

Effect of Silicon on Morpho-Physiological Attributes, Yield and Cadmium Accumulation in Two Maize Genotypes with Contrasting Root System Size and Health Risk Assessment

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Research Article

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Abstract

Background and aims Cadmium (Cd) contamination is a serious threat to plants and humans. Silicon (Si) was reported to have some alleviative effects on plant tolerance to Cd stress. However, whether Si alleviates Cd toxicity in maize genotypes with contrasting root system size are unknown.

Methods Effects of Si applications (0 and 200 mg kg⁻¹ soil) on shoot and root growth, Cd uptake and transportation under Cd treatments (0 and 20 mg kg⁻¹ soil) were assessed at the silking and maturity of maize genotypes Zhongke11 (large-rooted) and Shengrui999 (small-rooted) in a pot experiment.

Results Root dry weight, plant height and root length were significantly affected by Si addition. Root volume and average root diameter were significantly positively correlated with root Cd concentration, bioaccumulation and translocation factor, respectively, of two maize genotypes at the silking stage. Addition of Si significantly increased Cd concentration, content, bioconcentration and translocation factor in roots of Zhongke11, but reduced the values of these parameters in Shengrui999 at both growth stages. Under Cd stress, grain Cd concentration in the Si treatment was decreased by 14.4% (Zhongke11) and 21.4% (Shengrui999) than that in non-Si treatment. Grain yield was significantly negatively correlated with root Cd accumulation. Moreover, addition of Si significantly reduced Cd daily intake and health risk index in maize.

Conclusions This study demonstrated that addition of Si reduced health risk by eliminating Cd accumulation in maize shoot and grain, and alleviated Cd stress with more profound effects in the small-rooted genotype Shengrui999.

Introduction

Cadmium (Cd) is an environmental threats and industrial pollutant with high cytotoxicity (Rehman et al. 2019; Zhang et al. 2019; Zhao and Wang 2019). Cd intake is a health risk to animals and humans while not phytotoxic at plant concentrations (Ngugi et al. 2021). Because Cd absorption by plants, it can be enriched in high trophic organisms along the food chain (An et al. 2021; Thind et al. 2021). Over 50% of all calories consumed in the human diet are derived from cereal crops, and these crops account for a high proportion of dietary Cd (Ma et al. 2021). The safety threshold of Cd content in cereal grains has been set to 0.4 mg kg⁻¹ for rice (0.2 mg kg⁻¹ in China), 0.2 mg kg⁻¹ for wheat, and 0.1 mg kg⁻¹ for maize and barley by the Codex Alimentarius Commission, a joint office of the United Nation's Food and Agriculture Organization and the World Health Organization (Codex Alimentarius Commission 2014). Therefore, it has become indispensable to adopt a mitigation strategy to diminish the Cd concentrations in plants especially in food crops including maize (Akhtar et al. 2017; Thind et al. 2021). For this reason, many remediation techniques have already been used, such as organic (biochar, amino acid) and inorganic (zinc, silicon) treatments to decrease the bioavailability of Cd in soil and its uptake by crops (Rizwan et al. 2017; Rehman et al. 2019; Rehman et al. 2020a). These amendments produced promising results in minimizing the distribution and mobility of Cd in the contaminated soil (Lukačová et al. 2013; Adrees et al. 2015).

Silicon (Si), the second most abundant element in the earth's crust, is a beneficial element for plant growth and development, especially under various biotic and abiotic stresses (Bhat et al. 2019). Studies found that Si supply improves plant tolerance to Cd stress in many crop species, including wheat (Rizwan et al. 2017; Wu et al. 2019), maize (Vaculík et al. 2009; Liu et al. 2020), and rice (Nwugo and Huerta 2008; Zhao et al. 2020; Zaman et al. 2021). Appropriate Si fertilization could be a practical strategy to inhibit the uptake of Cd in maize organs to reduce Cd in Cd-contaminated farmland (Liang et al. 2005; Liu et al. 2020). Some reports claimed that Si application improved plant growth, yield and increased Cd accumulation in shoots, whereas others have refuted such claims (Coskun et al. 2019). In particular, we need to pay attention to the concentration and distribution of Cd in grains following Si application, aiming to increase

the content of Cd in non-edible parts, control or reduce the content of Cd in grains, and promote the phytoremediation of Cd pollution soil while ensuring the food safety of maize grains.

Maize (*Zea mays* L.) is a valuable cereal crop and provides food for humans, fodder for the livestock and bioconversion to clean energy ethanol (Gupta and Verma 2015; Dawid and Grzegorz 2021). It is widely adopted for phytomanagement of Cd-contaminated soils due to its high biomass production and Cd tolerance (Xu et al. 2014; Rizwan et al. 2017). Root morphology plays an important role in Cd uptake and translocation (Redjala et al. 2011; Kubo et al. 2015). Seed Cd concentration is influenced by the differences among cultivars in ease of translocation of Cd to seed and in Cd accumulation capacity of roots (Sugiyama et al. 2007). Liang et al. (2005) described that addition of Si into the soil experimentally polluted by Cd induced a significant increase in maize biomass (Liang et al. 2005). Lukačová Kuliková and Lux (2010) reported varied responses to Si application in root length and dry weights among five maize hybrids under Cd stress (Lukačová Kuliková and Lux 2010). Cadmium transfer from roots to grain during the post-flowering are important determinants of Cd concentrations in rice (Rodda et al. 2011; Chen et al. 2019) and wheat (Tavarez et al. 2015; Yan et al. 2018). Using two maize genotypes with constructing root system size selected from a root phenotyping study (Qiao et al. 2019), the objectives of this study were to assess (i) the role of Si in alleviating Cd stress and Cd accumulation in maize, (ii) genotypic variation in response to Si and Cd applications, and (iii) Cd health risks in grains following Si addition.

Materials And Methods

Experimental design, plant materials and soil

This experiment was conducted at the Institute of soil and water Conservation, Chinese Academy of Sciences, and Northwest A&F University during June and October 2019. The light/dark temperature was set at 35°C /25°C with relative humidity between 50 and 70%. A completely randomized block design was used consisting of two maize genotypes (Zhongke11 and Shengrui999), two Si levels (Si0, nil and Si1, 200 mg kg⁻¹ soil), and two Cd levels (Cd0, nil and Cd1, 20 mg kg⁻¹ soil), two harvests (at silking and maturity) and four replicates per treatment with a total of 64 pots. Three randomized replicates out of four replicates were taken for measurements.

A loessal soil collected from a maize farmland in Yangling was used in this study. The soil was air-dried, sieved (2 mm) and mixed well before putting into the plastic pots (diameter 30 cm, depth 30 cm), 20 kg per pot. The soil physical and chemical properties were analyzed, providing in Table 1. Fertilizers of N (46% urea), P₂O₅ (16% superphosphate) and K₂O (60% potassium chloride) at a rate of 0.1 (N), 0.15 (P), 0.05 (K) g kg⁻¹ was applied. Urea was applied in solution; superphosphate and potassium chloride were mixed into the soil before potting.

