Variability in cadmium uptake in common wheat: impact of genetic variation and silicon supplementation

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Abstract

Purpose To decrease the transfer of cadmium (Cd) to the food chain, it is essential to select wheat (*Triticum aestivum* L.) germplasm that accumulates the least amount of Cd and to develop management practices that promote a reduction in Cd uptake. This requires knowledge of factors controlling Cd accumulation in wheat plants, which are not fully understood. The aim of this study was thus to investigate variations in Cd accumulation, translocation, and subcellular distribution in response to supplemental Si in two wheat cultivars that have different Cd accumulation capacities.

Methods Cd uptake and distribution in two common wheat cultivars, high-Cd 'LCS Star' and low-Cd 'UI Platinum' were evaluated at two levels of Cd (0 and 50 μM) and Si (0 and 1.5 mM) in a hydroponic experiment.

Results LCS Star and UI Platinum were not different in root Cd accumulation but differed in Cd concentration in the shoot, which agreed with the variation between the two cultivars in their subcellular Cd distributions (i.e., cell wall and organelle and soluble fractions) as well as induced glutathione synthesis in response to Cd addition. Supplemental Si reduced Cd uptake and accumulation and suppressed Cd-induced glutathione synthesis.

Conclusions The differences between the wheat cultivars in Cd accumulation in shoot mainly derive from root-to-shoot translocation, which is related to subcellular Cd distribution and Cd-induced glutathione synthesis. Exogeneous Si could decrease Cd translocation from root to shoot to alleviate Cd toxicity in common wheat.

Introduction

Cadmium (Cd) is one of the most toxic contaminants and is a risk to the environment and the quality of our food (Centers for Disease Control and Prevention 2011). Cd can accumulate in cereal grains (e.g., rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and barley (*Hordeum vulgare* L.)) and transfer to the food chain via the consumption of cereal-derived food products (Greger and Lofstedt 2004; Jafarnejadi et al. 2011). Therefore, developing cereal germplasm with low Cd accumulation capacities could be a promising approach to reducing Cd transfer to the food chain and subsequent Cd toxicity. This approach requires an improved understanding of mechanisms controlling Cd accumulation and Cd tolerance in cereal plants.

After being absorbed into the root epidermis, Cd is transported through the cortex and endodermis and then enters the stele of roots for long-distance transport to shoot (Clemens et al. 2013; Dong et al. 2017; Uraguchi et al. 2009; Uraguchi and Fujiwara 2013). Root-to-shoot translocation has been identified as the major process determining Cd accumulation (Uraguchi et al. 2009), and there are genetic differences in root-to-shoot translocation. For instance, transgenic *Arabidopsis* enhanced root-to-shoot Cd translocation and Cd accumulation in shoot compared with the wild type (Gong et al. 2003). High-Cd genotypes had a greater percentage of Cd translocation from root to shoot (or translocation factor) in hot pepper

(*Capsicum annuum* L.) (Xin et al. 2013) and durum wheat (*Triticum turgidum* ssp. durum) (Harris and Taylor 2013; Perrier et al 2016).

After entering plant cells, Cd can be sequestered in the cell wall by binding to phosphates, proteins, peptides, and polysaccharides (Lu et al. 2021; Parrotta et al. 2015; Wei et al. 2021). For detoxification and tolerance of the stress, Cd can also be transported to vacuole as free Cd or Cd-phytochelatin complexes (Hu et al. 2021; Luo and Zhang 2021). Phytochelatins are usually synthesized from glutathione in response to heavy metals such as Cd (Farooq et al. 2016; Nakamura et al. 2020). Glutathione also acts as a scavenger of reactive oxygen species to alleviate the redox imbalance caused by Cd toxicity (Li et al. 2021; Lu et al. 2021; Hasanuzzaman et al. 2017). Genetic variations have been found in Cd subcellular distribution (e.g., cell wall, organelles, and cytosol) and syntheses of glutathione and phytochelatins. In common wheat, more Cd may be sequestered in the cell wall than in organelle and cytosol, but genotypes of different sensitivities to Cd stress may differ in Cd concentration in individual subcellular fractions (Jian et al. 2020; Lu et al. 2021). A Cd-tolerant genotype of common wheat may have a greater glutathione concentration compared with the Cd-sensitive genotype (Lu et al. 2021), whereas the wild-type *Arabidopsis* had smaller concentrations of glutathione and phytochelatins and lower Cd accumulation in shoot compared with transgenic plants with enhanced ability to synthesize glutathione (Nakamura et al. 2020).

Exogeneous Si has shown ameliorative effects on Cd-stressed plants via modulation of multiple processes and pathways in Cd uptake and accumulation. Si addition can decrease Cd uptake by an increase in root exudates (e.g., oxalate) that bind to Cd and prevent its absorption into the plant root, thus reducing Cd bioavailability (Wu et al. 2016). Greger and Landberg (2008) showed that Cd accumulation in wheat grain was reduced in response to supplemental Si due to a decrease in translocation of Cd from root to shoot. Si addition also decreased Cd accumulation in subcellular fractions (e.g., cell wall, organelles, and cytosol) in the shoot of rice (Shi et al. 2005) and peanut (*Arachis hypogaea* L.) (Shi et al. 2010), and root of common wheat (Wu et al. 2016). Cd-induced oxidative damage (e.g., membrane lipid peroxidation and reactive oxygen species) can be alleviated by supplemental Si because Si regulates the ascorbate-glutathione cycle and improves antioxidant defense against Cd toxicity (Farooq et al. 2013, 2016; Hasanuzzaman et al. 2017). Exogeneous Si can regulate phytochelatin synthesis by regulating the phytochelatin synthase gene (Farooq et al. 2016; Greger et al. 2016).

