).

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Proline, pigments, and dry weight measurment

Free proline in 0.1 g of leaf samples were measured according to Bates (1973). Leaf chlorophyll and cartenoid were extracted by acetone and measured spectrophotometrically using Arnon's equation (Arnon 1959). For dry weight determination, samples were oven dried at 70°C for 72 h and then weighed.

Relative water content measurement

The percentage of relative water content (RWC) was calculated as:

$$RWC = (Wi − Wd)/ (Wf − Wd) \times 100$$

Variables were the fresh weight of harvested leaves, which were cut to 1-cm segments (Wi); the weight of leaf segments soaked in water at 4°C in the dark for 24 h (Wf); and dry weight of the segments baked at 80°C for 24 h (Wd).

Cadmium uptake

For determination of Cd the harvested plant samples were rinsed with deionized water, oven dried at 70°C for 48 h. The dried material was ashed at 550°C for 24 h. The ash residue was incubated with 65% HNO_3 for 4 h. Then, HNO_3 was added until a clear solution was obtained. Cd was quantified using an atomic absorption spectrophotometer (Varian, spectra AA-220 model) at 228.8 nm according to Wickliff method (1980).

Statistical analysis and computations

All experiment set-ups were randomized complete block with three replicates each. Raw data were imported to Microsoft Excel program for calculations and graphical representation. SPSS version 17.0 was used for analysis of variance and comparison of means by Duncan's multiple range test at $α \leq 0.05$.

RESULTS

Effects on MDA shoot content

Exposure of seedlings to 100 μM Cd increased 70% of MDA shoot content. Treatment of maize stressed seedling with Si at three concentrations of 50, 100, 150 mM decreased significantly ($P < 0.05$) MDA shoot content by about 16.5, 36.2, and 55.22%, respectively, in comparison with cadmium-stressed seedlings. The MDA shoot contents of seedlings without Cd treatment and with 50, 100, 150 mM Si were similar (**Fig. 1**).

Effects on proline and pigments content

Proline accumulated in Cd treated seedlings and increased to 55.2%. However, exposure of seedlings to 50, 100, 150 mM Si decreased significantly ($P < 0.05$) free proline by about 33.7, 44.8 and 50.5% as compared to Cd-stressed seedlings. The proline contents of seedlings without Cd treatment and with 50, 100, 150 mM Si were similar (**Fig. 2**).

Cd treatment reduced 41.2 and 29.5% the total chlorophyll and carotenoid content respectively, in leaves seedlings compared to untreated seedlings. It was observed that exposure to 50, 100, 150 mM Si increased significantly ($P < 0.05$) 18.3, 30, and 34.1% the total chlorophyll and 19.36, 27.23, and 30.18% the carotenoid respectively, as compared to cadmium-stressed seedlings. Pigments content of Si-treated seedlings exposed to 50, 100, 150 mM Si increased by about 17.5, 19.9, and 20.3% in chlorophyll and 25.3, 28.5, 28.7% in carotenoid in comparison to control (**Figs. 3, 4**).
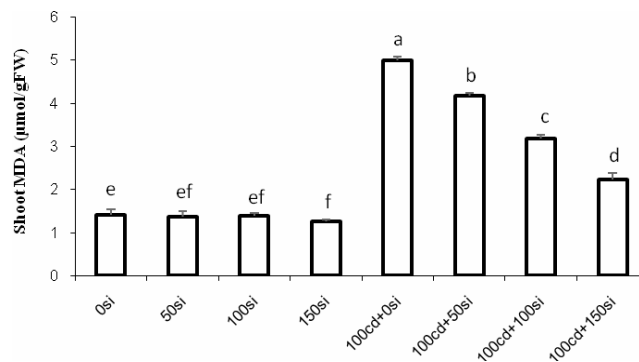

Fig. 1 Effects of Si in the presence and absence of Cd (100 μM) on maize seedlings MDA content. Values represent mean ± standard error (SE).
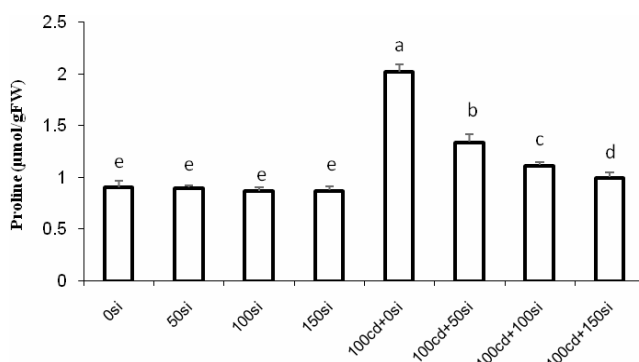

