Effects of silicon nutrition on growth and oxidative stress related parameters in rice plants exposed to moderate Zn toxicity

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Abstract

Zinc toxicity is a growth limiting factor in rice plants, however, silicon application may mitigate heavy metal stress. The present study has investigated the effect of silicon nutrition on alleviation of Zn toxicity in rice plants. Plants were cultured hydroponically with three different Zn concentrations including 10, 100 and 150 \(\mu\)gL\(^{-1}\) Zn as zinc sulfate and two levels of Si including 0, 1.5 mM as sodium silicate. Excess Zn imposed a significant retardation in growth along with a reduction of chlorophyll, carotenoids, lignin and soluble proteins under 150 \(\mu\)gL\(^{-1}\) Zn. Also, Zn toxicity decreased the activity of catalase in shoot and soluble peroxidase in both root and shoot. Consequently, \(H_2O_2\) content and lipid peroxidation level increased under Zn stress compared with the control plants. On the contrary, Si application reduced Zn accumulation, \(H_2O_2\) content and lipid peroxidation level under 150 \(\mu\)gL\(^{-1}\) Zn. Therefore, the Si-nourished plants showed an increased amount of chlorophylls, carotenoids and soluble proteins to the same level as that of the control plants. It appears that improved growth of Zn-intoxicated rice plants following Si application is mainly due to the suppression of oxidative stress.

Keywords: Zink toxicity, Silicon nutrition, Rice, Oxidative stress and Enzymes

Introduction

Zinc (Zn) is an essential micronutrient for all plants and next to iron, the second most abundant transition metal in plants (Broadley 2006). Not participating in redox reactions, Zn metabolic functions are mediated through its ability to form ligands with O, N and especially S in proteins involved in enzymatic reactions (Story 2007, Vallee and Auld 1990, Marchner 1995). Zn is involved in many cellular processes like protein synthesis, DNA replication and transcription, auxin synthesis and breakdown and many other enzymatic activities (Marchner 1995). Although Zn is vital for plant growth and development, excess Zn in soil can be toxic to plants. Mostly, soil pollution with Zn is the result of human activities such as production and release of sewage into environment, use of waste compost as fertilizer and spread of mining activities (Kiekens 1990). Excess Zn in plants may lead to inappropriate intracellular ligand formation or its competition with other metal ions for active sites of some enzymes and carrier proteins. Zn toxicity can also produce oxidative stress due to superoxide anion and hydroxyl radicals production (Weckx and Clijsters 1997). Zn toxicity retards root and shoot elongation in rice and causes chlorosis of older leaves (Song et al., 2011). Zn phytotoxicity may also induce magnesium, iron and manganese deficiency accompanied with decreased Rubisco and PSII activities and restricts photosynthesis (Alloway 2008, Story 2007).

Silicon is an immobile and non-essential element in most plants but it is necessary for normal growth and development of some higher plants (Epstein and Bloom 2005, Ma 2004). Silicon content of soil...
may be as high as 1 to 45% but soluble Si which can be absorbed by plants has a concentration range of 0.1 to 0.6 mM (Epstein 1994, Sommer et al., 2006). The Si content of plants is substantially different, so that members of Poaceae, Equisetaceae and Cyperaceae can accumulate Si up to 4% or even more while most plant species have little Si absorption and accumulation (Ma and Takahashi 2002, Hodson et al., 2005). It is well known that metal toxicity in several species can be mitigated by Si; however, the mechanisms involved are still poorly understood. It is possible that Si mitigates metal stresses in plants due to the following mechanisms: 1- an increase in the activity of antioxidant enzymes (Shi et al., 2005, Rogalla and Romheld 2002; Kayani et al., 2012) 2- a reduction in apoplastic absorption of toxic elements resulting from Si deposition in Casparian strip of roots (Da Cunha and do Nascimento 2009, Shi et al., 2005, Kiani et al., 2012) and/or 3- a decline in cuticular transpiration due to subcuticular Si deposition that lower the uptake of metals through transpiration stream (Ma and Takahashi 2002, Ma 2004, Ma and Yamaji 2006, Mehrabanjoubani et al., 2015b). Useful effects of Si on gas exchange, chlorophyll and carotenoid contents and membrane integrity in maize under Zn toxicity have been reported (Paula et al., 2015, Kaya et al., 2009).