Table 1
Physio-chemical physiochemical
parameters of soil

Soil parameters	Value
Textural class	Sandy loam
Sand (%)	52.12
Silt (%)	25.43
Clay (%)	22.45
pH (water)	8.40
Organic matter (g kg ⁻¹)	13.5
Total N (g kg ⁻¹)	0.92
Available N (mg kg ⁻¹)	18.45
Available P (mg kg ⁻¹)	13.5
Available K (mg kg ⁻¹)	190.5
Total Cd (mg kg ⁻¹)	0.16
Effective Cd (mg kg ⁻¹)	0.02

Planting and maintenance

Maize seeds were surface sterilized using 1.5% hypochlorite bleach solution for 20 mins and then washed four times with distilled water. Three seeds were sown in each pot and thinned to one seedling per pot at three leaves, about 13 days after sowing (DAS). Si was added as Na₂SiO₃·9H₂O (952 mg kg⁻¹ soil; 21% SiO₂) and Cd was added as CdCl₂·2.5 H₂O (40.6 mg kg⁻¹ soil). The Si was mixed with soil before potting; Cadmium solutions was supplied to the pots designated for Cd1 treatments, respectively, started from 15 DAS and thereafter every day for 20 days, and the total amounts of Cd added to each pot was 20 mg kg⁻¹ soil. All pots were placed in a rain-shed nursery and the soil water content was maintained at 80% ± 5% of the pot water by regular weighing method during the experiment.

Plant harvesting and measurement

At the silking stage (R1, 61 DAS) and maturity stage (R6, 102 DAS), plants of three replicates were harvested by separating roots, stem, leaf and ear at the silking stage, and root, stem, leaf and grain at the maturity stage. Ear parameters including 100-seed weight, ear length, ear thickness and bare top length (the part lack of seeds in the ear) were obtained. The roots were washed free of soil, and soaked in CaCl₂, then repeatedly washed with distilled water to remove the ions on the root surfaces. The root samples were scanned with a desktop scanner (Epson Perfection V800, Long Beach, CA, USA), and root morphological parameters (root length, root surface area, root volume and averaged root diameter) were generated by analyzing root images, using WinRhizo (v2009, Regent Instruments, Montreal, QC, Canada) at the silking stage. All plant tissues were dried at 75°C to a constant weight to determine dry weight (DW) for each organ.

Tissue Cd accumulation and translocation

The dried plant organ was digested using di-acid mixture (Liu et al. 2020). Briefly, 0.5 g of plant samples was digested in HNO₃: HClO₄ (4:1) mixture. Subsequently, Cd was determined using atomic absorbance spectrometry (PinAAcle 900H, Perkin Elmer, USA). Root, stem, leaf and grain Cd concentrations were expressed as µg g⁻¹ dry weight. The Cd content in each organ was calculated by multiplying Cd concentration in each organ and biomass of the respective organ.

Tolerance index (%) was used to assess plant tolerance to Cd toxicity, and calculated as dry weight (DW) percentage of Cd stressed treatment (Cd1) over the control (Cd0) (Wilkins 1978):

$$\text{Tolerance index (\%)} = \frac{[\text{Cd1}]_{\text{DW}}}{[\text{Cd0}]_{\text{DW}}} \times 100$$

The Cd bioconcentration factor (BCF) and translocation factor (TF) in each organ were calculated based on Cd concentration ([Cd1]) in the respective organs and soil as follows (Liu et al. 2020; Rehman et al. 2020a):

$$\text{Root (or stem/leaf/grain) Cd BCF} = \frac{[\text{Cd1}]_{\text{root / stem / leaf / grain}}}{[\text{Cd1}]_{\text{soil}}}$$

$$\text{Root Cd TF} = \frac{[\text{Cd1}]_{\text{root}}}{[\text{Cd1}]_{\text{soil}}}$$

$$\text{Stem (or leaf) Cd TF} = \frac{[\text{Cd1}]_{\text{stem / leaf}}}{[\text{Cd1}]_{\text{root}}}$$

$$\text{Grain Cd TF} = \frac{[\text{Cd1}]_{\text{grain}}}{[\text{Cd1}]_{\text{stem}} + [\text{Cd1}]_{\text{leaf}}}$$

Health risk index

Human Cd health risk index (HRI) at maturity stage of maize was calculated according to (Liu et al. 2020; Rehman et al. 2020b) as follows:

$$\text{DIM (daily intake of metal)} = \frac{[\text{Cd1}] \text{C (factor)} \text{DFI (dailyfoodintake)}}{\text{ABW (averagebodyweight)}}$$

$$\text{HRI (health risk index)} = \frac{\text{DIM}}{\text{ORDC (oralreferenceddoseofCd)}}$$

Where DIM is daily intake of metal, [Cd1] is the Cd concentration in grains (µg g⁻¹), C (factor) is a correction factor, the value is 0.085, DFI was set at 0.4 kg person day according to the FAO/WHO-proposed provisional tolerable daily intake. ABW was set at 70 kg assuming an average human adult body weight. ORDC is 0.001 mg kg day according to the U.S. EPA (1985).

Soil Cd concentration and pH

Post-harvest (at the silking and maturity stages) soil Cd concentration was determined following Liu et al. (2020) and expressed as mg kg⁻¹ soil. Soil samples were ground to homogeneity and passed through a 2 mm sieve; and 0.5 g soil was placed in a digestion tube. A mixture acid (HCl: HNO₃=3:1) was added to each tube with simultaneous gentle shaking. The tubes, after overnight stay, were then placed on a hot-plate set to 160°C for 1h and cooled. Next, 4 mL HClO₄ was added to each tube and digestion was performed at 230°C until the digested solution samples had turned colorless. The supernatant was assessed with an atomic absorption spectrometer (PinAAcle 900H, Perkin Elmer, USA) to measure Cd in the soil.

Soil pH was measured in soil: water=1:5, then a variable speed reciprocal shaker for 0.5 h (Apparatus Co. Ltd. Changzhou, China), determined with a pH meter (Mettler-Toledo AG 8603 Schwerzenbach, Switzerland).

Data analysis

The normal distribution and homogeneity of variance of all data was tested using SPSS 12.5 (IBM, USA). All data was subjected to three-way ANOVA and Duncan's multiple range tests for the main factors (genotype, Cd and Si treatments) and their interactions at $P \leq 0.05$. Pearson's correlation coefficient was used to analyze the relationship between dry weight, root traits and Cd concentration, content, bioaccumulation factor, translocation factor of root, stem, leaf and soil at the silking stage; Ear traits and Cd concentration, content, bioaccumulation factor, translocation factor of root, stem, leaf and grain at the maturity stage also were used to Pearson's correlation coefficient analyze. Boxplots were performed with "ggplot2" and principal component analysis (PCA) was performed with "prcomp" with the software package R.

