# **REGULAR ARTICLE**



# 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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## 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 Cd stress in plants. However, whether Si alleviates Cd toxicity in maize genotypes with contrasting root system size are unknown.

*Methods* Effects of Si application (200 mg kg<sup>-1</sup> soil) on shoot and root growth, Cd uptake and transportation under Cd stress (20 mg kg<sup>-1</sup> soil) were assessed at the silking and maturity stages of maize genotypes Zhongke11 (deep-rooted) and Shengrui999 (shallow-rooted) in a pot experiment.

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Y. Gao · Q. Kuang · Y. Wu College of Resources and Environment, Northwest A&F University, Yangling 712100, Shaanxi, China Results Application of Si significantly increased root dry weight, plant height and root length. 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 Shengrui9999 at both growth stages. Grain Cd concentration in the combined Cd and Si treatment was decreased by 14.4% (Zhongke11) and 21.4% (Shengrui999) than that in Cd 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

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Y. Chen The UWA Institute of Agriculture, & School of Agriculture and Environment, The University of Western Australia, Perth 6009, Australia accumulation in maize shoot and grain, and alleviated Cd stress with more profound effects in the shallow-rooted genotype Shengrui999.

**Keywords** Maize · Root parameters · Cd stress · Cd bioconcentration and translocation · Silicon application

# Introduction

In China, 16.1% of arable land is polluted and inorganic pollutants, and about 7% of arable land is polluted by cadmium (Cd) (MEP 2014). Cd is an environmental threats and industrial pollutant with high cytotoxicity (Rehman et al. 2019; Zhang et al. 2019; Zhao and Wang 2019; Xu et al. 2020). Cd intake is a health risk to animals and humans although some plants do not show toxic symptom grown in Cd contaminated soil (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. 2021b). The safety threshold of Cd content in cereal grains has been set to 0.4 mg kg<sup>-1</sup> for rice  $(0.2 \text{ mg kg}^{-1} \text{ in China}), 0.2 \text{ mg kg}^{-1}$  for wheat, and  $0.1 \text{ mg kg}^{-1}$  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; Wang 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. 2017a; Rehman et al. 2020a, b). 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. 2017a; 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. 2017b). 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. Lukačová Kuliková and Lux (2010) reported varied responses to Si application in root length and dry weights among five maize hybrids under Cd stress. 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 contrasting root system size (deep-rooted vs shallow-rooted) selected from our recent root phenotyping study (Qiao et al. 2019), the objectives of this study were to assess (i) variation between the deep-rooted and shallow-rooted genotypes in response to Si and Cd applications, (ii) the role of Si in alleviating Cd stress and Cd accumulation in maize, and (iii) Cd health risks in grains following Si addition.

### Materials and methods

Experimental design, plant materials and soil

This experiment was conducted in a rain shed at the Institute of Soil and Water Conservation, Chinese Academy of Sciences, and Northwest A&F University during June and October 2019. The day/night temperature varied from 25 to 35 °C with relative humidity between 50 and 70%. A randomized completely block design was used consisting of two maize genotypes (Zhongke11 and Shengrui999), two Si levels (0 and 200 mg kg<sup>-1</sup> soil), and two Cd levels (0 and 20 mg kg<sup>-1</sup> soil), two harvests (the silking and maturity stages) and four replicates per treatment with a total of 64 pots. The codes of the four treatments were Control (no addition of Cd and Si), (2) Cd (20 mg kg<sup>-1</sup> soil), (3) Si (200 mg kg<sup>-1</sup> soil), (4) Cd+Si. 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 airdried, sieved (2 mm) and mixed well before putting into the plastic pots (diameter 30 cm, depth 30 cm), 20 kg per pot with a density of 1.25 g cm<sup>-3</sup>. The soil physical and chemical properties were analyzed, providing in Table 1. Fertilizers of N (46% urea), P<sub>2</sub>O<sub>5</sub> (16% superphosphate) and K<sub>2</sub>O (60% potassium chloride) at a rate of 0.1 (N), 0.15 (P), 0.05 (K) g kg<sup>-1</sup> 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 Sandy loam			
Textural class				
Sand (%)	52.12			
Silt (%)	25.43			
Clay (%)	22.45			
pH (water)	8.40			
Organic matter (g $kg^{-1}$ )	13.5			
Total N (g kg <sup><math>-1</math></sup> )	0.92			
Available N (mg kg <sup>-1</sup> )	18.45			
Available P (mg kg $^{-1}$ )	13.5			
Available K (mg kg <sup>-1</sup> )	190.5			
Total Cd (mg kg <sup>-1</sup> )	0.16			
Effective Cd (mg kg <sup>-1</sup> )	0.02			

