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Responses of two kidney bean (*Phaseolus vulgaris*) cultivars to the combined stress of sulfur deficiency and cadmium toxicity

Dongqin Li · Guikui Chen · Qin Lu · Yongtao Li · Jinjin Wang · Huashou Li

Abstract Plants suffer from combined stress of sulfur deficiency and cadmium toxicity in some agricultural lands. However, little is known about the reaction in plants, such as responses in antioxidant enzymes and non-protein thiol compounds, to such combined stress. Therefore, in this study, four treatments, S-sufficiency (T_{S-Cd}), S-deficiency (T_{-S-Cd}), Cd stress (T_{S+Cd}) and combined stress of S-deficiency and Cd stress (T_{-S+Cd}), were set up to investigate (1) the effects of sulfur deficiency or sulfur sufficiency on Cd toxicity to kidney bean cultivar seedlings and the related mechanisms, and (2) the responses of two kidney bean cultivars to combined stress of S-deficiency and Cd-tolerance. The results showed significant increases in hydrogen peroxide (H_2O_2) and malondialdehyde contents and significant increases in antioxidant enzyme (superoxide dismutase, catalase, peroxidase, and glutathione S-transferase) activities and non-protein thiol compounds (non-protein thiols, reduced glutathione, phytochelatins) synthesis in the plants in T_{S+Cd} and T_{-S+Cd}. On the tissue level, higher proportion of Cd was found to be immobilized/deposited in roots, while on the sub-cell level, higher proportion of Cd was located in cell walls and vacuole fractions with lower in cell organelles. Taken together, the results indicated that Cd detoxification was achieved by the two kidney bean cultivars through antioxidant enzyme activation, non-protein thiol compound synthesis and sub-cellular compartmentalization. In addition, the results indicated that sufficient S supply helped to relieve Cd toxicity, which is of special significance for remediation or utilization of Cd-contaminated soils as S is a plant essential nutrient.

Keywords Antioxidant enzyme · Cadmium · Non-protein thiol compound · Oxidative damage · Sub-cellular distribution

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Introduction

Cadmium (Cd) is a naturally-occurring trace element and a highly toxic pollutant as well (Nazar et al. 2012). Cadmium contamination in agricultural soils has been dramatically increasing worldwide as a result of anthropogenic activities, such as improper disposal of industrial effluent and mining waste, and abuse of phosphate fertilizers, pesticides and sewage sludge (Li et al. 2014). According to the report of the Ministry of Environmental Protection and the Ministry of Land

and Resources of the People's Republic of China (2017), Cd pollution area of agricultural soils in China has reached more than 2.8×10^5 hm^2. Cadmium is highly mobile in soil and readily taken up by plants. Once into plants, it can cause oxidative stress, increase membrane permeability, disturb mineral nutrition, and inhibit photosynthesis and structural changes when in high concentration (Gallego et al. 2012; Gill et al. 2012; Nazar et al. 2012; Rui et al. 2016) and poses a serious threat to human health through the food chain (Nazar et al. 2012).

To prevent the deleterious effect of Cd, plants employ various strategies such as sub-cellular Cd compartmentalization, antioxidation enzyme recovery and Cd chelation and/or sequestration. Nazar et al. (2012) observed higher activities of antioxidative enzymes, such as catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), peroxidase (POD) and superoxide dismutase (SOD), in plants suffered from Cd stress. Likewise, more CAT, SOD, POD and GR were produced under Cd stress (Singh and Shah 2014).

Sulfur is an essential element for plant growth. Research shows that sulfur plays an important role in Cd tolerance of plants (Bashir et al. 2013). At the tissue level, S can decrease Cd accumulation in shoots and alleviate Cd toxicity in plants via the formation and precipitation of Cd-S (Nocito 2002). At the cellular level, S supply can decrease the proportion of Cd in cell walls but increase the proportion in vacuoles, a metabolically inactive fraction, thus, reducing Cd biotoxicity (Zhang et al. 2014). In addition, plant defense mechanisms, such as improved growth, increased antioxidative capacity and reduced reactive oxygen species (ROS) and lipid peroxidation, can be initiated by S (Gill and Tuteja 2011; Khan et al. 2015; Mostofa et al. 2015). Sulfur might lead to increases in the synthesis of non-protein thiols (NPT) substances, including phytochelatins (PCs) and reduced glutathione (GSH), which directly chelate and sequestrate Cd in vacuoles (Gallego et al. 2012; Sharma et al. 2016). Zhang et al. (2013) reported that S supply protected rice from Cd toxicity by increasing the synthesis of the thiol substances (such as PCs and GSH) to decrease Cd availability.

However, sulfur deficiency is one of the major problems in agriculture production (Tombuloğlu et al. 2016). In Chinese agricultural soils, the likeliness of the occurrence of sulfur deficiency has increased (Li

et al. 2001; Zhou et al. 2005). Kidney bean (*Phaseolus vulgaris*), a leguminous plant widely cultivated in South China, has high nutritional value, grows rapidly, and is of low production cost. As a leguminous plant, kidney bean needs more sulfur for growth as compared with non-leguminous plants, and in turn is more likely to be faced with sulfur deficiency. On the other hand, agricultural soils in South China have been severely polluted by Cd due to nonferrous metal smelting operations without strict pollution control (Hu et al. 2013). Therefore, kidney bean might suffer from the combined stress of sulfur deficiency and cadmium toxicity, and sulfur deficiency might exacerbate cadmium toxicity. This study was aimed to investigate (1) the effects of sulfur deficiency/sufficiency on Cd toxicity to kidney bean plants and the related mechanisms, and (2) the responses of two kidney bean cultivars to combined stress of S-deficiency and Cd-tolerance. The results from this study are expected to be helpful in kidney bean production in the Cd-contaminated soils in South China.