About two-thirds of calorie intake of two billion people in Asia comes from rice. This plant is also a major source of protein for a large population in the world. This annual plant is the most common crop in tropical and humid regions and mostly cultivated where annual precipitation is higher than 1000 mm. Rice is a Si accumulator plant in which the SiO₂ content accounts for about 10% of its dry shoot weight (Ma and Takahashi 2002). Silicon accumulation in rice is due to its ability to actively absorb Si (Takahashi et al., 1990, Menzies et al., 1992). Unlike other elements, high accumulation of Si is not toxic to rice, because silicic acid is polymerized and deposited in its cell walls (Ma and Takahashi 2002). The deposition rate and thickness of Si layers in epidermal cell walls increase as the Si supply increases in the nutrient solution (Sang 2002). Mitigation of extreme Zn toxicity (2 mM i.e. 130 mg L⁻¹) in short terms (3 and 7 days) in rice following Si application has been attributed to the improvement of antioxidant defense capacity, reduced damage to photosynthesis and increased expression of some photosynthesis-related genes (Song et al., 2011, Song et al., 2014). Also, a reduction in the absorption of excess Zn and binding of Zn in the cell wall of less bioactive tissues especially in sclerenhyma of root contributes to Si-assisted Zn tolerance of rice (Gu et al., 2011). The positive effects of Si on vegetative and reproductive growth of rice plants grown under excess Zn is also due to increased ionic homeostasis evidenced by increased K and Fe contents (Mehrabanjoubani et al., 2015a). To the best of authors knowledge, until now there is no report on the impact of Si nutrition on rice plants exposed to long terms and moderate levels of Zn toxicity. According to, growth and some ROS scavenging related parameters were studied in rice plants cultured under different concentrations of Zn from adequate to toxicity zone in the presence or absence of silicon. Our study could provide a better understanding of the damaging effects of moderate Zn toxicity in rice culture for long terms and its management to enhance plant yield through increased tolerance to Zn.

Materials and methods

Plant culture and growth conditions

Seeds of rice (Oryza sativa L. cv. fajr) were obtained from Iran Rice Research Institute. After screening for uniform size and color, they were surface sterilized with a 2.5 % sodium hypochlorite solution for 15 min, incubated in a moistened paper towel and germinated in darkness at 25 ±5° C for 48 h. Healthy seedlings of uniform size were transplanted into 10 L black plastic containers for hydroponic culture already filled with Yoshida nutrient solution (Yoshida et al., 1976). The experiments were performed as factorial in a completely randomized design with 5 replications. The first factor was Zn, applied to the root medium as ZnSO₄ (10, 100 and 150 μgL⁻¹) and the second factor was silicon nutrition supplied as sodium silicate (0 and 1.5 mM).
Treatments were started one week after transfer of seedling to the hydroponic culture. The pH of the nutrient solution was adjusted daily at 6.0 ± 0.2 and the nutrient solution was renewed weekly. The cool-white fluorescent lamps were used to create a light intensity of about 550 µmol photons m⁻² s⁻¹ under a day/night photoperiod of 16/8 h. Day and night temperatures were adjusted at 25 and 18º C, respectively, and relative humidity was at 50%-65%. Plants were harvested 4 weeks after starting the treatments and used for the assessment of growth parameters and other chemical analyses. Plant samples from either shoots or roots were weighed, oven-dried for 3 days at 70 ºC, re-weighed and ground to determine the mineral contents. Freshly harvested or deep-frozen samples were also used for the biochemical assays.