#### Planting and maintenance

Maize seeds were surface sterilized using 1.5% hypochlorite bleach solution for 20 min 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 and Cd was added as CdCl<sub>2</sub>·2.5 H<sub>2</sub>O. The selected Si rate of 200 mg kg<sup>-1</sup> was based on literature study (Khan et al. 2021; Tubana et al. 2016), which was equivalent to 500 kg Si ha<sup>-1</sup>. Cd treat of 20 mg kg<sup>-1</sup> was used based on the published work in maize including two local studies (He et al. 2013; Hui and Dang 2013). The Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O powder was directly mixed with soil before potting; CdCl<sub>2</sub>·2.5 H<sub>2</sub>O solutions was supplied to the pots designated for Cd 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<sup>-1</sup> soil. The soil pH was not adjusted after Si addition. All pots were placed in a rain-shed nursery and the soil water content was maintained at  $80\% \pm 5\%$  of the pot water by regular weighing method during the experiment.

#### Plant harvesting and measurement

Plants of three replicates were harvested by separating roots, stem, leaf and ear at the silking stage (R1, 61 DAS), and root, stem, leaf and grain at the maturity stage (R6, 102 DAS). Ear parameters and yield components including ear rows, number of grains per row, 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 20 mM CaCl<sub>2</sub> solution for a few minutes, then repeatedly washed with distilled water to remove ions on the root surface. 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 HNO<sub>3</sub>: HClO<sub>4</sub> (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  $\mu g g^{-1}$  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 the percentage in plant dry weight (DW) of Cd stressed treatments (i.e. Cd, and Cd+Si) over the Control (Wu et al. 2010; Wilkins 1978):

Tolerance index(%) = 
$$\frac{DW_{Cd \text{ or } Cd+Si}}{DW_{control}} \times 100$$

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

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

 $\operatorname{Root} \operatorname{Cd} \operatorname{TF} = \frac{\left[\operatorname{Cd}\right]_{\operatorname{root}}}{\left[\operatorname{Cd}\right]_{\operatorname{soil}}}$ 

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

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

## 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:

 $DIM (daily intake of metal) = \frac{[Cd] \times C (factor) \times DFI (daily food intake)}{ABW (average body weight)}$ 

HRI (health risk index) =  $\frac{\text{DIM}}{\text{ORDC} (\text{oral referance dose of Cd})}$ 

where DIM is daily intake of metal, [Cd] is the Cd concentration in grains ( $\mu g g^{-1}$ ), C (factor) is a

correction factor, the value is 0.085, DFI was set at 0.4 kg person<sup>-1</sup> day<sup>-1</sup> according to the FAO/WHOproposed provisional tolerable daily intake. ABW was set at 70 kg assuming an average human adult body weight. ORDC is 0.001 mg kg<sup>-1</sup> day<sup>-1</sup> 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<sup>-1</sup> 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:  $HNO_3=3:1$ ) 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 1 h and cooled. Next, 4 mL HClO<sub>4</sub> 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 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 were tested using SPSS 12.5 (IBM, USA). All data were subjected to three-way ANOVA and Duncan's multiple range tests for the main factors (genotype, Cd and Si treatments) and their interactions at  $P \le 0.05$ . 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, 100-seed weight 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 by G, Cd, Si and G×Cd ( $P \le 0.01$ , Fig. 1a). Compared to Control, Si additions (both Si and Cd+Si treatments) significantly reduced root dry weight in Zhongke11; while Cd and Cd+Si treatments significantly increased root dry weight in Shengrui999. Aboveground dry weight was significantly affected by G and Cd ( $P \le 0.05$ ; Fig. 1b). Compared to Control, the Cd treatment significantly increased aboveground dry weight in Zhongke11. Plant height was significantly affected by G, Cd, Si, G×Cd and G×Cd×Si ( $P \le 0.01$ ; Fig. 1c). Compared to Control, the three Cd/Si treatments significantly increased plant height in Zhongke11; while in Shengrui999, Cd addition (Cd, and Cd+Si treatments) significantly reduced plant height. Less variation in stem diameter was found among the treatments with a significant increase in Cd treatment only when compared to Control (Fig. 1d). Root length was significantly affected by Si ( $P \le 0.05$ ; Fig. 1e), resulting a decline by 35.3% than Control in Zhongke11; while Cd treatment had higher root length in Shengrui999. There were significant differences in root surface area between genotypes (G) and Cd treatments (Cd) ( $P \le 0.05$ ; Fig. 1f)

