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Effects of sulfur supply on cadmium transfer and concentration in rice at different growth stages exposed to sulfur-deficient but highly cadmium-contaminated soil

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Abstract

Background Sulfur (S) has been widely used to alleviate cadmium (Cd) toxicity and control Cd accumulation in rice under waterlogging conditions. However, the results are contradictory, and the reasons remain unclear. This could be because most studies rarely simultaneously monitor the processes of S-induced soil Cd bioavailability and Cd accumulation in rice throughout its growth period. A pot experiment was conducted to investigate the influence of two sulfur levels (0, and 30 mg S kg^{-1}) on Cd concentration and translocation in rice at three growth stages (booting, filling and maturity) under waterlogging conditions. Paddy soil deficient in S but contaminated with Cd (10.16 mg Cd kg^{-1}) was used for the pot experiment.

Results S application increased concentrations of Cd in grain at the filling stage partially because S induced the promotion of Cd transfer from roots to stems, leaves, and grains, and S induced the accumulation and fixation of Cd in iron plaques at the filling stage. However, the application of S significantly reduced Cd concentrations in brown rice at the maturity stage, which could be attributed to three aspects, as described below. First, S supply reduced the availability of Cd in soil and iron plaque on the root surface by reducing dissolved Cd in soil pore water and transferring Cd from iron plaque on the root surface to roots. Second, S supply inhibited the transfer of Cd in other tissues to brown rice based on Cd transfer factors from roots, stems, leaves, and husks to brown rice, which were obviously lower with S supply than without S supply at the maturity stage. Third, S induced the dilution of Cd in brown rice because the application of S significantly increased brown rice biomass by 215%.

Conclusions A S-induced decline in Cd accumulation in brown rice was related to S-regulated Cd transfer among rice plants, S-induced promotion of rice growth and a decrease in Cd bioavailability in S-deficient but Cd-contaminated paddy soil under waterlogging conditions. This study provides valuable information for growing rice in low-S and Cd-contaminated paddy soil and reducing the risk of Cd in rice to humans.

Keywords Cadmium, Rice (*Oryza sativa* L.), Sulfur, Cd translocation, Dilution effect

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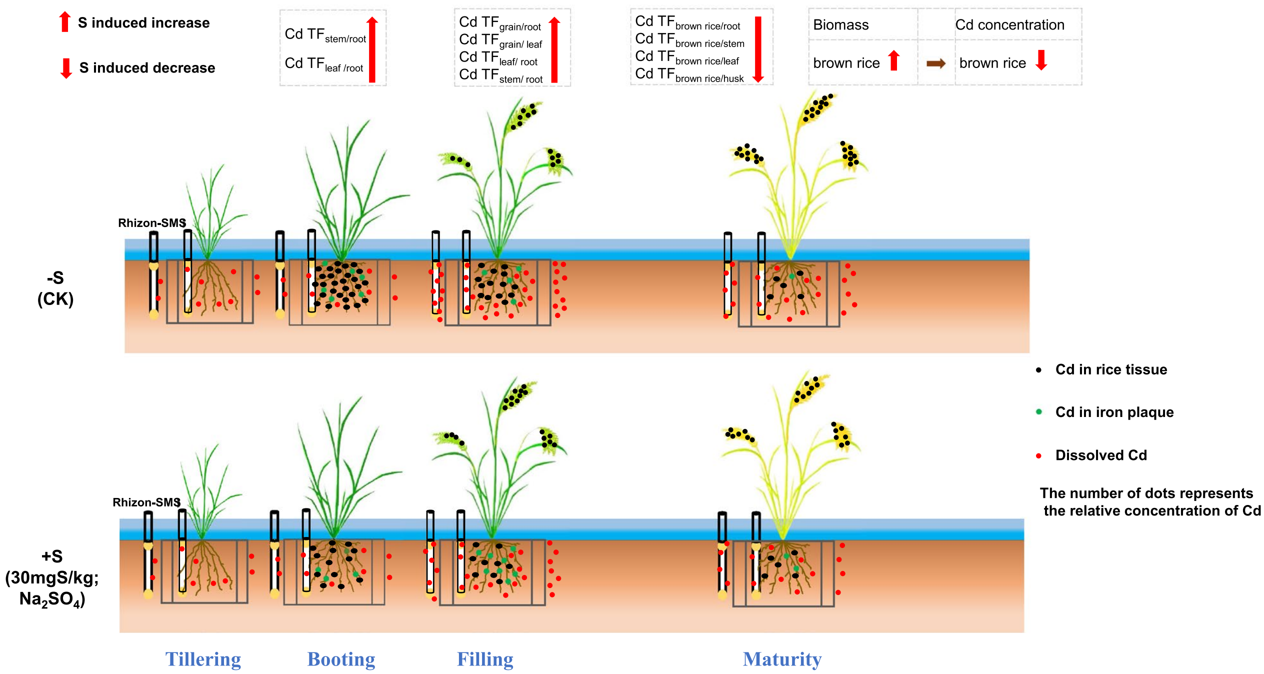
Graphical abstract



Background

Rice (*Oryza sativa* L.), as an important food crop in many countries, is a major route of Cd intake for people [1, 2]. The issues with Cd contamination in rice are increasingly recognized in many regions of the world, including China, India, Thailand, and Japan [3, 4]. One-third of paddy soils in the main rice-growing areas in China were reported to be contaminated with Cd [5]. The concentrations of Cd in rice grains increased from 2002 to 2019, with the maximum concentration of Cd in rice grains reaching 1.21 mg kg^{-1} [6], which indicates that some paddy fields with high Cd contamination are still planted with rice. Therefore, the control of Cd transfer and accumulation in rice is of great importance to promote food safety.