Fig. 2 Effects of Si in the presence and absence of Cd (100 μM) on maize seedlings proline content. Values represent mean ± standard error (SE).
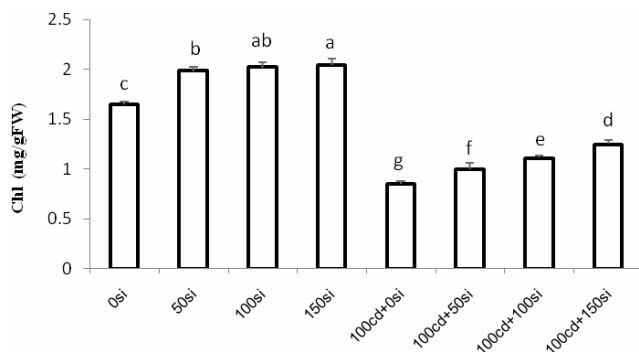

Fig. 3 Effects of Si in the presence and absence of Cd (100 μM) on maize seedlings chlorophyll content. Values represent mean ± standard error (SE).
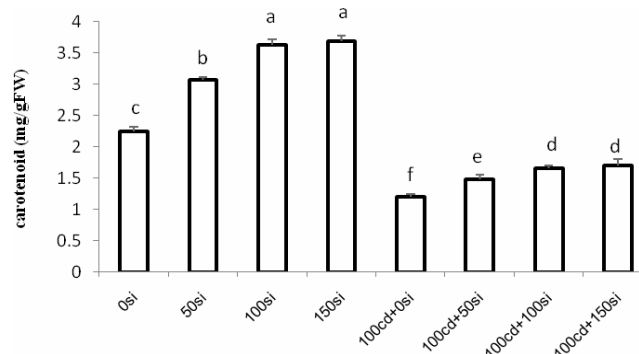

Fig. 4 Effects of Si in the presence and absence of Cd (100 μM) on maize seedlings carotenoid content. Values represent mean ± standard error (SE).

Effects on root and shoot fresh and dry weight and RWC

As **Figs. 5** to **8** shows, exposures of maize seedlings to 100 μM Cd resulted in dramatic decrease in both root and shoot fresh weight (40 and 21%, respectively) and root and shoot dry weight (44 and 28.8%, respectively), all significant at $P < 0.05$. In addition, three concentrations of Si treatment increased significantly ($P < 0.05$) the root and shoot dry and fresh weight.

Cd treatments reduced 4% of the RWC compared to the control (**Fig. 9**). However, Si treatment at concentrations of 50, 100, 150 mM increased RWC percent (1.5, 2, and 2.8%, respectively) compared to cadmium-stressed seedlings. It was observed that exposure of maize seedlings to 50, 100, 150 mM Si, significantly increased shoot and root fresh weight and RWC as compared to non-silicon treated seedlings (**Fig. 5-9**).

Effects on cadmium uptake

Cd concentration of root and shoot after 100 μM Cd treatment were 0.37 and 0.078 mg/gDW, respectively. Treatment with 50, 100, 150 mM Si decrease 42.2, 61.4 and 83.1% of Cd concentration in root, significantly ($P < 0.05$) and 56.8, 67 and 86.8% in shoot compared to non- treated seedlings (**Fig. 10**).

DISCUSSION

As the results showed, Cd influences some physiological parameters but Si can alleviate the toxicity of Cd and reduced Cd uptake in maize. These were reported by other investigations (Liang *et al*. 2005; Cunha *et al*. 2008).

Based on the MDA levels in the present study, lipid peroxidation of leaves increased with the Cd addition and then decreased after intraction with Si. An enhanced content of MDA in leaves of maize exposed to Cd indicates that the heavy metal may have caused oxidative stress and membrane damage (Lozano-Rodríguez *et al*. 1997; Cui and Wang 2006) and Si can alleviate these stress and damage.

Increasing of free proline under heavy metal stress have been reported by others (Schat *et al*. 2006; Choudhary *et al*. 2007). Proline has been attributed to up-regulation of Δ^1-pyrroline-5-carboxylate synthetase encoding gene expression (Hong *et al*. 2000) and decrease in proline consumption (Raymond and Smirnoff 2002). Proline accumulation may serve as a means of osmotic adjustment and storing carbon and nitrogen when stress leads to slower growth (Bohnert and Jensen 1996). Increase in both proline and MDA content with increasing heavy metal concentration is indicative of a correlation between free radical generation and proline accumulation (Choudhary *et al*. 2007).