Fig. 1 Shoot and root attributes of maize genotypes (Zhongke11 and Shengrui999) at the silking stage in response to silicon  $(Si, 200 \text{ mg kg}^{-1})$  and cadmium (Cd, 20 mg  $kg^{-1}$ ) applications. For each attribute across genotypes, mean data ( $\pm$ SE, n=3) with different letters indicate significant difference ( $P \le 0.05$ ). 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 (\*,  $P \le 0.05; **, P \le 0.01)$ 



with a 31.0% reduction in Si treatment in Zhongke11. Effect on root volume mirrored that of root surface area (Fig. 1g). Cd addition (both Cd and Cd+Si treatments) significantly increased average root diameter by 23.0% than Control in Zhongke11 ( $P \le 0.05$ ; Fig. 1h).

# Plant growth at the maturity stage

At maturity, root dry weight was significantly affected by genotype (G) and G×Cd ( $P \le 0.01$ ; Table 2). Root dry weight was significantly declined in Zhongke11 but increased in Shengrui999 under Cd addition (Cd and Cd+Si treatments). Aboveground dry weight was significantly affected by genotype (G) and Si ( $P \le 0.01$ ; Table 2). Si addition (Si, and Cd+Si treatments) resulted in a significant increase in the aboveground biomass in Shengrui999. Grain yield was significantly affected by Cd and Si ( $P \le 0.05$ ; Table 2). Increased grain yield was found in Si treatment for Zhongke11, and in Si and Cd+Si treatments for Shengrui999.

Cd, Si and Cd×Si significantly affected 100 seed weight ( $P \le 0.05$ ; Table 2). Cd treatment significantly reduced 100 seed weight compared to Control

in both genotypes (5-8% reduction). Combined Cd and Si had no significant difference in 100 seed weight with Control, but significantly increased it by 7.39% (Zhongke11) and 5.19% (Shengrui999) compared to Cd treatment. Bare top length was significantly affected by Cd, Si,  $G \times Cd$ ,  $G \times Si$  and  $Cd \times Si$  $(P \le 0.05; \text{ Table 2})$ . In Zhongke11, Cd treatment significantly increased bare top length by 58.3% than Control; Si treatment significantly reduced bare top length by 55.8% than Control; Cd+Si treatment significantly reduced bare top length than Control and Cd treatment. In Shengrui999, Cd+Si significantly reduced bare top length compared to Cd treatment (Table 2; Fig. S1). There was no significant difference in ear thickness and ear row number in all factors and their interactions, except for ear rows between genotypes with more ear row number in Zhongke11 than Shenrui999 ( $P \le 0.05$ ; Table 2).

# Cd concentration and accumulation

At the silking stage, root Cd concentration was significantly affected by G, Cd, G×Cd, G×Si and G×Cd×Si ( $P \le 0.05$ ; Fig. 2a); stem Cd concentration

Genotype	Treatment	Dry weight		Grain	100 seed	Ear length	Ear	Ear rows	Bare top
		Root (g plant <sup>-1</sup> )	Aboveground (g plant <sup>-1</sup> )	Yield (g plant <sup>-1</sup> )	weight (g)	(cm)	thickness (cm)		length (mm)
Zhongke11	Control	28.5 a	205 ab	99.7 bc	24.3 ab	18.4	47.6	14.7 ab	16.3 bc
	Cd	22.6 bc	200 bc	96.9 bc	23.0 c	17.1	47	14.7 ab	25.8 a
	Si	25.7 ab	218 a	110 a	24.0 bc	17.5	48.9	15.3 a	7.21 e
	Cd+Si	22.8 bc	207 ab	99.3 bc	24.7 ab	18.3	47.5	13.3 abc	11.0 de
Shengrui999	Control	13.5 d	188 cd	96.8 bc	24.5 ab	19.2	46.4	12.7 bc	17.4 bc
	Cd	18.7 c	185 d	90.0 c	23.1 c	17.1	45.6	12.0 c	18.7 b
	Si	12.7 d	200 bc	107 ab	25.5 a	18.4	48	13.3 abc	14.4 bcd
	Cd+Si	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	*
	$G \times Cd \times Si$	ns	ns	ns	ns	ns	ns	ns	ns

