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ORIGINAL PAPER

# Silicon nutrition potentiates the antioxidant metabolism of rice plants under iron toxicity

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**Abstract** Iron toxicity reduces growth of rice plants in acidic lowlands. Silicon nutrition may alleviate many stresses including heavy metal toxicity in plants. In the present study, the ameliorating effects of silicon nutrition on rice (*Oryza sativa* L.) plants under toxic Fe levels were investigated. Plants were cultivated in greenhouse in hydroponics under different Fe treatments including 10, 50, 100, and 250 mg $L^{-1}$ as Fe-EDTA and silicon nutrition including 0 and 1.5 mM sodium silicate. Iron toxicity imposed significant reduction in plant fresh weight, tiller, and leaf number. The activity of catalase, cell wall, and soluble peroxidases, and polyphenol oxidase in shoots decreased due to moderate Fe toxicity (50 and 100 mg $L^{-1}$), but increased at greater Fe concentration. Ascorbate peroxidase activity increased in both roots and shoots of Fe-stressed plants. Iron toxicity led to increased tissue hydrogen peroxide and lipid peroxidation. Silicon nutrition improved plant growth under all Fe treatments and alleviated Fe toxicity symptoms, probably due to lower Fe concentration of Si-treated plants. Silicon application could improve the activity of antioxidant enzymes such as catalase, ascorbate peroxidase, and soluble peroxidase under moderate Fe toxicity, which resulted in greater hydrogen peroxide detoxification and declined lipid peroxidation. Thus, silicon nutrition could ameliorate harmful effects of Fe toxicity possibly through reduction of plant Fe concentration and improvement of antioxidant enzyme activity.

**Keywords** Antioxidant enzymes · Fe toxicity · Rice · Silicon

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## Introduction

Iron is the fourth most common element in the earth crust. The total Fe concentration of soil varies from 1 to 20 %, but its usual concentration in plants is only 0.005 % of their dry mass. Iron is an essential element for plants and involved in basic redox reactions of photosynthesis and respiration. It is part of the prosthetic groups of several enzymatic systems such as cytochromes, catalase (CAT), peroxidase and iron–sulfur proteins including ferredoxin, aconitase, and superoxide dismutase (SOD) (Marschner 1995; Audebert 2006). In addition, it has important roles in chloroplast development, chlorophyll biosynthesis, and unsaturation of fatty acids. Iron can be absorbed by the roots either in the form of $Fe^{2+}$ or $Fe^{3+}$. The emergence of Fe toxicity in plants is related to uptake of large amounts of $Fe^{2+}$ by roots and its transport to shoot. Free $Fe^{2+}$ accelerates the formation of reactive oxygen species (ROS) including singlet oxygen, superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical (OH·) in plant cells via Fenton reaction (Becana et al. 1998). ROS are toxic and directly correlated with the damage to lipids, proteins, and nucleic acids (Molassiotiset al. 2006; Da Silveira et al. 2007). Plants have established several protective enzymatic and non-enzymatic mechanisms to

scavenge ROS and mitigate its harmful effects. ROS-scavenging enzymes including CAT, peroxidases, ascorbate peroxidase, SOD, and glutathione reductase as well as a number of antioxidants such as glutathione, ascorbate, and carotenoids perform ROS detoxification in plants (Tiryakioglu et al. 2006).

Silicon (Si) is one of the most abundant elements in soils (Gong et al. 2006). Plants take up Si mostly as silicic acid [$Si(OH)_4$], its concentration in soils generally varies from 0.1 to 0.6 mM (Epstein 1994). The Si concentration of plant tissues varies from 1 to 10 % of the dry material depending on the plant species (Currie and Perry 2007). While Si is not generally categorized as an essential nutrient, its beneficial effects on the alleviation of abiotic and biotic stresses such as metal toxicity, salinity, drought, and temperature stresses has been documented in many plants (Liang et al. 2007; Farshidi et al. 2012; Hashemi et al. 2010). Amelioration of heavy metals like Mn, Cd, Al, and Zn toxicity following Si application has been reported and the possible mechanisms for its action has been discussed (Neumann and Zur Nieden 2001; Shi et al. 2005, 2010; Zhang et al. 2008; Doncheva et al. 2009). The possible mechanisms for mitigating heavy metal stress in plants by Si are toxic ion precipitation, reduction of its transport from roots to shoots, sequestration within plants, and the improvement of the ROS-scavenging capacity in plants (Ma 2004; Shi et al. 2005; Liang et al. 2007).

Rice (*Oryza sativa* L.) is the most important staple food for a large part of the world's population. It is cultivated in many countries in diverse ecosystems including irrigated, rain fed lowland, upland, and flood-prone area (Fageria et al. 2008). Iron toxicity is the prevalent nutritional disorder in lowland (flooded) rice and in acidic soils where increased solubility of $Fe^{2+}$ occurs under these conditions (Becker and Asch 2005). To the best of our knowledge, there is no report on the interaction of Fe toxicity and Si nutrition in rice plants and how the beneficial effects of Si in Fe—stressed rice plants (if any) is exerted. Since Fe toxicity leads to the increased oxidative stress with the resultant growth reduction of rice plants (Becana et al. 1998; Sahrawat 2004; Mehraban et al. 2008), it was of interest therefore, to evaluate the effects of silicon nutrition on these aspects of plant response to Fe toxicity. Accordingly, the present work was planned to examine the ability of Si in alleviating Fe toxicity symptoms of rice plants. In this regard, growth, changes in Fe concentration, the contents of soluble protein, chlorophyll, carotenoids, malondialdehyde (MDA), and hydrogen peroxide and the activities of some ROS-scavenging enzymes were studied in rice plants under different Fe concentration supplied with or without sodium silicate.