Sulfur (S) is an essential nutrient for plant growth and acts as a resistance factor against biotic and abiotic stress in plants [7]. S has been widely used to alleviate Cd toxicity and control the accumulation of Cd in rice under waterlogging conditions [1, 8–14], but the existing results of Cd transfer and accumulation in rice vary widely and even conflicting [15–18]. First, the existing effects of S application on rice biomass vary widely and even conflicting, which are related to the degree of soil Cd contamination and soil S levels. Previous studies reported that excess S supply (60–300 mg S kg^{-1}) in moderately Cd-contaminated soils (3.72 and 5 mg Cd kg^{-1}) increased rice biomass [8, 13]. Similarly, the application of S in low Cd-contaminated (0.79 and 1.5 mg Cd kg^{-1}) and low S-containing soils also increased rice biomass [9, 12]. However, the application of S in Cd-contaminated (2.22 mg Cd kg^{-1}) but high S-containing (797 mg S kg^{-1}) soil reduced rice biomass [8]. Second, the existing effects of S application on the concentration of Cd in rice vary widely and even conflict, which is related to soil S levels. Previous studies have shown that the application of S in low S-containing (total S < 200 mg kg^{-1}, or Ca $(H_2PO_4)_2$ extractable S < 26.7 mg kg^{-1}) soils significantly reduced the concentration of Cd in rice plants [9, 10, 12, 13]. However, Zhang et al. [15] observed that the application of S (114–458 mg S kg^{-1}) in S-rich soils increased the concentrations of Cd in roots, stems, leaves and grains. Third, previous reports have suggested that the translocation of Cd from roots to shoots in rice plants was the most common physiological factor leading to Cd accumulation in shoots and rice grains [8, 19, 20]. The mechanism of S-regulated transfer and distribution of Cd in rice plants has been increasingly elucidated, and the conclusions are generally consistent. Sulfate significantly induced the formation of glutathione (GSH) in rice plants, which is a cysteine (Cys)-rich peptide and a direct precursor/substrate for the synthesis of phytochelatins (PCs) [21–23]. As the major reservoir of nonprotein thiols, PCs can sequester Cd to form PC–Cd complexes and sequester it into vacuoles [8, 21, 24], subsequently decreasing Cd transportation from both roots and stems to rice grains. However, the existing effects of S application on the transfer factors of Cd from roots to aboveground plant parts of rice vary widely and are related to S supply levels, soil Cd contamination levels, and rice growth stages [12, 13, 16, 17]. Moreover, there is little available information about the effect of S application on the transfer factor of Cd from iron plaque on the rice root surface to the roots.

Therefore, the results of sulfur regulation on Cd accumulation in rice are inconsistent, and the reasons for the contradictory results are not fully understood. This could be because most studies rarely simultaneously monitor the effects of S application on rice biomass and the processes of S-regulated Cd uptake, transfer, redistribution, and accumulation in rice throughout its growth period. Zhou et al. [18] suggested that a large amount of Cd was taken up and accumulated in leaves at the tillering and booting stages, and then translocated in brown rice at the maturity stage. Therefore, it is necessary to investigate the mechanism by which sulfur regulated the uptake, transfer and accumulation of Cd in rice throughout its growth period to develop targeted measures to control the accumulation of Cd in rice.

In this study, a pot experiment was designed to investigate the effect of sulfur application on the transfer and concentration of Cd in rice plants at different growth stages of rice exposed to sulfur-deficient but highly Cd-contaminated soil. The specific objectives of this study were to (1) investigate the effects of sulfur application on rice growth under high Cd stress; (2) investigate the effect of sulfur application on the transfer of Cd in rice plants; and (3) investigate the effect of sulfur application on the concentration of Cd in rice plants under high Cd stress.

Methods

Soil

The uncontaminated top-layer soil (0–20 cm) of the paddy field used for preparing Cd-contaminated soil was sampled from Huaining city, Anhui Province, China. The soil was air-dried and passed through a 2-mm mesh. The $CdCl_2$ solution was sprayed onto the soil, thoroughly mixed and stabilized for 4 weeks at a 60% water holding capacity to reach a soil Cd content of 10.16 mg kg^{-1}. Subsequently, the Cd-contaminated soil was air-dried and passed through a 2-mm mesh for the pot experiment. The main physical and chemical properties were determined as follows: soil pH, 6.56; soil organic carbon (SOC) content, 10.20 mg g^{-1}; total S and Ca $(H_2PO_4)_2$-extractable S contents, 343 and 16.5 mg kg^{-1}, respectively; total Fe and DTPA-extracted Fe contents, 38.4 g kg^{-1} and 48.2 mg kg^{-1}, respectively; and DTPA-extractable Cd content, 8.96 mg kg^{-1}.

Pot experiments

The pot experiment included two treatments: Cd-contaminated soil without amendment (–S) and S addition (+S, 30 mg kg^{-1}) as Na_2SO_4; 30 mg S kg^{-1} was used based on our previous studies [16, 17]. Each treatment was conducted in triplicate. A total of 7 kg of Cd-contaminated soil was placed in each PVC pot (24 cm side length and 20 cm height) with a rhizosphere box (12 cm side length and 20 cm height) made from 400 mesh (30 μm) nylon meshes. A soil basal fertilizer comprising KCl (0.2 g kg^{-1}), $KH_2PO_4 \cdot H_2O$ (0.15 g kg^{-1}) and $CO(NH_2)_2$ (0.2 g kg^{-1}) was applied to each pot. All pots were placed in a greenhouse and stabilized for 1 week under natural climate conditions. The *japonica* rice cultivar Zhongzuo 2002 was used for the experiment. Rice seeds were sterilized in 10% NaClO (*v*/*v*) solution for 20 min and washed with deionized water. Then the seeds were germinated on moist filter paper at 37 ℃ and grown in sterilized moist quartz sand for 3 weeks. Afterward, one uniformly sized rice seedling was transplanted into the rhizosphere box of each pot. During the experiment, the soil in the pots was submerged to a water depth of approximately 2 cm.