**Determination of Zn and Si contents**

Zn content of plants was determined after burning 100 mg powder of the oven-dried tissue samples at 450º C for 4 h. The obtained ashes were dissolved in 6 mol L⁻¹ HCl overnight and the acidic extract was filtered. The content of Zn in the filtrated extract was quantified using an atomic absorption spectrometer (AA-7000, Shimadzu Corporation, Japan). Si content in plant tissues was determined spectrophotometrically according to Narayanaswamy and Prakash (2009) after digestion of the plant material with H₂O₂ and NaOH in an autoclave (Elliott and Snyder 1991).

**Hydrogen peroxide and lipid peroxidation**

The tissue lipid peroxidation was measured as described by Du and Bramlage (Du et al., 1992). Fresh tissues (0.1 g) were homogenised in 3 mL 0.1% TCA (trichloroacetic acid) solution and centrifuged at 2,500 g for 10 min. The amount of malondealdyde (MDA) was assayed in the supernatant. The tissue hydrogen peroxide content was quantified colorimetrically in TCA extract, as described by Sergive et al., (1997).

**Assay of enzymatic activity**

Tissue extracts for the enzyme assays were prepared, as described by Kar and Mishra (1976). The fresh tissue (0.05 g) were homogenized with 2 ml phosphate buffer (0.1 M, pH 6.8) and centrifuged at 17000 ×g for 20 min. The clear supernatant was subsequently used as an enzyme source to assay catalase, soluble peroxidase and polyphenol oxidase. The remaining pellets were washed four times with the extraction buffer and mixed with 1 M NaCl solution. The supernatant mixture containing cell wall proteins was centrifuged and saved for the assay of cell wall bound peroxidase. Soluble and wall-bound peroxidase activities were measured using the guaiacol-peroxidase reaction. The activities of peroxidase, catalase, and polyphenol oxidase were determined as described earlier (Hashemi et al., 2010).

**Other biochemical assays**

Measurement of soluble proteins was performed according to Bradford (1976). For measurement of lignin, 0.1 g of powdered dried tissue samples was extracted with ethanolic HCl (absolute ethanol: 1 mol L⁻¹ HCl; 1:1; v/v) after pre-extracting the plant tissues three times with 50% methanol at 60ºC to remove any phenolic compounds (Zimmer 1999). The lignin content was determined spectrophotometrically at 488 nm using Phloroglucinol according to Zimmer (1999). Chlorophylls and carotenoids were assayed, as described by Lichtenthaler (1987).

**Statistical analyses**

Statistical analyses of the data were performed using SAS statistical software (SAS Institute 2003, version 9.1). All data were subjected to ANOVA and a comparison of the means was carried out using a least significant difference (LSD) test.

**Results**

**Growth and pigment contents**

The highest shoot and root fresh and dry weights were observed in 10 µgL⁻¹ Zn treatment (Table 1). Excess Zn (150 µgL⁻¹) resulted in significant reduction in root and shoot dry weights. Application of silicon led to significant increase in fresh weights of roots and shoots as well as dry weight of
shoot. The shoot/root ratios did not change due to Zn or Si treatments.

The leaf chlorophyll content declined significantly due to excess Zn in the root medium. The contents of chlorophyll a, chlorophyll b and carotenoids were 29%, 34% and 44% less, respectively, in 150 \( \mu \text{gL}^{-1} \) Zn treated plants compared with 10 \( \mu \text{gL}^{-1} \) Zn treated ones (Fig. 1). Under 150 \( \mu \text{gL}^{-1} \) Zn, Si application could recover the leaf pigment contents of plants so that the contents of chlorophyll a, chlorophyll b and carotenoids were 48%, 43% and 72% more, respectively, in the Si-fed plants compared with plants grown without Si.