## Materials and methods

### Plant culture and growth conditions

Seeds of rice (*Oryza sativa* L. cv. Tarem) were surface sterilized with a 2.5 % sodium hypochlorite solution after screening for uniform size and color. They were then incubated in moistened paper towels and germinated in darkness at 25 ± 5 °C for 48 h. Healthy seedlings of uniform size (shoot length of about 3 cm) were transplanted into 10 L black plastic boxes filled with Yoshida nutrient solution (Yoshida et al. 1976). The plants were grown hydroponically in a greenhouse at the Golestan University. The experiments were performed as factorial in a completely randomized design. Factor one was Fe concentration (10, 50, 100, and 250 mg $L^{-1}$) applied to the cultivation medium as Fe-EDTA (Fluka Chemie AG, Buchs, Switzerland), and factor two was silicon nutrition, which supplied as sodium silicate solution (0 and 1.5 mmol $L^{-1}$) (Merck KGaA 64271 Darmstadt, Germany). Treatments were initiated 2 weeks after transplanting seedlings in hydroponic culture. Analytical grade materials were purchased from Sigma-Aldrich and Fluka Chemical Co. The pH of the nutrient solution after the addition of sodium silicate increased to about 7.5, which was subsequently adjusted to 6.0 by the addition of 1 N HCl and it was monitored daily during the experiment. The nutrient solution was changed weekly. The concentrations of Fe and Si in nutrient solution were quantified before seedlings transplanting and 1 week after plant cultivation (Table 1). The expected and measured concentration of Fe was almost similar in the nutrient solution before seedlings transplanting and reduced slightly due to iron absorption 1 week after plant cultivation. The measured Si concentration in nutrient solution was initially very close to the expected figure; however, it declined 1 week after plant cultivation. During the experiments, the mid-day irradiance in the greenhouse was approximately 900 µmol photons $m^{-2}$ $s^{-1}$, maximum and minimum air temperatures were 32 and 19 °C, respectively, and the mean relative humidity was 70 %. Plants were harvested 3 weeks after starting the treatments and used for the assessment of growth parameters and chemical analyses. Freshly harvested or deep-frozen samples were used for biochemical analyses. The plant Fe concentration was quantified after digesting 100 mg powder of the oven-dried tissues at 175 °C in a mixture of concentrated nitric acid and perchloric acids (3:1; V/V) using Atomic absorption spectrometer Shimadzu AA7000.

### Enzyme extraction and assay

Tissue extracts for the enzymatic assays were prepared as described by Liu and Huang (2000). Fresh leaf and root

**Table 1** Measured concentrations of both Fe and Si in the nutrient solution before seedlings transplanting and 1 week after plant cultivation

| Applied Fe concentration (mg $L^{-1}$) | Fe concentration (mg $L^{-1}$) | | Si concentration (mg $L^{-1}$) | |
|---|---|---|---|---|
| | −Si | +Si | −Si | +Si |
| 10 | | | | |
| Fresh solution | 13.87 | 12.77 | 0 | 42.24 |
| After 1 week | 12.77 | 12.06 | 0 | 19.69 |
| 50 | | | | |
| Fresh solution | 51.59 | 50.49 | 0 | 42.66 |
| After 1 week | 50.49 | 48.29 | 0 | 15.93 |
| 100 | | | | |
| Fresh solution | 98.79 | 92.36 | 0 | 38.91 |
| After 1 week | 96.03 | 91.69 | 0 | 16.59 |
| 250 | | | | |
| Fresh solution | 247.18 | 237.66 | 0 | 40.83 |
| After 1 week | 231.33 | 221.63 | 0 | 21.93 |

samples (0.05 g) were homogenized with 2 mL phosphate buffer (100 mmol $L^{-1}$, pH 6.8) and centrifuged at 17,000*g* for 15 min. The clear supernatant was used as an enzyme source of catalase, soluble peroxidase, and polyphenol oxidase. The remaining pellets were rinsed four times with the extraction buffer until no peroxidase activity could be identified. The pellet from the last centrifugation step was mixed with 1 mol $L^{-1}$ NaCl solution (1 mL) and centrifuged for 20 min at 4 °C. The supernatant containing cell wall peroxidase was saved.

The activities of peroxidase, catalase, and polyphenol oxidase were determined as described earlier (Mehraban et al. 2008; Hashemi et al. 2010). Ascorbate peroxidase activity was assayed according to Nakano and Asada (1981). The reaction mixture in a total volume of 2 mL contained, 250 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 1.2 mM hydrogen peroxide, and aliquots from the 17,000 g supernatant as the enzyme source. The hydrogen peroxide-dependent oxidation of ascorbic acid was followed by monitoring the decrease in absorbance at 290 nm assuming an extinction coefficient of 2.8 $mM^{-1}$ $cm^{-1}$.

### Other biochemical assays

For chlorophyll and carotenoid assays, 100 mg of the leaf tissues were ground with chilled 100 % acetone solution and the absorbance was measured at 470, 645, and 662 nm as described by Lichtenthaler (1987). The content of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids were then calculated. The tissue lipid peroxidation was quantified as described by Heath and Packer (1968) and expressed as the amount of MDA per gram fresh weight. The tissue hydrogen peroxide content was quantified colorimetrically as described by Jana and Choudhuri (1982).

### Statistical analyses

The experiments were planned in a completely randomized design. Each hydroponic vessel of 10 L contained nine plants in triplicate. Statistical analyses of the data were carried out using SAS statistical software (SAS Institute 2004). All data were subjected to ANOVA and a comparison of the means was carried out using the Least Significant Difference test.

