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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

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ABSTRACT

A series of hydroponics experiments were performed to investigate roles of silicon (Si) in enhancing cadmium (Cd) tolerance in two pakchoi (*Brassica chinensis* L.) cultivars: i.e. cv. Shanghaiqing, a Cd-sensitive cultivar, and cv. Hangyoudong, a Cd-tolerant cultivar. Plants were grown under 0.5 and 5 mg Cd L^{-1} Cd stress without or with 1.5 mM Si. Plant growth of the Cd-tolerant cultivar was stimulated at the lower Cd level, but was decreased at the higher Cd level when plants were treated with Cd for one week. However, Plant growth was severely inhibited at both Cd levels as stress duration lasted for up to three weeks. Plant growth of the Cd-sensitive cultivar was severely inhibited at both Cd levels irrespective of Cd stress duration. Addition of Si increased shoot and root biomass of both cultivars at both Cd levels and decreased Cd uptake and root-to-shoot transport. Superoxide dismutase, catalase and ascorbate peroxidase activities decreased, but malondialdehyde and H_2O_2 concentrations increased at the higher Cd level, which were counteracted by Si added. Ascorbic acid, glutathione and non-protein thiols concentrations increased at the higher Cd level, which were further intensified by addition of Si. The effects of Si and Cd on the antioxidant enzyme activity were further verified by isoenzyme analysis. Silicon was more effective in enhancing Cd tolerance in the Cd-tolerant cultivar than in the Cd-sensitive cultivar. It can be concluded that Si-enhanced Cd tolerance in *B. chinensis* is attributed mainly to Si-suppressed Cd uptake and root-to-shoot Cd transport and Si-enhanced antioxidant defense activity.

1. Introduction

Cadmium concentrations of uncontaminated soils are usually below 0.5 mg kg^{-1}, but can reach up to 3.0 mg kg^{-1} depending on the soil parent materials [1]. More recently, substantial amounts of heavy metals including cadmium have been released by geological activities or by anthropogenic impacts and industrialization such as the combustion of fossil fuels, mining, smelting activities, release of wastes and sewage water, and the use of fertilizers and pesticides [2–5]. Cadmium (Cd) is a toxic heavy metal with high mobility in the environment. It can cause serious problems to microbes, plants and animals even at trace concentrations, and can be highly toxic to human beings through its bioaccumulation in the food chain [6]. Previous studies have demonstrated that some plants such as cabbage and tobacco can detoxify Cd via gene-encoded, low-molecular-weight phytochelatins, resulting in Cd accumulation at a level higher than safety standard without visible phytotoxic symptoms [7]. Furthermore, vegetables are prone to heavy-metal exposure in some vegetable-grown soils that were amended repeatedly with organic manures, especially chicken and swine manures, and sewage sludge containing substantial amounts of heavy metals including zinc, copper and cadmium [8,9].

Cadmium can be taken up and accumulated in plants, which can disturb the metabolism in multiple ways. Many studies have indicated that excess Cd not only causes the deficiency of iron, magnesium, and calcium [10] and the reduction of chlorophyll content, but also inhibits plant growth and respiration, causes the ultrastructural damage of plant cells such as cell nucleus, chloroplast and mitochondria and alters the activity and quantity of the key enzymes of various metabolic pathways [11]. Recently, evidence has suggested that these disturbances are closely related to the accumulation of heavy metals and the excessive production of reactive oxygen species (ROS) in plants, such as superoxide radical (O_2^-), hydrogen peroxide, singlet oxygen and hydroxyl rad-

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icals (·OH), which are inevitably generated naturally via a number of cell metabolic pathways, such as photosynthesis, photorespiration, fatty acid oxidation and senescence [12]. ROS are highly reactive and can induce damage to cell structure and function, consequently leading to unbalanced nutritional status and abnormal plant growth. Moreover, ROS can also induce oxidative damage to the bio-molecules such as lipid, and protein, leading to cell membrane peroxidation and loss of ions. Plants have developed a complex antioxidative defense system, including low-molecular-weight antioxidants as well as antioxidative enzymes, such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) [13–15]. Maintaining high activities of the antioxidant enzymes and high contents of the non-enzymatic constituents is very important for plants to survive under stressful conditions such as heavy metals stress.

Although Silicon (Si) is not considered an essential element for higher plants, there is an increasing body of evidence showing that Si has multiple direct and indirect beneficial effects on plant growth and development [16–19]. Si can increase the resistance of some plant species to toxic metals [4,20]. So far, numerous studies have demonstrated that Si can enhance resistance and/or tolerance to Al [21], Mn [22] and salt toxicity in plants [16,23–25]. By contrast, less is known about the role of Si in Cd tolerance, although Chen et al. [26] found that application of silicon-containing steel sludge and furnace slag could decrease Cd uptake by rice and cabbage. However, the mechanisms behind this phenomenon are not fully understood. Liang et al. [4] reported that Si could enhance Cd tolerance in maize grown in a low pH soil experimentally contaminated with cadmium. However, few reports are available on Si-mediated Cd toxicity relating particularly to ROS metabolism and oxidative damage. More importantly, attempts have focused mainly on the roles of Si in alleviating heavy metal toxicity in graminaceous plants such as rice [27,28] and maize [4] that are known as Si-accumulators. In contrast, less work has been done on the possible roles of Si in dicots such as pakchoi, bean and strawberry that generally accumulate much less amount of Si in their shoot tissues [18].

Based on the review of the current literature, we hypothesize that Si may also ameliorate toxic effects of Cd in the non-Si-accumulating dicots including vegetable crops by enhancing growth and antioxidant defense capacity, as well as by regulating Cd uptake and distribution in *Brassica chinensis*. To test this hypothesis, we investigated the impacts of silicon on Cd detoxification with regard to antioxidative enzyme activities, isoenzymatic informs and non-enzymatic antioxidants, plant growth and Cd uptake and accumulation in two contrasting *B. chinensis* (pakchoi) cultivars that differ greatly in response to Cd exposure. Based on our preliminary studies, cultivar Hangyoudong (cadmium-tolerant, HYD) and cultivar Shanghaiqing (cadmium-sensitive, SHQ) were chosen in the present studies.