Materials and methods

Treatment set up and sampling

Seeds of two kidney bean (*P. vulgaris*) cultivars, Youxuan and Texuan, were sterilized with 10% H_2O_2 for 10 min and germinated at 25 °C in an artificial climate incubator in plastic pots (length $\times$ wide $\times$ height: 20 cm $\times$ 10 cm $\times$ 8 cm) filled with sterilized sands. After cotyledons fully stretched, uniform seedlings were transferred into plastic pots (25 cm in diameter) filled with 1 kg reconstituted sterilized sands and divided into two groups: $+$ S and $-$ S. The $+$ S group was watered with an S-sufficient nutrient medium containing 102.20 g/L KNO$_3$, 189.00 g/L Ca(NO$_3$)·4H$_2$O, 98.00 g/L MgSO$_4$·7H$_2$O, 27.20 g/L NH$_4$H$_2$PO$_4$, 2.86 g/L H$_3$BO$_3$, 1.55 g/L MnSO$_4$·4H$_2$O, 0.22 g/L ZnSO$_4$·7H$_2$O, 0.08 g/L CuSO$_4$·5H$_2$O, 8.42 g/L EDTA-Fe–Na, 0.03 g/L CoCl$_2$·6H$_2$O, and 0.08 g/L MoO$_3$. In contrast, the $-$ S group was watered with the same nutrient medium but with the SO$_4$$^{2-}$ salts being replaced by equimolar amounts of corresponding Cl$^-$ salts. After 7 days, three pots from each of the two groups were sacrificed for seedling collecting for later analyses, and then each of the two groups was further divided

into another two groups: +Cd and −Cd, obtaining a total of 4 treatments of T_{S+Cd}, $T_{S−Cd}$, $T_{−S+Cd}$, and $T_{−S−Cd}$. For the T_{+S+Cd} and $T_{−S+Cd}$ treatments, Cd (in the form of $CdCl_2·2.5H_2O$) was added at a rate of 8 mg/kg soil on a dry weight basis. After another 7 days, plants were harvested from all the treatments.

For the plants harvested before and after Cd treatment, the roots and shoots were separated. The roots were soaked in 20 mM EDTA for 20 min to desorb the metal ions bound to the roots. Then, the roots were washed with deionized water, blot-dried, frozen in liquid nitrogen and kept at −80 °C in an ultra-low temperature freezer (Haier, DW-86L338, China) before being used for biochemical assays and cadmium sub-cellular accumulation measurements within a week after harvesting and freezing.

Determination of antioxidant enzyme activities

Fresh samples of leaves (0.2 g) were ground in a chilled mortar and enzymes were extracted with 4 mL ice-cold 50 mM potassium phosphate buffer (pH 7.2) containing 1% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at $12,000×g$ and 4 °C for 15 min. The supernatants were collected for enzyme assays. The activities of SOD, POD, and CAT were determined using an ultraviolet spectrophotometer UV-2501PCS (Shimadzu, Japan) as described by Chen and Wang (2006). Glutathione S-transferase (GST) activity was determined using a kit from Nanjing Jiancheng Bioengineering Research Institute. Determination of protein content was carried out using Coomassie brilliant blue, with bovine serum albumin (BSA) as standard.

Assessment of oxidative damage

To assess the extent of membrane damage in leaves, lipid peroxidation was measured and expressed as malondialdehyde (MDA) content (μmol/g FW) and H_2O_2 content (μmol/g FW). MDA was determined according to Chen and Wang (2006). Briefly, fresh leaf samples (0.2 g) were homogenized in 2 mL solution that contained 0.5% (w/v) thiobarbituric acid (TBA) and 10% (w/v) trichloroacetic acid (TCA). The mixture was subjected to a hot water bath at 95 °C for 20 min and then immediately to an ice bath to stop the reaction. The mixture was then centrifuged at $3000×g$ and 4 °C for 10 min, and MDA concentration

was measured spectrophotometrically at 532 nm, 600 nm and 450 nm. H_2O_2 in leaves was determined following the procedure of Loreto and Velikova (2001). Point 5 g of fresh samples was homogenized with 5 mL of 0.1% (w/v) TCA in an ice bath. The homogenate was centrifuged at $12,000×g$ for 15 min; then, 1 mL of the supernatant was added to a mixture of 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide. The absorbance at 390 nm was read. H_2O_2 concentration was calculated according to the standard curve.

Determination of NPT, GSH and PCs contents

Fresh leaf samples (1 g) were ground in liquid nitrogen and homogenized in 8 mL 5% sulfosalicylic acid (SSA). The homogenate was centrifuged at $10,000×g$ and 4 °C for 15 min and the supernatant was stored at −80 °C before used for NPT analyses. NPT were determined following the method described by Rama Devi and Prasad (Rama Devi and Prasad 1998). One millilitre of the supernatant was added to 2 mL of a reaction medium consisting of 0.5 mL 0.2 M Tris–HCL (pH 8.2) and 0.15 mL 10 mM 2-nitrobenzoic acid (DTNB). The absorbance at 412 nm was read after 20 min. For control, no DTNB was added. GSH was determined also using a kit from Nanjing Jiancheng Bioengineering Research Institute. The amount of PCs was estimated using the following formula: PCs = NPT-GSH (Bhargava et al. 2005).

Tissue fractionation and Cd analysis

Frozen roots,stems and leaves were homogenized in a pre-cold extraction buffer containing 50 mM Tris–HCl (pH 7.5), 250 mM sucrose and 1.0 mM $C_4H_{10}O_2S_4$ and 10 mM cysteine. Cells were separated into the following three fractions: cell wall, organelle and vacuole, using the differential centrifugation technique (Wang et al. 2008). The homogenate was centrifuged at $3000×g$ for 15 min and the precipitation was designated as 'cell wall' mainly consisting of cell walls and cell wall debris. The resulting supernatant was further centrifuged at $15,000×g$ for 30 min. The resulting deposition and supernatant were the "organelle" fraction and the "vacuole" fraction, respectively. The entire separation procedure was performed at 4 °C. The cell wall, organelle and vacuole fractions were digested with concentrated

HNO$_3$–HClO$_4$ (3:1, v/v), and Cd concentration was determined by atomic absorption spectrophotometry (ZEEnit700P, Analytik Jena AG, Germany). Cd partition coefficient in sub-cellular fractions was estimated using the following formula:

$$\text{Partition coefficient } (\%) = \text{Cd}_i / (\text{Cd}_{cellwall} \\ + \text{Cd}_{organelle} \\ + \text{Cd}_{vacuole}) 100$$

where Cd$_i$ is the Cd concentration in sub-cellular fraction i, i can be cell wall, organelle or vacuole.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) with SPSS 17.0 and Microsoft Excel 2003, and figures were plotted using Sigma Plot 12.0. The results were presented as mean ± S.E. (standard error) in tables and figures. Significance of differences was determined at the 0.05 confidence level and treatment means were separated by Duncan's multiple range test.