## Results

### Growth parameters, Si and Fe concentrations of rice plants

Fe concentration that leads to the greatest plant biomass was found to be 10 mg $L^{-1}$ (Fig. 1). The increment of Fe concentration in the cultivation medium up to 250 mg $L^{-1}$ led to gradual growth reduction. Silicon application caused increased growth parameters in all Fe treatments (Fig. 1). In plants grown under 250 mg $L^{-1}$ Fe and treated with 1.5 mM Si, shoot, and root fresh masses and tiller and leaf numbers were greater by 54.2, 39.2, 64.7, and 57.3 %, respectively, compared to plants grown without Si.

The root and shoot Fe concentrations of plants increased significantly as Fe concentration was increased in the cultivation medium (Fig. 2). Silicon application did not affect significant changes in the Fe concentration of plants at 10 mg $L^{-1}$ Fe treatment. At greater Fe concentrations, i.e., 50 and 100 mg $L^{-1}$, the root Fe concentration was not affected by Si nutrition, however, it led to significant decrease in the shoot Fe concentration. The Fe concentration in both roots and shoots decreased markedly due to supplemental silicon under excess Fe, i.e., 250 mg $L^{-1}$ Fe treatment. A significant increase in Si concentration in both roots and shoots occurred when Si was supplied to the plants (Fig. 2). Iron treatments did not affect the Si concentration except under 250 mg $L^{-1}$ Fe treatment.

### Biochemical assays

The hydrogen peroxide contents of both roots and shoots increased significantly due to Fe toxicity (Fig. 3) and in general the hydrogen peroxide concentration of shoots was greater than roots. Supplemental Si decreased the amount of hydrogen peroxide under all Fe treatments in rice plants and it was found to be more efficient in shoots compared to roots. The hydrogen peroxide in shoots decreased by 31.7,

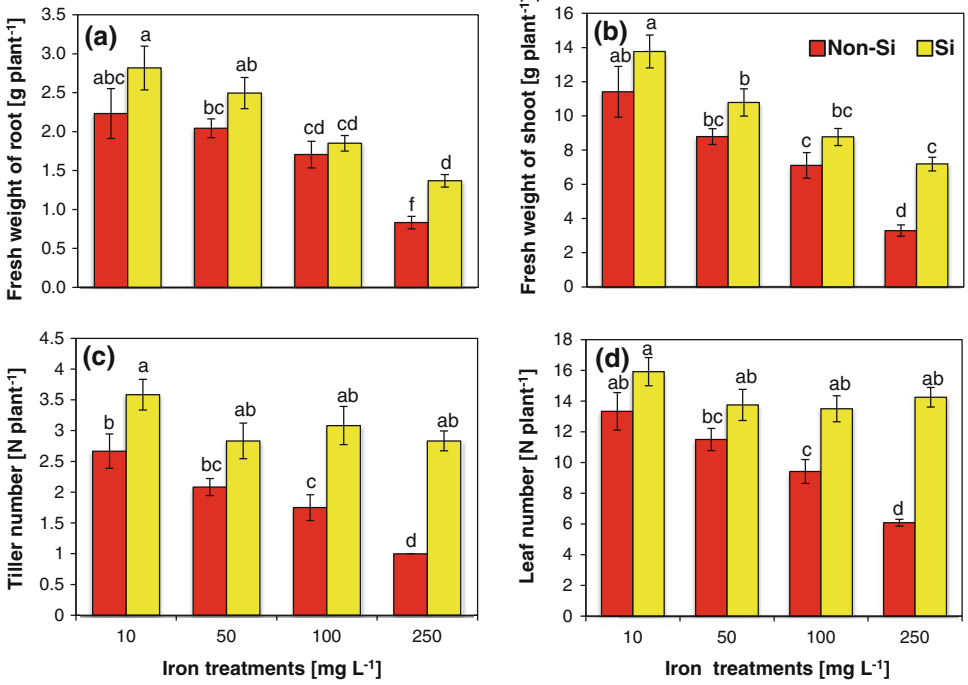


**Fig. 1** Changes in fresh weight of root (**a**) and shoot (**b**), tiller number (**c**), and leaf number (**d**) of rice plants in the presence or absence of 1.5 mM Si. The values are means of five replicates ± standard error (SE). Different *small letters* on histograms represent statistically significant differences at $P \leq 0.05$