2. Materials and methods

2.1. Plant materials, growth conditions and treatments

2.1.1. Experiment A

This experiment was performed in a growth chamber (where daily photoperiod was 14 h, 8:00–22:00 with controlled temperature 25 °C/18 °C, day/night, 70% relative humidity all day, and photosynthetic photon flux density (PPFD) in the range of 400 mmol m^{-2} s^{-1}). Seeds were surface-sterilized with H_2O_2 (10%) for 30 min, followed by rinsing thoroughly with distilled water and germinated on moist filter paper for 30 h in an incubator at 25 °C. The germinated seeds were sown in plastic containers filled with quartz sand and watered with 1/2-strength Hoagland nutrient solution. The composition of the basic nutrient solution was (mg L^{-1}): $MgSO_4{\cdot}7H_2O$ 490, KNO_3 510, $Ca(NO_3)_2{\cdot}4H_2O$ 1180, KH_2PO_4 140, Fe-citrate 0.02, $MnCl_2{\cdot}4H_2O$ 1.81, $ZnSO_4{\cdot}7H_2O$ 0.22, $CuSO_4{\cdot}5H_2O$ 0.08, HBO_3 2.86, $H_2MoO_4{\cdot}4H_2O$ 0.09. The solution pH was adjusted to 5.8 with HCl or NaOH daily. Uniform twenty-five-day-old plants were transferred to 5-L plastic pots (16 plants per pot).

Plants were exposed to three concentrations of Cd (0, 0.5, 5.0 mg L^{-1}, which were referred to as CTRL, Cd1 and Cd2, respectively) without or with 1.5 mM Si by adding $CdSO_4$ and/or $K_2SiO_3{\cdot}nH_2O$ to the nutrient solution. Additional K introduced by $K_2SiO_3{\cdot}nH_2O$ was subtracted from KNO_3 and the resultant nitrate loss was supplemented with dilute nitric acid. All solutions were renewed every other day. In total, there were six treatments with each of them replicated six times. After having been treated for seven and twenty-one days, plants were harvested to measure root and shoot biomass, respectively.

2.1.2. Experiment B

This experiment was designed to study Cd and Si uptake and distribution in plant organs. The experimental design and growth conditions were the same as described in Section 2.1.1. After having grown for seven days, plants were harvested and samples were collected for determination of Si and Cd concentrations.

2.1.3. Experiment C

This experiment was designed to study the antioxidant defense activity and the corresponding isoenzyme profiles. The experimental design and growth conditions were the same as described in Section 2.1.1. After having grown for seven days, plants were harvested and samples were collected to assay antioxidant enzyme activity.

2.2. Determination of plant growth and Cd concentration

Plants of each treatment were harvested and separated into shoots and roots. The shoots were washed thoroughly with distilled water. To remove the ions in the root free space, we washed the roots with 0.5 mM $CaCl_2$ for 30 min and rinsed them thoroughly with distilled water according to Guo's method [27]. The pretreated plant tissues were oven-dried for 72 h at 70 °C, and then weighted and ground to pass a 1.0-mm sieve, and parts of plant tissues were digested in a nitric-perchloric (4:1) medium and subjected to determination of Cd concentrations using an atomic absorption spectrophotometer (Perkin-Elmer).

2.3. Determination of Si concentration

After plant samples were microwave-digested Si concentration in the digest solution was determined by the colorimetric molybdenum blue method as described by Ma et al. [29].

2.4. Antioxidative enzyme extraction and assays

Leaf fragments were ground in a potassium phosphate buffer (pH 7.8) containing 0.2 mM EDTA. The homogenate was centrifuged at 15,000 × *g* for 20 min at 4 °C, and the supernatants were stored at 4 °C for analysis of enzyme activity and soluble protein concentration. The protein concentrations in the extracts were determined by the method of Bradford [30], using bovine serum albumin (BSA, Sigma) as a standard.

SOD activity was assayed using the procedure as described by Sgherri et al. [31]. One unit SOD activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of NBT (nitro blue tetrazolium chloride) reduction. The specific enzyme activity was expressed as the enzyme unit per mg of protein.

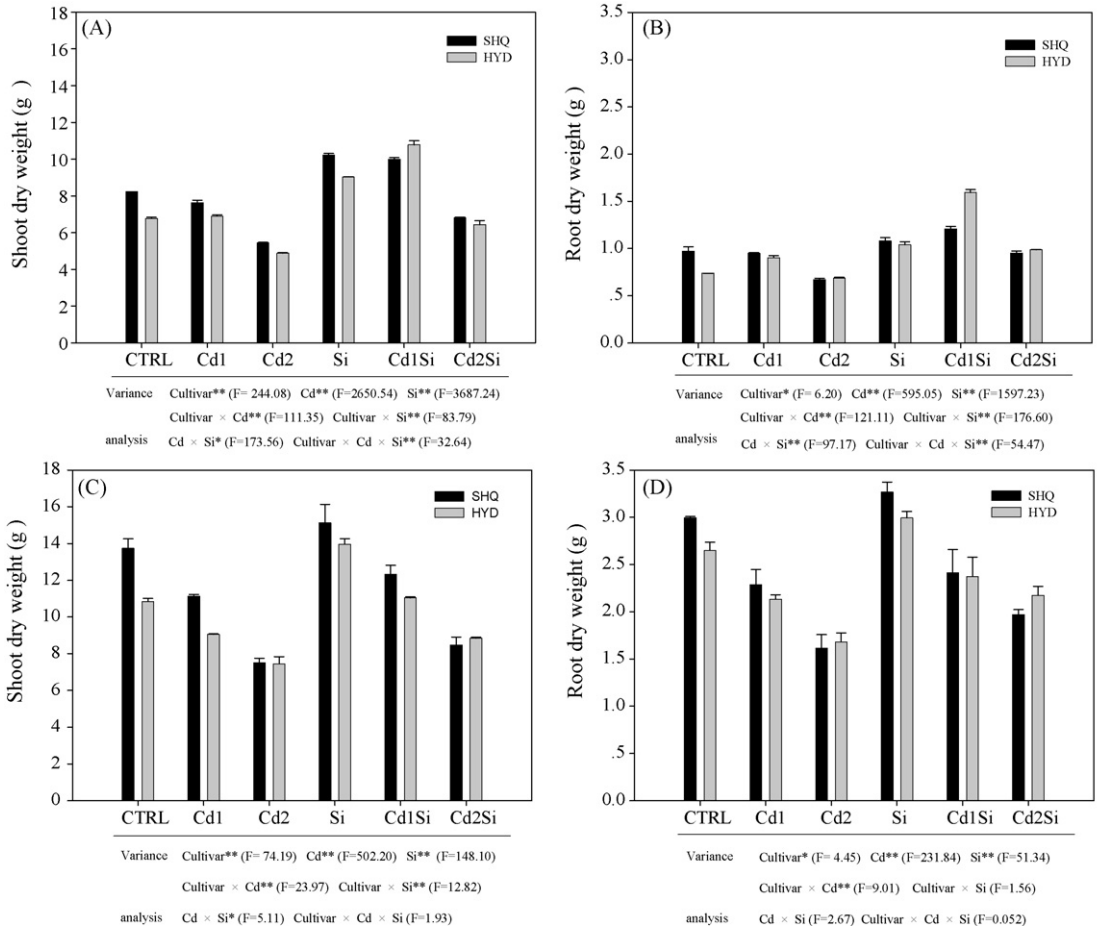

Fig. 1. Shoot and root dry weight of *B. chinensis* plants grown hydroponically with various levels of Cd with or without 1.5 mM Si for seven (A and B) or twenty-one days (C and D). Data are means ± S.D. ($n = 3$). *P*-value indicates significance level based on three-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$. CTRL: treatment with neither Cd nor Si; Cd1: treatment with 0.5 mg L^{-1} Cd; Cd2: treatment with 5.0 mg L^{-1} Cd; 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.