Table 1. Effect of different Zn treatments on the fresh and dry weights and shoot / root ration of rice plants grown for four weeks with or without supplementary silicon as Na\(_2\)SiO\(_3\) in the nutrient solution

<table>
<thead>
<tr>
<th>Treatment</th>
<th>10 µg Zn</th>
<th>100 µg Zn</th>
<th>150 µg Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot 0 mM Si</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>3.48(^a)</td>
<td>3.86(^a)</td>
<td>2.66(^b)</td>
</tr>
<tr>
<td>Root</td>
<td>2.80(^a)</td>
<td>2.67(^a)</td>
<td>1.95(^b)</td>
</tr>
<tr>
<td>Total</td>
<td>6.28(^a)</td>
<td>6.53(^a)</td>
<td>4.61(^b)</td>
</tr>
<tr>
<td>Zn concentration (µgL(^{-1}))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root 1.5 mM Si</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>0.44(^a)</td>
<td>0.48(^a)</td>
<td>0.35(^b)</td>
</tr>
<tr>
<td>Root</td>
<td>0.18(^a)</td>
<td>0.23(^a)</td>
<td>0.11(^b)</td>
</tr>
<tr>
<td>Total</td>
<td>0.59(^ab)</td>
<td>0.71(^a)</td>
<td>0.46(^b)</td>
</tr>
<tr>
<td>Dry weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>1.25(^a)</td>
<td>1.45(^a)</td>
<td>1.47(^a)</td>
</tr>
<tr>
<td>Root</td>
<td>1.47(^a)</td>
<td>1.43(^a)</td>
<td>1.13(^b)</td>
</tr>
<tr>
<td>Shoot /root ratio</td>
<td>1.25(^a)</td>
<td>1.45(^a)</td>
<td>1.47(^a)</td>
</tr>
</tbody>
</table>

Means followed by the same letter within each row are not significantly different according to a least significant difference (LSD) test (P < 0.05).

Figure 1. Effect of different Zn treatments on the (A) chlorophyll a, (A) chlorophyll b and (C) carotenoids contents of rice plants grown for four weeks with or without supplementary silicon. Error bars represent the standard error. DW, dry weight. Different small letters on histograms represent statistically significant differences by LSD test (P < 0.05).
**Zn and Si contents**
The Zn contents of root and shoot increased significantly as Zn concentration increased in the nutrient solution (Fig. 2). Silicon application did not affect the content of Zn in plants raised under 10 mg L\(^{-1}\) Zn. However, a significant reduction in the Zn concentration in both roots and shoots was observed following application of silicon under excess Zn, i.e., at 150 mg L\(^{-1}\) Zn treatment. The Si concentration in both roots and shoots were significantly increased when Si was supplied to plants (Fig. 2). Zn treatments did not affect the Si concentration in both root and shoot.

![Figure 2. Effect of different Zn treatments on the Si and Zn contents in the (A, C) roots and (B, D) shoots of rice plants grown for four weeks with or without supplementary silicon. Error bars represent the standard error. DW, dry weight. Different small letters on histograms represent statistically significant differences by LSD test (P < 0.05).]

**Activity of antioxidant enzymes**
The greatest catalase activity in shoot was observed under 10 µgL\(^{-1}\) Zn treatment (Table 2). Irrespective of Si application, excess Zn above 10 µgL\(^{-1}\) led to a significant reduction in catalase activity (Table 2). Si application decreased catalase activity in shoot under 10 µgL\(^{-1}\) Zn treatment; however, at higher Zn concentration, the enzyme activity was not affected by Si. Root catalase activity under all Zn and Si treatments remained more or less unchanged.

The activity of soluble peroxidase was higher in roots than shoots. In shoots, the enzyme activity declined as Zn concentration increased in nutrient solution, and silicon application furthermore decreased the enzyme activity in all treatments. In roots, the greatest enzyme activity occurred at 50 µgL\(^{-1}\) Zn and further increase of Zn reduced the enzyme activity. In contrast to 10 and 100 µgL\(^{-1}\) Zn treatments, silicon increased soluble peroxidase activity under 150 µgL\(^{-1}\) Zn.