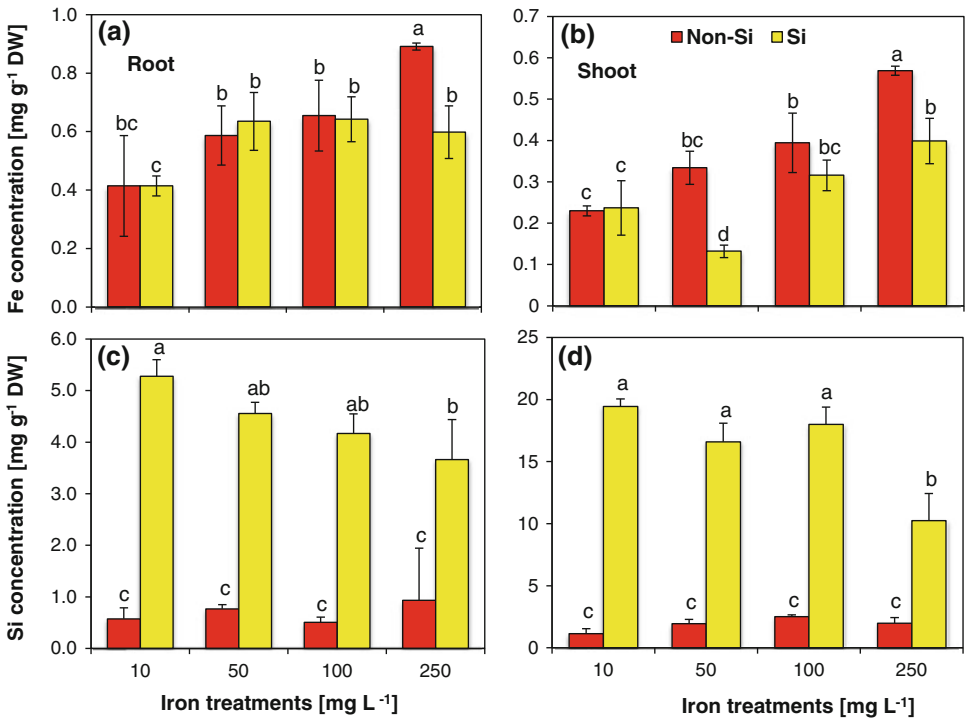


**Fig. 2** Changes in iron (**a**, **b**) and silicon (**c**, **d**) contents in root (**a**, **c**) and shoot (**b**, **d**), of rice plants in the presence or absence of 1.5 mM Si. The values are means of five replicates ± standard error (SE). Different *small letters* on histograms represent statistically significant differences at $P \leq 0.05$

36.2, 56.7, and 34.8 %, in 10, 50, 100, and 250 mg $L^{-1}$ Fe treatments, respectively, following Si application, whereas the corresponding figures for roots were 24, 15.3, 11, and 9.1 %.

Malondialdehyde has commonly been used as a proper marker of lipid peroxidation in plants. Increased Fe nutrition of roots up to 100 mg $L^{-1}$ led to significant accumulation of MDA in this organ and it remained unchanged at greater Fe supply, i.e., 250 mg $L^{-1}$ (Fig. 3). The MDA content of shoots was significantly greater than roots. The content of MDA in roots remained nearly unaffected as Fe supply increased from 10 to 250 mg $L^{-1}$, however, significant increase in the MDA content of shoots occurred at 250 mg $L^{-1}$ iron nutrition. In both root and shoot of rice plants grown under different Fe regimes, the amount of MDA was significantly lower following Si application.

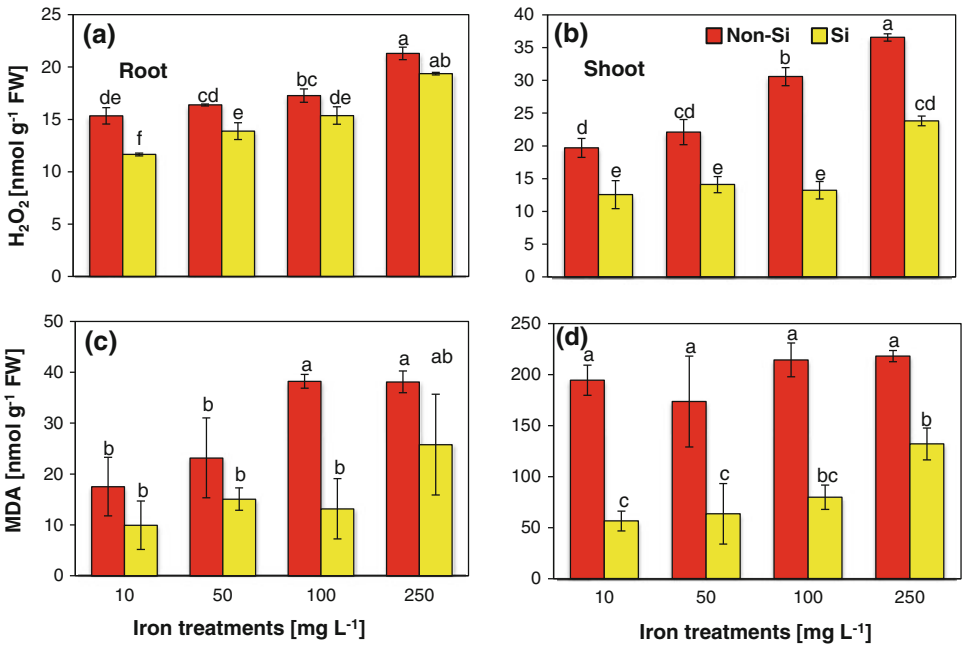


**Fig. 3** Changes in $H_2O_2$ concentrations (**a**, **b**) and lipid peroxidation (MDA) (**c**, **d**) in root (**a**, **c**) and shoot (**b**, **d**) of rice plants in the presence or absence of 1.5 mM Si. The values are means of five replicates ± standard error (SE). Different *small letters* on histograms represent statistically significant differences at $P \leq 0.05$

The concentration of chlorophyll *a* in shoot did not change by Fe treatments except at Fe concentration of 250 mg $L^{-1}$ where it caused significant reduction (Fig. 4). Si application resulted in marked increase in chlorophyll *a* in 250 mg $L^{-1}$ Fe treatment. Iron treatments >10 mg $L^{-1}$ caused significant increase of chlorophyll *b* concentration. Supplemental Si decreased chlorophyll *b* in 50 and 100 mg $L^{-1}$ Fe treatments, but it was ineffective at greater Fe concentrations. The chlorophyll *a*:chlorophyll *b* ratio decreased by increment of Fe in the cultivation medium and the supplemental Si increased this ratio to the level observed for the control plants, i.e., those grown under 10 mg $L^{-1}$ Fe. Neither Fe treatments nor Si application affected significantly carotenoids content of plants.

