Title
Silicon-mediated alleviation of cadmium toxicity in roots of Brassica chinensis is mainly attributable to silicon-enhanced antioxidant defense capacity and silicon-suppressed oxidative damage

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Introduction
Cadmium (Cd) is not essential but highly toxic for higher plants even at a trace level, with the clear exception of certain hyperaccumulator plant species (Schickler and Caspi 1999).

There is an increasing body of evidence showing that Si has many direct and indirect beneficial effects on the growth of plants subjected to various forms of abiotic stress including Cd stress (Epstein, 1994; Chen et al., 2000; Liang et al., 2005). So far, numerous studies have demonstrated that Si can enhance resistance or tolerance to Al (Liang et al. 2001), Mn (Rogalla and Römheld 2002) and salt toxicity in plants (Liang et al. 1999). By contrast, less work has been done about possible roles of Si in Cd tolerance, although Chen et al. (2000) reported that application of silicon-containing steel sludge and furnace slag could decrease Cd uptake by wetland rice. More recently, we have shown that Si-mediated alleviation of Cd toxicity in pakchoi could be attributed to Si-suppressed Cd uptake and root-to-shoot transport (Song et al., 2009). However, the underlying mechanisms are still poorly understood. More importantly, studies have been focused mainly on the roles of Si in alleviating heavy metal toxicity in Si-accumulating graminaceous plant species such as rice (Zhang et al. 2008) and maize (Liang et al. 2005), while less work has been done on the possible roles of Si in dicots such as pakchoi, bean and strawberry that do not accumulate much amount of Si in their tissues (Liang et al. 2007). In this study, we show that Si was able to mitigate Cd toxicity in roots of pakchoi, with respect to antioxidant enzymes and non-enzymatic antioxidants, and histochemical characterization using two contrasting pakchoi cultivars that differ greatly in response to Cd exposure.

Materials and methods
Plant Material, growth conditions and treatments
Based on our preliminary studies, two pakchoi (Brassica chinensis L.) cultivars differing in Cd tolerance were used in this investigation: i.e. cv. Hangyoudong, a cadmium-tolerant cultivar (HYD) and cv. Shanghaiqing, a cadmium-sensitive cultivar (SHQ). Plantlets were grown hydroponically at 3 Cd levels (0, 0.5, 5.0 mg L⁻¹, which were referred to as Cd0, Cd1 and Cd2, respectively) without or with 1.5 mM Si in all possible treatment combinations by adding CdSO₄ and/or K₂SiO₃·nH₂O to the nutrient solution. Addition of K introduced by K₂SiO₃·nH₂O was subtracted from KNO₃ and the resultant nitrate loss was supplemented with dilute nitric acid. All solutions were renewed every other day. In total, there were 6 treatments with four replicates each.

Two independent experiments were performed in this study, one to study root and shoot biomass and histochemistry, and the other to study antioxidant defense activity and lipid peroxidation. The roots were harvested seven days after cadmium treatments and used for biochemical and histochemical analysis immediately.

Statistical analyses
All the experiment data presented in the paper was examined statistically by analysis of variance. Means of three replicates were subjected to the three-way analysis of variance at 0.05 probability
level using Sigmastat for Windows Version 2.03 (SPSS Inc.)

Results

Root length of pakchoi plants under Cd stress

Root length of both cultivars used was decreased in both Cd treatments (Table 1). For the Cd-sensitive cultivar (SHQ), root length decreased to 86.8 and 50.3% compared with the control in both Cd treatments, respectively. For the Cd-tolerant cultivar (HYD), root length decreased to 98.3 and 75.6% compared with the corresponding control at both Cd levels. Root length in the treatment with Cd plus Si increased by 16.6 and 9.6% in the Cd-sensitive cultivar, compared with the corresponding Cd treatment alone, and by 30.1 and 17.4% in the Cd-tolerant cultivar, compared with the corresponding Cd treatment alone.