CAT activity was measured as the reduction in absorbance at 240 nm due to the reduction of H_2O_2, which was assayed following the method of Aebi [32]. The enzyme activity was expressed as ΔOD_{240} $min^{-1} \times g^{-1}$ protein.

APX activity was measured according to the method of Nakano and Asada [33]. The assay depended on the decrease in absorbance at 290 nm as ascorbates were oxidized. The enzyme activity was expressed as ΔOD_{290} $min^{-1} \times mg^{-1}$ protein.

2.5. Gel electrophoresis

The isoenzymes of SOD and CAT were separated on discontinuous polyacrylamide gels (PAGE) under the non-denaturing conditions. The stacking and separating gels contained 4.5% and 10% polyacrylamide, respectively. Proteins were electrophoretically separated at 4 °C and 80 V in the stacking gel followed by 120 V in the separating gel. For detection of SOD isoenzymes, the gels were soaked in a 50 mM PBS (pH 7.8) containing 1.125 mM NBT in darkness for 20 min, followed by soaking in the 36 mM PBS (pH 7.8) containing 28 mM N, N, N′, N′-tetramethylet hylenediamine (TEMED) and 28 μM riboflavin [34]. SOD activity was detected by illuminating the gel submerged in 50 mM PBS (pH 7.8) with 0.1 mM EDTA.

The gels for visualizing CAT activity were measured according to the method described by Woodbury et al. [35]. To visualize CAT activity, the gels were first incubated in 0.3% H_2O_2 for 20 min. After 1 min of gentle washing in water, they were developed in a 1% (w/v) ferricyanide and 1% ferric chloride solution (w/v) for 15 min.

2.6. Determination of malondialdehyde (MDA) and H_2O_2 concentrations

The concentration of MDA was determined according to the method of Heath and Packer [36]. Root tissues (500 mg) were homogenised in 3 mL 0.1% TCA (trichloroacetic acid) solution. The homogenate was centrifuged at 2500 *g* for 10 min and the supernatant was assayed for MDA concentration. Hydrogen peroxide (H_2O_2) levels were determined by reading the absorbance at 390 nm according to Velikova et al. [37].

2.7. Determination of glutathione (GSH), non-protein thiols (NPT) and AsA concentrations

GSH concentration was determined by reading the absorbance at 412 nm according to Guri et al. [38]. NPT concentrations were determined by reading the absorbance at 412 nm according to Metwally et al. [39]. AsA concentration was measured based on the reduction of Fe^{3+} to Fe^{2+} by AsA, and Fe^{2+} was quantified spectrophotometrically at 534 nm for 90 min at 30 °C following the method described by Law et al. [40].

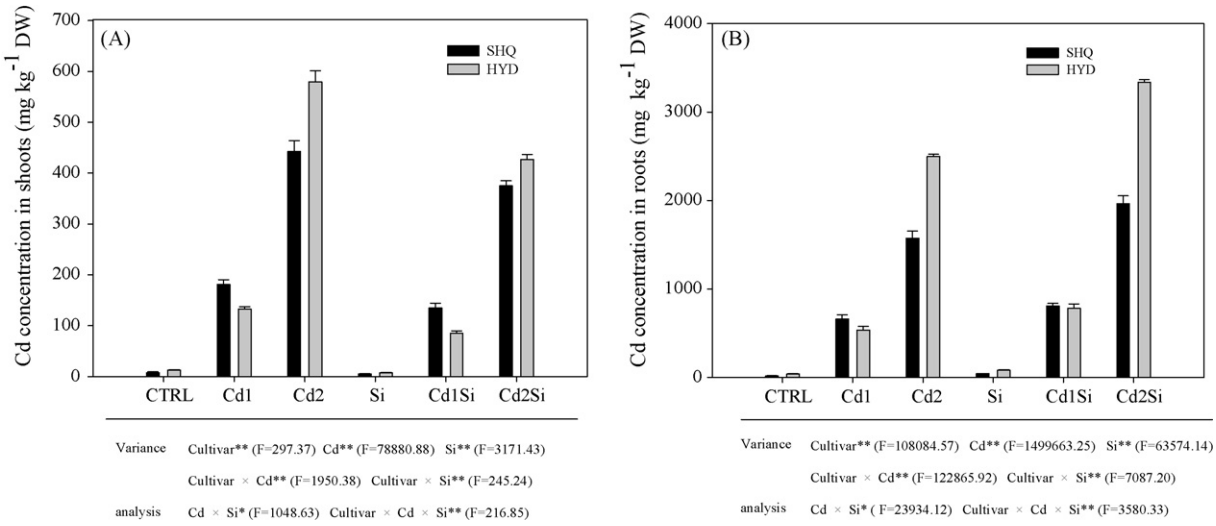

Fig. 2. Cd concentrations in shoots (A) and roots (B) of *B. chinensis* plants grown hydroponically with various levels of Cd with or without 1.5 mM Si for seven days. Data are means ± S.D. ($n = 3$). *P*-value indicates significance level based on three-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$. CTRL: treatment with neither Cd nor Si; Cd1: treatment with 0.5 mg L^{-1} Cd; Cd2: treatment with 5.0 mg L^{-1} Cd; 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.

2.8. Statistical analyses

All the experimental data presented in this paper were statistically examined by three-way analysis of variance. Statistical significance of the means of three replicates was compared at 0.05 probability level using Sigmastat for Windows Version 2.03 (SPSS Inc.).