## Activity of some ROS scavenging enzymes

The ascorbate peroxidase activity was significantly greater in shoots than roots. It increased in roots up to 50 mg $L^{-1}$ Fe nutrition and remained unchanged thereafter. In shoots, however, the enzyme activity increased gradually as Fe concentration of the growth medium increased to 250 mg $L^{-1}$ (Fig. 5). Si application significantly enhanced the activity of this enzyme in all Fe treatments in shoots. On the contrary, no significant changes in the activity of ascorbate peroxidase occurred in roots by the added Si except at 250 mg $L^{-1}$ Fe treatment (Fig. 5).

The pattern of catalase activity was different in roots compared to shoots under Fe treatments (Fig. 5). In roots, this activity increased gradually as the Fe in the cultivation medium increased, however, in shoots the activity of this enzyme was high at 10 mg $L^{-1}$ Fe treatment, decreased markedly in moderate Fe treatments (50 and 100 mg $L^{-1}$ Fe) and increased again at 250 mg $L^{-1}$ Fe treatment (Fig. 5). Silicon application did not show any significant effects on catalase activity of roots but in shoots, it induced significant increase in the enzyme activity at 10 and 50 mg $L^{-1}$ Fe treatments (Fig. 5).

The plant soluble peroxidase activity decreased when Fe supply in the growth medium increased from 10 to 50 mg $L^{-1}$ and increased at greater Fe concentrations, i.e., 100 and 250 mg $L^{-1}$ (Fig. 5). In general, the soluble peroxidase activity of roots was greater than shoots. Silicon application increased the soluble peroxidase in all Fe treatments in both plants organs.

The activity of cell wall peroxidase increased in roots due to Fe excess in the medium and did not change significantly by Si application (Fig. 6). In shoots, maximal cell wall peroxidase activity was observed at 10 mg $L^{-1}$ Fe treatment. The enzyme activity was much lower at 50 and 100 mg $L^{-1}$ Fe treatments and increased again at 250 mg $L^{-1}$ Fe treatment to the nearly similar level found for 10 mg $L^{-1}$ Fe. Supplemental Si increased the shoot cell wall peroxidase activity under 50 and 100 mg $L^{-1}$ Fe treatments to that level observed for 10 mg $L^{-1}$ Fe.

No significant differences in polyphenol oxidase activity of roots occurred under different Fe regimes (Fig. 6). However, in shoots changes in enzyme activity under different Fe treatments were similar to that observed for peroxidase and catalase activity as these enzymes showed their greatest activities at 10 mg $L^{-1}$ Fe treatment and in extreme Fe levels (250 mg $L^{-1}$ Fe). Si application increased the polyphenol oxidase activity in 250 mg $L^{-1}$

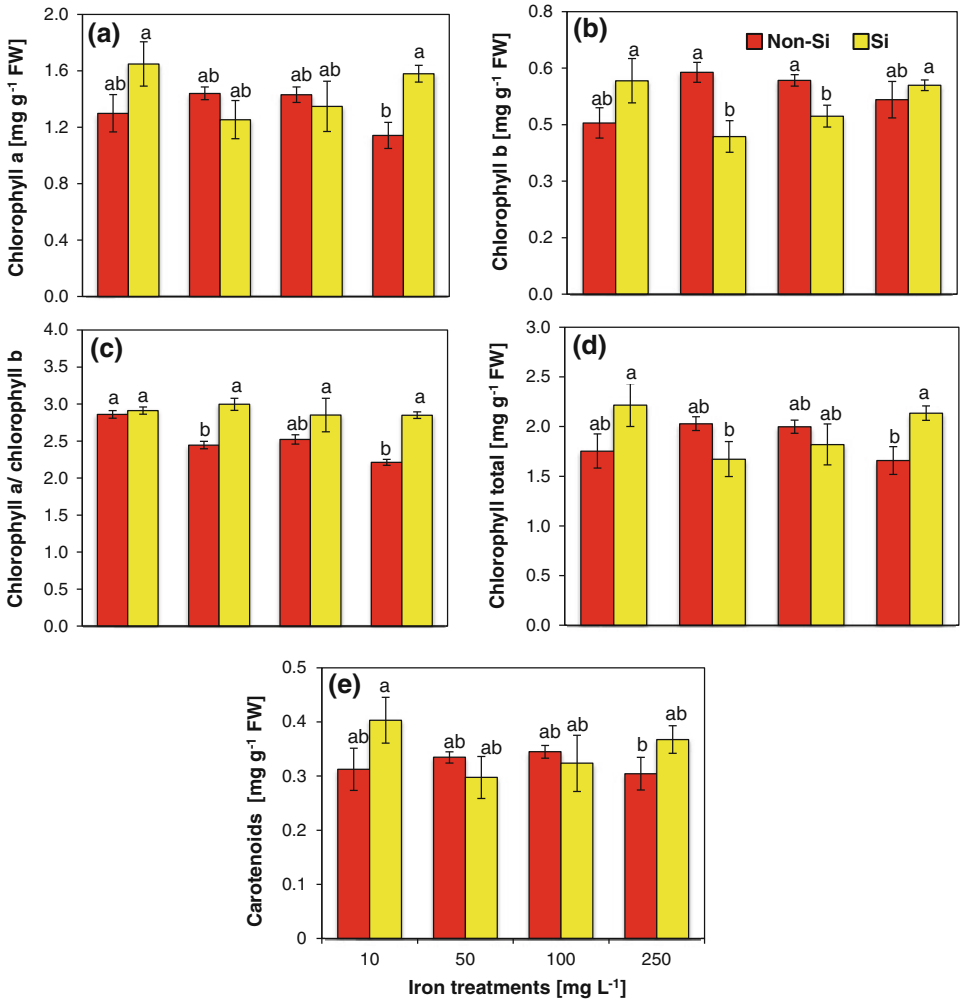


**Fig. 4** Changes in chlorophyll *a* (**a**), chlorophyll *b* (**b**), ratio of chlorophyll *a*/chlorophyll *b* (**c**), total chlorophyll (**d**), and carotenoids (**e**) of rice plants in the presence or absence of 1.5 mM Si. The values are means of five replicates ± standard error (SE). Different *small letters* on histograms represent statistically significant differences at $P \leq 0.05$

Fe treatments in roots and 50 and 100 mg $L^{-1}$ Fe treatments in shoots (Fig. 6).