Results

Plant growth at the silking stage

At the silking stage, root dry weight was significantly affected ($P \leq 0.01$) by G, Cd, Si and G · Cd (Fig. 1a). Under Cd0, Si1 significantly declined root dry weight by 15.2% in Zhongke11; under Si0 and Si1, Cd1 stress significantly increased root dry weight by 25.9% and 25.8% in Shengrui999. Aboveground dry weight was significantly affected ($P \leq 0.05$) by G and Cd (Fig. 1b), under Si0, Cd stress significantly increased aboveground dry weight by 7.9% in Zhongke11. Plant height was significantly affected ($P \leq 0.01$) by G, Cd, Si, G · Cd and G · Cd · Si (Fig. 1c), under Cd0, Si1 significantly increased plant height by 9.4% in Zhongke11. In Shengrui999, under Si0, Cd1 significantly reduced plant height by 13.5%; Under Cd stress, Si1 significantly increased plant height by 9.4%. Root length was significantly affected ($P \leq 0.05$) by Si (Fig. 1e), under Cd0, Si1 significantly declined root length by 35.3% in Zhongke11. Root surface area was significantly affected ($P \leq 0.05$) by G and Cd (Fig. 1f), under Cd0, Si1 significantly declined root surface area by 31.0% in Zhongke11. Root volume was significantly affected ($P \leq 0.01$) by G and Cd (Fig. 1g), under Si1, Cd1 significantly increased root volume by 61.1% in Zhongke11. Average root diameter was significantly affected ($P \leq 0.05$) by Cd and G · Cd (Fig. 1h), under Si0, Cd stress significantly increased average root diameter by 23.0% in Zhongke11.

Plant growth at the maturity stage

At maturity, root dry weight was significantly affected ($P \leq 0.01$) by genotype (G) and G · Cd (Table 2). Under Si0, Cd stress significantly declined root dry weight by 20.8% in Zhongke11; but Cd stress significantly increased root dry weight by 38.9% and 57.9% under Si0 and Si1 in Shengrui999, respectively. Aboveground dry weight was significantly affected ($P \leq 0.01$) by genotype (G) and Si (Table 2). Under Cd stress, Si application resulted in a significant increase the aboveground biomass by 11.9% in Shengrui999. Grain yield was significantly affected ($P \leq 0.05$) by Cd and Si (Table 2). Under nil Cd, the application of Si resulted in a significant increase the grain yield by 10.5% in Zhongke11; Under Si1, Cd stress significantly declined grain yield by 9.82% in Zhongke11. Under Cd stress, Si application significantly increased grain yield by 14.4% in Shengrui999.

Table 2

Dry weight and ear characteristics of maize genotypes (Zhongke11 and Shengrui999) at the maturity stage in response to silicon (Si0, 0 mg kg⁻¹; Si1, 200 mg kg⁻¹) and cadmium (Cd0, 0 mg kg⁻¹; Cd1, 20 mg kg⁻¹) applications.

Genotype	Treatment	Dry weight		Grain Yield (g plant ⁻¹)	100 seed weight (g)	Ear length (cm)	Ear thickness (cm)	Ear rows	Bare top length (mm)
		Root (g plant ⁻¹)	Aboveground (g plant ⁻¹)						
Zhongke11	Cd0Si0	28.5 a	205 ab	99.7 bc	24.3 ab	18.4	47.6	14.7 ab	16.3 bc
	Cd1Si0	22.6 bc	200 bc	96.9 bc	23.0 c	17.5	47	14.7 ab	25.8 a
	Cd0Si1	25.7 ab	218 a	110 a	24.0 bc	17.1	48.9	15.3 a	7.21 e
	Cd1Si1	22.8 bc	207 ab	99.3 bc	24.7 ab	18.3	47.5	13.3 abc	11.0 de
Shengrui999	Cd0Si0	13.5 d	188 cd	96.8 bc	24.5 ab	19.2	46.4	12.7 bc	17.4 bc
	Cd1Si0	18.7 c	185 d	90.0 c	23.1 c	18.4	45.6	12.0 c	18.7 b
	Cd0Si1	12.7 d	200 bc	107 ab	25.5 a	17.1	48	13.3 abc	14.4 bcd
	Cd1Si1	20.0 c	207 ab	103 ab	24.3 ab	18.6	48.4	13.3 abc	12.9 cd
ANOVA	G	**	**	ns	ns	ns	ns	**	ns
	Cd	ns	ns	*	**	ns	ns	ns	**
	Si	ns	**	**	**	ns	ns	ns	**
	G · Cd	**	ns	ns	ns	ns	ns	ns	**
	G · Si	ns	ns	ns	ns	ns	ns	ns	**
	Cd · Si	ns	ns	ns	*	ns	ns	ns	*
	G · Cd · Si	ns	ns	ns	ns	ns	ns	ns	ns
For each parameter across genotypes, mean data (± SE, n=3) with different letters indicate significant difference ($P \leq 0.05$). ANOVA results for the main factors (genotype, G; silicon, Si; cadmium Cd) and their interactions (G · Cd, G · Si, Cd · Si and G · Cd · Si) are given for each parameter (*, $P \leq 0.05$; **, $P \leq 0.01$; ns, non-significant).									

Cd, Si and Cd · Si was significant affected ($P \leq 0.05$) 100-seed weight (Table 2). Under Si0, Cd stress significant reduced 100-seed weight by 5.6% and 5.8% in Zhongke11 and Shengrui999, respectively. Under Cd stress, Si application significant increased 100-seed weight by 7.7% and 5.3% in Zhongke11 and Shengrui999, respectively. Bare top length was significant affected ($P \leq 0.05$) by Cd, Si, G · Cd, G · Si and Cd · Si (Table 2). In Zhongke11, under Si0, Cd stress significant increased bare top length by 58.1%; Under Cd0, Si application significant reduced bare top length by 55.8%; Under Cd1, Si application significant reduced bare top length by 57.3%. In Shengrui999, under Cd stress, Si application significant reduced bare top length by 30.6% (Table 2) (Fig. S1).

Cd concentration and accumulation

At the silking stage, root Cd concentration was significant affected ($P \leq 0.05$) by G, Cd, G · Cd, G · Si and G · Cd · Si (Fig. 2a); Stem Cd concentration was significant affected ($P \leq 0.05$) by G, Si and Cd · Si (Fig. 2b); Leaf Cd concentration was significant affected ($P \leq 0.05$) by G, Cd, Si, G · Cd, and Cd · Si (Fig. 2c). Under Cd1, Si significantly reduced stem Cd concentration by 18.3%, but significantly increased leaf Cd concentration by 110% in Zhongke11. Under Cd1, Si significantly reduced root and stem Cd concentration by 15.5% and 19.0%, respectively, but significantly increased leaf Cd concentration by 15.5% in Shengrui999.

At the maturity stage, root Cd concentration was significant affected ($P \leq 0.05$) by Cd, Si, G · Si and G · Cd · Si (Fig. 2e); Stem Cd concentration was significant affected ($P \leq 0.05$) by G, Cd, Si, G · Cd and Cd · Si (Fig. 2f); Leaf Cd concentration was significant affected ($P \leq 0.01$) by G, Cd, Si, G · Cd, G · Si, Cd · Si and G · Cd · Si (Fig. 2g); Grain Cd concentration was significant affected ($P \leq 0.01$) by Cd, Si, and Cd · Si (Fig. 2h). Under Cd1, Si significantly reduced leaf and grain Cd concentration by 31.6% and 14.4%, respectively, but significantly increased root Cd concentration by 57.1% in Zhongke11. Under Cd1, Si significantly reduced root, stem, leaf and grain Cd concentration by 23.6%, 17.2%, 9.1% and 21.4%, respectively, in Shengrui999. Cd concentration in seeds ranged from 0.05–0.06 $\mu\text{g g}^{-1}$ when grown in Cd amended soil, which is under the safety threshold for human health in maize (0.1 mg kg^{-1}) (Codex Alimentarius Commission 2014).