Results

Oxidative damage

H$_2$O$_2$ increased significantly by 133 and 110% in T$_{-S+Cd}$ and T$_{S+Cd}$, respectively for Youxuan and significantly by 129 and 140% in T$_{-S+Cd}$ and T$_{S+Cd}$, respectively for Texuan after Cd treatment as compared with before Cd treatment (Fig. 1). In addition, H$_2$O$_2$ content in T$_{-S+Cd}$ and T$_{S+Cd}$ was significantly higher than that in T$_{-S-Cd}$ and T$_{S-Cd}$, respectively: 86 and 68%, respectively for Youxuan and 50 and 64%, respectively for Texuan. H$_2$O$_2$ content in T$_{S-Cd}$ and T$_{-S-Cd}$ did not increased significantly after 7 days of Cd treatment. It was not significantly different between T$_{-S+Cd}$ and T$_{S+Cd}$ though it was higher in the former and was not significantly different between T$_{-S-Cd}$ and T$_{S-Cd}$, either. The two cultivars showed similar H$_2$O$_2$ contents and changing patterns.

MDA showed a similar increasing pattern to that of H$_2$O$_2$ (Fig. 2). It increased significantly by 183 and 92% in T$_{-S+Cd}$ and T$_{S+Cd}$, respectively for Youxuan and significantly by 188 and 109% in T$_{-S+Cd}$ and T$_{S+Cd}$, respectively for Texuan after Cd treatment as compared with before Cd treatment. In addition, MDA content in T$_{-S+Cd}$ and T$_{S+Cd}$ was significantly higher than that in T$_{-S-Cd}$ and T$_{S-Cd}$, respectively: 71 and 101%, respectively for Youxuan and 57 and 47%, respectively for Texuan. MDA content did not increased significantly in T$_{S-Cd}$ but increased significantly in T$_{-S-Cd}$ after 7 days of Cd treatment. MDA content was not significantly different between T$_{-S+Cd}$ and T$_{S+Cd}$ though it was higher in the former and was not significantly different between T$_{-S-Cd}$ and T$_{S-Cd}$, either. The two cultivars showed similar H$_2$O$_2$ contents and changing patterns.

ROS scavenging antioxidant system

In order to prevent or minimize lipid peroxidation damage, activities of various antioxidant enzymes in plants are increased to scavenge ROS. In this study, the activities of the following antioxidant enzymes were focused on: SOD, CAT, POD, and GST.

SOD activity in Youxuan (Fig. 3a) and Texuan (Fig. 3b) increased significantly by 125 and 194%, respectively in T$_{-S+Cd}$ and by 67 and 108%, respectively in T$_{S+Cd}$ after Cd treatment as compared with before Cd treatment. SOD activity in Youxuan (364.41 U/g FW) and Texuan (510.85 U/g FW) was higher after Cd treatment in T$_{-S+Cd}$ than in T$_{S+Cd}$ where SOD activity was 349.73 U/g FW and 399.52 U/g FW, respectively. SOD activity increased by 125% for Youxuan and 194% for Texuan after Cd treatment compared with before Cd treatment in T$_{-S+Cd}$. For T$_{S+Cd}$, SOD activity increased by 67% for Youxuan and 108% for Texuan after Cd treatment compared with before Cd treatment.

POD activity was induced by Cd and increased significantly after Cd treatment in the two cultivars, especially when sulfur was limited (T$_{-S+Cd}$) (Fig. 4). However, activity increase was more pronounced in Youxuan than in Texuan, by 229 and 351% in Youxuan, and just 131 and 249% in Texuan after Cd treatment in the T$_{S+Cd}$ and T$_{-S+Cd}$ treatments, respectively as compared with before Cd treatment. Compared with that in T$_{S-Cd}$ and T$_{-S-Cd}$, POD activity in T$_{S+Cd}$ and T$_{-S+Cd}$ was 66 and 154%, respectively higher in Youxuan and 47 and 100%, respectively higher in Texuan after Cd treatment.