Plant accumulation of Cd is highly variable due to differences in uptake, translocation, and tolerance capacity among different plant species and genotypes. Many studies have shown contradictory Cd bioaccumulation, distribution, and toxicity effects, which likely is a result of diversities in strategies and mechanisms to tolerate Cd toxicity (Greger et al. 2016; Jian et al. 2020; Lu et al. 2021; Nakamura et al. 2020; Wu et al. 2016). Despite plenty of evidence on exogenous Si improving plant tolerance of Cd stress (Greger et al. 2016; Hussain et al. 2015; Naeem et al. 2015; Wu et al. 2016), there is a lack of investigations of the alleviatory role of Si in diverse genetic backgrounds of common wheat. This information is essential for selecting common wheat germplasm with low Cd accumulation and developing management practices to reduce Cd accumulation in common wheat. Thus, the objective of

the current study was to investigate variations in Cd accumulation, translocation, and subcellular distribution in response to supplemental Si in wheat cultivars of different Cd accumulation capacities.

Materials And Methods

Plant materials and experimental design

A greenhouse experiment was conducted in a hydroponic system at the Aberdeen Research and Extension Center, the University of Idaho in Aberdeen, Idaho (40.95°N, 112.83°W; elevation 1342 m). Wheat seeds were surface sterilized in 0.5% NaOCl solution for 20 min and placed on filter paper saturated with deionized water in Petri dishes for five days. Four seedlings of similar sizes were selected and transferred to a 2.5-L plastic pot containing modified Hoagland solution with 5 mM $Ca(NO_3)_2$, 5mM KNO_3, 1 mM KH_2PO_4, 2 mM $MgSO_4$, 45 µM H_3BO_3, 10 µM $MnCl_2$, 20 µM EDTA-Fe, 0.8 µM $ZnSO_4$, 0.3 µM $CuSO_4$, and 0.4 µM Na_2MoO_4. The pH of the nutrient solution was measured weekly and adjusted to 6.0 ± 0.2 by adding 1 M HCl.

The experiment followed a randomized complete block design with four replications and was repeated twice in 2018 (from February 19 to March 21 and from April 9 to May 9). Two levels of Si (0 and 1.5 mM) and Cd (0 and 50 µM) were applied to two spring wheat cultivars: low-Cd ‘UI Platinum’ and high-Cd ‘LCS Star’ (Liang et al. 2017). The level of Cd addition was to create toxicity in plants, and the level of Si addition was to create significant impacts on Cd uptake in plants subject to Cd stress (Greger et al. 2016; Shi et al. 2005; Wu et al. 2016). The Si and Cd treatments were initiated seven days after seedings were transferred to the hydroponic pots and supplied as Na_2SiO_3 and $CdCl_2$, respectively. An equivalent amount of Na as Na_2SO_4 was added to the zero Si treatment to compensate for the Na content of 1.5 mM Si-treated plants. The hydroponic solution in each pot was continuously aerated using an air pump and replaced with a new solution every seven days. After exposure to the Si and Cd treatments for 21 days, plants were harvested at an early booting stage (Zadoks 39–41) (Zadoks et al. 1974). The average air temperature was 24.4°C during the first repeat and 24.0°C during the second repeat in the greenhouse.

Biomass and root morphology

The shoot and root of each plant were separated at harvest. Roots were soaked in ice-cold 10 mM $CaCl_2$ solution for 10 min to displace extracellular Cd, rinsed in deionized water, and dried with tissue paper. The fresh roots were scanned using a high-resolution scanner (Expression STD4800, Epson America, Inc., USA), and the images were analyzed using the WinRHIZO Pro 2013 software (Regent Instrument Inc., Quebec, CA) for root morphological characteristics including root length, surface area, volume, and average diameter. A part of the fresh shoots and roots were frozen in liquid nitrogen and stored at -80°C until further analysis. The rest shoot and root samples were dried in an oven at 75°C until the constant weight was achieved.

Cd concentration and subcellular distribution in shoot and root

Dried shoots and roots were ground to a fine powder using a cyclone sample mill (UDY Corporation, Fort Collins, CO) and digested following the method of Huang and Schulte (1985). In detail, 0.5 g of plant tissue was added to 5 mL of concentrated HNO_3 and pre-digested at 60°C for 30 min using a block digestor (Environmental Express, Charleston, SC). Then 3 mL of 30% H_2O_2 was added to the warm samples. Samples were further digested at 120°C for 90 min. The final digested solution was filtered through Whatman No. 42 filter paper (GE Healthcare, Chicago, IL), diluted to 50 mL using deionized water, and analyzed for total Cd using an inductively coupled plasma (ICP) spectrometer standardized using certified Cd standards (iCAP 6500, Thermo Scientific, Waltham, MA).