3. Results

3.1. Plant growth

Compared with the corresponding controls, treatment with Cd1 for one week decreased shoot biomass in the Cd-sensitive pakchoi, but had no significant impact on shoot biomass in the Cd-tolerant pakchoi (Fig. 1A). The treatment with Cd1 did not significantly decrease root biomass in the Cd-sensitive pakchoi but significantly increased it in the Cd-tolerant pakchoi (Fig. 1B). Compared with the controls, treatment with Cd2 for one week reduced shoot and root biomass by 33.8% and 30.6% in the Cd-sensitive cultivar, respectively (Fig. 1A and B), compared to 45.3% and 46.1% reduction as the experiment lasted for three weeks (Fig. 1C-D). However, addition of 1.5 mM Si to Cd treatments significantly increased shoot and root biomass regardless of both cultivar and Cd level tested as compared with the corresponding Cd treatments alone. For examples, shoot and root dry weights of the Cd-sensitive plants treated with SiCd2 for one week were 24.9% and 41.1% higher than those of plants treated with Cd2 alone (Fig. 1A and B), compared to 12.8% and 22.2% increase as the experiment lasted for three weeks (Fig. 1C and D). For the Cd-tolerance cultivar HYD, very similar changes were found (Fig. 1) with an exception that stimulative effects of lower Cd (Cd1) on HYD shoots and roots growth were noted after having been treated for one week compared with the corresponding control (Fig. 1A and B), and the alleviative effect of Si on plant growth was more significant in the Cd-tolerant cultivar than in Cd-sensitive cultivar (Fig. 1).

3.2. Cadmium concentration

As shown in Fig. 2, Cd concentrations in both shoots and roots of the two cultivars increased with increasing Cd concentration in the nutrient solution. Cadmium concentration was significantly higher in roots than in shoots. Si added significantly decreased shoot Cd concentrations in both cultivars at the two levels of Cd used, which was genotype-dependent (Fig. 2A). For example, compared with the Cd treatment alone, addition of Si decreased shoot Cd concentrations by 25.4% and 15.2%, respectively, in the sensitive cultivar (SHQ), compared to 35.6% and 26.3% in the tolerant cultivar (HYD). On the contrary, addition of Si considerably increased root Cd concentrations in both cultivars, especially in the tolerant cultivar (HYD) (Fig. 2B). For example, with supply of Si, root Cd concentrations in the sensitive cultivar (SHQ) were increased by 22.1% at the lower level of Cd and by 24.7% at the higher level of Cd, compared with Cd treatments alone. However, the corresponding values for the tolerant cultivar (HYD) were 45.6% and 33.6%.

3.3. Si concentration

For the Cd-sensitive cultivar (SHQ), Si concentration in shoots and roots was 7.2 and 8.0 times as high in the Si treatment alone as in the corresponding control treatment, respectively (Table 1). Si concentration in both shoots and roots decreased with increasing Cd level from 0.5 to 5.0 mg L^{-1}. A similar tendency of Si concentration in shoots and roots as affected by Cd was found in the Cd-tolerant cultivar (HYD). However, the Si root/shoot ratio was higher in the Cd-tolerant cultivar than in the Cd-sensitive cultivar.

3.4. H_2O_2 concentration

For the Cd-sensitive cultivar (SHQ), H_2O_2 concentrations were increased by 28.7 and 59.0% at the lower and higher levels of Cd, respectively, compared with the control ($P < 0.05$) (Fig. 3). However, H_2O_2 concentration decreased with increasing Si concentrations. Addition of Si decreased H_2O_2 concentration in Cd-stressed plants by 28.0% and 27.8% at the lower and higher levels of Cd, respectively, compared with the corresponding Cd treatment alone. Very similar changes were also observed in the Cd-tolerant cultivar (HYD) (Fig. 3).

3.5. MDA concentration

For the Cd-sensitive cultivar (SHQ), increasing Cd exposure level significantly increased shoot MDA concentrations compared with the control ($P < 0.05$) (Fig. 4). The treatment with Si was effective in

Table 1
Si concentration in shoots and roots of Cd-sensitive (SHQ) and Cd-tolerant (HYD) *B. chinensis* grown hydroponically with various levels of Cd.

	Si mM	Cd mg × L^{-1}	Si mg g^{-1}	
			SHQ	HYD
Shoot				
		0	1.47 ± 0.06	1.74 ± 0.06
	0	0.5	1.38 ± 0.09	1.73 ± 0.02
		5.0	1.24 ± 0.07	1.67 ± 0.00
		0	10.50 ± 0.26	11.20 ± 0.09
	1.5	0.5	8.69 ± 0.21	8.95 ± 0.42
		5.0	5.87 ± 0.20	6.36 ± 0.46
Root				
		0	2.49 ± 0.09	3.38 ± 0.28
	0	0.5	2.04 ± 0.02	3.47 ± 0.16
		5.0	1.84 ± 0.09	3.34 ±0.08
		0	19.96 ± 0.03	22.10 ± 1.00
	1.5	0.5	17.00 ± 0.15	19.35 ± 0.17
		5.0	12.18 ± 0.18	15.72 ± 0.11

		Shoot			Root		
		df	*P*	$LSD_{0.05}$	*df*	*P*	$LSD_{0.05}$
Source of variation	Cultivar	1	<0.001	0.15	1	<0.001	0.22
	Cd	2	<0.001	0.18	2	<0.001	0.27
	Si	1	<0.001	0.15	1	<0.001	0.22
	Cultivar × Cd	2	0.548	0.26	2	0.003	0.38
	Cultivar × Si	1	0.351	0.21	1	<0.001	0.31
	Cd × Si	2	<0.001	0.26	2	<0.001	0.38
	Cultivar × Cd × Si	2	0.344	0.37	2	0.111	0.54

Pakchoi seedlings were grown hydroponically with various levels of Cd with or without 1.5 mM Si for seven days, and plants were then harvested to measure the Si concentrations of shoots and roots. Data are means ± S.D. of three replicates.

decreasing MDA concentrations. The MDA concentration in the Si treatment alone in the Cd-sensitive cultivar was 82.6% that of the control. The MDA concentration in both SiCd1 and SiCd2 treatments was 85.9% and 80.0% that of the corresponding Cd1 and Cd2 treatments, respectively. Very similar results were also observed in the Cd-tolerant cultivar (HYD) (Fig. 4). Cadmium-induced increment in MDA concentrations was lower in the Cd-tolerant cultivar (HYD) than in the Cd-sensitive cultivar (SHQ). Silicon was more effective in decreasing MDA concentrations in the tolerant plants than in the sensitive plants. For example, at Cd2 level, MDA concentration in the sensitive cultivar was 1.6-fold as high as that of the control, compared to 1.4-fold in the tolerant cultivar. In the sensitive cultivar, MDA concentrations at Cd1 and Cd2 levels were decreased by Si by 14.1% and 9.8%, respectively, compared with the corresponding Cd treatment alone. In contrast, in the tolerant cultivar, MDA concentration in the Cd1 and Cd2 treatment with Si were decreased by 21.5% and 13.0%, respectively, compared with the corresponding Cd treatment alone (Fig. 4).