Sampling and harvest

Rice plants (roots, stems, leaves and grain) were collected at three growth stages (booting, filling and maturity), equivalent to 53 days, 82 days and 112 days of transplanting rice seedlings. Part of the plant samples were stored at − 80 ℃ for the determination of thiol compound contents after smashing to fine powder under liquid nitrogen. Part of the fresh root samples were used to extract iron plaque on the root surface. Rice was harvested on the 112th day of transplanting, and rice plants were separated into roots, leaves, stems, husks and brown rice for the determination of various tissue biomasses after drying at 70 ℃ for 48 h to a constant weight. Part of the dry plant samples were used to determine Cd and S.

Chemical analysis

Dried plant samples were digested following the method of Matusiewicz et al. [25]. Briefly, 0.2 g of each plant sample was weighed in Teflon digestion vessels, and 8 mL of HNO_3 and 3 mL of 30% H_2O_2 were added. The vessels were placed in a microwave oven

(Mar 6, CEM Corporation, North Carolina, USA) and digested at a temperature of 190 ℃ (5 min ramp, and then held for 35 min). The digested solution was diluted to 50 mL with deionized water and then filtered through a 0.45-μm polyethersulfone filter. The Cd concentrations in the solution were determined by ICP-MS, and S was determined by ICP-OES. The frozen rice leaves, stems and grains at the booting and filling stages were ground to fine powder under liquid nitrogen to determine thiol compounds [glutathione (GSH) and cysteine (Cys)]. The GSH contents were determined according to the DTNB [5,50-dithiobis-(2-nitrobenzoic acid)] method [26]. The Cys content in plant material was estimated by using acid–ninhydrin reagent [27].

Statistical analysis

The transfer factor (TF) was defined as the ratio of the Cd concentration in tissue A compared to that in tissue B, indicating the ability of Cd translocation from A to B [16]. The differences in the physicochemical characteristics of the plant among the two S treatments or rice growth stages were evaluated using one-way analysis of variance with a least significant difference test at a 0.05 significance level. Statistical analyses were performed using SPSS 23.0 (IBM, USA).

Results

Effect of S supply on rice biomass at the maturity stage

The application of S significantly ($p<0.05$) increased the dry weights of brown rice, husks and stems by 215%, 102% and 10%, respectively, compared with the –S treatment (Table 1), implying that the application of S to S-deficient soil could increase rice yield even under severe soil Cd stress. This could be because S supply effectively alleviated Cd-induced inhibition of rice growth and development, especially grain growth.

Effect of S supply on rice S, GSH and Cys concentrations at different growth stages

The concentrations of S, GSH, and Cys in rice were related to S supply and growth stages (Table 2; Fig. 1). The application of S increased the concentrations of S in the roots, stems and leaves of rice at all three growth stages except for the roots at the booting stage, as compared with the –S treatment (Fig. 1). The application of S also increased the concentrations of S in grain at the filling stage, as well as in brown rice and husk at the maturity stage (Fig. 1a). The application of S significantly

Table 1 Effect of sulfur addition on biomass ($g \cdot kg^{-1}$ DW) in the tissues at the mature of rice exposed to Cd-contaminated soil

		Brown rice	Husk	Stem	Leaf	Root
Biomass (g pot^{-1} DW)	–S	5.48 ± 0.32b	9.98 ± 0.68b	15.12 ± 0.26b	7.24 ± 0.41a	4.39 ± 0.49a
	+S	17.28 ± 0.38a	20.2 ± 1.66a	16.69 ± 0.54a	6.95 ± 1.18a	4.32 ± 0.52a

Values are mean ± standard deviations. Values followed by different letters within a column indicate significance at $p < 0.05$ *(LSD)* for sulfur application

–S: without S supply, *+S* with S supply

Table 2 Effects of sulfur addition on content of GSH and Cys in the stem and leaf of rice exposed to Cd-contaminated soil

		Booting Stem	Leaf	Filling Stem	Leaf	Grain
GSH(mg·kg^{-1})	–S	216.3 ± 17.1a	212.7 ± 4.7b	214.9 ± 17.0a	343.6 ± 7.3a	223.8 ± 7.1a
	+S	185.2 ± 8.6b	316.5 ± 22.1a	194.4 ± 3.8a	295.4 ± 18.5b	206.4 ± 7.8b
Cys (mg·kg^{-1})	–S	310.8 ± 21.2a	630.2 ± 22.3b	396.9 ± 19.6a	1081.1 ± 41.3a	277.8 ± 26.8b
	+S	286.1 ± 4.5a	934.2 ± 62.8a	446.9 ± 32.4a	983.5 ± 5.6b	395.5 ± 19.4a
Difference of indexes among two growth stages						
GSH	–S	ns	**			
	+S	ns	ns			
Cys	–S	**	**			
	+S	**	ns			