The translocation factor (TF) of Cd was calculated as Cd concentration in shoot divided by Cd concentration in root multiplied by 100. Cd content in shoot or root biomass was expressed as μg $plant^{-1}$, which resulted from multiplying Cd concentration (μg g^{-1}) by either shoot or root biomass (g $plant^{-1}$). Cd uptake per unit root length (μg m^{-1}) was calculated as the amount of Cd accumulated in the whole plant (shoot and root) divided by root length (m $plant^{-1}$).

Subcellular distribution of Cd was determined according to Weigel and Jäger (1980) with slight modifications. Briefly, lyophilized shoots and roots were homogenized in a fractionation solution containing 250 mM sucrose, 50 mM Tris-HCl buffer (pH 7.5), and 1 mM dithioerythritol (DTE). The homogenized solution was filtered through cheesecloth, and the residue on the cheesecloth was washed three times with the same fractionation solution. To obtain the total cell wall, the filtrate was centrifuged at 300 g for 30 s. The gained pellet plus the plant residues retained on the cheesecloth was regarded as cell wall fraction. The filtrate was then centrifuged at 20,000 g for 45 min, and the obtained pellet was considered as organelle fraction, while the supernatant was the soluble fraction. The whole procedure was accomplished at 4°C. The concentration of Cd in the soluble fraction was determined directly using the ICP spectrometer, whereas the cell wall and organelle fractions were firstly digested using the above-described method and then determined using the ICP spectrometer.

Apoplastic bypass flow

Apoplastic bypass flow was estimated by analyzing the transport of fluorescence tracer trisodium 8-hydroxypyrene-1,3,6-trisulfonate (PTS) from root to shoot following the method of Yeo et al (1987). Immediately prior to harvest, one seedling from each pot was transferred to the same nutrient solution with the corresponding Si and Cd levels containing 30 mg L^{-1} PTS. After 24 h, 1 g of the fresh shoot was cut into small pieces and extracted in 10 mL of deionized water at 90°C for 2 h. The fluorescence was measured at 403 nm excitation and 510 nm emission (Biotek Epoch 2, BioTek Instruments, Inc., Winooski, VT, USA).

Glutathione in shoot and root

Concentrations of glutathione and glutathione disulfide in shoot and root were determined according to Dempsey et al (2012) with slight modifications. 0.2 g of frozen plant tissue was homogenized with 1 mL of 0.1 M HCl and 0.1 mM EDTA (assay buffer) and centrifuged at 20,000 g for 15 min to get a clear

supernatant. Then a fresh working solution, containing the assay buffer, glutathione reductase (500 U, 20 μL mL^{-1} assay buffer), and DTNB (1.5 mg mL^{-1} dimethyl sulfoxide, w/v) in a ratio of 9.1:1:1 was prepared. For total glutathione (glutathione + glutathione disulfide), 20 μL of the supernatant was mixed thoroughly with 80 μL of the working solution and 80 μL of an NADPH solution (0.25 mg reduced NADPH mL^{-1} assay buffer, w/v). For glutathione disulfide, 4 μL of 10% 2-vinyl pyridine in the assay buffer (v/v) was mixed with 20 μL of the supernatant and incubated at room temperature for 60 min. Then 13 μL of 9% triethanolamine (TEA) in the assay buffer (v/v) was added. After incubation at room temperature for another 10 min, 80 μL of the working solution and 80 μL of the NADPH solution were added and thoroughly mixed. The absorbance was measured at 412 nm and the concentrations of total glutathione and glutathione disulfide were expressed as glutathione equivalents. The difference between total glutathione and glutathione disulfide is presented as the glutathione concentration.

Statistical analysis

Data were analyzed using the generalized linear mixed model (proc glimmix) in SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). Fixed effects included Cd level, Si level, wheat cultivar, and their interactions, and repeat and block were treated as random effects. Since the Cd treatments were significant in most variables and had interactive effects with other factors, data analysis in all variables was performed by Cd level. All variables (or variable transformations) were visually analyzed for variance homogeneity and passed the Shapiro-Wilk W test ($P > 0.05$) for normality. Treatment effects were considered significant at $P \leq 0.05$, and pairwise comparisons were made using the lsmeans statement with the Fisher's Least Significant Difference method at a significance level of 0.05. Spearman's correlation analysis was conducted using the corrplot package in R (R Core Team 2013). Preparation of all figures was performed using R.

Results

Shoot and root biomass and root morphology

Without Cd addition, UI Platinum + Si produced greater shoot biomass than UI Platinum–Si and LCS Star regardless of Si level (Fig. 1a). Under Cd stress, there was no difference in shoot biomass between the two wheat cultivars, but Si supplementation improved shoot growth (Fig. 1b). No difference in root biomass was found between the two cultivars at either Cd level (Fig. 1c and 1d). Si supplementation improved root growth in Cd-treated plants, but root biomass of the -Cd + Si treatment was lightly smaller than -Cd-Si plants.

LCS Star had greater root length, surface area, and volume than UI Platinum without Cd addition (Table 1). Under Cd stress, the two cultivars showed minimal differences in root morphological characteristics, but supplemental Si increased root length and surface area and decreased average diameter. UI Platinum + Si and LCS Star + Si had greater root volume than LCS Star–Si and UI Platinum–

Si when received Cd addition. No significant difference in Cd content in shoot or root was found between cultivars or Si levels at either Cd level (Fig. 1e-h).