Figure 5 shows that CAT activity in the two cultivars increased significantly after Cd treatment in all treatments but T$_{S-Cd}$. CAT activity in both
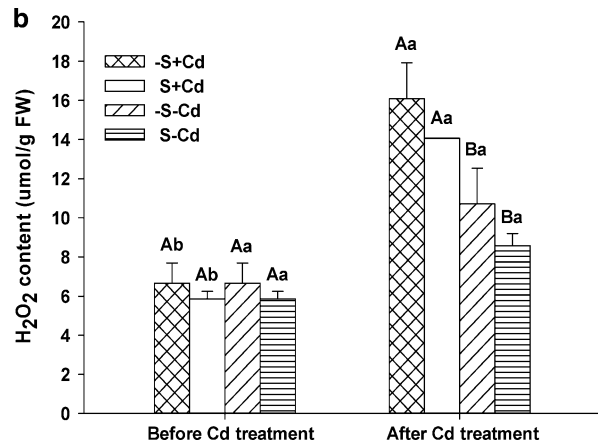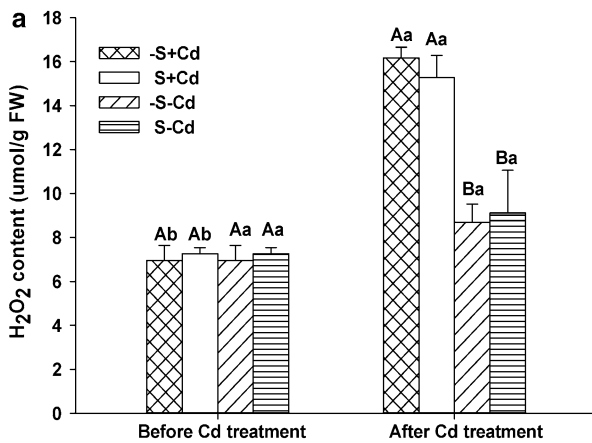
Fig. 1 The changes of hydrogen peroxide (H_2O_2) content in the leaves of Youxuan (**a**), and Texuan (**b**). The same capital letters indicate no significant differences ($P \geq 0.05$) between the treatments for a same sampling time, while the same lowercase letters indicate no significant differences ($P \geq 0.05$) between sampling times for a same treatment. The values are mean $\pm$ SE and n = 3. S + Cd, Cd stress; S − Cd, S-sufficient; − S − Cd, S-deficient; − S + Cd, combined stress of S-deficiency and Cd stress
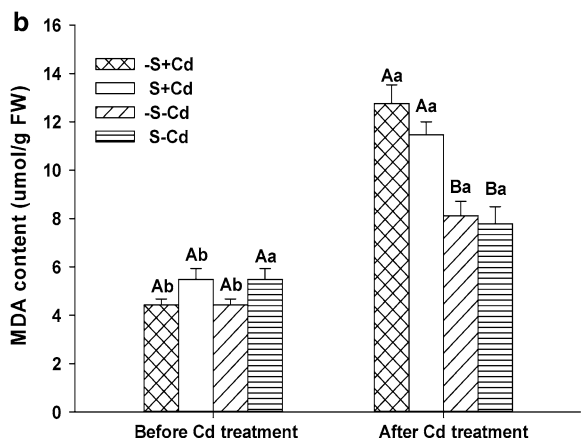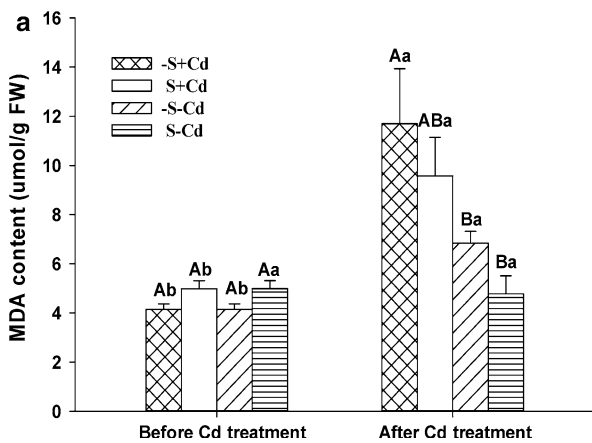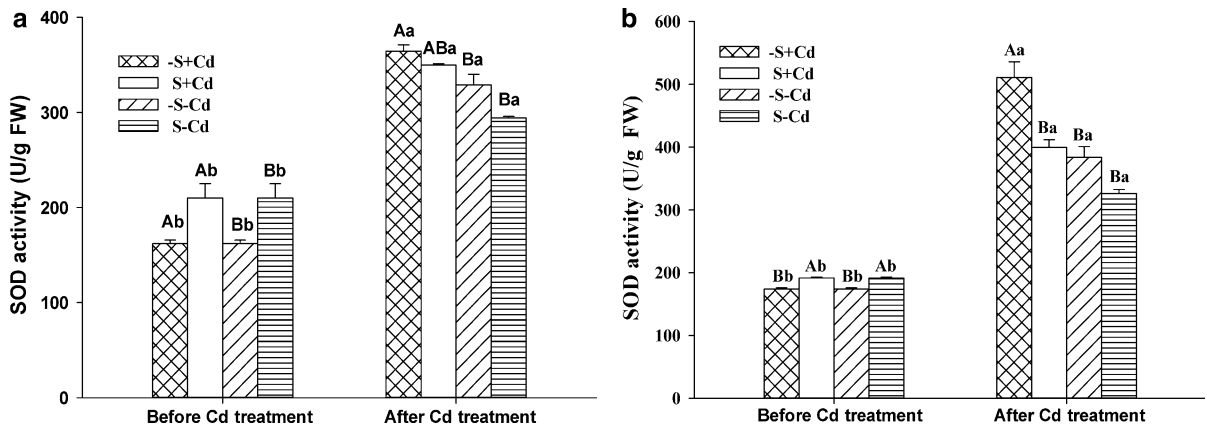

Fig. 2 The changes of malondialdehyde (MDA) content in the leaves of Youxuan (**a**), and Texuan (**b**). The same capital letters indicate no significant differences ($P \geq 0.05$) between the treatments for a same sampling time, while the same lowercase letters indicate no significant differences ($P \geq 0.05$) between sampling times for a same treatment. The values are mean $\pm$ SE and n = 3. S + Cd, Cd stress; S − Cd, S-sufficient; − S − Cd, S-deficient; − S + Cd, combined stress of S-deficiency and Cd stress

cultivars was greater in S-deficient treatments (T_{-S+Cd}, T_{-S-Cd}) than in S-sufficient treatments (T_{S+Cd}, T_{S-Cd}) after Cd treatment. And there were no significant differences among T_{-S+Cd}, T_{S+Cd} and T_{-S-Cd} for Youxuan while for Texuan, CAT activity in T_{-S+Cd} was significantly higher than in T_{S+Cd}, T_{-S-Cd} and T_{S-Cd}. CAT activity increased by 272 and 409% in Texuan and 419 and 602% in Youxuan after Cd treatment compared with that before Cd treatment in T_{S+Cd} and T_{-S+Cd}, respectively. Compared with that in T_{S-Cd} and T_{-S-Cd}, CAT activity after Cd

treatment in T_{S+Cd} and T_{-S+Cd} increased by 231 and 38%, respectively in Youxuan, and by 215 and 186%, respectively in Texuan.

Similarly, GST activity was greater in the treatments with Cd addition than in those without Cd addition (Fig. 6). But unlike other enzyme activities, GST activity was larger in S-sufficient treatments (T_{S+Cd}/T_{S-Cd}) than in the corresponding S-deficient ones (T_{-S+Cd}/T_{-S-Cd}) for both cultivars. GST activity increased much more in Texuan (by 218% in T_{S+Cd} and 176% in T_{-S+Cd}) than in Youxuan (by 95% in