## Discussion

Maximum plant biomass was occurred in 10 mg $L^{-1}$ Fe supply and greater Fe in the growth medium induced Fe toxicity as evidenced by stepwise growth retardation (Fig. 1). The iron concentration range used in this study was adopted essentially from Mehraban et al. (2008). As an essential micronutrient, Fe improves plant growth at low concentrations but at high concentration it can retard plant growth (Sahrawat 2004; Becker and Asch 2005; Dorlodot et al. 2005; Mehraban et al. 2008). Si application promoted the growth of rice plants in all Fe treatments. It appears that silicon application has mitigated the deleterious effects of excess Fe on rice plants as the Si-fed plants showed greater shoot and root fresh masses and leaf and tiller numbers (Fig. 1). The positive effect of Si application on ameliorating Fe toxicity symptoms has been reported in rice, recently (You-Qiang et al. 2012). Also, several investigators have reported the improvement of plant growth under toxic levels of Al, Mn, Cd, and boron (Neumann and Zur Nieden 2001; Shi et al. 2005, 2010; Gunes et al. 2007; Zhang et al. 2008; Doncheva et al. 2009). The Fe concentration of plants increased significantly as Fe supply increased (Fig. 2) in congruence with the former study (Mehraban et al. 2008) and the exogenously applied Si prevented Fe accumulation especially in shoot. The observed lower Fe concentration in shoots treated with 50 mg $L^{-1}$ Fe + Si was un-expected and it needs further investigation. You-Qiang et al. (2012) reported the reduced formation of iron plaque on roots of rice plants following Si application that might be associated with occupying the binding site of Fe by Si and reducing Fe precipitation on the surface of rice roots. Other mechanisms like the Si inhibition of transport of other metals in Gramineae including the thickening of endodermis, xylem, and pericycle cell walls by lignin and Si deposition might be accounted for the Si mitigation of iron toxicity (Da Cunha and Do Nascimento 2009; Vaculík et al. 2012). It has been

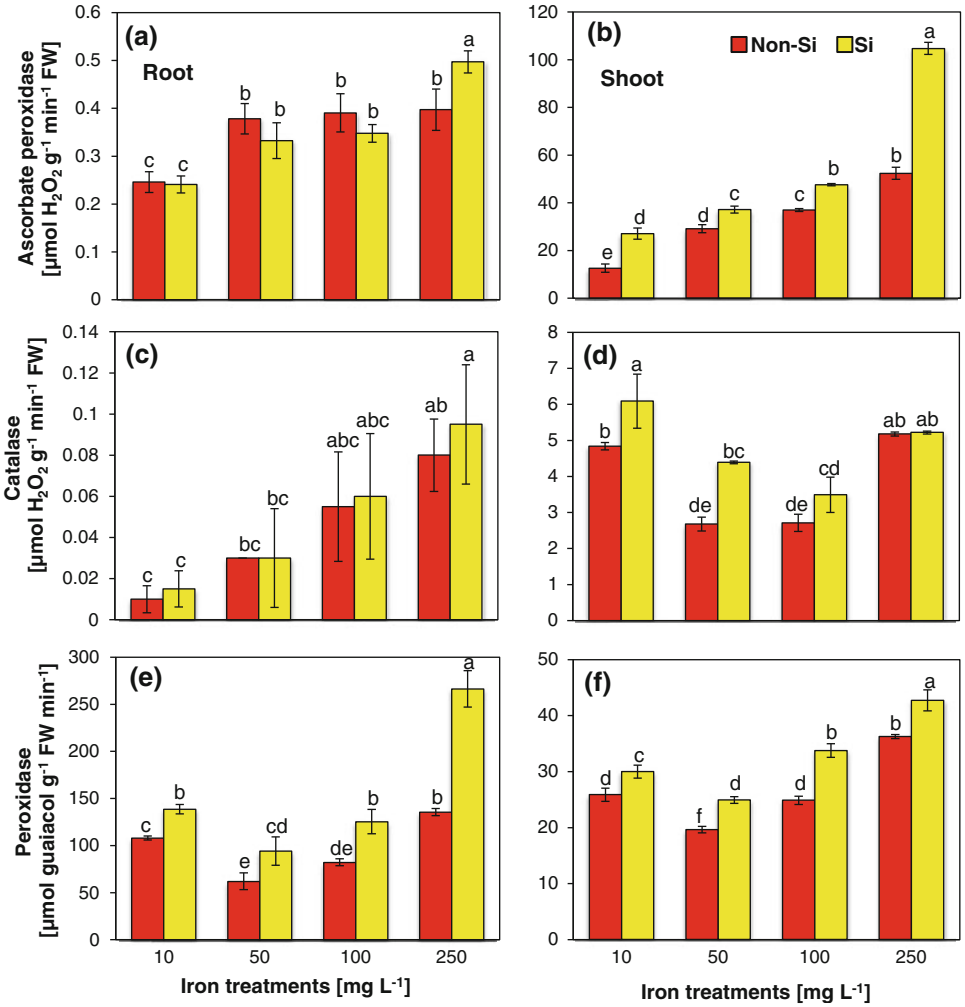


**Fig. 5** Changes in enzyme activities, ascorbate peroxidase (**a**, **b**), catalase (**b**, **c**), soluble peroxidase (**e**, **f**) in root (**a**, **c**, **e**), and shoot (**b**, **d**, **f**) of rice plants in the presence or absence of 1.5 mM Si. The values are means of five replicates ± standard error (SE). Different *small letters* on histograms represent statistically significant differences at $P \leq 0.05$