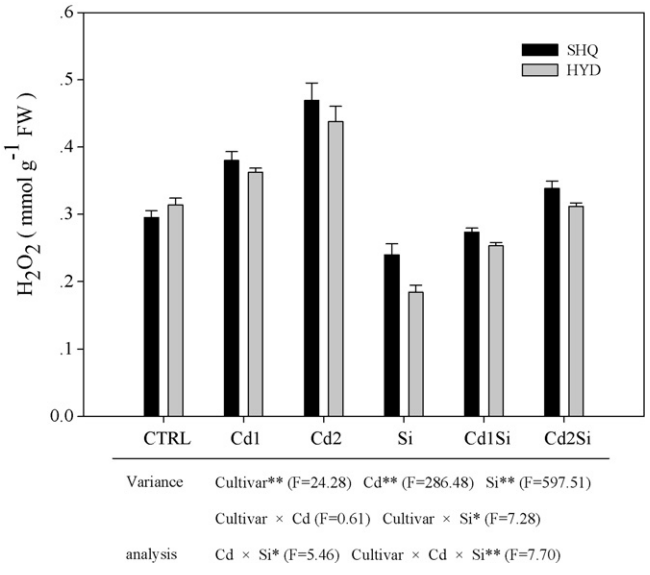

Fig. 3. H_2O_2 concentration in *B. chinensis* plants grown hydroponically with various levels of Cd with or without 1.5 mM Si for seven days. Data are means ± S.D. ($n = 3$). *P*-value indicates significance level based on three-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$. CTRL: treatment with neither Cd nor Si; Cd1: treatment with 0.5 mg L^{-1} Cd; Cd2: treatment with 5.0 mg L^{-1} Cd; 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.

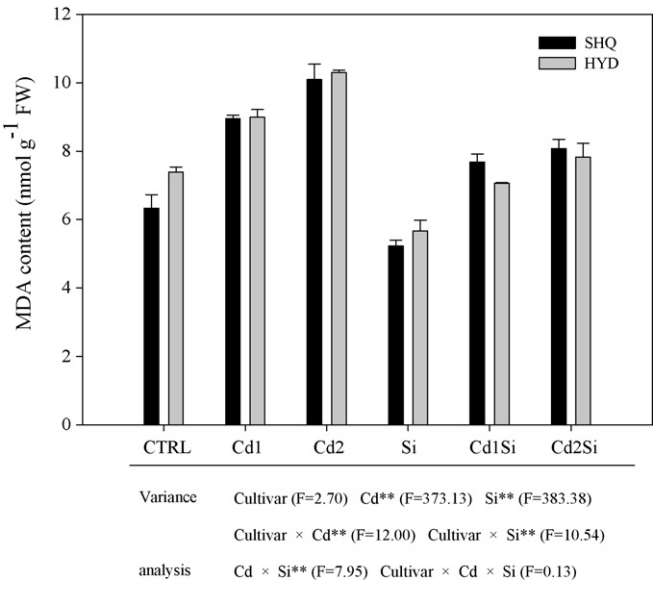

Fig. 4. MDA concentration in *B. chinensis* plants grown hydroponically with various levels of Cd with or without 1.5 mM Si for seven days. Data are means ± S.D. ($n = 3$). *P*-value indicates significance level based on three-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$. CTRL: treatment with neither Cd nor Si; Cd1: treatment with 0.5 mg L^{-1} Cd; Cd2: treatment with 5.0 mg L^{-1} Cd; 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.