Fig. 3 The changes of superoxide dismutase (SOD) activity in the leaves of Youxuan (**a**), and Texuan (**b**). The same capital letters indicate no significant differences ($P \geq 0.05$) between the treatments for a same sampling time, while the same lowercase letters indicate no significant differences ($P \geq 0.05$) between sampling times for a same treatment. The values are mean ± SE and n = 3. S + Cd, Cd stress; S − Cd, S-sufficient; − S − Cd, S-deficient; − S + Cd, combined stress of S-deficiency and Cd stress
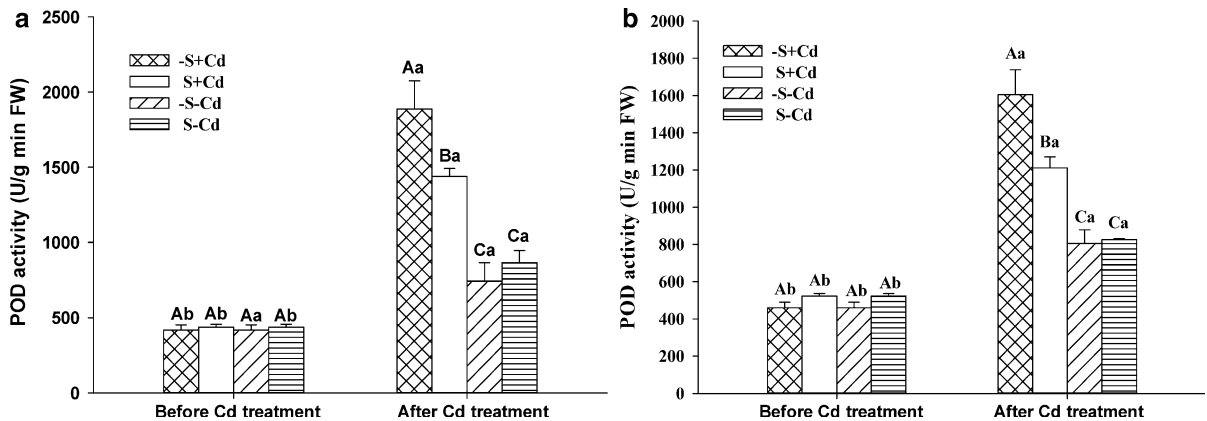
Fig. 4 The changes of peroxidase (POD) activity in the leaves of Youxuan (**a**), and Texuan (**b**). The same capital letters indicate no significant differences ($P \geq 0.05$) between the treatments for a same sampling time, while the same lowercase letters indicate no significant differences ($P \geq 0.05$) between sampling times for a same treatment. The values are mean ± SE and n = 3. S + Cd, Cd stress; S − Cd, S-sufficient; − S − Cd, S-deficient; − S + Cd, combined stress of S-deficiency and Cd stress

T_{S+Cd} and 98% in T_{-S+Cd}) after Cd treatment as compared with before Cd treatment. GST activity after Cd treatment in T_{S+Cd} and T_{-S+Cd} was 17 and 195% higher than in T_{S-Cd} and T_{-S-Cd}, respectively for Youxuan, and 69 and 56% higher, respectively for Texuan.

Sulfur-assimilation compounds

For both cultivars after Cd treatment, NPT content was higher in T_{S-Cd} than in T_{-S-Cd} and was significantly higher in T_{S+Cd} than in T_{-S+Cd} (Fig. 7). For T_{S+Cd},

NPT content increased more in Youxuan (by 434%) than in Texuan (by 358%), but for T_{-S+Cd}, more in Texuan (by 320%) than in Youxuan (by 272%) after Cd treatment as compared with before Cd treatment. NPT content after Cd treatment in T_{S+Cd} and T_{-S+Cd} was 146 and 57%, respectively higher in Youxuan and 221 and 56%, respectively higher in Texuan than in T_{S-Cd} and T_{-S-Cd}, respectively.

GSH content (Fig. 8) and PCs content (Fig. 9) showed similar increasing trends to that of NPT for both cultivars.
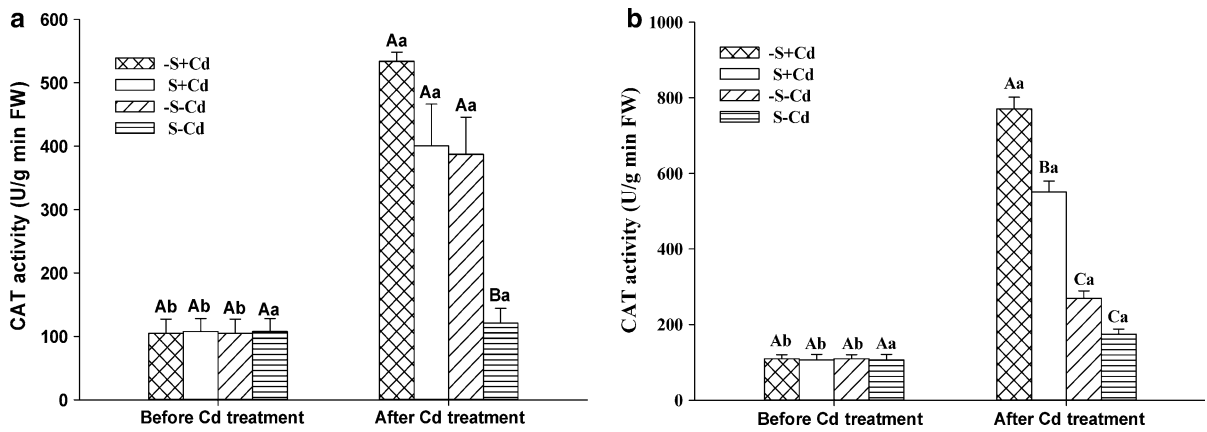
Fig. 5 The changes of catalase (CAT) activity in the leaves of Youxuan (**a**), and Texuan (**b**). The same capital letters indicate no significant differences ($P \geq 0.05$) between the treatments for a same sampling time, while the same lowercase letters indicate no significant differences ($P \geq 0.05$) between sampling times for a same treatment. The values are mean $\pm$ SE and n = 3. S + Cd, Cd stress; S − Cd, S-sufficient; − S − Cd, S-deficient; − S + Cd, combined stress of S-deficiency and Cd stress
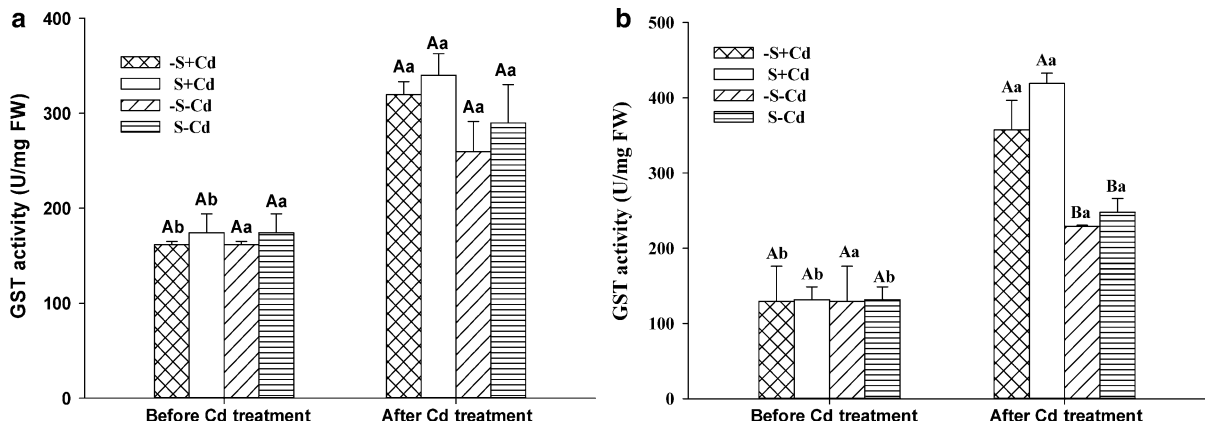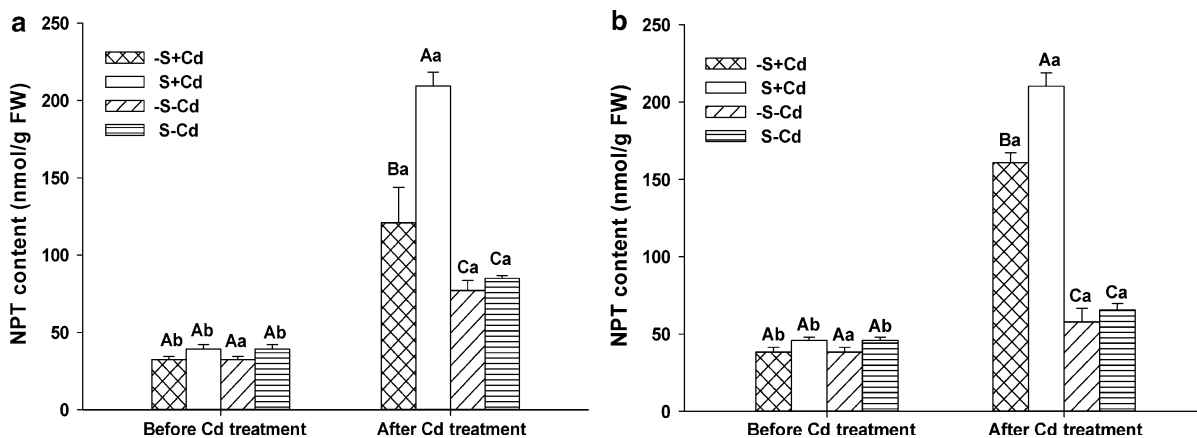