At the silking stage, root Cd accumulation was significant affected ($P \leq 0.05$) by G, Cd, Si, G · Cd, G · Si, Cd · Si and G · Cd · Si (Fig. S2, Table. S1); Stem Cd content was significant affected ($P \leq 0.05$) by Cd, Si and Cd · Si; Leaf Cd content was significant affected ($P \leq 0.01$) by G, Cd, Si, G · Cd, and Cd · Si. Under Cd1, Si1 significantly reduced stem Cd content by 24.9%, but significantly increased root and leaf Cd content by 37.9% and 108.5%, respectively, in Zhongke11. Under Cd1, Si1 significantly reduced root and stem Cd content by 11.3% and 15.8%, respectively, but significantly increased leaf Cd content by 20.8% in Shengrui999.

At the maturity stage, root Cd accumulation was significant affected ($P \leq 0.01$) by G, Cd, Si, G · Cd, G · Si, Cd · Si and G · Cd · Si (Fig. S2, Table S1); Stem Cd content was significant affected ($P \leq 0.05$) by Cd; Leaf Cd content was significant affected ($P \leq 0.01$) by G, Cd, Si, G · Cd, G · Si, Cd · Si, and G · Cd · Si; Grain Cd content was significant affected ($P \leq 0.05$) by Cd, Si, and Cd · Si; Under Cd1, Si significantly reduced leaf and grain Cd content by 30.5% and 12.2%, respectively, but significantly increased root Cd content by 88.0% in Zhongke11. Under Cd1, Si significantly reduced root and grain Cd content by 18.6% and 10.2%, respectively, in Shengrui999.

Cd bioconcentration and translocation factors

At the silking stage, Si supply increased the Cd bioconcentration factor (BCF) in leaf in Zhongke11 by 200% (Fig. 3c), but reduced the BCF in root (Fig. 3a), stem (Fig. 3b) and leaf (Fig. 3c) by 35.2%, 37.1% and 18.2%, respectively, in Shengrui999. At the maturity stage, Si application increased root (Fig. 3e) Cd BCF by 69.5%, but reduced leaf (Fig. 3g) Cd BCF by 26.2% in Zhongke11; Si application reduced root (Fig. 3e), stem (Fig. 3f) by 29.8%, leaf (Fig. 3g) and grain (Fig. 3h) Cd BCF 35.1%, 29.8%, 22.8% and 33.3%, respectively, in Shengrui999. Roots had significantly higher Cd BCF than other organs at the silking and maturity stages.

At the silking stage, under Cd stress, Si supply increased the leaf Cd translocation factor (TF) in Zhongke11 and Shengrui999 by 85.2% and 50.0%, respectively (Fig. 4c), but reduced stem Cd TF by 29.4% in Zhongke11 (Fig. 4b), and reduced root Cd TF by 35.2% in Shengrui999 (Fig. 4a). At the maturity stage, Si application increased root Cd TF by 69.5%, reduced stem and leaf Cd TF by 39.3% and 55.9%, respectively, in Zhongke11 (Fig. 4f, g); and reduced root Cd TF by 35.1%, increased leaf Cd TF by 18.9% in Shengrui999 (Fig. 4e, g). Roots had significantly higher Cd TF than other organs at the silking and maturity stages (Fig. 4).

Soil Cd concentration and pH

At the silking and maturity stages, soil Cd concentration was significantly affected ($P \leq 0.01$) by Cd, G · Si and G · Cd · Si (Fig. 5a, c); Si supply reduced soil Cd concentration in Zhongke11, but lowered in Shengrui999. At the both growth stages, pH was significantly affected ($P \leq 0.05$) by Si and G · Cd · Si (Fig. 5b, d). In Shengrui999, under Cd stress, Si supply increased soil pH at the both growth stages, but soil without Si had lower pH.

Plant Cd tolerance, daily intake and health risk assessment

At the silking stage, under Cd stress, Zhongke11 increased Cd tolerance by 6.0% under Si0 and no effect under Si1, and Shengrui999 increased Cd tolerance by 6.0% (Si0) and 5.6% (Si1) (Fig. 6a). At maturity, under Cd stress, Zhongke11 reduced Cd tolerance index by 5.1% (Si0) and 6.0% (Si1); Shengrui999 increased Cd tolerance index by 1.2% (Si0) and 7.1% (Si1) (Fig. 6c). Shengrui999 showed higher tolerance to Cd stress than Zhongke11 at the both silking and maturity stages (Fig. 6a, c).

The application of Si significantly decreased both daily intake of Cd (DIM) and health risk index (HRI) under Cd stress. The daily intake of Cd by adults ranged from $2.34E^{-05}$ (Cd1Si1) to $2.98E^{-05}$ (Cd1Si0) in Shengrui999 (Fig. 6b). A similar trend was observed for Cd health risk index. Under Cd stress, the application of Si decreased Cd health risk index by 14.4% (Zhongke11) and 21.4% (Shengrui999) (Fig. 6d).

Correlations between root and ear parameters and Cd accumulation

At the silking stage, the correlation among root parameters (root length, root surface area, root volume and average root diameter), plant organs biomass, Cd concentration, bioaccumulation factor, translocation factor was analyzed (Table S2). Pearson's correlation analysis showed that root parameters (root length, root surface area, and root volume) had significant negative correlation with pH ($P \leq 0.05$). Root volume had significant positive correlation with root, stem and soil Cd concentration, root and stem bioaccumulation and translocation factor ($P \leq 0.05$). Average root diameter had significant positive correlation with root, stem and soil Cd concentration, root bioaccumulation and translocation factor ($P \leq 0.05$).

At the maturity stage, the correlation among grain yield, ear parameters (100-seed weight, ear length, ear thickness and bare top length), plant organs Cd concentration, bioaccumulation factor and translocation factor were analyzed (Table S3). Pearson's correlation analysis showed that grain yield had significant negative correlation with root, stem, leaf and grain Cd concentration; root, stem, leaf and grain bioaccumulation factor, root and grain translocation factor, DIM and HRI ($P \leq 0.05$); 100-seed weight had significant negative correlation with stem, leaf, grain and soil Cd concentration, stem, leaf and grain bioaccumulation and translocation factor, DIM and HRI ($P \leq 0.05$). 100-seed weight and ear thickness had significant positive correlation with pH. Bare top length had significant negative correlation with pH ($P \leq 0.05$).

Principal component analysis of growth and physiological traits

At the silking stage, the PCA identified four principal components (PCs) (Table S4). PC1 and PC2 accounted for 54.9% and 16.7% of the variation, respectively. PC1 separated the effects of Cd treatment, and PC2 separated the effects of genotype treatment (Fig. S3a). The Cd concentration (root, stem, leaf and soil), content (root, stem, leaf and soil), bioaccumulation factor (root, stem and leaf), and translocation factor (root and stem) were the key factors in PC1. Root biomass, plant height, total root surface area, total root volume, pH, and leaf translocation factor were the key factors in PC2 (Table S4).

At the maturity stage, PCA identified five PCs (Table S5). PC1 and PC2 accounted for 63.2% and 11.0% of the variation, respectively (Fig. S3b). PC1 separated the effects of Cd treatment. The Cd concentration (stem, grain and soil), content (stem and grain), translocation factor (stem, leaf and grain), daily intake of metal and Cd health risk index were the key factors in PC1. Stem biomass, grain yield and ear rows were the key factors in PC2 (Table S5).