Table 1
Root morphological characteristics affected by cultivar and Si level without and with Cd addition.

Treatment	Root length	Root surface area	Root volume	Root average diameter
	m plant^{-1}	cm^2 plant^{-1}	cm^3 plant^{-1}	mm
No Cd applied				
Cultivar				
UI Platinum	24.70 B*	234.3 B	1.778 B	0.301
LCS Star	28.39 A	262.6 A	1.953 A	0.293
Si level				
No Si applied	26.6	253.3	1.938	0.302
Si applied	26.5	243.6	1.797	0.291
Source of variance				
Cultivar	< 0.001	0.001	0.037	0.243
Si	0.927	0.252	0.097	0.077
Cultivar × Si	0.592	0.538	0.525	0.833
Cd applied				
Cultivar				
UI Platinum	6.699	78.46	0.747	0.389
LCS Star	6.837	79.47	0.741	0.384
Si level				
No Si applied	4.188 B	54.25 B	0.56	0.415 A
Si applied	9.348 A	103.7 A	0.925	0.359 B
Source of variance				
Cultivar	0.770	0.830	0.881	0.521
Si	< 0.001	< 0.001	< 0.001	< 0.001
Cultivar × Si	0.130	0.060	0.028	0.780

*Means followed by different letters within a column differ significantly by cultivar or Si level ($P \leq 0.05$).

Cd concentration and subcellular distribution in shoot and root

In plants without Cd addition, the two cultivars did not differ in Cd concentration in the shoot, but supplemental Si reduced Cd concentration in the shoot (Fig. 2a). In the subcellular distribution, no difference was observed between cultivars in Cd concentration in the cell wall, organelle, or soluble fractions. Supplemental Si decreased Cd concentration in the organelle fraction.

Under Cd stress, LCS Star–Si had the highest Cd concentration in shoot followed by UI Platinum–Si and the two cultivars with Si supplementation (Fig. 2b). Cultivars were not different in Cd concentration in the cell wall fraction, but supplemental Si reduced the concentration. Cd concentrations in the organelle and soluble fractions in LCS-Star–Si was greater than in the other three treatments, but the differences between UI Platinum–Si and UI Platinum + Si in both fractions were not significant.

In the root of plants without Cd addition, no difference was observed in Cd concentration in the whole tissue or the organelle fraction (Fig. 2c). LCS Star–Si had a greater Cd concentration in the cell wall fraction than UI Platinum–Si and LCS Star + Si. Greater Cd concentration in the soluble fraction was observed in UI Platinum + Si followed by LCS Star + Si, LCS Star–Si, and UI Platinum–Si.

In Cd-treated plants, cultivars were not different in Cd concentration in the root or any subcellular fraction, and supplemental Si decreased Cd concentration in root tissue and the organelle and soluble fractions (Fig. 2d).

Glutathione (GSH) and glutathione disulfide (GSSG)

The concentration of GSH or GSSH in the shoot was not different between the two cultivars or Si levels in plants that were not treated with Cd (Table 2). In Cd-treated plants, supplemental Si failed to change the concentration of GSH or GSSG in the shoot, whereas UI Platinum had smaller concentrations of both thiol products in the shoot compared with LCS Star. In the root, the concentration of GSH or GSSG did not differ between cultivars at either Cd level, and supplemental Si reduced the concentration of GSH in the root regardless of Cd level.

Table 2
Concentrations of glutathione (GSH) and glutathione disulfide (GSSG) in shoot and root, translocation factor (TF), Cd uptake per unit root length, and apoplastic bypass flow affected by Si level and cultivar without and with Cd addition.

Treatment	Shoot		Root		TF	Cd uptake per unit root length	Apoplastic bypass flow
	GSH	GSSG	GSH	GSSG			
	nmol g^{-1} fw		nmol g^{-1} fw		%	µg m^{-1}	nmol PTS g^{-1} fw
No Cd applied							
Cultivar							
UI Platinum	1046	527.2	8.391	1.282	7.174	0.078	13.34 A
LCS Star	891.7	572.6	6.031	1.882	6.352	0.080	8.538 B
Si level							
No Si applied	905.6	562.5	10.63 A	1.406	8.296A	0.092	12.74 A
Si applied	1033	603.1	3.79 B	1.757	5.494 B	0.068	9.137 B
Source of variance							
Cultivar	0.443	0.308	0.477	0.273	0.493	0.894	0.002
Si	0.546	0.953	0.050	0.516	0.026	0.120	0.015
Si × Cultivar	0.294	0.864	0.289	0.882	0.892	0.118	0.151
Cd applied							
Cultivar							
UI Platinum	916.6 B*	583.4 B	13.36	1.598	1.754 B	27.85	2.710
LCS Star	1598 A	756.0 A	15.47	1.908	2.200 A	26.59	3.075
Si level							
No Si applied	1280	684.4	18.86 A	3.050	1.677 B	44.39 A	4.214 A
Si applied	1235	655.0	9.963 B	2.846	2.302 A	16.68 B	1.571 B

*Means followed by different letters within a column differ significantly by cultivar or Si level ($P \leq 0.05$).