Fig. 6 The changes of glutathione S-transferase (GST) activity in the leaves of Youxuan (**a**), and Texuan (**b**). The same capital letters indicate no significant differences ($P \geq 0.05$) between the treatments for a same sampling time, while the same lowercase letters indicate no significant differences ($P \geq 0.05$) between sampling times for a same treatment. The values are mean $\pm$ SE and n = 3. S + Cd, Cd stress; S − Cd, S-sufficient; − S − Cd, S-deficient; − S + Cd, combined stress of S-deficiency and Cd stress

### Cd accumulation in tissues and its sub-cellular distribution

After 7 days of Cd exposure, more Cd accumulated in plant tissues in T_{-S+Cd} than in T_{S+Cd} (Table 1). More Cd was deposited in the roots of Youxuan than in the roots of Texuan whether in T_{-S+Cd} or T_{S+Cd}, while an opposite trend was found in the stems of the two cultivars (Table 1). A larger proportion of Cd was translocated from roots to shoots in Texuan than in Youxuan at different periods with or without the supply of sulfur (Table 2).

Cd sub-cellular distribution in the two cultivars in different treatments are exhibited in Fig. 10. It can be seen that in the plant leaves in any of the four treatments, Cd was predominantly (approximately 80%) immobilized/deposited in cell walls, while in the roots and stems, comparable amounts of Cd were deposited in cell walls and vacuoles. This result suggests that vacuolar sequestration played a vital role in Cd detoxification in both cultivars. The proportion of Cd in the organelles of different tissues in treatment T_{-S+Cd} was greater than in T_{S+Cd}.

Fig. 7 The changes of non-protein thiols (NPT) content in the leaves of Youxuan (**a**), and Texuan (**b**). The same capital letters indicate no significant differences ($P \geq 0.05$) between the treatments for a same sampling time, while the same lowercase letters indicate no significant differences ($P \geq 0.05$) between

sampling times for a same treatment. The values are mean $\pm$ SE and n = 3. S + Cd, Cd stress; S − Cd, S-sufficient; − S − Cd, S-deficient; − S + Cd, combined stress of S-deficiency and Cd stress
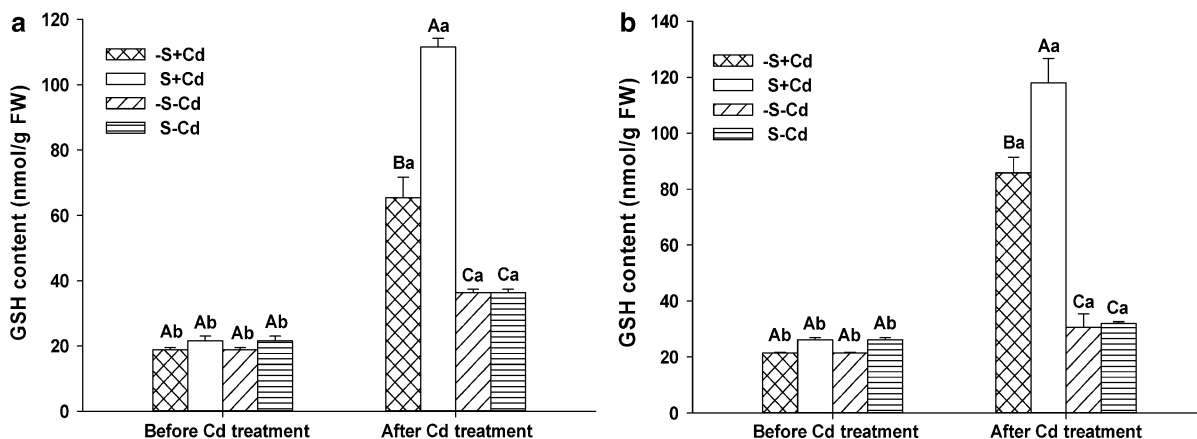
Fig. 8 The changes of reduced glutathione (GSH) content in the leaves of Youxuan (**a**), and Texuan (**b**). The same capital letters indicate no significant differences ($P \geq 0.05$) between the treatments for a same sampling time, while the same lowercase letters indicate no significant differences ($P \geq 0.05$)