In the absence of Si, the activity of wall bound peroxidase was not affected due to Zn treatments in shoots but decreased under 100 µgL\(^{-1}\) Zn in the root (Table 2). Supplemental Si significantly increased wall bound peroxidase activity in shoots under 10 µgL\(^{-1}\) Zn only, whereas Si nutrition decreased the enzyme activity in roots under all Zn treatments.

The plant polyphenol oxidase activity, in non-Si fed plants, was highest in roots and shoots under 100 µgL\(^{-1}\) Zn treatment (Table 2). In roots, Si application led to a significant increase of polyphenol oxidase activity under 10 and 150 µgL\(^{-1}\) Zn.
Zinc toxicity is an important stress that reduces growth and yield of rice plants. Our results showed that the optimum Zn supply was 10 µg L\(^{-1}\) under Zn treatments only.

Increased Zn levels in the root medium up to 150 µg L\(^{-1}\) led to a significant and gradual accumulation of MDA in both root and shoot (Fig. 3). Similar to H\(_2\)O\(_2\), the MDA content was significantly greater in shoots than it was in roots. Si nutrition decreased the amount of hydrogen peroxide in rice shoots under all Zn treatments, whereas, it decreased hydrogen peroxide in roots under 150 µg L\(^{-1}\) Zn treatment only.

Increased Zn levels in the root medium up to 150 µg L\(^{-1}\) led to a significant and gradual accumulation of MDA in both root and shoot (Fig. 3). Similar to H\(_2\)O\(_2\), the MDA content was significantly greater in shoots than it was in roots. Si nutrition decreased the amount of hydrogen peroxide in rice shoots under all Zn treatments, whereas, it decreased hydrogen peroxide in roots under 150 µg L\(^{-1}\) Zn treatment only.

### Lipid peroxidation and H\(_2\)O\(_2\) content

The hydrogen peroxide contents of both roots and shoots increased significantly under 150 µg L\(^{-1}\) Zn compared to control (Fig. 3), and generally the H\(_2\)O\(_2\) concentration was greater in shoots than it was in roots. Si nutrition decreased the amount of hydrogen peroxide in rice shoots under all Zn treatments, whereas, it decreased hydrogen peroxide in roots under 150 µg L\(^{-1}\) Zn treatment only.

Neither Zn treatments nor Si nutrition brought about significant changes in the soluble protein content of root. At greater Zn concentrations, i.e., 100 and 150 µg L\(^{-1}\), the shoot soluble protein content was not affected by Si nutrition, however it was significantly increased in Si-fed plants under 10 µg L\(^{-1}\) Zn treatment.

### Discussion and Conclusion

Zinc is a trace element known to be an essential nutrient for plant growth and development and can be toxic when supplied in excess (Becker and Asch 2005, Song et al., 2011). Zinc toxicity is an important stress that reduces growth and yield of rice plants. Our results showed that the optimum Zn supply was 10 µg L\(^{-1}\) whereas 100 and 150 µg L\(^{-1}\) Zn treatments led to toxicity, as evidenced by significant reduction in fresh and dry weights of rice plants. Exogenous application of 1.5 mM Si improved the growth of rice under 100 and 150 µg L\(^{-1}\) Zn treatments. Under Zn-toxic condition, root and shoot fresh weights increased in Si-fed plants in comparison to those of non Si-fed ones (Table 1). Similar to our study, other studies have reported the ameliorating effects of Si on extreme but short term Zn toxicity in rice (Song et al., 2011, Song et al., 2014). In rice plants exposed to excess Zn, an improvement in

### Table 2. Enzymatic activity in the roots and shoots of rice plants grown for four weeks at different Zn treatments with or without supplementary silicon.