reported that heavy metals are mainly deposited in the endodermis and epidermis of roots, where Si deposition is high (Shi et al. 2005). A possible mitigation mechanism of Si during Fe stress is the reduction of Fe accumulation as reported for other heavy metals such as Mn (Maksimović et al. 2012) and Cd (Shi et al. 2005). Significant reduction of Fe concentration especially in shoot parts of rice plants grown under excess Fe (Fig. 2) might suggest that the decreased Fe accumulation in shoot is possibly mediated by restriction of Fe transport from root to shoot. The formation of colloidal Si in the growth medium, its precipitation and interaction with iron, which leads to changes in the concentration of both Si and Fe ions in our experiment was ruled out. The concentration of Si in our study was 1.5 mM (corresponding to 43 mg $L^{-1}$) while the colloidal Si formation occurs at concentrations above 2 mM (Raven 1983) and it was even further reduced 1 week after plant culture (Table 1). This reduction is mainly due to Si absorption by rice plants and to a lesser extent might be explained by its polymerization and does not appear to affect significantly plants as the $K_m$ for Si absorption in rice plants is much lower (Ma et al. 2004) than the measured concentration of Si in our nutrient solution even 1 week after plant culture. Furthermore, it has been shown that the concentrations of minor and major nutrients in the growth medium remain unaltered following Si application (Yeo et al. 1999).

Increase in the activity of ascorbate peroxidase, i.e., a ROS scavenging enzyme, occurred in both roots and shoots of rice plants as Fe concentration increased in the cultivation medium (Fig. 3). The enhancement of ascorbate peroxidase activity following short term Fe toxicity has been reported earlier (Kampfenkel and Montagu 1995; Sinha and Saxena 2006). In the ascorbate–glutathione cycle, ascorbate peroxidase reduces $H_2O_2$ using ascorbate as an electron donor. Catalase is an important antioxidant enzyme which decomposes $H_2O_2$ to water and molecular oxygen (Zhu et al. 2004). Increase of catalase activity in Fe intoxicated rice plants may reduce $H_2O_2$ content. However, a gradual increase in catalase activity accompanying Fe nutrition occurred only in roots (Fig. 5). In shoots catalase activity initially declined as Fe concentration increased in

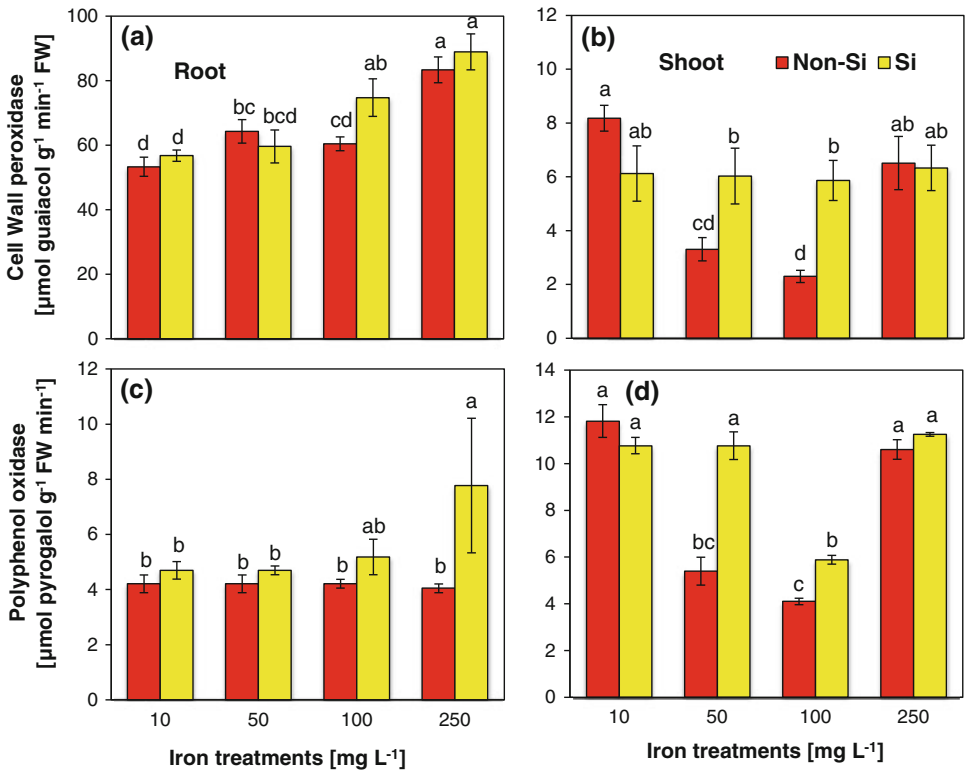


**Fig. 6** Changes in enzyme activities, cell wall peroxidase (**a**, **b**), and polyphenol oxidase enzymes activity (**b**, **c**) in root (**a**, **c**) and shoot (**b**, **d**) of rice plants in the presence or absence of 1.5 mM Si. The values are means of five replicates ± standard error (SE). Different *small letters* on histograms represent statistically significant differences at $P \leq 0.05$