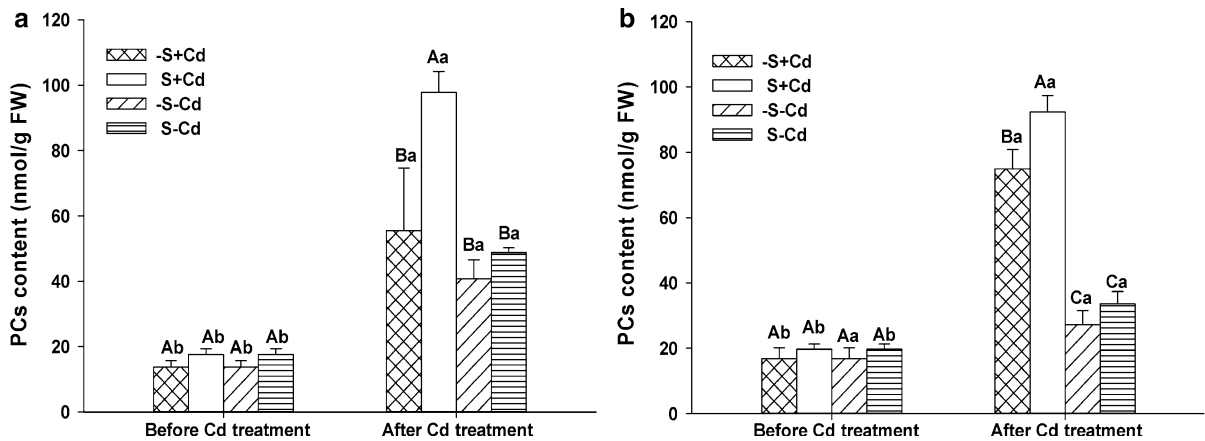between sampling times for a same treatment. The values are mean $\pm$ SE and n = 3. S + Cd, Cd stress; S − Cd, S-sufficient; − S − Cd, S-deficient; − S + Cd, combined stress of S-deficiency and Cd stress

Discussion

In stressful environments, reactive oxygen species (ROS), e.g., superoxide anion (O_2^-), hydroxyl radicals ($\cdot OH$), and hydrogen peroxide (H_2O_2), are commonly produced. Significant increase of H_2O_2 content in T_{-S+Cd} and T_{s+Cd} (Fig. 1) indicated a Cd pollution level of 8 mg/kg had caused a stressful effect on the plants. In contrast, no significant changes of H_2O_2 content in T_{-S-Cd} implied that S deficiency did not play a significant role in H_2O_2 production. ROS

such as H_2O_2 are strong oxidant agents and they can cause lipid peroxidation and subsequent membrane injury as well as protein and nucleic acid damage (Ercal et al. 2001). MDA content, a direct measure of lipid peroxidation, is a good indicator for oxidative stresses (Armas et al. 2015). Similarly, significant increases of MDA content in T_{-S+Cd} and T_{s+Cd} (Fig. 2) indicated occurrence of lipid peroxidation in the plants under Cd stress. But it was worthy of noting that in the plants not subjected to Cd stress, significant increase in MDA content was observed in

Fig. 9 The changes of phytochelatins (PCs) content in the leaves of Youxuan (**a**), and Texuan (**b**). The same capital letters indicate no significant differences ($P \geq 0.05$) between the treatments for a same sampling time, while the same lowercase letters indicate no significant differences ($P \geq 0.05$) between sampling times for a same treatment. The values are mean $\pm$ SE and n = 3. S + Cd, Cd stress; S − Cd, S-sufficient; − S − Cd, S-deficient; − S + Cd, combined stress of S-deficiency and Cd stress

Table 1 Dynamic changes of total Cd accumulated in the different organs of the two kidney bean cultivars

The same letters in a row indicate no significant differences ($P \geq 0.5$) between the two treatments for a cultivar

Tissue	Cd concentration (mg/kg FW)			
	Youxuan		Texuan	
	T_{S+Cd}	T_{-S+Cd}	T_{S+Cd}	T_{-S+Cd}
Root	6.05 ± 0.11 a	6.33 ± 0.12 a	5.89 ± 0.03 a	5. 90 ± 0.33 a
Stem	2.18 ± 0.02 b	2.48 ± 0.01 a	2.46 ± 0.07 b	2.83 ± 0.03 a
Leaf	0.07 ± 0.00 b	0.10 ± 0.00 a	0.09 ± 0.00 b	0.12 ± 0.00 a

Table 2 Cd translocation factors (TF) of two kidney bean cultivars

Youxuan		Texuan	
T_{S+Cd}	T_{-S+Cd}	T_{S+Cd}	T_{-S+Cd}
0.37	0.41	0.43	0.50

TF was calculated according to the following equation: $IF = (Cd_{stem} + Cd_{leaf})/Cd_{root}$

those in T_{-S-Cd} but not in T_{S-Cd}, implying that S deficiency might also lead to lipid peroxidation due to production of other ROS but H_2O_2. Increased generation of MDA and H_2O_2 indicated that Cd provoked cellular disruption in the two cultivars through the induction of oxidative damage. The most severe oxidative damage was observed in Texuan under Cd stress (Figs. 1, 2).