<table>
<thead>
<tr>
<th></th>
<th>10 µg Zn</th>
<th>100 µg Zn</th>
<th>150 µg Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Mm Si</td>
<td>1.5 Mm Si</td>
<td>0 Mm Si</td>
</tr>
<tr>
<td>Catalase (µM min(^{-1}) g(^{-1}) Fw)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>7.45(^a)</td>
<td>5.60(^b)</td>
<td>4.32(^c)</td>
</tr>
<tr>
<td>Root</td>
<td>0.05(^a)</td>
<td>0.02(^a)</td>
<td>0.06(^b)</td>
</tr>
<tr>
<td>Soluble peroxidase (µM min(^{-1}) g(^{-1}) Fw)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>44.89(^a)</td>
<td>37.14(^b)</td>
<td>38.38(^c)</td>
</tr>
<tr>
<td>Root</td>
<td>130.38(^a)</td>
<td>135.49(^b)</td>
<td>209.77(^a)</td>
</tr>
<tr>
<td>Cell wall peroxidase (µM min(^{-1}) g(^{-1}) Fw)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>5.86(^c)</td>
<td>9.42(^c)</td>
<td>5.65(^b)</td>
</tr>
<tr>
<td>Root</td>
<td>40.15(^a)</td>
<td>12.08(^b)</td>
<td>41.77(^c)</td>
</tr>
<tr>
<td>Polyphenol oxidase (µM min(^{-1}) g(^{-1}) Fw)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>0.026(^c)</td>
<td>0.030(^a)</td>
<td>0.040(^b)</td>
</tr>
<tr>
<td>Root</td>
<td>0.010(^c)</td>
<td>0.020(^a)</td>
<td>0.020(^b)</td>
</tr>
</tbody>
</table>

Means followed by the same letter within each row are not significantly different according to a least significant difference (LSD) test (P < 0.05).

### Lignin and soluble protein contents

In the absent of Si, as Zn concentration was increased the lignin contents of roots was decreased progressively (Fig. 3). Supplemenat Si increased the lignin content of both root and shoot under all Zn treatments to nearly the same extent.

Regardless of supplemental Si, the soluble protein content of shoot decreased significantly as Zn concentration was increased in the cultivation medium.
the vegetative and reproductive growth by Si has also been reported (Gu et al., 2011, Mehrabanjoubani et al., 2015a). In addition, Paula et al., (2015) and Kaya et al., (2009) have stated the beneficial effects of Si on growth of maize plants grown under Zn toxicity.

![Figure 3](image-url)

**Figure 3.** The hydrogen peroxide and malondialdehyde concentrations in the (A, C) roots and (B, D) shoots of rice plants grown for four weeks under different Zn treatments with or without supplementary silicon. Error bars represent the standard error. DW, dry weight. Different small letters on histograms represent statistically significant differences by LSD test (P < 0.05).

In congruence with the former studies (Mehrabanjoubani et al., 2015a, Song et al., 2011), as the Zn concentration was increased in the root medium, so was the Zn content in rice plants (Fig. 2). Song et al., (2011) reported a much higher concentration of Zn in roots than in shoots. In contrast, we found no significant differences between the amounts of Zn in roots and shoots. This could be related to the short term (7 days) exposure of plants to extreme Zn toxicity (2 mM Zn) in their study compared with the long term (4 weeks) exposure to moderate Zn toxicity (150 µg L⁻¹) in here. The growth reduction of rice plants under excess Zn was possibly due to a high Zn concentration of plants that may impose Zn toxicity. Binding excess Zn to inappropriate ligands or its competition with other metals in the active sites of enzymes and carrier proteins along with producing superoxide anion and hydroxyl radicals bring about development of oxidative stress in plants (Weckx and Clijsters 1997). Supplemental Si reduced the level of toxicity probably by inhibiting Zn accumulation in both root and shoot (Fig. 2). Gu et al., (2012) have reported a decreased Zn concentration in both root and shoot of rice seedlings following the application of Si. Restriction in the absorption of metals due to thickening of cell walls of endodermis, pericycle and xylem by lignin and Si deposition might be explained the Si mitigation of Zn toxicity (Da Cunha and Do Nascimento 2009, Vaculík et al., 2012). Also, the concentration of biologically active Zn decreases due to co-localization of Zn and Si in the cell wall of metabolically less active tissues, e.g., sclerenchyma of root (Gu et al., 2011).