Values are mean ± standard deviations. Values followed by different letters within a column indicate significance at $p < 0.05$ (LSD) for sulfur application at the same growth stage

ns no difference, *–S* without S supply, *+S* with S supply

** indicate difference of index between booting and filling stage at $p < 0.01$

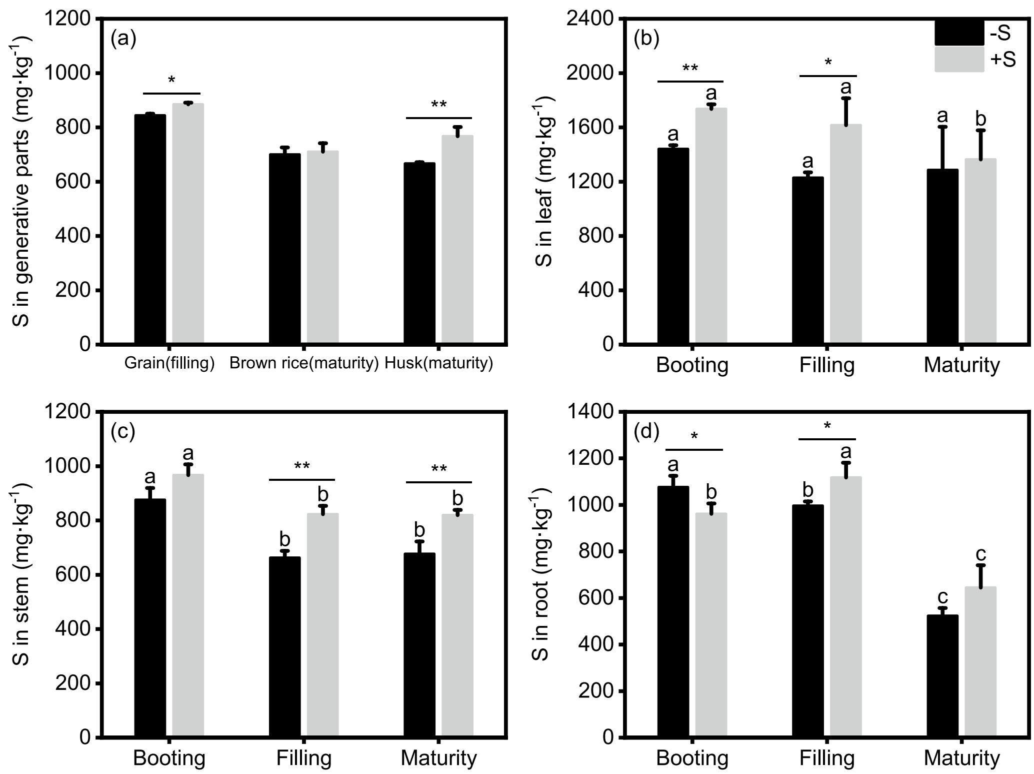


Fig. 1 Effect of sulfur addition on content of sulfur (mg·kg^{-1}) in grain (filling) and brown rice (maturity) (**a**), husk (**a**), leaves (**b**), stems (**c**) and roots (**d**) at the booting, filling and maturity stage of rice exposed to Cd-contaminated soil. Values are mean ± standard deviations. Different letters indicate significance at *$p < 0.05$ (LSD)* for different stage under the same treatment. *, ** indicate difference of index between –S and + S treatments at *$p < 0.05, 0.01$*, respectively. *–S* without S supply, *+ S* with S supply

decreased the GSH and Cys content in stems but increased that in leaves at the booting (Table 2). At the filling stages, the application of S significantly ($p < 0.05$) decreased the GSH content in leaves and grains, as compared with the –S treatment (Table 2). The application of S significantly ($p < 0.05$) decreased Cys content in leaves but increased that in grain at the filling stage (Table 2).

Effect of S supply on rice plant Cd concentrations and TFs at different growth stages of rice

Concentrations of Cd in rice

Obvious differences in Cd concentrations in rice plants were observed in dependence on rice tissues, growth stages and S levels (Fig. 2). The concentrations of Cd in roots showed a decreasing trend from the booting stage to the maturity stage irrespective of S supply (Fig. 2d). The application of S significantly ($p < 0.05$) decreased the concentrations of Cd in roots at the booting and filling stages, as compared with the –S treatment (Fig. 2d). The concentrations of Cd in stems increased from the filling stage to the maturity stage irrespective of S supply, but had a decrease from the booting stage to the filling stage without S supply (Fig. 2c). Compared with the –S treatment, the application of S significantly increased the concentrations of Cd in stems at the filling ($p < 0.05$) and maturity ($p < 0.01$) stages (Fig. 2c). The concentrations of Cd in leaves increased from the filling stage to the maturity stage irrespective of S supply (Fig. 2b). The application of S significantly ($p < 0.05$) decreased the concentrations of Cd in leaves at the filling stage, as compared with the –S treatment (Fig. 2b). The application of S significantly ($p < 0.05$) decreased the concentrations of Cd in brown rice at the maturity stage, as compared with the –S treatment (Fig. 2a). Grain at the filling stage showed a similar trend, but husk at the maturity stage showed an opposite trend.