Table 1 Effect of Si on growth of roots of pakchoi grown under different Cd levels

<table>
<thead>
<tr>
<th>Si (mM)</th>
<th>Cd mg ·L⁻¹</th>
<th>Root length (cm)</th>
<th>Mean (Cd)</th>
<th>Mean (Si)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cd-sensitive Cultivar</td>
<td>SHQ</td>
<td>Cd-tolerant Cultivar</td>
<td>HYD</td>
</tr>
<tr>
<td>0</td>
<td>11.81 ± 0.18</td>
<td>7.88 ± 0.19</td>
<td>11.17 (Cd0)</td>
<td>8.27</td>
</tr>
<tr>
<td>0.5</td>
<td>10.25 ± 0.09</td>
<td>7.75 ± 0.25</td>
<td>10.01 (Cd1)</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>5.94 ± 0.22</td>
<td>5.96 ± 0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14.04 ± 0.04</td>
<td>10.95 ± 0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>11.96 ± 0.95</td>
<td>10.08 ± 0.08</td>
<td>6.35 (Cd2)</td>
<td>10.09</td>
</tr>
<tr>
<td>5.0</td>
<td>6.51 ± 0.01</td>
<td>7.00 ± 0.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pakchoi seedlings were grown hydroponically with various levels of Cd and/or Si for seven days, and roots were then harvested to measure the root length. Data is mean ± SD of three replicates.

Lipid peroxidation in roots under Cd stress

The concentration of MDA increased in both pakchoi cultivars exposed to Cd stress compared with the control (Table 2). The MDA concentrations in the Cd1 plus Si treatment and Cd2 plus Si treatment were 78.6 and 84.1% of the corresponding Cd1 and Cd2 treatments, respectively (Table 2). Very similar changes were also observed in the Cd-tolerant cultivar (HYD) (Table 2).
Table 2 Effect of Si on MDA concentration in roots of pakchoi grown under different Cd levels

<table>
<thead>
<tr>
<th>Si (mM)</th>
<th>Cd (mg·L⁻¹)</th>
<th>Cd-sensitive Cultivar SHQ MDA concentration (nmol g⁻¹FW)</th>
<th>Cd-tolerant Cultivar HYD</th>
<th>Mean (Cd)</th>
<th>Mean (Si)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>6.66 ± 0.32</td>
<td>7.38 ± 0.15</td>
<td>6.15 (Cd0)</td>
<td>8.65</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>9.49 ± 0.28</td>
<td>7.96 ± 0.01</td>
<td>7.65 (Cd1)</td>
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</tr>
<tr>
<td></td>
<td>5.0</td>
<td>10.30 ± 0.07</td>
<td>10.09 ± 0.45</td>
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<tr>
<td></td>
<td>0</td>
<td>5.33 ± 0.47</td>
<td>5.22 ± 0.02</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>0.5</td>
<td>7.46 ± 0.12</td>
<td>5.68 ± 0.03</td>
<td>9.24 (Cd2)</td>
<td>6.71</td>
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<tr>
<td></td>
<td>5.0</td>
<td>8.66 ± 0.22</td>
<td>7.91 ± 0.12</td>
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<td></td>
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</tbody>
</table>

Mean (Cultivar) 7.98 7.37

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Df</th>
<th>P</th>
<th>LSDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar</td>
<td>1</td>
<td>&lt;0.001</td>
<td>0.17</td>
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<tr>
<td>Cd</td>
<td>2</td>
<td>&lt;0.001</td>
<td>0.20</td>
</tr>
<tr>
<td>Si</td>
<td>1</td>
<td>&lt;0.001</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Pakchoi seedlings were grown hydroponically with various levels of Cd and/or Si for seven days, and roots were then harvested to measure MDA concentration. Data is mean ± SD of three replicates.