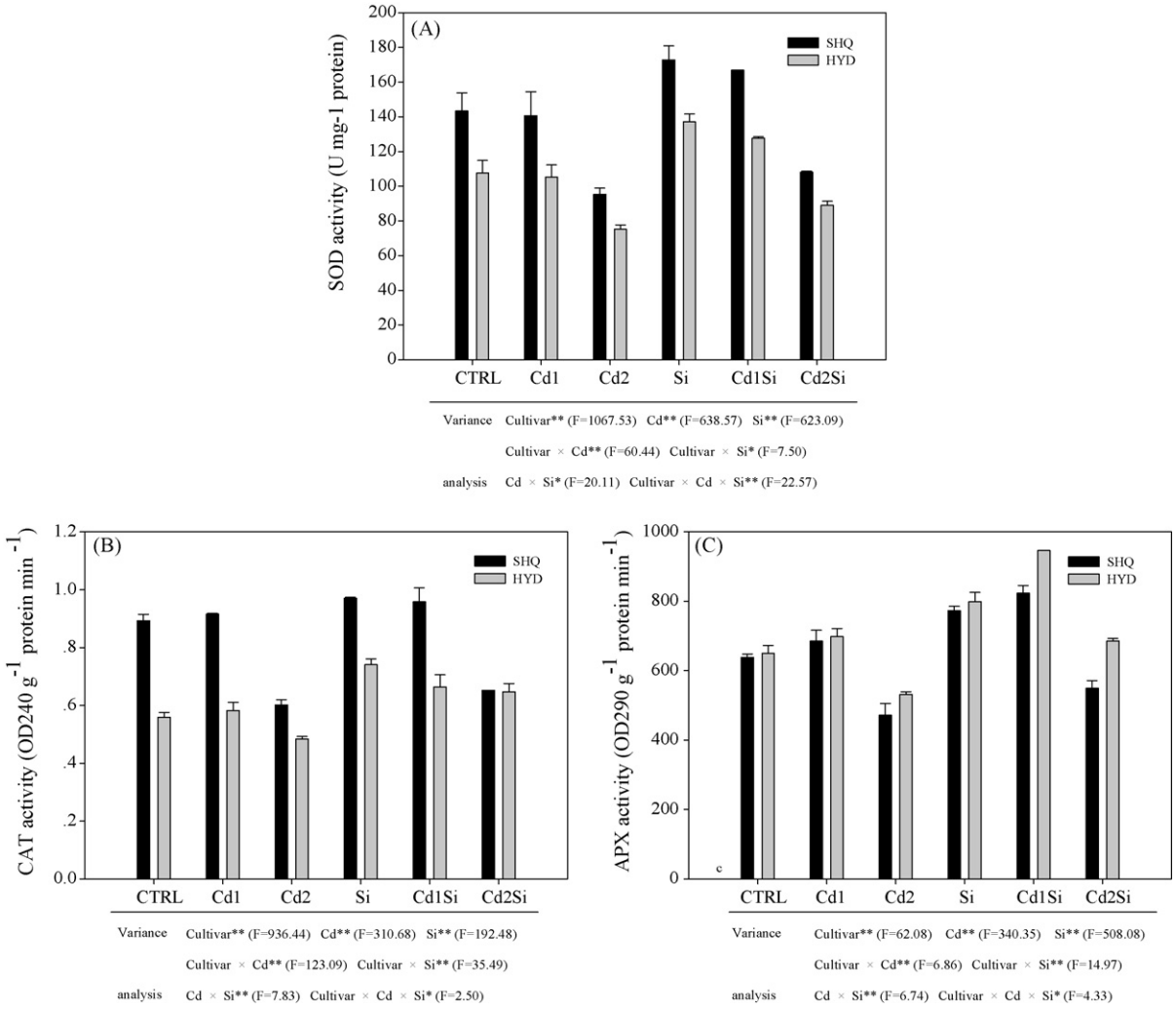

Fig. 5. SOD (A), CAT (B) and APX (C) activities in *B. chinensis* plants grown hydroponically with various levels of Cd with or without 1.5 mM Si for seven days. Data are means ± S.D. ($n = 3$). *P*-value indicates significance level based on three-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$. Control: treatment with neither Cd nor Si; Cd1: treatment with 0.5 mg L^{-1} Cd; Cd2: treatment with 5.0 mg L^{-1} Cd; 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.

3.6. SOD, CAT and APX activities

For the Cd-sensitive cultivar (SHQ), SOD activities in shoot was not significantly affected by addition of 0.5 mg L^{-1} Cd (Cd1) but was significantly decreased by addition of 1.5 mg L^{-1} Cd (Cd2) compared with the control. ($P < 0.05$) (Fig. 5A). However, SOD activity was increased by addition of Si by 20.6%, 18.6% and 13.4%, respectively, in shoots of pakchoi grown at 0, 0.5 and 5.0 mg L^{-1} Cd. For the Cd-tolerant cultivar (HYD), very similar results were noted in SOD activity in the Cd treatments or Cd plus Si treatments, with an exception that significant increase of SOD activity in shoots was found in SiCd2 treatment compared with the Cd2 alone treatment (Fig. 5A).

For the sensitive cultivar (SHQ), no significant differences in CAT activities in shoots were found between the Cd1 and control treatments (Fig. 5B). With increasing Cd concentrations, CAT activity significantly decreased in the Cd2 treatment, compared with the control. Addition of Si significantly increased CAT activity in Cd-stressed pakchoi shoots compared with Cd treatment alone throughout the whole experiment (Fig. 5B). For example, CAT activities were increased by addition of Si by 108.8%, 104.6% and 108.4%, respectively, at 0, 0.5 and 5.0 mg L^{-1} Cd, compared with the corresponding Cd treatments. For the Cd-tolerant cultivar (HYD), very similar results were obtained of CAT activities in the Cd treatment 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. 5B).

For the sensitive cultivar SHQ, addition of Si significantly increased APX activities in shoots by 21.0% compared with the control. The activity of APX was 20.1% higher in the SiCd1 treatment than in the Cd1 treatment alone, compared to 16.6% at the Cd2 level (Fig. 5C). For the Cd-tolerant cultivar (HYD), very similar changes were observed in APX activities in the Cd treatments with or without Si (Fig. 5C).

3.7. SOD and CAT isoform activities

We further analyzed SOD isoform activity profiles using non-denaturing PAGE to illuminate Si effect on SOD activity of both pakchoi plants. For the sensitive cultivar (SHQ), there were three SOD isoforms detected in leaves, but only isoenzyme SOD1 and SOD2 were substantially visualized (Fig. 6A). Treatment with lower Cd (Cd1) increased SOD1 activity, with the SOD1 band more substantially visualized, and SOD2 disappeared. In contrast, SOD1 and SOD2 appeared more clearly in the treatment with Si alone or in the treatment with Cd plus Si, compared with the corresponding Cd treatment alone (Fig. 6A). SOD3 did not change in the treatment with Cd and/or with Cd plus Si. For the Cd-

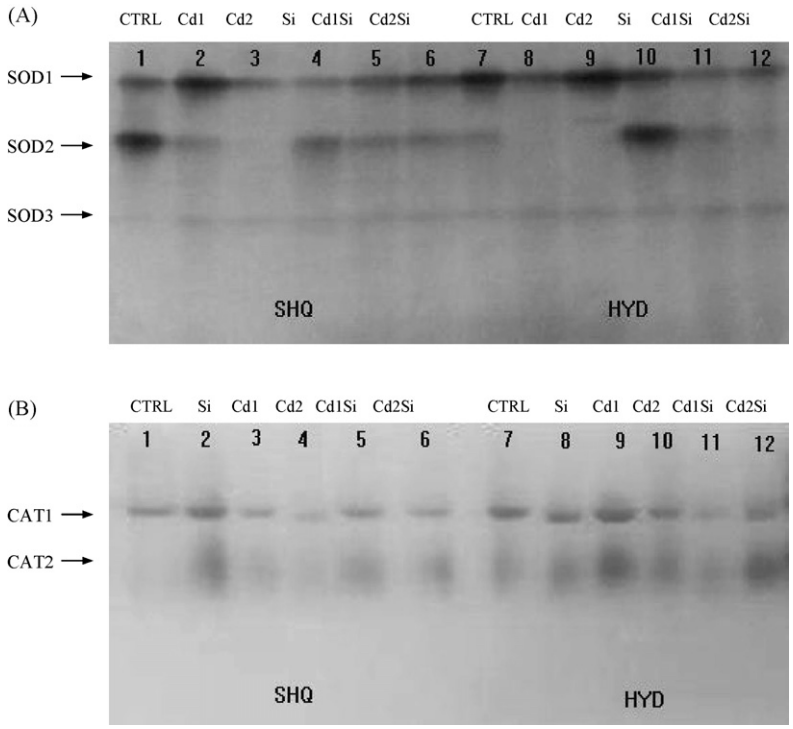

Fig. 6. SOD (A) and CAT (B) isoform activities in leaves of two *B. chinensis* plants grown hydroponically with various levels of Cd with or without 1.5 mM Si for seven days. Proteins form leaves were extracted and loaded into the native PAGE. Following the electrophoresis, the gels were stained and photographed. Lanes 1–6 represent the Cd-sensitive cultivar (SHQ), while lanes 7–12 represent the Cd-tolerant cultivar (HYD). CTRL: treatment with neither Cd nor Si; Cd1: treatment with 0.5 mg L^{-1} Cd; Cd2: treatment with 5.0 mg L^{-1} Cd; 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.

tolerant cultivar (HYD), very similar changes were noted in SOD isoforms in the Cd treatments or Cd plus Si treatments, with an exception that SOD1 was substantially visualized in the Cd2 treatment, which was in accordance with the change of total enzyme activities.