Cd transfer factors at different growth stages of rice

Obvious differences in Cd transfer factors in rice plants were determined in dependence on growth stages, S levels and among tissues (Table 3). The root/iron plaque Cd-TFs decreased from booting stage to the maturity stage irrespective of the S supply, especially the S supply. In contrast, Cd-TFs for stem/root and leaf/root increased from the booting stage to the maturity stage irrespective of S supply (Table 3). The brown

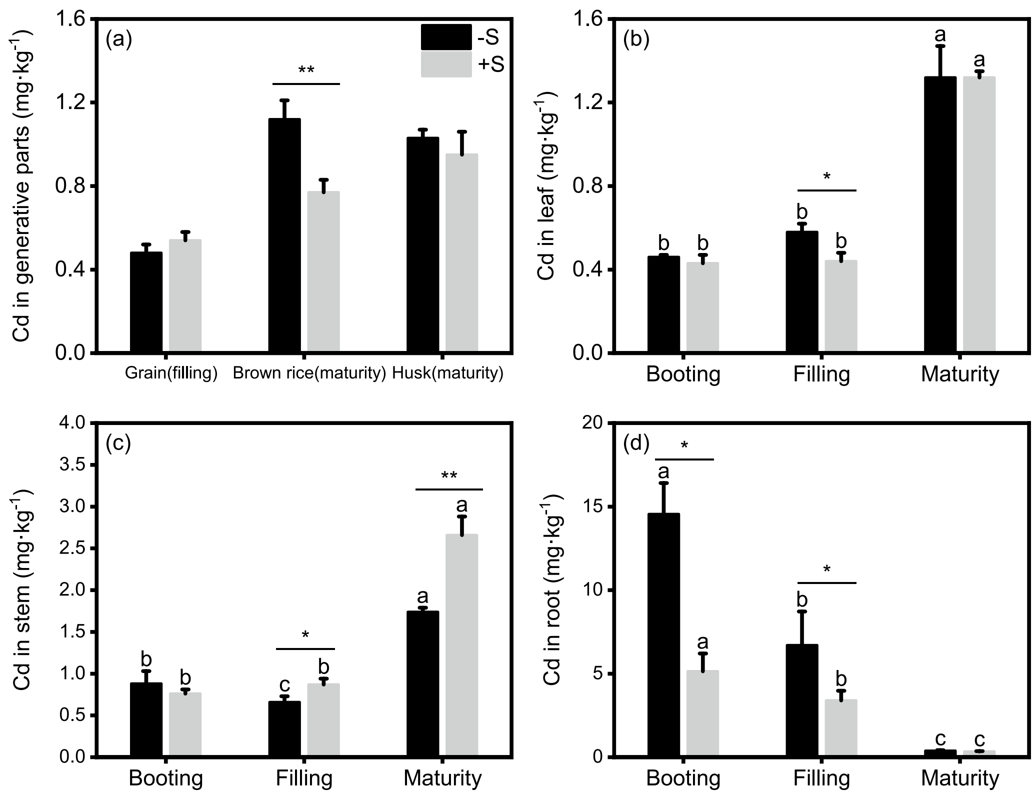


Fig. 2 Effect of sulfur addition on content of cadmium (mg·kg^{-1}) in grain (filling) and brown rice (maturity) (**a**), husk (**a**), leaves (**b**), stems (**c**) and roots (**d**) at the booting, filling and maturity stage of rice exposed to Cd-contaminated soil. Values are mean ± standard deviations. Different letters indicate significance at *p* < *0.05 (LSD)* for different stage under the same treatment. *, ** indicate difference of index between -S and + S treatments at *p* < *0.05, 0.01*, respectively. *–S* without S supply, + *S* with S supply

rice/stem Cd-TFs decreased from the filling stage to the maturity stage irrespective of S supply, especially S supply (Table 3). The brown rice/leaf Cd-TFs decreased from the filling stage to the maturity stage of rice with S supply, and there was no difference without S supply (Table 3). S supply increased the Cd-TFs from roost to stems, leaves, and husks, while decreased the Cd-TFs from stems to leaves and husks and from leaves to husks at the maturity stage (Table 3). Meanwhile, S supply increased the Cd-TFs from iron plaques, roots, stems, leaves and husks to brown rice at the maturity stage (Table 3).

Effect of S supply on distribution of Cd and S in rice plants at maturity stage of rice

Compared with the –S treatment, the application of S significantly reduced the distribution of Cd in the roots and leaves of rice ($p < 0.05$), but had the opposite trend for other tissues (brown rice, husks and stems), with a difference at $p < 0.05$ for brown rice (Table 4).

Compared with the –S treatment, the application of S significantly reduced the distribution of S in the roots, stems and leaves of rice ($p < 0.05$), but the opposite trend was observed for brown rice and husks, with a difference at $p < 0.05$ for brown rice (Table 4).

Discussion

Effects of S supply on rice grain biomass under Cd stress

Sulfur plays a vital role in plant growth and development and acts as a resistance factor against biotic and abiotic stress in plants [7]. Previous studies have shown that the effects of S application on rice biomass are controversial and the effect could be related to the degree of soil Cd contamination and S levels [8, 13]. Fan et al. [8] found that the application of S in Cd-contaminated but high S soil (2.22 mg Cd kg^{-1}, 797 mg S kg^{-1}) reduced rice biomass. However, excessive S supply (60–300 mg S kg^{-1}) in moderately Cd-contaminated soils (3.72 and 5 mg Cd kg^{-1}) was reported to increase the biomass of rice [8, 13]. Similarly, the application of S in low Cd-contaminated (0.79 and 1.5 mg Cd kg^{-1}) and low S soils was also observed to increase rice biomass [9, 12]. In the present study, the application of S (30 mg S kg^{-1}) in Cd-contaminated (available S of 16.5 mg kg^{-1}, total Cd of 10.16 mg kg^{-1}) soil significantly increased the rice

Table 3 Effects of sulfur addition on TFs for Cd in the different tissues at the three growth stages of rice exposed to Cd-contaminated soil