Our results indicated that an increase in Zn stress led to the accumulation of
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hydrogen peroxide and lipid peroxidation level in rice (Fig. 1). Apparently, insufficient detoxification by antioxidant enzyme under Zn toxicity caused reactive oxygen species (ROS) and oxidative stress in root. Enhanced accumulation of various reactive oxygen species (ROS) such as hydrogen peroxide, superoxide, hydroxyl and oxygen radicals has been implicated as a major event after the exposure of plants to heavy metals (such as Zn) causing tissues injury (Song et al., 2011, Lasina et al., 2005). In fact, following Zn toxicity, an increase in various ROS leads to degradation of lipids, proteins and nucleic acids, reducing plant growth. In the case of extreme toxicity, cell death may occur (Weckx and Clijsters 1997, Molassiotis et al., 2006, Mithofer et al., 2004). Increased lipid peroxidation has been reported under the influence of heavy metals (Mithofer et al., 2004, Chen et al., 2000). Application of Si did not change the catalase activity in shoot and root or even decreased the shoot soluble peroxidase in the toxic level of Zn supply. Only the root soluble peroxidase activity under 150 mg L\(^{-1}\) was increased following Si nutrition. However, exogenous Si application reduced the amount of lipid peroxidation and hydrogen peroxide under Zn-toxic levels (Fig. 2). It seems other ROS scavenger pathways beside those studied here are involved in detoxifying ROS under excess Zn following Si application. The activity of superoxide dismutase, catalase, and ascorbate peroxidase increased in roots following Si application under Zn toxicity (Song et al., 2011). The reduced lipid peroxidation and increased antioxidant enzyme activities by Si have been reported in peanut under Cd toxicity (Shi et al., 2010), in Cucumber under Mn toxicity (Shi et al., 2005), and in spinach, potatoes and barley under B toxicity (Gunes et al., 2007, Molassiotis et al., 2006, Karabal et al., 2003).

The chlorophylls a, b and carotenoids decreased and soluble proteins declined as Zn supply increased in the root medium (Fig. 1, 3). It may have been related to oxidative stress or direct effects of excess Zn in plant tissues caused by consequent impair of photosynthesis. Song et al., (2014) reported the reduction of photosynthetic parameters, including net photosynthetic rate, and chlorophyll concentration in rice under short term and extreme Zn toxicity. Si application however, increased the amount of chlorophylls a, b and carotenoids to the same level observed in the control plants. This might be related to a reduction in Zn content or bioactive Zn in plants and reduced oxidative stress following Si application. Indeed, Si mitigates the Zn-induced damage to photosynthesis parameters including chlorophyll concentration in rice (Song et al., 2014). Similarly, preservation of higher chlorophyll a contents in Si-fed Mn-stressed maize plants have been reported (Doncheva et al., 2009).

Excess Zn in the root medium decreased the lignin content in root of rice plants (Fig. 4). The lignin contents increased in rice plants, particularly in roots following the application of Si. Gu et al., (2012) suggested the formation of Zn-Si precipitates and the change of Zn into non-phytotoxic forms in the cell wall of inactive tissues such as sclerenchyma of root. An increase in the lignin of root may contribute to the formation of Zn-Si precipitates.

In conclusion, data reported in the present study suggest the beneficial effects of supplemental silicon in rice plants grown under long term but moderate Zn toxicity. There are several reasons which may contribute to this event: the decreased Zn content in Si-supplemented rice plants grown under excess Zn accompanied with reduced oxidative as evidenced by lower levels of H\(_2\)O\(_2\), lipid peroxidation and higher pigments.
Figure 4. Changes in (A, B) lignin and (C, D) soluble proteins in root (a, c) and shoot (b, d) of rice plants grown for four weeks under different Zn treatments in the presence or absence of 1.5 mM Si. Error bars represent the standard error. DW, dry weight. Different small letters on histograms represent statistically significant differences by LSD test (P < 0.05).

References
Kayani, G., Bdlzadh, A., Sadeghipour, H.F.,


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