the growth medium and increased only at extremely high (250 mg $L^{-1}$) Fe nutrition. Considering the fact that the highest ascorbate peroxidase and catalase activities at all Fe nutritional levels occurred in shoots and roots, respectively, of rice plants may suggest that ascorbate peroxidase is the major ROS-detoxifying enzyme in the former organ while catalase might have a similar role in the latter organ. Soluble peroxidase also catalyzes the oxidation of several organic compounds using hydrogen peroxide and the Fe toxicity-induced increment of its activity might reduce tissue $H_2O_2$ content (Ekmekci et al. 2008). The stepwise increase in hydrogen peroxide of rice plants following their exposure to excess Fe (Fig. 3) might be related to changes in cell-free radical metabolism toward the production of more ROS (Imlay 2003). Apparently, the increase in the activity of ROS scavenging enzymes would have not been sufficient in confronting the oxidative stress caused by high Fe concentrations. Thus, the content of $H_2O_2$ increased and Fe toxicity symptoms appeared in plants. The increased lipid peroxidation of rice plants especially in roots (Fig. 3) and their growth retardation under Fe toxicity might suggest dysfunction of ROS-scavenging mechanisms.

Si application significantly enhanced the activities of ascorbate peroxidase in shoot, soluble peroxidase in both root and shoot and catalase in shoot up to 100 mg $L^{-1}$ Fe treatment (Fig. 5). Peroxidases and catalase are major ROS scavenging enzymes (Hertwig et al. 1992) and increment of their activities by Si application may decrease oxidative stress cussed by Fe toxicity. Consequently, significant decrease in the $H_2O_2$ levels of Fe-stressed rice plants (Fig. 3) following Si application might have partly been related to the greater activities of these enzymes. It appears that Si application potentiates the ROS scavenging metabolic pathways under Fe toxicity. Although, there is no report on Si effect in Fe-stressed plants, decrease in the apoplastic concentration of $H_2O_2$ in cucumber plants following exposure to excess Mn have been reported by the added Si (Maksimović et al. 2012). A consequence of free oxygen radical accumulation in plant cells under stress is lipid peroxidation of unsaturated fatty acids leading to membrane damage and electrolyte leakage (Marschner 1995; Molassiotis et al. 2006). Exogenously added Si considerably decreased MDA contents in both roots and shoots under all Fe treatments (Fig. 3). Similarly, Si application could decrease lipid peroxidation in *Brassica chinensis* and peanut plants under cadmium toxicity (Song et al. 2009; Shi et al. 2010) and in wheat plants under boron toxicity (Gunes et al. 2007). Reduced lipid peroxidation of Si-fed plants under Fe toxicity might be justified by the greater activities of the above mentioned enzymes. Apparently, ROS-scavenging pathway is more efficient in Si-supplied rice plants under Fe toxicity possibly due to their lower Fe concentration.

The chlorophyll *a* to chlorophyll *b* ratio decreased by the increment of Fe in the growth medium (Fig. 4). It may impair photosynthesis; however, Si nutrition increased this

ratio to the same level observed for the control plants. Similarly, reduction in chlorophyll *a* to chlorophyll *b* ratio under drought stress (Ashraf et al. 1994) and maintenance of higher chlorophyll *a* contents in Si-fed Mn-stressed maize plants have been reported (Doncheva et al. 2009).

Except at 250 mg $L^{-1}$ Fe treatment, the activities of cell wall peroxidase and polyphenol oxidase of shoots decreased by increment of Fe in the cultivation medium; however, Si nutrition increased these activities to the level observed for the control plants grown under 10 mg $L^{-1}$ Fe (Fig. 6). The cross linking of cell wall proteins and pectins as well as the oxidation of cinnamyl alcohols for lignin and suberin formation have been attributed to the activity of wall bound peroxidases (Amaya et al. 1999; Whetten et al. 1998). The activity of polyphenol oxidase leads to the oxidation of mono- or di-phenols into o-quinone (Escobar and Shilling 2008). The resulting quinone product then interacts with amino group moieties of proteins and thus enhances the formation of black or brown pigment deposits (Vaughn and Duke 1984). The higher activities of polyphenol oxidase and cell wall peroxidase in Si-fed Fe-stressed plants possibly led to the higher polymerization of polyphenols which enhance heavy metals like Fe to be detoxified through direct chelation or trapping, thus it results to lower Fe toxicity following Si application (Lavid et al. 2001). Similarly, Vaculík et al. (2012) have reported the alleviation of Cd toxicity by Si through enhanced binding of Cd to the apoplastic fraction of maize shoots. The increased polyphenol oxidase activity of Si-supplied plants under 250 mg $L^{-1}$ Fe might have not been easily explained only by Fe and Si interactions, rather the possible effects of excess iron on the other plant physiological processes might be involved. It is noteworthy that these increased responses were also shown in roots for the two other ROS-scavenging enzymes, i.e., soluble peroxidase.

In conclusion, data reported here suggest the beneficial effects of silicon application on rice plants grown under Fe toxicity. The reduced Fe concentration of Si-fed rice plants grown under excess Fe along with the other mechanisms like improvement of ROS-scavenging capacity as evidenced by higher antioxidant activity and balancing chlorophyll a to chlorophyll b ratio might be considered as ways through which Si exerts its beneficial effects in plants under Fe toxicity.

**Author contribution** All experiments were carried out by Zahra Kiani Chalmardi. The project was planned and supervised by Ahmad Abdolzadeh. Biochemical data were interpreted by Hamid Reza Sadeghipour.

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