		Root/iron plaque	Brown rice/root	Husk/root	Leaf/root	Stem/root	Brown rice/stem	Husk/stem	Leaf/stem	Brown rice/Leaf	Husk/Leaf	Brown rice/Husk
Booting	–S	0.22	–	–	0.03	0.06	–	–	0.54	–	–	–
	+ S	0.10	–	–	0.09	0.15	–	–	0.56	–	–	–
Filling[a]	–S	0.13	0.076	–	0.09	0.11	0.72	–	0.87	0.83	–	–
	+ S	0.05	0.16	–	0.13	0.26	0.63	–	0.50	1.24	–	–
Maturity	–S	0.03	2.87	2.61	3.37	4.45	0.64	0.59	0.75	0.85	0.79	1.10
	+ S	0.02	2.27	2.82	3.94	7.89	0.29	0.36	0.50	0.58	0.72	0.81

Values are mean ± standard deviations. TFs $_{(A/B)}$ was defined as the ratio of Cd concentration (mg Cd/kg) in tissue A and B

–S without S supply, + *S* with S supply

[a] Grain/root at filling stage

Table 4 Effect of sulfur addition on distribution of Cd and S in the tissues at the mature of rice exposed to Cd-contaminated soil

		Brown rice	Husk	Stem	Leaf	Root
Cd (%)	–S	11.36 ± 0.17b	19.01 ± 2.30a	48.86 ± 1.70a	17.57 ± 0.83a	3.19 ± 0.34a
	+ S	15.13 ± 0.84a	21.99 ± 3.02a	50.64 ± 1.64a	10.58 ± 2.06b	1.66 ± 0.14b
S (%)	–S	11.94 ± 1.02b	20.74 ± 2.62b	31.74 ± 1.13a	28.46 ± 3.65a	7.12 ± 0.66a
	+ S	22.90 ± 0.64a	28.93 ± 2.57a	25.59 ± 2.25b	17.42 ± 1.29b	5.16 ± 0.46b

Values are mean ± standard deviations. Values followed by different letters within a column indicate significance at *$p < 0.05$ (LSD)* for sulfur application

–S without S supply, + *S* with S supply

grain biomass although it had no obvious effect on the root, leaf and stem biomass (Table 1), implying that the application of S could improve rice grain biomass under high Cd stress and S-deficient. The current results are consistent with those reported by [9, 12]. This is because the application of S in S-deficient soils provided sulfur for rice growth and development. Additionally, the application of S reduced the availability of Cd in non-rhizosphere/rhizosphere soils as well as in iron plaque on the rice root surface (Additional file 1: Figs. S1, S3), consequently alleviating the toxicity of Cd to rice. Another reason for this observation could be that application of S could reduce Cd transportation from iron plaque to roots and from both roots and stems to rice grains (Table 3) due to S-induced formation of PCs–Cd complexes and sequestration of Cd into vacuoles [8, 21, 24].

Effects of S supply on the translocation of Cd in rice under Cd stress

Translocation from roots to aboveground plant parts of rice has been suggested as the leading and most common physiological factor for Cd accumulation in shoots and rice grains [19, 20, 28]. The Cd-TF values indicated the ability of Cd translocation and distribution in different rice tissues [16], which were strongly affected by S under Cd stress in the present study (Table 3). Our results indicated that the application of S tends to decrease the root/iron plaque Cd-TFs at the booting, filling and maturity stages (Table 3). The current results are not consistent with those reported by Huang et al. [16] and Huang et al. [17], who observed that the application of S increased the root/iron plaque Cd-TFs at the maturity stage. The S-induced decrease in the root/iron plaque Cd-TFs could be attributed to the decline in Cd contents in roots rather than the increase in Cd contents in iron plaque (Fig. 2; Table 3). Moreover, the application of S increased ACA-extractable Fe at the booting stage (Additional file 1: Fig. S3).

In the present study, obvious differences in the Cd-TFs from roots to aboveground plants of rice were observed between the –S treatment and + S treatment and depended on the rice growth stages and aboveground tissues of rice (Table 3). At the filling stage, the application of S increased the Cd-TFs from roots to stems, leaves, husk and grain (Table 3). This result is due to the decrease in Cd contents in roots and the increase in Cd contents in stems and grain rather than the increase in Cd contents in leaves at the filling stage (Fig. 2). In addition, the application of S increased the Cd-TFs from leaves to grain but reduced the Cd-TFs of Cd from stems to leaves and from stems to grain at the filling stage (Table 3).

At the maturity stage, the application of S also increased the Cd-TFs from roots to stems, leaves and husks, but reduced the Cd-TFs from roots to brown rice (Table 3). This is due to the decrease in Cd contents in roots, husk and brown rice and the increase in Cd contents in stem (Fig. 2). The current results are not consistent with those reported by Zhang et al. [13], who observed that application of S (150 and 300 mg S kg^{-1}) in Cd contaminated soil (total of Cd 5 mg Cd kg^{-1}; total S of 167.08 mg S kg^{-1}) significantly decreased the Cd-TFs from roots to stems and leaves at the maturity stage. The current results are also not completely consistent with those reported by Zhang et al. [12], who observed that application of S (10 mg S kg^{-1}) in Cd-contaminated soil (total Cd of 0.79 mg Cd kg^{-1}; $Ca(H_2PO_4)_2$ extractable S of 26.7 mg kg^{-1}) increased the Cd-TFs from roots to stems, but further increasing the level of sulfur application (40–160 mg S kg^{-1}) reduced the Cd-TFs from roots to stems at the maturity stage [12]. This could be attributed to the differences in soil Cd contamination levels because the tested soil had a heavy Cd contamination level (10.16 mg Cd kg^{-1}) and resulted in high Cd contents (2.5 mg Cd kg^{-1}) in stems at the maturity stage (Fig. 2), which was obviously greater than results reported by Zhang et al. [13] (approximately 0.5 mg Cd kg^{-1}). In addition, application of S reduced the Cd-TFs from leaves to brown rice and from stems to brown rice, husk and leaves at the maturity stage (Table 3), implying that the application of S interfered with Cd transfer from aboveground tissues to brown rice at the maturity stage. This result shows that application of S in S-deficient but highly Cd contaminated soil generally obstructs the translocation of Cd from roots to brown rice and the re-translocation of Cd from aboveground vegetative tissue to reproductive tissue at the maturity stage.