Discussion

Effects of Si application and Cd stress on plant root parameters

In our study, cadmium stress increased average root diameter considerably in Zhongke11 under Si0 (Fig. 1h), which is consistent with the results of Romdhane et al. (2021) and Ur Rahman et al. (2021a) (Romdhane et al. 2021; Ur Rahman et al. 2021a). Cadmium increased root volume in Zhongke11 regardless Si application as the case in Ur Rahman et al. (2021a) who found that Si application increased root volume under Cd50 in a hydroponic wheat (Ur Rahman et al. 2021a). Silicon application reduced root length considerably in Zhongke11 under Cd0 (Fig. 1e), which is consistent with the results of Lukačová Kuliková and Lux (2010), who found that Si application reduced the primary seminal root length of five maize hybrids in a hydroponic facility (Lukacova Kulikova and Lux 2010). However, Si had no effects on root length in Shengrui999 regardless of Cd stress (Fig. 1e), Wu et al. (2016) found that Cd treatment did not affect root length in wheat under Si treatment in a hydroponic wheat (Wu et al. 2016). Ur Rahman et al. (2021a) claimed that Si addition significantly increased root length compared with no Si treatment and Cd50 in a hydroponic (Ur Rahman et al. 2021a).

In the present study, maize root dry matter exhibited mixed responses in various growth traits in different growth stages when grown in Cd-Si amended soil. Application of Si significantly reduced root dry weight in Zhongke11 under Cd0 (Fig. 1a). Bokor et al. (2013) found that Si (5 mmol L⁻¹) reduced maize root and shoot dry weight than non-Si control (Bokor et al. 2013). However, the application of Si did not significantly affect root dry weight in Shengrui999 under Cd0 and Cd1 at the silking and maturity stages (Fig. 1a, Table 2), Farooq et al. (2016) found no effect of Si (0.6 mmol L⁻¹) on rice root dry weight (Farooq et al. 2016). However, application of Cd significantly increased root dry weight in Shengrui999 at the both growth stages regardless Si status (Fig. 1a, Table 2), Si application significantly increased maize root dry weight under antimony (Vaculíková et al. 2014), and nickel stress (Vaculík et al. 2021). Zhang et al. (2008) found rice root and shoot dry weights of Si treatment were significantly increased 15 days under Cd (0 and 4 mmol L⁻¹) (Zhang et al. 2008). Rizwan et al. (2012) found wheat root dry decreased first and then increased with increasing the rates of Si from 0 to 15 ton ha⁻¹ (Rizwan et al. 2012). These findings showed that Si had different effects on dry weight under different heavy metal species or concentrations, and plants may be also in a transition between adaptation and toxicity in responses to Si application under low heavy metals stress (Rizwan et al. 2017; Khan et al. 2021).

In our study, correlation analysis indicated that root length, root surface area and root volume were positively correlated with root biomass, which also found in soybean seedlings under 9 µM and 23 µM Cd (Wang et al. 2016). Wang et al. (2016) found that root volume was positively correlated with root biomass; root length, root surface area and root volume were negatively correlated with root Cd concentration, and the translocation factor of Cd from root to shoot was not correlated with root length, root surface area, root volume and average root diameter under 45 µM Cd. Our study indicated that root volume and average root diameter were positively correlated with root Cd concentration, bioaccumulation and translocation factor under 20 mg kg⁻¹ Cd (Table S2). Huang et al. (2015) found that root length, root surface area, root average diameter, root volume in hot pepper were not correlated with root, and shoot Cd concentration, and shoot Cd translocation factor under 2 µM Cd, but root surface and root volume were positively correlation with root and shoot Cd concentration and shoot Cd translocation factor under 10 µM Cd (Huang et al. 2015).

Lu et al. (2013) found that root average diameter in peanut was negatively correlated with root and stem Cd concentration, but not correlated with shoot Cd translocation factor under 2 and 20 μM Cd (Lu et al. 2013). Variations in these correlations may attribute to species and Cd concentration treatment.

Si application affects Cd concentration, bioaccumulation and translocation factors in different organs

Our results showed that maize roots had the highest cadmium concentration and content among all plant organs (Figs. 2, S2). Cadmium easily infiltrates the root via the cortical tissue; the growing root part is covered with exudation (carbohydrates, amino acid, enzyme) excreted by the root cap and rhizodermal cells, which can bind Cd (Seregin and Kozhevnikova 2011; Bali et al. 2020). Cadmium, like the essential nutrients, follows the same apoplastic and symplastic pathways to move radially across the root layers (Clemens et al. 2002). Apoplasmic movement of Cd to the xylem can be restricted by the development of the exodermis, endodermis, and other extracellular barriers; symplasm movement is thought to be restricted by the production of phytochelatins and the sequestration of Cd-chelates in vacuoles (Shi et al. 2005; Ur Rahman et al. 2021b). Silicon application can attribute not only to Cd immobilization (Fig. 5a, c) but also to its low bioavailability arising from pH rise (Fig. 5b, d) in soil (Vaculík et al. 2009; Cai et al. 2020), which may restrict Cd transfer from root to grain (Fig. 2) (Liu et al. 2013; Khan et al. 2021). Si-supplied plants also enhanced binding of Cd to the cell walls, existing in the form of [Si-hemicellulose matrix] Cd complexation (Ma et al. 2015), restricted the apoplastic transport of Cd and reduced the transporting of Cd into aboveground organs (Song et al. 2009; Ye et al. 2012). Moreover, Si influences the oxidative status of plants by modifying the activity of various antioxidants, improves membrane stability, and acts on transporter gene expression (Ma et al. 2015; Vaculík et al. 2020; Khan et al. 2021).

Aboveground organs Cd concentrations are determined largely by Cd entry to the root, sequestration within root vacuoles, transpiration steam in the xylem, dilution within the aboveground tissues during the growth (Hart et al. 2006; Vaculík et al. 2009; Lukačová et al. 2013). Silicon application reduced stem, leaf and grain Cd concentration in Shengrui9999 at the maturity stage (Fig. 2). From silking to maturity, the Cd concentration, content, bioconcentration and translocation factor of root were reduced, but stem, leaf and grain were increased (Figs. 2–4, S2). The possible reason is involved for Cd transport during grain maturation; Cd is transported to the stems, leaves and the outer parts of panicles, then followed by remobilized to grains through phloem (Fujimaki et al. 2010; Rodda et al. 2011).

Some studies reported that Si inhibits Cd uptake and accumulation in maize root, shoot and grain (Lukačová et al. 2013; Liu et al. 2020), others reported maize treated with Si displayed increased Cd uptake in roots and shoots (Da Cunha and Do Nascimento 2008; Vaculík et al. 2009). However, in our study, Si increased total Cd content in Zhongke11, and reduced that in Shengrui999 (Fig. S2). The content of Cd in the root, the mobility of Cd in the soil and the transportation to aboveground organs depend on the concentration of Si and Cd in the soil-plant system (Liang et al. 2005; Da Cunha et al. 2008; Ji et al. 2017).