Antioxidation enzyme recovery is one of the various protective strategies employed by plants subjected to environmental stress. Antioxidation enzymes including SOD, CAT, POD and GST are very effective in scavenging the harmful ROS. SOD is induced primarily and responsible for the detoxification of O_2^-; CAT and POD are two major systems to promote H_2O_2 conversion to H_2O and O_2 in plant cells (Torres et al. 2008); GST is a protein superfamily of multifunctional enzymes, whose members play a crucial role in protecting plants against Cd toxicity (Corticeiro et al. 2013). Under Cd stress, production of CAT, SOD and POD was reported to be more significantly triggered with increasing Cd concentration (Singh and Shah 2014). Deng et al. (2010) observed higher CAT, APX, GR, POD and SOD activities with increasing Cd stress (Deng et al. 2010). Findings from this study were consistent with those reported in the literature: Antioxidation enzyme
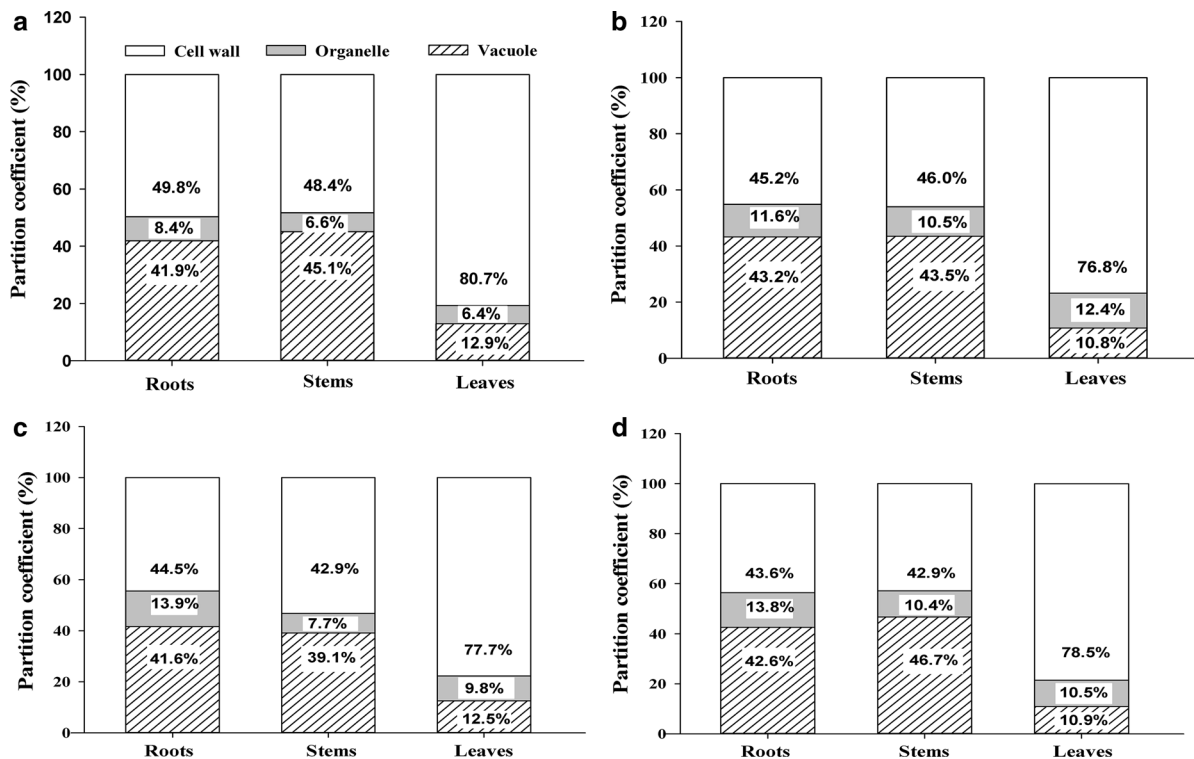
Fig. 10 Partition coefficient of Cd in the tissues of Youxuan with Cd stress (T_{S+Cd}) (**a**), Youxuan under combined stress of S-deficiency and Cd stress (T_{-S+Cd}) (**b**), Texuan under Cd stress (T_{S+Cd}) (**c**), and Texuan under combined stress of S-deficiency and Cd stress (T_{-S+Cd}) (**d**). The values are mean $\pm$ SE and n = 3

activities increased significantly in plants under Cd stress (Figs. 3, 4, 5, 6).

Cadmium rapidly induces the synthesis of PCs' thiol-based complexing substances through the up-regulation of GSH biosynthesis by PCs synthase (Cobbett 2000), which explains the significant increase in NPT substances in the treatments with Cd application (Figs. 7, 8, 9). This is one of the detoxification mechanisms adopted by plants under Cd as NPT substances can directly chelate Cd and sequestrate it into vacuoles, a metabolically inactive fraction of plant cells (Gallego et al. 2012; Sharma et al. 2016), thus reducing the biotoxicity of Cd. Unlike H_2O_2 content, MDA content and the antioxidation enzyme activities, the contents of NPT compounds were significantly higher in T_{s+Cd} than in T_{-S+Cd}. This is because S is an element in these compounds, and sufficient S was clearly favorable for their synthesis. As a result, Cd bioavailability was reduced, less ROS were produced, and lower antioxidation enzyme activities were found in T_{s+Cd} compared with in $T_{-S+Cd.}$

For the two cultivars tested in this study, higher Cd accumulation was found in roots than in other tissues (Table 1), and S-deficiency caused more Cd to be transported from the roots to the above-ground parts (Table 2), which is consistent with the findings of others (Gallego et al. 2012; Liang et al. 2016; Sharma et al. 2016). The results indicated that the application of S can reduce Cd translocation and relieve the oxidative damage of kidney bean leaves during Cd exposure in this study. Cd accumulation in Youxuan roots was higher than in Texuan roots. In contrast, Texuan had a higher translocation factor than Youxuan (Table 2). This suggests that Cd accumulation and translocation are not only related to S nutrient status, but also to genotype.

Sub-cellular partitioning of metals is one of the possible internal mechanisms for heavy metal detoxification in plants (Sharma et al. 2016). Cd mainly accumulated in the metabolically inactive fractions of cell walls and vacuoles, with an extremely small percentage of Cd deposited in the metabolically active cell fraction of organelles (Fig. 10). There are polyose

(including cellulose, hemicellulose, and pectin) and proteins within cell walls to bind Cd ions and stop Cd from entering protoplasm (Fu et al. 2011; Van Belleghem et al. 2007; Zhang et al. 2014). For the part of Cd that does enter protoplasms, it would be transferred into vacuoles and converted into inactive chemical forms, thus achieving Cd detoxification (Su et al. 2014; Whiting et al. 2000). The organelles are very metal-sensitive (Zhang et al. 2014). For both cultivars, a higher proportion of Cd was found in organelles in the S-deficient treatment compared with the S-sufficient treatment, further supporting the Cd detoxification mechanism of NPT chelation and subsequent compartmentalization of the Cd-PCs complexes in vacuoles (Nocito 2002; Van Belleghem et al. 2007).

Conclusions

Cadmium stress led to significant increase in ROS (i.e., H_2O_2) content and prominent lipid peroxidation in both cultivars no matter S was sufficient or not. In response to Cd stress, mainly three detoxification mechanisms were adopted by the cultivars: (1) increasing the activities of antioxidation enzymes (i.e., SOD, CAT, POD and GST) to scavenge the ROS so as to minimize lipid peroxidation and membrane damage; (2) synthesizing more NPT to chelate and compartmentalize Cd especially when S was sufficient; (3) on the tissue level, keeping Cd from translocating from roots to the above-ground parts; on the sub-cell level, keeping Cd in cell walls and vacuoles instead of organelles.

The results clearly showed that sufficient S supply can alleviate Cd toxicity in plants. This is of special significance as S is an essential nutrient for plants and in addition, it is of low cost compared with other soil amendments used to remediate soil Cd contamination or other remediation technologies.

Additionally, the two cultivars showed some differences in their responses to Cd stress. Genotype difference can be further explored in the future.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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