Treatment	Shoot		Root		TF	Cd uptake per unit root length	Apoplastic bypass flow
	GSH	GSSG	GSH	GSSG			
Source of variance							
Cultivar	0.008	0.050	0.164	0.323	0.050	0.702	0.582
Si	0.850	0.730	< 0.001	0.153	0.010	< 0.001	< 0.001
Si × Cultivar	0.863	0.608	0.645	0.331	0.748	0.504	0.080

*Means followed by different letters within a column differ significantly by cultivar or Si level ($P \leq 0.05$).

Translocation factor, Cd uptake, and apoplastic bypass flow

LCS Star had a greater translocation factor than UI Platimum under Cd addition; supplemental Si increased translocation factor under Cd stress but decreased the factor without Cd addition (Table 2). Cd uptake per unit root length did not differ between cultivars or Si levels in plants that did not receive Cd addition. Under Cd stress, supplemental Si reduced Cd uptake per unit root length, whereas no difference was found between cultivars. Si supplementation significantly reduced apoplastic bypass flow at both Cd levels. UI Platinum had greater apoplastic bypass flow than LCS Star in plants without Cd addition, while the cultivars had similar apoplastic bypass flow under Cd stress.

Correlations

Without Cd addition, Cd concentration in the shoot was positively correlated with Cd concentrations in root and all three subcellular fractions of the shoot, and Cd uptake per unit root length (Fig. 3a). Cd concentration in root was positively correlated with Cd concentrations in the cell wall and organelle fractions of root and cell wall and soluble fractions of the shoot, and Cd uptake per unit root length, but negatively correlated with translocation factor.

Under Cd stress, Cd concentration in the shoot was positively correlated with Cd concentrations in the root, all three subcellular fractions of the shoot, and soluble and organelle factions of the root, as well as Cd uptake per unit root length, GSSG concentration in the shoot, and GSH concentration in the root (Fig. 3b). Cd concentration in root was positively correlated with Cd concentrations in all subcellular fractions of root and shoot, apoplastic bypass flow, Cd uptake per unit root length, and GSH concentration in the root, but negatively correlated with translocation factor.

Discussion

After being absorbed into the epidermis, Cd is transported through the cortex and endodermis and then enters the stele of roots for long-distance transport to shoot (Clemens et al. 2013; Dong et al. 2017; Uraguchi et al. 2009; Uraguchi and Fujiwara 2013). In the current study, the two wheat cultivars were not

different in Cd concentration in the root, but greater Cd concentration was found in the shoot of LCS Star than UI Platinum in the treatment of + Cd-Si (Fig. 2b and 2d). LCS Star also had a greater translocation factor under Cd addition (Table 2). Thus, the differences between cultivars in Cd accumulation in shoot mostly derived from root to shoot translocation, and the low-Cd cultivar can restrict Cd translocation from root to shoot efficiently compared with the high-Cd cultivar (Perrier et al. 2016). The positive correlation between shoot and root Cd concentrations (Fig. 3) also suggests the contribution of root-to-shoot translocation to Cd accumulation in the shoot. Our findings agree with Dong et al. (2017) that tall fescue and Kentucky bluegrass had similar Cd concentrations in the root, but Kentucky bluegrass transported more Cd into its root stele and had greater leaf Cd concentration. These differences could be related to transporters at the endodermis and vascular bundles, such as natural resistance-associated macrophage proteins (NRAMP) (e.g., OsNramp5 in rice) and heavy-metal ATPase (HMA) transporters (e.g., OsHMA2 in rice and HMA4 in *Arabidopsis halleri*) (Clemens et al. 2013; Courbot et al. 2007; Sasaki et al. 2012; Takahashi et al. 2012; Uraguchi and Fujiwara 2013).

Compartmentation of heavy metals into tissues that are less metabolically active (e.g., cell wall and vacuole) is an important mechanism of tolerance to heavy metal toxicity (Clemens 2006; Clemens et al. 2013). In the current study, LCS Star and UI Platinum accumulated more shoot Cd in their organelle fraction, followed by the cell wall and soluble fraction, and cultivar differences in shoot Cd concentration under Cd addition mainly derived from differences in Cd accumulation in organelle and soluble fraction (Fig. 2b and 2d). However, Wu et al. (2016) found that most Cd accumulated in the soluble fraction and least in the organelle fraction in common wheat. In another study on common wheat, the genotype insensitive to Cd stress accumulated the least Cd in the organelle, whereas the least in the soluble fraction for the sensitive genotype; both genotypes had the most Cd accumulated in the cell wall (Jian et al. 2020). The compartment of Cd in the cell wall accounted for more subcellular Cd accumulation than organelle and soluble fractions in pepper cultivars regardless of their differences in Cd accumulation capacities (Hu et al. 2021). Thus, plant species and genotypes may have various intracellular distributions and thus different mechanisms to tolerate Cd toxicity (Jian et al. 2020; Hu et al. 2021; Wu et al. 2016).