Si diminishes Cd concentrations in plants root, stem, leaf and grain in Zhongke11 (Fig. 2), by reducing upward translocation from soil to root and by decreasing metal bioaccumulation in stem leaf and grain tissues (Fig. 4–5), which the results of Liu et al. (2020) found that Si application also reduced root, stem, leaf and grain Cd concentration under Cd stress (Liu et al. 2020). Differences in organs physiological and biochemical properties may result in differences in Cd uptake, accumulation and translocation under Si application (Lukačová et al. 2013; Yu et al. 2020; Ma et al. 2021). Application of Si reduced grain Cd concentration, daily intake of metals (DIM) and health risk index (HRI) in maize (Fig. 6b, d), which is consistent with the results in rice (Zaman et al. 2021), wheat (Rizwan et al. 2017) and maize (Liu et al. 2020). The exogeneous application of Si may be a feasible approach in these contexts. We observed a significant negative correlation between grain yield and DIM and HRI (Table S3).

Genotypic variation in response to Si application under moderate Cd stress

Great differences among plant species and genotypes of the same species in response to Cd and Si were observed (Lukačová Kuliková and Lux 2010; Rizwan et al. 2017), root to shoot translocation of Cd (Harris and Taylor 2013; Tavarez et al. 2015), and the accumulation of Cd in grain (Naeem et al. 2015). Tolerant genotypes with smaller biomass and higher Cd concentration of root were evidenced in previous studies (Ekmekci et al. 2008; Guo et al. 2019). In our study, cadmium tolerant Shengrui999 (small root system) had less dry weight, root length, root surface area, and root volume than Cd-sensitive Zhongke11 (large root system) (Fig. 1, Table 2). However, Cd concentration in roots was adverse regardless of Si (Fig. 2). The capacity to translocate elements to shoots is an important factor involved in tolerance (Harris and Taylor 2013; Yan et al. 2018). Shengrui999 had higher stem and leaf Cd concentration, BCF and TF efficiency than Zhongke11 regardless of Si at maturity (Figs. 2, 4, 5). Silicon application significantly reduced Shengrui999 Cd concentration and BCF in root, stem, leaf and grain compared to non-Si treatment (Figs. 2, 3). Silicon was more effective in enhancing Cd tolerance in the Cd-tolerant genotype than in the Cd-sensitive genotype, through suppressing Cd uptake and root to shoot transport (Song et al. 2009).

Conclusion

Both maize genotypes accumulated more Cd in roots than in the aboveground parts with the least in grains. Application of Si had significant effects on Cd concentration and BCF in stem and leaf, soil pH, daily intake of Cd and Cd health risk index under Cd stress (Fig. 7). Genotype Shengrui999 (small-rooted) was more effective response to Si application under Cd stress than Zhongke11 (large-rooted). Therefore, the present study advocates for addition of Si with small-rooted maize when grown in soils contaminated with Cd as a practical approach to alleviating Cd-induced toxicity and diminishing Cd health risk. Future studies are required to reveal the underlying mechanisms of the role of Si in alleviating plant tolerance to Cd stress at physiological and molecular aspects under different Si species and more genotypes.

Declarations

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Not applicable

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Figures

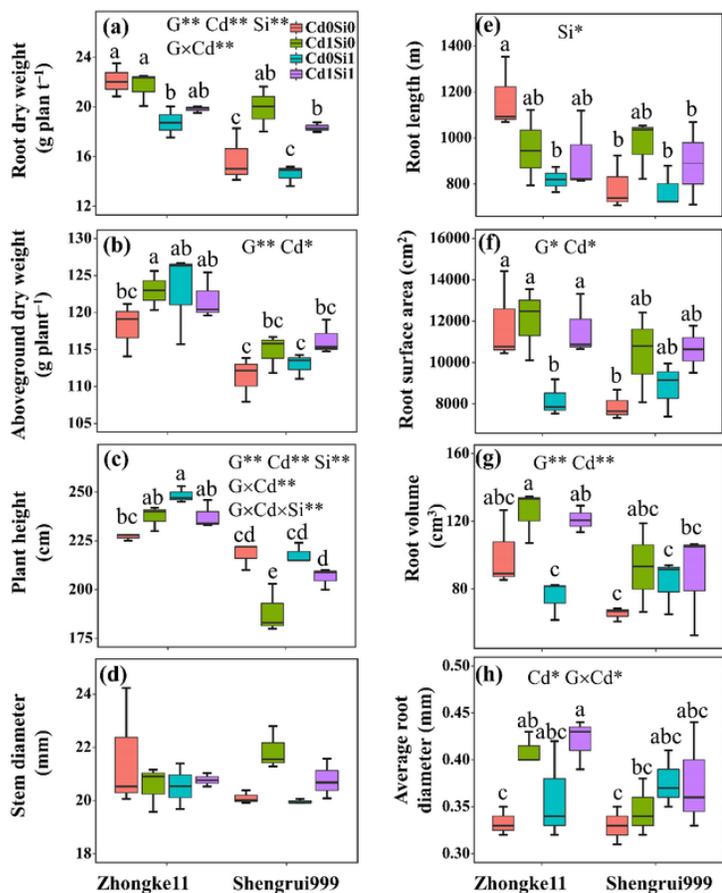


Figure 1

Shoot and root attributes of maize genotypes (Zhongke11 and Shengrui999) at the silking stage in response to silicon (Si0, 0 mg kg⁻¹; Si1, 200 mg kg⁻¹) and cadmium (Cd0, 0 mg kg⁻¹; Cd1, 20 mg kg⁻¹) applications. For each attribute across genotypes, mean data (\pm SE, n=3) with different letters indicate significant difference ($P \leq 0.05$). ANOVA results for the main factors (genotype, G; silicon, Si; cadmium Cd) and their interactions (G \times Cd, G \times Si, Cd \times Si and G \times Cd \times Si) are given for each attribute if significantly different (*, $P \leq 0.05$; **, $P \leq 0.01$).