The mechanism of S-regulated Cd transfer in rice during growth stages could be explained by increased S assimilation and glutathione metabolism in plants with S supply [29–31]. S-induced formation of phytochelatins decreases Cd transfer from rice plants to brown rice [1, 8] due to phytochelate-driven sequestration of Cd in vacuoles [1, 31, 32]. Glutathione is the precursor of phytochelates in plants [31, 33]. Fan et al. [8] reported that sulfur application increased the formation of GSH in the leaves of rice at the maturity stage under Cd stress (2.22 mg Cd kg^{-1} and 3.75 mg Cd kg^{-1}). Yang et al. [24] also observed that the application of S induced GSH formation in leaves of rice at the maturity stage under Pb stress. In the present study, at the booting stage, the application of S increased the GSH content in leaves but decrease the GSH content in stems (Table 2), suggesting that Cd was more readily sequestered in leaves than in stems and subsequently facilitated the transfer of Cd from stems to leaves. The leaf/stem Cd-TF was higher with S supply than without S supply (Table 3). At the filling stage, the application of S decreased the GSH content in leaves, which may indirectly reduce the formation of PCs. This was not conducive to the sequestration of Cd in leaves, which increase the transfer of Cd from leaves to grains. An obviously increase in the grain/leaf Cd-TF was observed with S supply than without S supply (Table 3). Additionally, small molecules, such as Cys, may increase the mobility of Cd in plants [16]. In the present study, the application of S decreased the Cys content in leaves but increased the Cys content in stems and grains (Table 2), which could be attributed to the promotion of Cys transfer from leaves to stems and grains, and subsequently promoted the retransfer of Cd from leaves to stem and then to grain (Table 3).

Effects of S supply on concentrations of Cd in rice under Cd stress

Previous studies reported that the application of S in low S (total S < 200 mg kg^{-1}, or $Ca(H_2PO_4)_2$ extractable S < 26.7 mg kg^{-1}) soils significantly reduced the concentration of Cd in rice plants [9, 10, 12, 13]. However, Zhang et al. [15] observed that application of S (114–458 mg S kg^{-1}) in S-rich soils increased the concentrations of Cd in roots, stems, leaves and grains. In the present study, the effects of S application on the Cd concentration in grain depended on the rice growth stages (Fig. 2a). The application of S tended to increase concentration of Cd in grain at the filling stage (Fig. 2a). The result was similar to that reported by Zhang et al. [15]. This could be predominantly attributed to S-induced promotion of Cd transfer from rice plants to grain at the filling stage, because the Cd-TFs from roots to stems, leaves and grain as well as from leaves to grain were obviously higher in the + S treatment than in the –S treatment at the filling stage (Table 3). In addition, S induced the accumulation of Cd in iron plaques and increased the source of Cd uptake by rice at the filling stage, because ACA-extractable Cd in iron plaques was greater in the + S treatment than in the –S treatment at the filling stage (Additional file 1: Fig. S3).

In the present study, the application of S significantly reduced the concentration of Cd in brown rice at the maturity stage, and a similar phenomenon was observed in the husk (Fig. 2a). The result was consistent with those reported by Zhang et al. [12] and Zhang et al. [13]. This could be attributed to three aspects of the mechanism. First, S induced a decline in the availability of Cd in soil and iron plaque on the root surface. The application of S reduced dissolved Cd in soil pore water and soil labile Cd fractions (exchangeable-Cd, carbonate-Cd) during the whole rice growth period (Additional file 1: Figs. S1, S2), which resulted in significant decline in soil Cd availability. Moreover, S induced the accumulation and sequestration of Cd in iron plaques at the filling and maturity stages (Additional file 1: Fig. S3). ACA-extractable Cd was negatively correlated with brown rice Cd ($r^2 = -0.946$, $p < 0.01$, $n = 6$) at maturity stage (Additional file 1: Table S2). In addition, S supply inhibited the transfer of Cd from iron plaque to roots, because the root/iron plaque Cd-TFs were obviously lower with S supply than without S supply during the whole rice growth period, and then reduced the uptake of Cd in roots (Table 3). Second, S supply inhibited the transfer of Cd in other tissues to brown rice. The Cd-TFs from roots, stems, leaves and husk to brown rice were obviously lower with S supply than without S supply at the maturity stage (Table 3). In addition, S supply inhibited the retransfer of Cd from stems to leaves during the whole rice growth stage. The Cd-TFs from stems to leaves were obviously lower with S supply than without S supply during the whole rice growth stage. Furthermore, S supply inhibited retransfer of Cd from stems/leaves to husks; the Cd-TFs from stems and leaves to husks were obviously lower with S supply than without S supply at the maturity of rice (Table 3). Third, S induced the dilution of Cd in brown rice because the application of S significantly increased brown rice biomass by 215%. The application of S alleviated the deficiency of S in rice, because the application of S increased the concentration of S rice plants grown under soil S deficiency ($Ca(H_2PO_4)_2$-extractable S content of 16.5 mg kg^{-1}) (Fig. 1). The application of S alleviated Cd stress and toxicity, because the application of S reduced the concentration of Cd in roots during the whole rice growth period, in leaves at the booting and filling stages, and in brown rice and husk at the maturity stage (Fig. 2). Ultimately, the application of S increased brown rice and husk biomass by 215% and 102%, respectively. This was one of the reasons for the decrease in Cd concentration in brown rice, although application of S increased the distribution of Cd in brown rice (Table 4).