In our study, glutathione concentration in the root was positively correlated with root Cd concentrations in Cd-treated plants (Fig. 3b), which agrees with findings that enhancing exogenous glutathione in the rhizosphere increases Cd accumulation in the root by forming phytochelatins and sequestering Cd in the root cell wall (Li et al. 2021; Nakamura et al. 2020). Furthermore, glutathione concentration in the root was not different between the two cultivars, but the concentration in the shoot of low-Cd UI Platinum was lower than high-Cd LCS Star (Table 2; Fig. 2b), suggesting that 1) UI Platinum might have low free Cd in the cytosol of the shoot as there was no increase in glutathione concentration in the shoot of UI Platinum between treatments with and without Cd addition (Table 2); 2) less Cd is translocated from root to shoot and thus induces less glutathione production in the shoot of UI Platinum. The cultivar differences in our study agree with Nakamura et al. (2020) that Cd concentration in the shoot was smaller in the wild-type Arabidopsis compared with transgenic plants with an enhanced ability to synthesize glutathione in the root. Glutathione and phytochelatins can act as long-distance carriers of Cd for translocation from root to

shoot (Mendoza-Cózatl et al. 2008). Therefore, high levels of glutathione in the root are likely to affect Cd translocation and accumulation in the shoot, e.g., the positive correlation between glutathione concentration in the root and Cd concentration in the shoot (Fig. 3b).

Between the Si levels, Si addition decreased Cd concentrations in both shoot and root as well as Cd uptake per unit root length and apoplastic flow (Fig. 2 and Table 2), which agree with previous studies on common wheat (Wu et al. 2016), durum wheat (Rizwan et al. 2012), rice (Nwugo and Huerta 2008; Shi et al. 2005), and cotton (*Gossypium arboreum* L.) (Farooq et al. 2013). Si deposition in the root cell wall reduces the porosity of inner root tissues, especially endodermis (Shi et al 2005). The apoplastic barriers reduce the amount of Cd entering the root stele and hence root-to-shoot translocation of Cd and eventually decrease Cd accumulation in the shoot (Qi et al. 2020). It also agrees with the association of low apoplastic flow with low Cd concentration in the root and low Cd uptake per unit root length in Cd-treated plants (Fig. 3b).

Added Si also decreases Cd concentration in shoot and root subcellular fractions (Fig. 2b and 2d). Si can form negatively charged complexes with hemicellulose of the cell wall that is capable of binding positively charged Cd cations and sequestering them in the cell wall (Ma et al 2015). Additionally, Si can limit Cd transportation from the vacuole to cytosol, promoting vacuole compartmentalization (Wei et al. 2021). In plants subject to Cd stress, Si also decreased glutathione concentration in roots (Table 2), which suggests reduced concentrations of free Cd in the cytosol to induce glutathione synthesis (Farooq et al. 2016). These agree with the Si-induced decrease of Cd concentration in the soluble fraction of root (Fig. 2d). Genetically, supplemental Si can mediate Cd-induced gene expression. For instance, Si downregulates the expressing level of genes encoding transporters (e.g., HMA2, LCT1, and Nramp5) that are involved in Cd uptake and translocation (Greger et al. 2016; Kim et al. 2014; Ma et al. 2016; Shao et al. 2017). Si can also regulate genes encoding glutathione-S-transferase (e.g., GSTU1 and 6 and GSTF14), phytochelatin synthase (e.g., PCS1), and stress-associated protein (e.g., SAP1 and 14) to detoxify Cd stress (Farooq et al. 2016; Ma et al. 2016; Nwugo and Huerta, 2011).

In this study, genetic differences in Cd accumulation in shoots mainly derive from root-to-shoot translocation, which agreed with the variation in subcellular Cd distributions (i.e., cell wall, organelle, and soluble fractions) as well as induced glutathione synthesis in response to Cd addition. Supplemental Si could effectively reduce Cd accumulation in shoot and root, suppress glutathione synthesis, and thus decrease Cd translocation from root to shoot. Variations in Cd accumulation are controlled by multiple processes, such as transmembrane transport mediated by transporters (e.g., Nramp5, HMA2, HMA3, and LCT1) and Cd-phytochelatin complexes (Clemens et al. 2013; Nakamura et al. 2020; Sasaki et al. 2012; Uraguchi and Fujiwara 2013). However, these transporters also regulate the translocation of other elements (e.g., manganese, iron, and zinc) bidirectionally (e.g., HMA3 for influx and Nramp3 for efflux to vacuole) (Clemens et al. 2013; Ishimaru et al. 2012; Luo and Zhang 2021; Mendoza-Cózatl et al. 2011; Sasaki et al. 2012). Moreover, glutathione and phytochelatins can also be involved in long-distance translocation (Mendoza-Cózatl et al. 2008 and 2011). Further research is thus needed to identify regulators (e.g., transporters and phytochelatins) of the most significant contributions to root-to-shoot

translocation of Cd in common wheat. This information will facilitate the development of common wheat germplasm with low Cd accumulation.

Declarations

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Conflicts of interest/Competing interests

The authors have no relevant financial or non-financial interests to disclose. The authors have no competing interests to declare that are relevant to the content of this article.