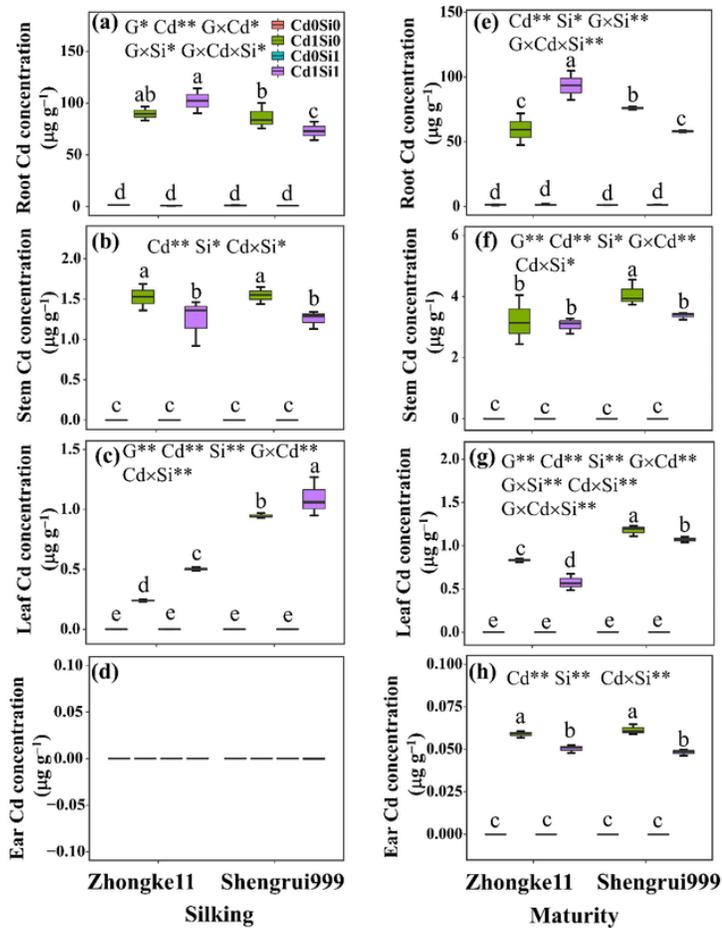


Figure 2

Cd concentration in roots (a, e), stems (b, f), leaves (c, g) and ears (d, h) of maize genotypes (Zhongke11 and Shengrui999) at the silking (a-d) and maturity (e-h) stages in response to silicon (Si0, 0 mg kg⁻¹; Si1, 200 mg kg⁻¹) and cadmium (Cd0, 0 mg kg⁻¹; Cd1, 20 mg kg⁻¹) applications. There were no ears Cd concentration data (d) at the silking stage. For each attribute across genotypes, mean data (\pm SE, n=3) with different letters indicate significantly different ($P \leq 0.05$). ANOVA results for the main factors (genotype, G; silicon, Si; cadmium, Cd) and their interactions (G \times Cd, G \times Si, Cd \times Si and G \times d \times Si) are given for each attribute if significantly different (*, $P \leq 0.05$; **, $P \leq 0.01$).

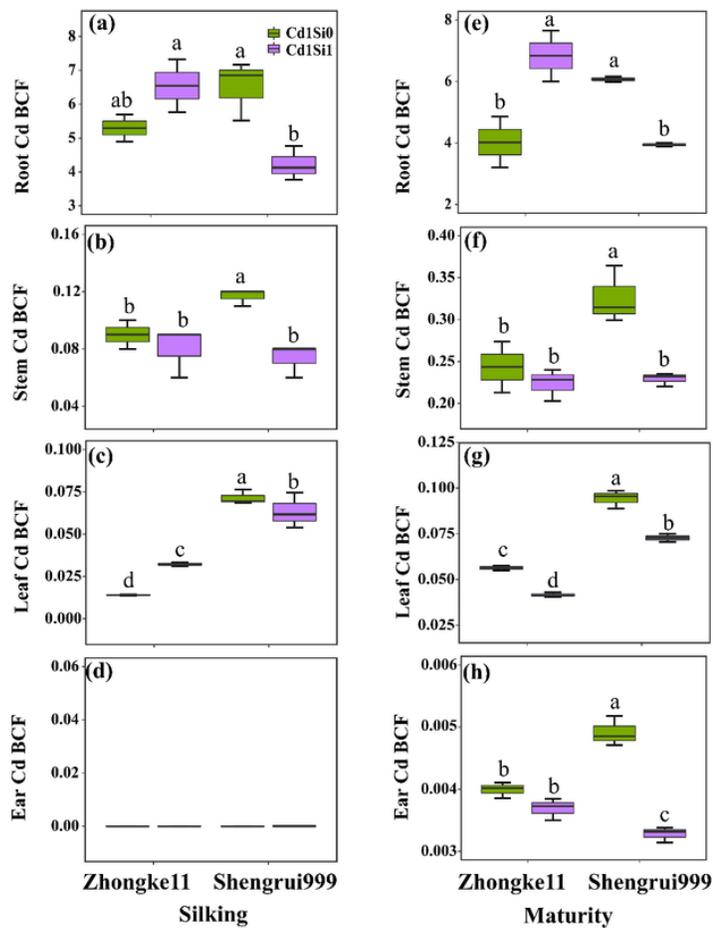


Figure 3

Cd bioconcentration factor (BCF) in roots (a, e), stems (b, f), leaves (c, g), and ears (d, h) of maize genotypes (Zhongke11 and Shengrui999) at the (a-d) silking and (e-h) maturity stages in response to silicon (Si0, 0 mg kg⁻¹; Si1, 200 mg kg⁻¹) and cadmium (Cd0, 0 mg kg⁻¹; Cd1, 20 mg kg⁻¹) applications. There were no ear Cd BCF data (d) at the silking stage. For each organ across genotypes, mean data (\pm SE, n=3) with different letters indicate significant difference ($P \leq 0.05$).

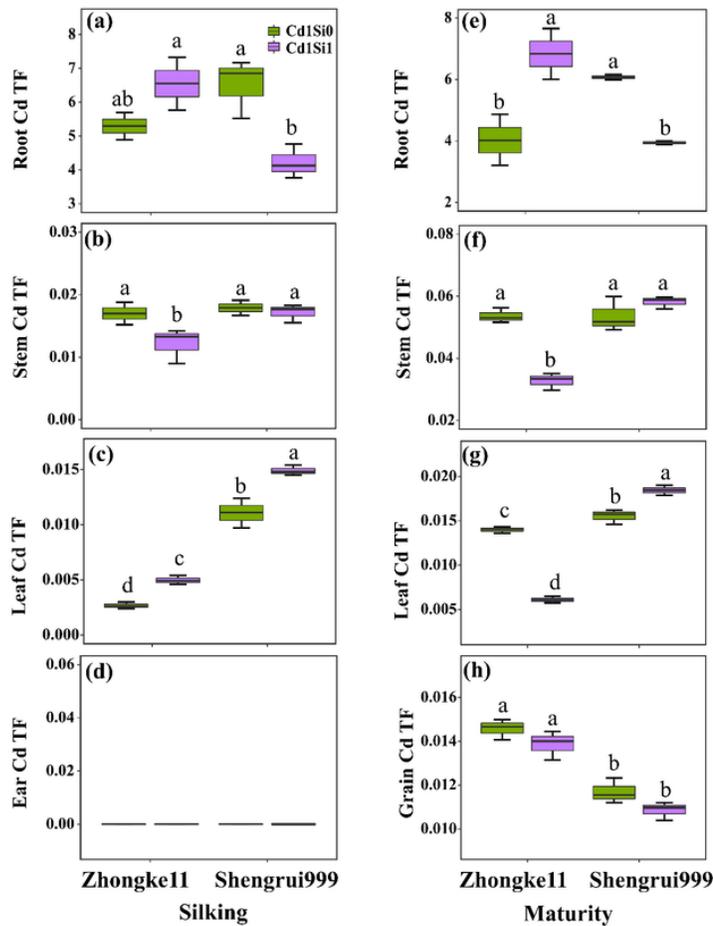


Figure 4

Cd translocation factor (TF) in roots (a, e), stems (b, f), leaves (c, g) and ears (d, h) of maize genotypes (Zhongke11 and Shengrui999) at the silking (a-d) and maturity (e-h) stages in response to silicon (Si0, 0 mg kg⁻¹; Si1, 200 mg kg⁻¹) and cadmium (Cd0, 0 mg kg⁻¹; Cd1, 20 mg kg⁻¹) applications. There were no ear Cd TF data (d) at the silking stage. For each organ across genotypes, mean data (\pm SE, n=3) with different letters indicate significant difference ($P \leq 0.05$).