Analysis of CAT by native PAGE showed two isoforms in gels in the leaves of both cultivars (Fig. 6B). For the sensitive cultivar (SHQ), the expression of CAT1 and CAT2 was restrained with increasing Cd concentration. However, addition of Si, increased the intensity of both CAT1 and CAT2, showing that Si could increase CAT activities under Cd stress. For the tolerant cultivar (HYD), both CAT1 and CAT2 were clearly visualized in both Cd1 and Cd2 treatments. Interestingly, band CAT2 was more substantially detected in the SiCd2 treatment than in the Cd2 treatment, while CAT1 and CAT2 were less clearly visualized in the SiCd1 treatment than in the Cd1 treatment (Fig. 6B).

3.8. GSH, AsA and NPT concentrations

For the Cd-sensitive cultivar (SHQ), Cd1 and Cd2 exposures increased GSH concentration by 137.7% and 303.6%, compared with the control (Fig. 7A). Addition of Si further increased the concentration of GSH. The maximum response in the GSH concentration occurred in the SiCd2 treatment, the GSH concentration being increased by 6.8-fold compared with the control. The same was true for the Cd-tolerant cultivar (HYD) with respect to the interactive effects of Cd and Si on GSH concentration, with an exception that GSH concentration was significantly higher in the Cd1 treatment than in the control (Fig. 7A).

There were no significant differences in NPT (non-protein thiols) concentrations between the Cd1 treatment and the control in the sensitive pakchoi. Both the Si and Cd2 treatments significantly increased NPT concentrations compared with the control. The NPT concentration was significantly higher in the SiCd2 treatment than in the Cd2 treatment alone. Very similar changes for the tolerant cultivar (HYD) were also noticed with respect to NPT concentration in all the treatments (Fig. 7B). Addition of Si increased NPT concentrations, the stimulating effect of Si being much higher in the sensitive cultivar (SHQ) than in the tolerant cultivar (HYD) (Fig. 7B). For example, NPT concentration in the SiCd2 treatment was 3.5-fold as high as that of control treatment in the tolerant pakchoi, compared to 1.7-fold in the sensitive pakchoi.

The same changes as the NPT concentrations were observed for the AsA concentrations in different Cd treatments in the sensitive cultivar (SHQ). Significant differences were induced by Si treatment. For example, AsA concentrations in the SiCd1 and SiCd2 treatments increased by 10.5% and 24.4%, respectively, compared with the corresponding Cd treatment. The same was true for the Cd-tolerant cultivar (HYD) (Fig. 7C). However, Si was more effective in increasing AsA concentrations in the Cd-tolerant pakchoi than in the Cd-sensitive pakchoi under Cd stress. For example, AsA concentrations in the SiCd1 and SiCd2 treatments were 1.4 and 1.5 times as high as those of their corresponding Cd treatment in the Cd-tolerant pakchoi, compared to 1.1 and 1.2 times in the Cd-sensitive pakchoi (Fig. 7C).

4. Discussion

In the present study, shoot and root dry weights of both pakchoi cultivars treated for seven and twenty-one days were all significantly decreased by Cd added at the higher concentration (Cd2) (Fig. 1), which was all significantly alleviated by addition of 1.5 mM Si. Also, it is noted that Si could significantly stimulate the growth of both pakchoi cultivars without Cd stress (Fig. 1). This is meaningful and particularly interesting. Firstly, it is suggested that Si is beneficial for the growth of not only Si-accumulators such as rice [18] and sugarcane [41], but also non-Si-accumulators including pakchoi, a dicotyledonous plant species. Secondly, Si is beneficial for