Availability of data and material
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Rui Yang and Xi Liang. The first draft of the manuscript was written by Rui Yang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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Figures

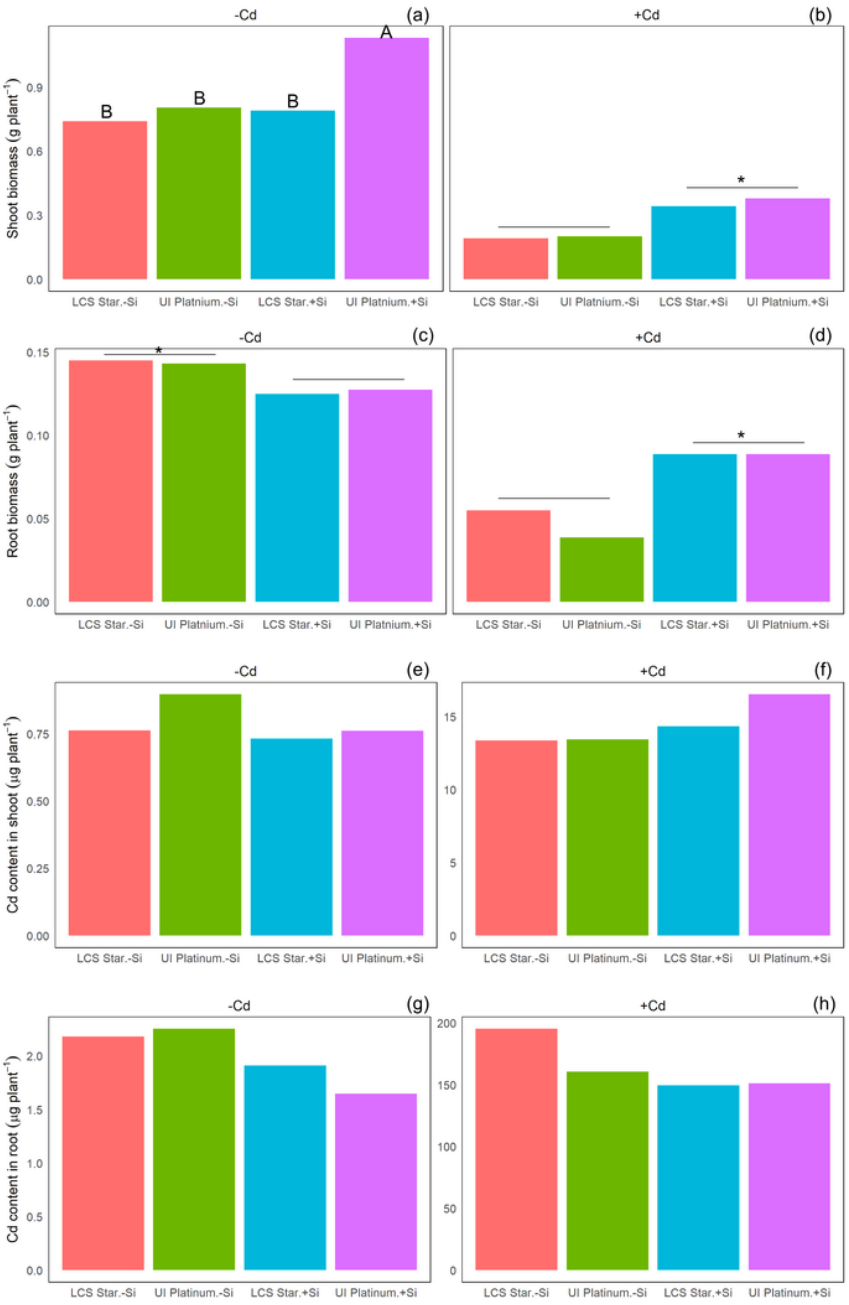

Figure 1

Shoot and root biomass (a-d) and Cd content (e-h) of LCS Star and UI Platinum in response to Si and Cd treatments. Different letters indicate significant differences in cultivar × Si level at α = 0.05. The symbol "*" indicates significant differences between Si levels at α = 0.05.