**Oxidative damage in roots under Cd stress**

To confirm Si-mediated roles in antioxidative defense system, we performed histochemical staining experiments with Evans blue and Schiff’s reagent (Fig. 1a, b). The Evans blue was applied to determine the loss of plasma membrane integrity and the Schiff’s reagent to determine the degree of peroxidation of membrane lipids (Wang and Yang 2005). For the Cd sensitive cultivar (SHQ), the roots treated with both concentrations of Cd alone were stained to different extents, and under the higher Cd (Cd2) treatment, the roots were stained extensively. And the roots became lighter straining in the treatment with Cd plus Si compared with the Cd treatment alone. For example, root tips were more heavily stained in the Cd1 treatment than in the Cd1Si treatment. For the Cd-tolerant HYD, very similar changes were also observed in the roots (Fig. 1). Furthermore, the Si beneficial effects on the protection of cell membrane against Cd-induced oxidative damage were more significant in the Cd-tolerant plant roots than in the Cd-sensitive plant roots.
Fig. 1 Effect of the Si on the Cd-induced loss of plasma membrane integrity (a) and lipid peroxidation (b) in the root tips of pakchoi. Seedlings were treated with Cd and/or Si for seven days. Afterwards, the roots were rinsed with 0.5 mM CaCl$_2$ (pH 4.5) solution and were stained with Evans blue (a) or Schiff’s reagent (b), and immediately photographed under a light microscope. The scale bar in the graph indicates 0.5 cm. CK: treatment with neither Cd nor Si; Cd1: treatment with Cd at 0.5 mg L$^{-1}$; Cd2: treatment with Cd at 5.0 mg L$^{-1}$; Si: treatment with 1.5 mM Si; Cd1Si: treatment with 0.5 mg L$^{-1}$ Cd plus 1.5 mM Si; Cd2Si: treatment with 5.0 mg L$^{-1}$ Cd plus 1.5 mM Si.

**Antioxidative enzyme activities in roots under Cd stress**

For the Cd-sensitive cultivar (SHQ), addition of Cd significantly decreased SOD activities in roots compared with the control, which was intensified with increasing Cd concentrations ($P<0.05$) (Fig. 2a). The activity of SOD was increased by 47.3%, 12.0% and 9.6% in the plants treated with Cd plus Si compared with the corresponding Cd treatments without Si, respectively (Fig. 2a). For the Cd-tolerant cultivar (HYD), very similar changes were noted in SOD activity in the Cd treatments with or without Si added, with an exception that no significant differences in SOD were found between the Cd1 treatment alone and the control (Fig. 2a). For the sensitive cultivar (SHQ), CAT activity in the Cd treatment significantly decreased with increasing Cd concentrations compared with the control. Addition of Si significantly increased CAT activity in Cd-stressed pakchoi roots compared with Cd treatment alone throughout the whole experiment (Fig. 2b). For example, addition of Si increased CAT activities by 3.7%, 28.4% and 25.7%,
respectively, at 0, 0.5 and 5.0 mg L\(^{-1}\) Cd, compared with the corresponding Cd treatments alone. For the Cd-tolerant cultivar (HYD), very similar results were obtained of CAT activities in the Cd treatments with or without Si, with an exception that addition of Si did not result in significant differences in CAT activities between the lower and the higher Cd treatments (Fig. 2b). For the Cd-sensitive cultivar, addition of Si significantly increased APX activities in roots by 55.1% compared with the control. The activity of APX was 16.7% higher in the Cd1 plus Si treatment than in the Cd1 treatment alone, compared to 11.4% at the Cd2 level (Fig. 2c). For the Cd-tolerant cultivar, very similar changes were observed in APX activities in the Cd treatments with or without Si, with an exception that significant increases in APX activity were found between the Cd plus Si treatment and the Cd treatment alone (Fig. 2c).

**Fig. 2** Effect of Si on SOD (a), CAT (b) and APX (c) activities in pakchoi roots grown hydroponically with various levels of Cd and/or Si for seven days. Data is mean ± S.D. of three replicates. Note: P-value indicates significance level based on three-way ANOVA. *P<0.05, **P<0.01, N: not significant. CK: treatment with neither Cd nor Si; Cd1: treatment with Cd at 0.5 mg L\(^{-1}\); Cd2: treatment with Cd at 5.0 mg L\(^{-1}\); Si: treatment with 1.5 mM Si; Cd1Si: treatment with 0.5 mg L\(^{-1}\) Cd plus 1.5 mM Si; Cd2Si: treatment with 5.0 mg L\(^{-1}\) Cd plus 1.5 mM Si.
Conclusions
In conclusion, Si can alleviate Cd stress, enhance plant root elongation and attenuate toxic symptom in the root tips under Cd stress. The possible mechanisms involved may be attributed to higher antioxidant defense activity and lower lipid peroxidation, which are acquired through Si-suppressed membrane lipid peroxidation and membrane integrity injury in root apexes. In order to gain a better insight into the function of Si in mediating Cd-induced oxidative damage in plants, further research is needed to determine the roles of Si in plant biology at the molecular level through transcriptome analysis.

Acknowledgements
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References