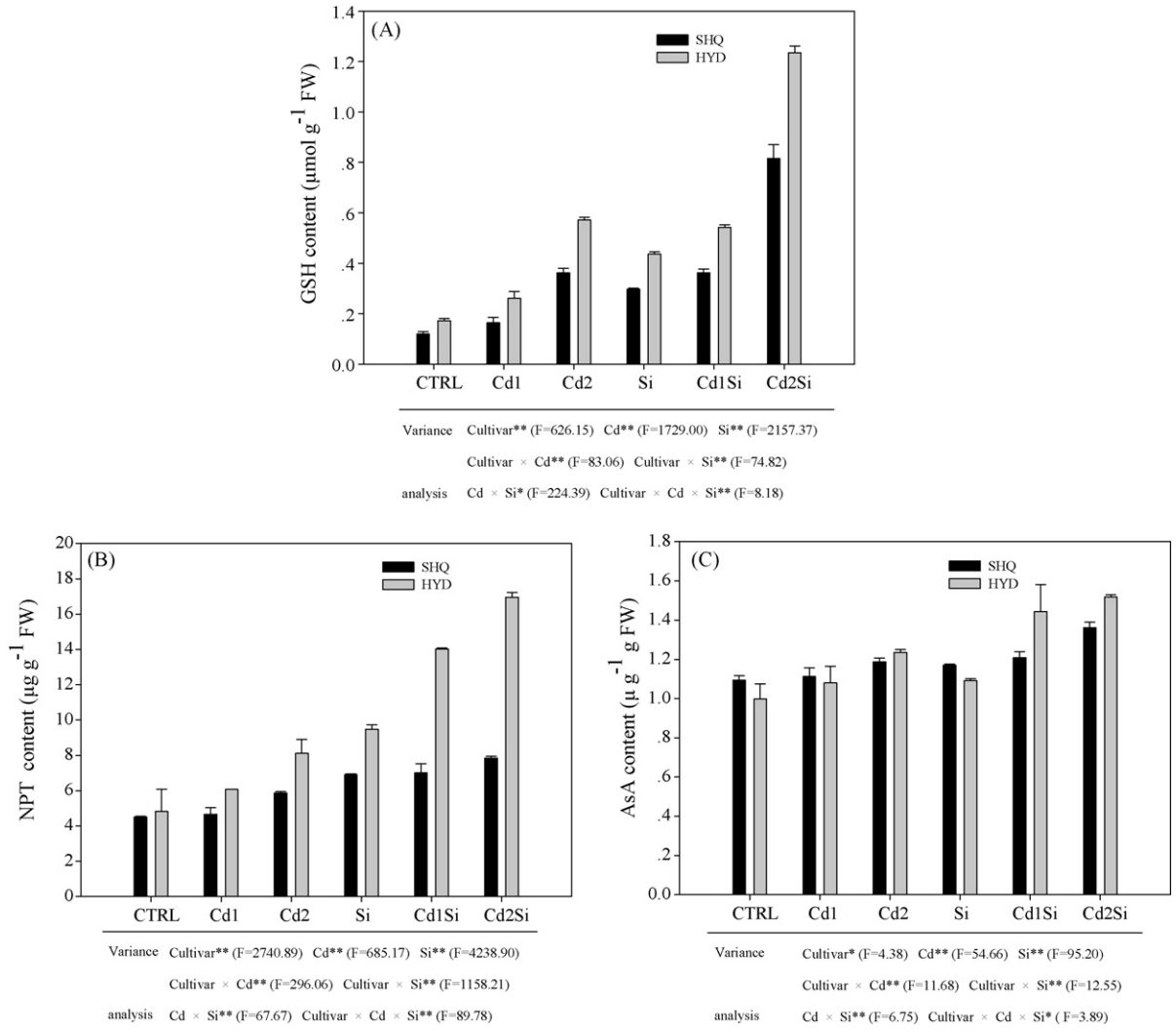

Fig. 7. GSH (A), NPT (B) and AsA (C) concentration in leaves of *B. chinensis* plants grown hydroponically with various levels of Cd with or without 1.5 mM Si for seven days. Data are means ± S.D. ($n = 3$). *P*-value indicates significance level based on three-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$. CTRL: treatment with neither Cd nor Si; Cd1: treatment with 0.5 mg L^{-1} Cd; Cd2: treatment with 5.0 mg L^{-1} Cd; 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.

the growth of plants subjected to either non-stressful or stressful conditions (Cd stress in this study).

Cd concentrations were 2–10 times higher in roots than in shoots in the two pakchoi plants grown hydroponically with various Cd concentrations (Fig. 2). Addition of Si decreased Cd concentration in shoots of both cultivars (Fig. 2), and partially alleviated the inhibitory effect of Cd on the biomass accumulation. The root/shoot Cd ratios were significantly higher in Si-amended Cd-stressed plants than in non-Si-amended Cd-stressed plants (Fig. 2). This is consistent with the reports that Si reduced uptake and transport of Cd in maize [4] and rice [42]. The main reason might be that Si could enhance uptake of calcium ion in plants [43], which competes with Cd for uptake sites in plant roots [44], and inhibits root-to-shoot Cd transport. These effects of Si on reducing Cd concentration in pakchoi shoots are practically important in particular with respect to vegetable safety. It can be expected that application of Si fertilizer in Cd-contaminated vegetable-grown soils can help reduce vegetable safety risks by inhibiting Cd uptake and transport into the edible parts. However, further pot and field studies using silicon fertilizers such as furnace slag amendments are needed to verify whether these results obtained in hydroponic can be repeated in field conditions. This is particularly important for food safety both in China and in the world since heavy metals contamination of soils, especially those for vegetable production, has received public concerns because of repeated use of heavy-metal-containing manures, sludge and pesticides.

Another important finding in this study is the Si-mediated enhancement of antioxidant defense system in pakchoi plants exposed to Cd stress. The activities of SOD and CAT in leaves of both pakchoi plants were significantly decreased under Cd stress (Fig. 5A and B; Fig. 6A and B). Therefore, ROS might accumulate in leaves of Cd-stressed pakchoi, causing lipid peroxidation as indicated by the elevated levels of MDA (Fig. 4). Interestingly, addition of Si increased antioxidant enzyme activities (Figs. 5 and 6) and consequently alleviated the lipid peroxidation as indicated by the decreased H_2O_2 level and MDA concentrations (Figs. 3 and 4). Such Si effect on alleviating Cd-induced oxidative damage was more significant in the Cd-tolerant cultivar than in the Cd-sensitive cultivar. This observation was also supported by the isozyme activity profiles using non-denaturing PAGE (Fig. 6).

In the ascorbate–glutathione circle cycle, APX plays an important role in removing H_2O_2. In the present study, APX activity increased in pakchoi plants exposed to the lower concentration of Cd but decreased with increasing Cd concentration (Fig. 5C),

suggesting the important role of APX in the detoxification of H_2O_2. However, addition of Si increased activities of APX under Cd stress (Fig. 5C), suggesting that Si could increase plant tolerance to oxidative stress under Cd toxicity by increasing the efficiency of ascorbate–glutathione cycle. Ascorbate and glutathione are important antioxidants which are redox buffering in the apoplasts, protecting the plasmalemma from oxidation [45]. In the present study, the presence of Cd significantly increased GSH concentrations, which was further increased by the addition of Si (Fig. 7A). It was reported that accelerated synthesis of GSH occurred in plant exposed to Cd stress [46], which might explain why GSH concentrations were observed to increase in response to Cd exposure (Fig. 7A). In the present study, AsA concentrations were also increased in Cd-stressed pakchoi plants (Fig. 7C), which was further increased by addition of Si. The enhancement of AsA levels might help cope with the Cd-induced oxidative damage. It seems to suggest that addition of Si enhanced the activity of ROS scavengers and increased Cd tolerance, therefore reducing the negative effect of Cd on growth inhibition (Fig. 1).

NPT also plays an essential role in Cd detoxification [39]. In the present study, addition of Si further increased levels of NPT in Cd-stressed pakchoi (Fig. 7B). The increase of NPT in plants might be an important defense response to Cd toxicity, which has also been reported in Cd-stressed *Phragmites australis* [47].

We previously reported that Si addition increased antioxidant defense activity and decreased oxidative damage in salt-stressed barley [17,24]. Such roles of Si in mediating antioxidant defense system and suppressing lipid peroxidation were subsequently confirmed in salt-stressed cucumber [48], drought-stressed wheat [49], excess Mn-stressed cucumber [19], excess B-stressed wheat [50] and spinach [51] and freezing-stressed wheat [52]. It appears that the Si-enhanced antioxidant defense activity is a universal mechanism for coping with various forms of abiotic stress in both Si-accumulating and non-Si-accumulating plants.

It is also interesting to note that H_2O_2 concentration along with MDA concentration was significantly lower in Si-treated plants than in the control (Figs. 3 and 4), suggesting that Si be also involved in the metabolism of ROS in plants that are not exposed to stressful environments. This phenomenon is also supported by the higher SOD, CAT and APX activities (Figs. 5 and 6) and higher GSH, NPT and AsA concentrations in Si-treated plants (Fig. 7).

5. Conclusion

In the present study we showed that the addition of Si alleviated the Cd phytotoxicity and significantly decreased Cd uptake and root-to-shoot transport in pakchoi plants grown hydroponically with Cd. The MDA and H_2O_2 concentrations in leaves were lower than in plants treated with Cd alone. Moreover, addition of Si enhanced the activity of SOD, CAT and APX, the concentrations of GSH, AsA and NPT, consequently reducing the Cd-induced oxidative damage. Simultaneously, the Si-enhanced tolerance to Cd stress was more significant in the Cd-tolerant pakchoi than in the Cd-sensitive pakchoi, showing significant genotypic differences.

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