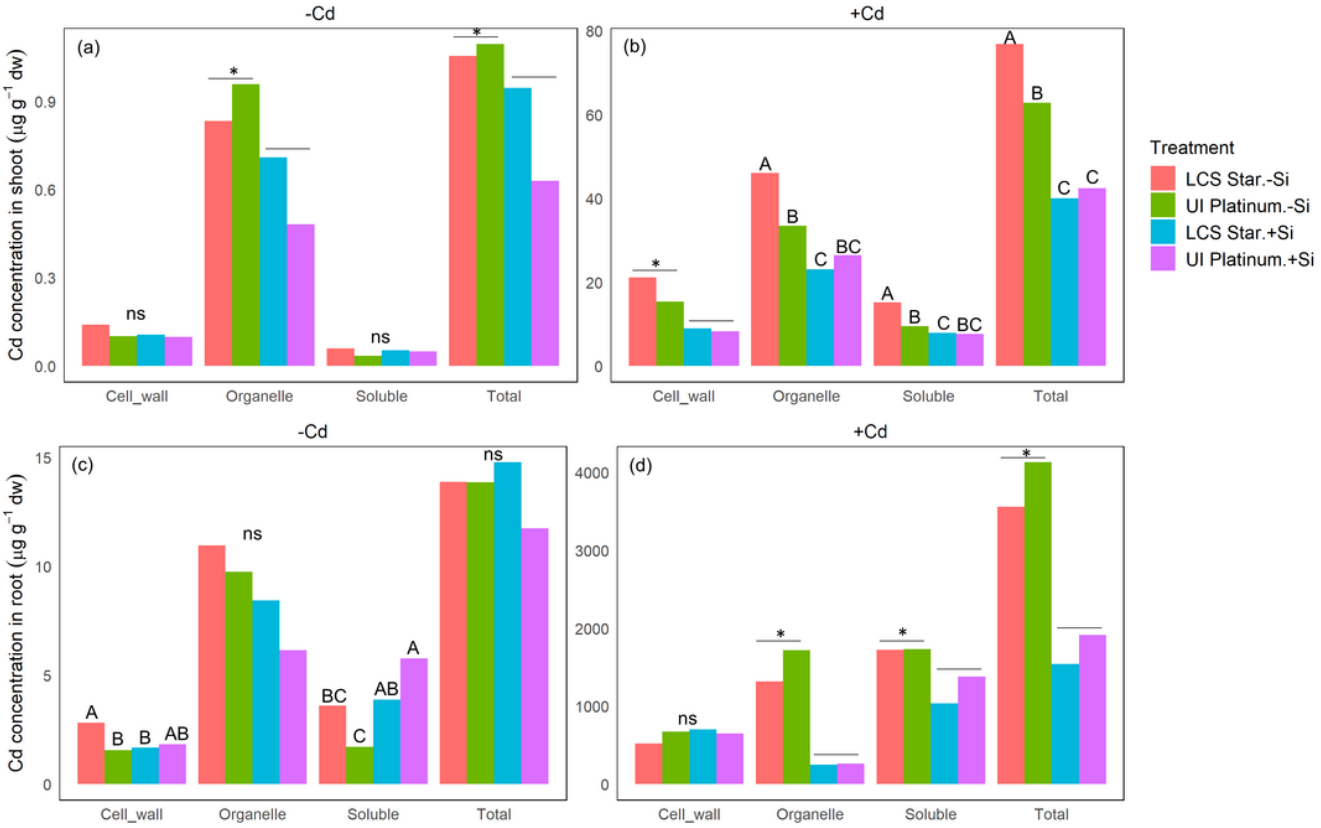

Figure 2

Subcellular distribution (i.e., cell wall, organelle, and soluble fractions) of Cd in the shoot (a and b) and root (c and d) of LCS Star and UI Platinum in response to Si and Cd treatments. Different letters indicate significant differences in cultivar × Si level at α = 0.05. The symbol "*" indicates significant differences between Si levels at α = 0.05. "ns" represents insignificant differences.

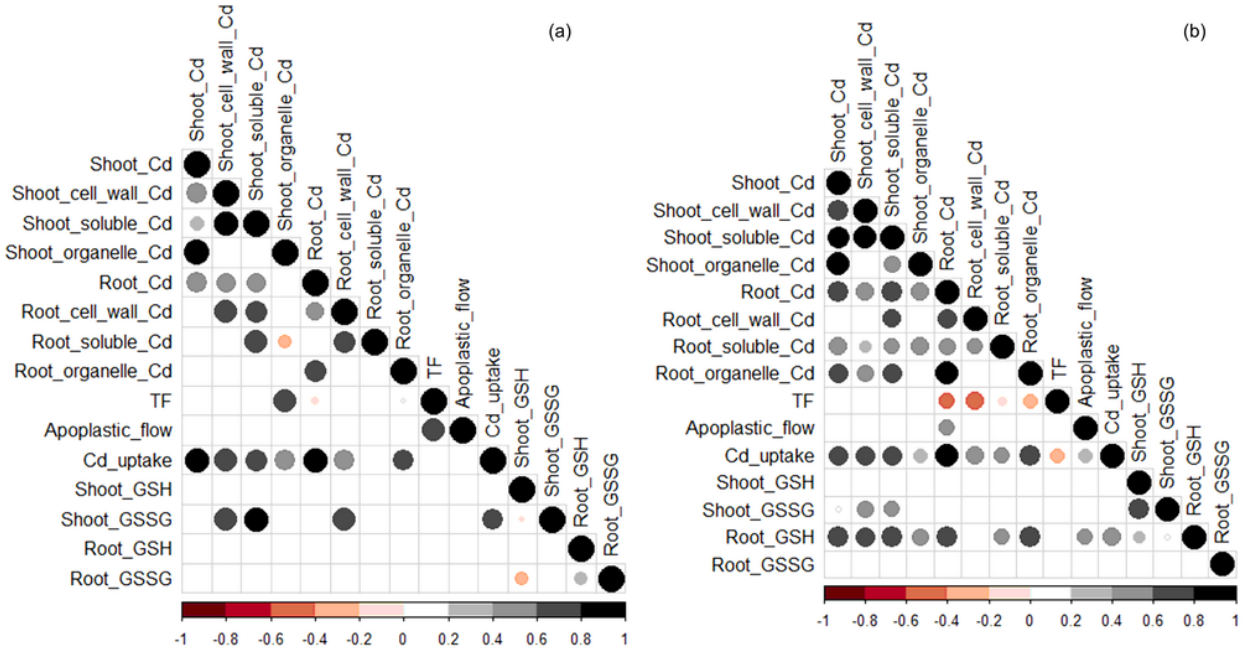

Figure 3

Correlations between Cd concentrations in the shoot and root and their subcellular distributions (i.e., cell wall and soluble and organelle fractions), translocation factor (TF), apoplastic bypass flow, Cd uptake per unit root length, and concentrations of glutathione (GSH) and glutathione disulfide (GSSH) in shoot and root in Cd-untreated (a) and -treated (b) plants. Blank cells indicate insignificant correlations ($P > 0.05$).