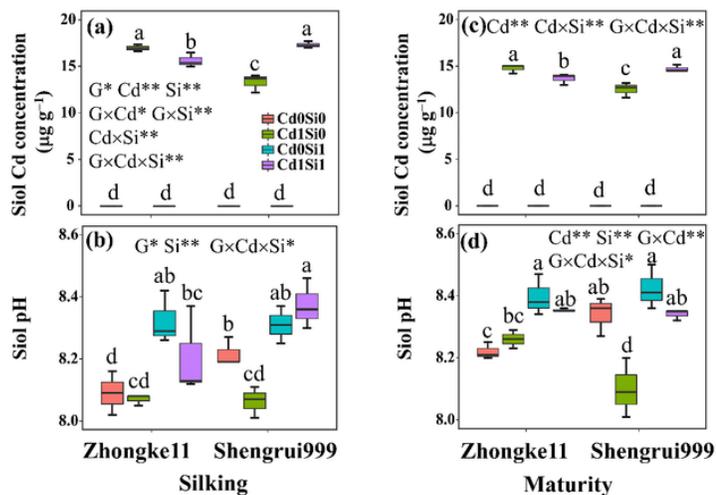


Figure 5

Soil Cd concentration (a, c) and soil pH (b, d) for maize genotypes (Zhongke11 and Shengrui999) at the silking (a, b) and maturity stages (c, d) stages in response to silicon (Si0, 0 mg kg⁻¹; Si1, 200 mg kg⁻¹) and cadmium (Cd0, 0 mg kg⁻¹; Cd1, 20 mg kg⁻¹) applications. For each attribute across genotypes, mean data (\pm SE, n=3) with different letters indicate significantly different ($P \leq 0.05$). ANOVA results for the main factors (genotype, G; silicon, Si; cadmium, Cd) and their interactions (G \times Cd, G \times Si, Cd \times Si and G \times Cd \times Si) are given for each attribute if significantly different (*, $P \leq 0.05$; **, $P \leq 0.01$).

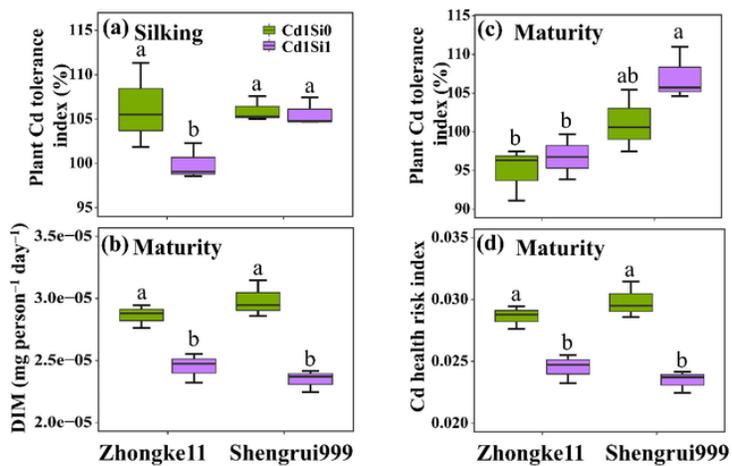


Figure 6

Plant Cd tolerance index (a, c), daily intake of metal (b, DIM) and Cd health risk index (d, HRI) of maize genotypes (Zhongke11 and Shengrui999) in response to silicon (Si0, 0 mg kg⁻¹; Si1, 200 mg kg⁻¹) and cadmium (Cd0, 0 mg kg⁻¹; Cd1, 20 mg kg⁻¹) applications. For each attribute across genotypes, mean data (\pm SE, n=3) with different letters indicate significant difference ($P \leq 0.05$).

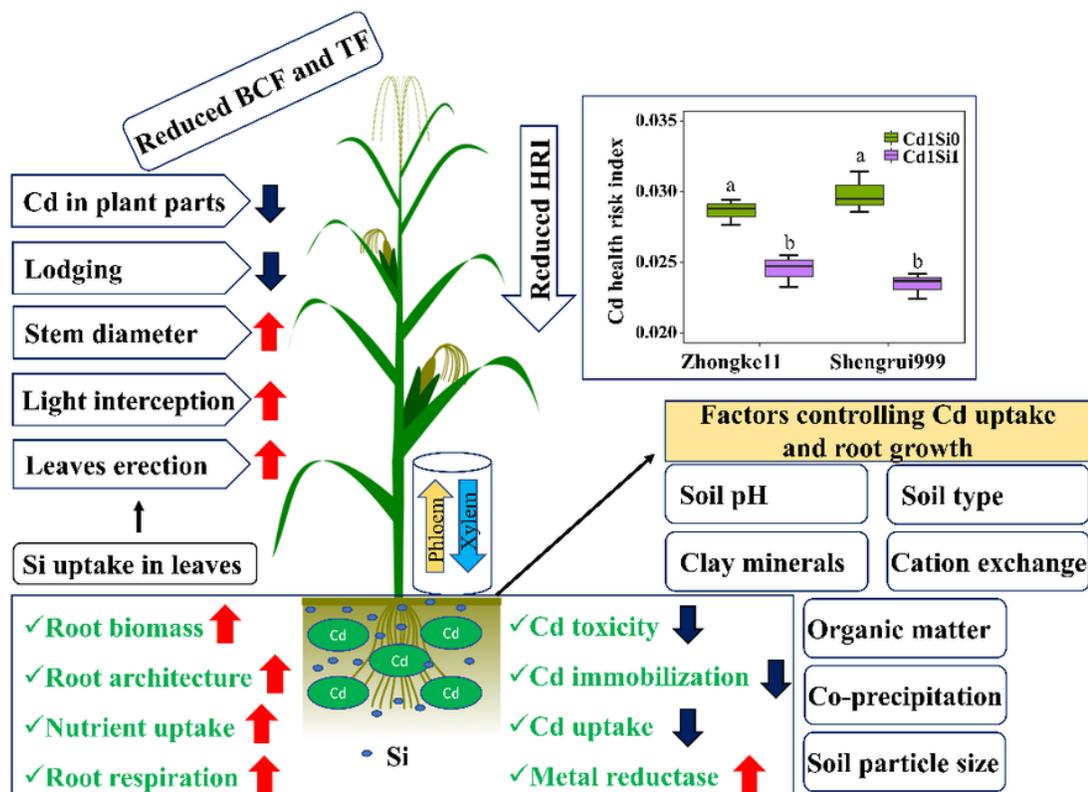


Figure 7

Schematic model of the mechanisms underlying the influence of Si in soil, root, stem, leaf and grain under Cd stress at the maturity stage. Soil attributes include soil pH, soil type, clay minerals, cation exchange, organic matter, co-precipitation, soil particle size; Si application increases root biomass, root architecture, nutrient uptake, root respiration and metal reductase under Cd stress; Si application reduce root toxicity, Cd immobilization and Cd uptake under Cd stress. Si and Cd can translocate into the xylem and transported to the shoots via the transpirational pull. Si application increases leaves erection, light interception and stem diameter under Cd stress; Si application reduces lodging, Cd uptake in plant parts, bioconcentration factor (BCF), translocation factor (TF) and health risk index (HRI). Arrow color: red, increase; dark blue, reduce.

Supplementary Files

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- [MaizeCdSiSupple.docx](#)