The present results showed that Cd toxicity decreased the total chlorophyll and carotenoid contents of the leaves of maize seedlings as the other reports in *Brassica napus* (Larsson *et al*. 1998; Baryla *et al*. 2001), and *Azolla imbricata* (Dai *et al*. 2006). Decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis. Cd was reported to affect chlorophyll biosynthesis and inhibit protochlorophyll reductase and aminolevulinic acid synthesis (Stobart *et al*. 1985). At lower Cd concentrations, an increase in proline, protein and sugar was observed in Duckweed plants (*Lemna polyrrhiza* L.) but at higher concentrations (above 30 mg/l) their decrease was noticed (John *et al*. 2008). Cd decreased the chlorophyll content and inhibited the growth of roots and shoots of rice seedlings (Huang *et al*. 2006) and Si alleviate the toxic effects of Cd in rice (Nwugo and Huerta 2008).

This study showed that application of Si could improve the pigments and water status of Cd-stressed maize seedlings. In addition, Si enhanced Cd tolerance in maize seedlings by improvement of root and shoots dry weight and reduction in root and shoot cadmium content (Liang *et al*.
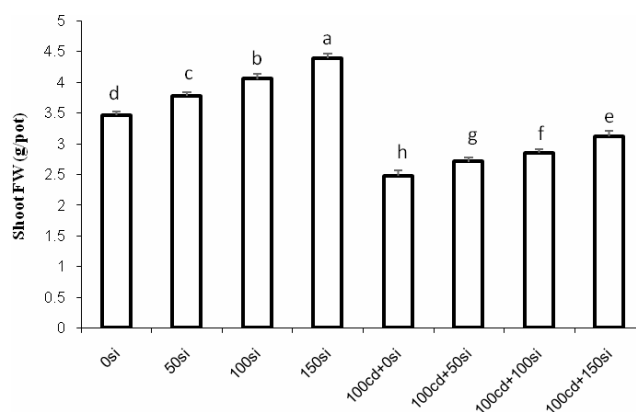
Fig. 5 Effects of Si in the presence and absence of Cd (100 μM) on maize seedlings shoot fresh weight. Values represent mean ± standard error (SE).
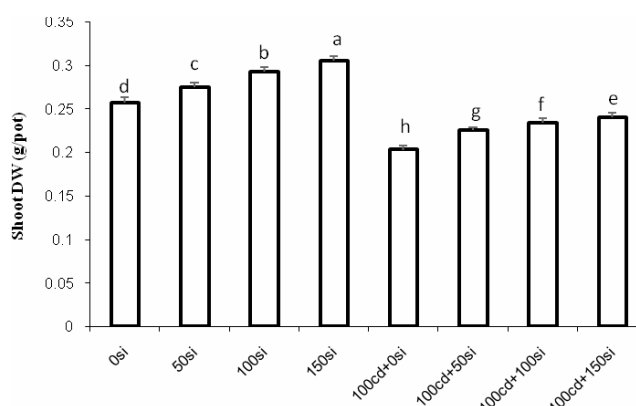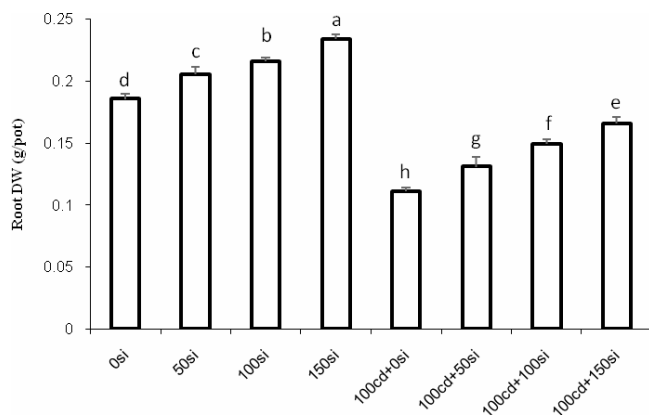

Fig. 6 Effects of Si in the presence and absence of Cd (100 μM) on maize seedlings shoot dry weight. Values represent mean ± standard error (SE).
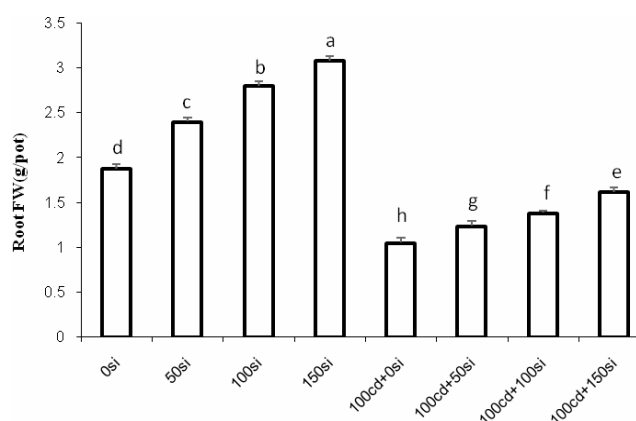
Fig. 7 Effects of Si in the presence and absence of Cd (100 μM) on maize seedlings root fresh weight. Values represent mean ± standard error (SE).