Implication of S-regulated accumulation of Cd in brown rice

In China, the accumulation of Cd in paddy soils and concentrations of Cd in rice have exhibited an increasing trend in the past 10 years [6]. The average Cd concentrations in paddy soils are in the range of 0.018–4.230 mg/kg [6], and some soil Cd concentrations are as high as 8.40 mg kg^{-1} and 27.3 mg kg^{-1} [14, 34]. The average Cd concentrations in rice were in the range of 0.002–1.212 mg/kg and the proportion of rice Cd content > 0.2 mg/kg (the National Food Safety Standard, GB 2762-2017) was 75% [6]. Implying that some high Cd polluted rice fields could still be used to cultivate rice due to limited land resources and the enormous food demand [35]. The present results show that the concentration of Cd in brown rice grown in 10 mg Cd kg^{-1} soil ranged from 0.77 mg kg^{-1} to 1.12 mg kg^{-1} (Fig. 1).

Sulfur is one of the essential nutrient elements for the growth and development of all plants. Soil sulfur has great spatial variability, and the concentration of total S in paddy soils is 102–895 mg kg^{-1} in China [35]. In some places of southern China, due to the high temperatures and abundant rainfall, organic S is easily mineralized and leached out of the soil, leading to soil S deficiency, especially in sandy soil [35]. The results obtained from the current study demonstrated that the application of S to rice under waterlogging conditions not only increased rice yield, but also decreased the concentration of Cd in brown rice grown in high-Cd contaminated paddy soil (Table 1, Fig. 2). Although the concentration of Cd in brown rice exceeded the national rice cadmium content standard (0.2 mg/kg, GB 2762-2017), it also showed that the application of sulfur fertilizer could reduce the risk of Cd to human health to a certain extent in deficit S but heavy Cd-contaminated paddy soil. This study provides valuable information for growing rice in low-S and Cd-contaminated paddy soil and reducing the risk of Cd in rice to humans.

Conclusions

Our results revealed that the application of sulfur had significant effects on Cd transfer and concentration in rice plants, which was related to the growth stages. The application of S tended to increase the concentration of Cd in grain at the filling stage, but significantly the reduced concentration of Cd in grain (brown rice and husk) at the maturity stage of rice. The S-induced decrease in brown rice Cd could be attributed to three aspects. First, S supply reduced the availability of Cd in soil and iron plaque on the root surface by reducing dissolved Cd in soil pore water and transferring Cd from iron plaque on the root surface to roots. Second, S supply inhibited the transfer of Cd in other tissues to brown rice based on Cd transfer factors from roots, stems, leaves, husks to brown rice, which was obviously lower with S supply at the maturity stage. Third, S induced the dilution of Cd in brown rice because the application of S significantly increased brown rice biomass by 215%. The current study demonstrated that the application of S not only increased rice yield but also decreased the concentration of Cd in brown rice grown in high-Cd contaminated paddy soil under waterlogging conditions. This study provides valuable information for growing rice in low-S and Cd contaminated paddy soil and reducing the risk of Cd in rice to humans.

Abbreviations

GSH	Glutathione
Cys	Cysteine
PCs	Phytochelatins
SOC	Soil organic carbon
DTPA	Diethylenetriaminepentaacetic acid
ICP-MS	Inductively coupled plasma-mass spectrometry
ICP-OES	Inductively coupled plasma optical omission spectrometer
TF	Transfer factor
ACA	Ascorbic–citric–acetic acid

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00388-6.

Additional file 1: Table S1. Correlation coefficients between ACA-Cd and content of Cd in rice tissue at filling stage. **Table S2.** Correlation coefficients between ACA-Cd and content of Cd in rice tissue at maturity stage. **Fig. S1.** Effect of sulfur addition on dynamic dissolved Cd in soil pore water at four growth stages of rice exposed to Cd-contaminated soil. rhizosphere-S: without S supply in rhizosphere soil; rhizosphere+S: with S supply in rhizosphere soil; non-rhizosphere-S: without S supply in non-rhizosphere soil; non-rhizosphere+S: with S supply in non-rhizosphere soil. **Fig. S2.** Effect of sulfur addition on distribution of Cd fractions in non-rhizosphere and rhizosphere soils at four growth stages of rice exposed to Cd-contaminated soil. -S: without S supply; +S: with S supply. **Fig. S3.** Effect of sulfur addition on ACA-extractable Fe (a) and Cd (b) (g kg^{-1} DW) in iron plaque on the surface of rice root exposed to Cd-contaminated soil at the booting, filling and maturity stage. Values are mean ± standard deviations. Different letters indicate significance at $p < 0.05$ *(LSD)* for different stage under the same treatment. *, ** indicate difference of index between -S and +S treatments at $p < 0.05, 0.01$, respectively. –S: without S supply; +S: with S supply.

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Author contributions

SL wrote the original draft and edited the manuscript. SL, GW, XY and XK carried out the experiment, collected and analyzed data. SL, LH, YL, YQ and MT prepared illustrations, figures, tables, and references. ZH designed and conceptualized the study, funding acquisition. ZH supervised, reviewed, and edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data are presented in the manuscript.

Declarations

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The manuscript is an original work that has not been published in other journals. The authors declare no experiments involving humans and animals.

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Competing interests
The authors declare that they have no competing interests.

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