2005; Cunha *et al*. 2008). Accumulation of Cd in plant tissues can be toxic at a cellular level limiting growth and development. Prevention of Cd uptake by plant roots is, therefore, an important strategy to minimize the adverse biological effects of Cd (McLauglin *et al*. 1999). Cd stress decreased RWC% in maize leaves as in other plant (Barcelo *et al*. 1986) and Si increased RWC% in Cd treatment maize seedlings.

One reasons for Si alleviation of Cd toxicity is deposition of Si in the several parts of plant especially in roots, reduces apoplastic bypass flow and provides binding sites for metals, resulting in decreased uptake and translocation of toxic metals and salts from the roots to the shoots. The other proposed mechanism has been associated with an in-

Fig. 8 Effects of Si in the presence and absence of Cd (100 µM) on maize seedlings root dry weight. Values represent mean ± standard error (SE).
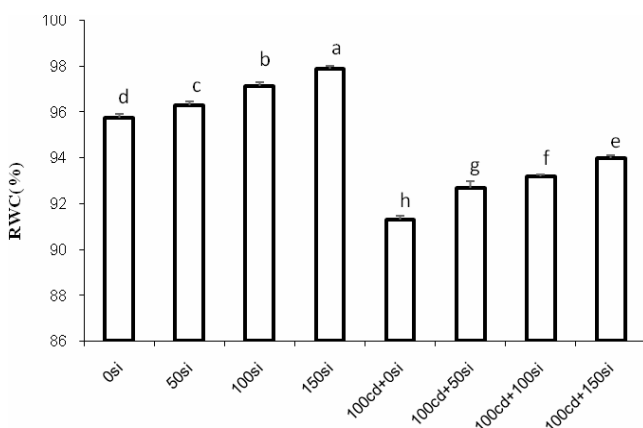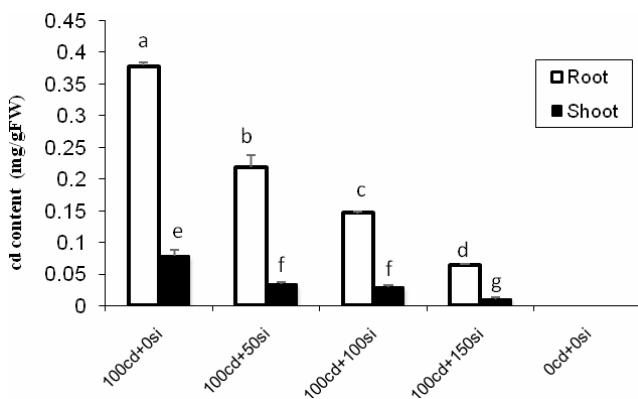

Fig. 9 Effects of Si in the presence and absence of Cd (100 µM) on maize seedlings RWC percentage. Values represent mean ± standard error (SE).

Fig. 10 Effects of Si on the cadmium content of roots and shoots of maize seedlings which were treated with 100 µM Cd. Values represent mean ± standard error (SE).

crease in antioxidant defense abilities (Liang *et al.* 2003; Zhu *et al.* 2004; Gong *et al.* 2005). In addition to the role of Si alleviation in this study, the results showed that Si has positive effects on some growth parameters. Si can improve light interception by keeping leaves erect and increase photosynthesis in rice (Ma and Yamaji 2006). It has been reported that Si promotes cell elongation but not cell division, probably because of Si-enhanced extensibility of the cell wall (Hossain *et al.* 2002). It was found that Si increased the extensibility of the cell wall in the growing zone and decreased cell-wall extensibility in the basal zone of isolated stellar tissues covered by endodermal inner tangential walls. In the roots of sorghum, implying that Si has a

role in enhancing root elongation and in protecting the stele as a mechanical barrier by hardening the cell wall of the stele and endodermal tissues (Hattori *et al.* 2003; Lux *et al.* 2003). The addition of 1 mM Si in high-Cu nutrient solutions significantly improved plant growth and reduced water loss preventing plant death related to Cu-excess (Oliva *et al.* 2011). The key mechanisms of Si-mediated alleviation of abiotic stresses in higher plants include: (1) stimulation of antioxidant systems in plants, (2) complexation or co-precipitation of toxic metal ions with Si, (3) immobilization of toxic metal ions in growth media, (4) uptake processes, and (5) compartmentation of metal ions within plants (Liang *et al.* 2007).

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