**Table 2** Dry weight and ear characteristics of two maize genotypes (Zhongke11 and Shengrui999) at the maturity stage in response to silicon (Si, 200 mg kg<sup>-1</sup>) and cadmium (Cd, 20 mg kg<sup>-1</sup>) applications

For each parameter across genotypes, mean data ( $\pm$  SE, n = 3) with different letters indicate significant difference ( $P \le 0.05$ ). 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 (\*,  $P \le 0.05$ ; \*\*,  $P \le 0.01$ ; ns, non-significant)

was significantly affected by G, Si and Cd×Si ( $P \le 0.05$ ; Fig. 2b); leaf Cd concentration was significantly affected by G, Cd, Si, G×Cd, and Cd×Si ( $P \le 0.05$ ; Fig. 2c). Cd concentration and contents in non-Cd treatments (Control, and Si treatment) were extremely low or not detected. Cd+Si treatment significantly reduced stem Cd concentration by 18.3% than Cd treatment, but significantly increased leaf Cd concentration by 110% in Zhongke11. Cd+Si significantly reduced root and stem Cd concentration by 15.5% and 19.0% than Cd treatment, respectively, but significantly increased leaf Cd concentration by 15.5% in Shengrui999.

At the maturity, root Cd concentration was significantly affected by Cd, Si, G×Si and G×Cd×Si ( $P \le 0.05$ ; Fig. 2e); stem Cd concentration was significantly affected by G, Cd, Si, G×Cd and Cd×Si ( $P \le 0.05$ ; Fig. 2f); leaf Cd concentration was significantly affected by G, Cd, Si, G×Cd, G×Si, Cd×Si and G×Cd×Si ( $P \le 0.05$ ; Fig. 2g); grain Cd concentration was significantly affected by Cd, Si, and Cd×Si ( $P \le 0.01$ ; Fig. 2h). In Zhongke11, Cd+Si significantly reduced leaf (31.6%) and grain (14.4%) Cd concentration than Cd treatment, but significantly increased root Cd concentration by 57.1%. In Shengrui999, Cd+Si treatment significantly reduced

Fig. 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 (Si, 200 mg kg<sup>-1</sup>) and cadmium (Cd, 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 ( $P \le 0.05$ ). ANOVA results for the main factors (genotype, G; silicon, Si; cadmium, Cd) and their interactions (G×Cd, G×Si,  $Cd \times Si$  and  $G \times Cd \times Si$ ) are given for each attribute if significantly different (\*,  $P \le 0.05; **, P \le 0.01)$ 



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root, stem, leaf and grain Cd concentration by 23.6%, 17.2%, 9.14% and 21.4% than Cd treatment, respectively. Cd concentration in seeds ranged from  $0.05 - 0.06 \ \mu g \ g^{-1}$  when grown in Cd amended soil, which is under the safety threshold for human health in maize (0.1 mg kg<sup>-1</sup>) (Codex Alimentarius Commission 2014).

At the silking stage, root Cd accumulation was significantly affected by G, Cd, Si, G×Cd, G×Si, Cd×Si and G×Cd×Si ( $P \le 0.05$ ; Fig. S2, Table. S1); stem Cd content was significantly affected by Cd, Si and Cd×Si ( $P \le 0.05$ ); leaf Cd content was significantly affected by G, Cd, Si, G×Cd, and Cd×Si ( $P \le 0.01$ ). Combined Cd and Si significantly reduced stem Cd content by 24.9% compared to Cd alone, but significantly increased root and leaf Cd content by 37.9% and 108.5%, respectively, in Zhongke11. Cd+Si treatment significantly reduced root and stem Cd content by 11.3% and 15.8% than Cd treatment, respectively, but significantly increased leaf Cd content, respectively, but significantly increased leaf Cd content by 20.8% in Shengrui999.

At the maturity, root Cd accumulation was significantly affected by G, Cd, Si, G×Cd, G×Si, Cd×Si and G×Cd×Si ( $P \le 0.01$ ; Fig. S2, Table S1); stem Cd content was significantly affected by Cd ( $P \le 0.05$ ); leaf Cd content was significantly affected by G, Cd, Si, G×Cd, G×Si, Cd×Si, and G×Cd×Si ( $P \le 0.01$ ); grain Cd content was significantly affected by Cd, Si, and Cd×Si ( $P \le 0.05$ ). In Zhongke11, Cd+Si treatment significantly reduced leaf and grain Cd content by 30.5% and 12.2% than Cd treatment, respectively, but significantly increased root Cd content by 88.0%. In Shengrui999, Cd+Si significantly reduced root and grain Cd content by 18.6% and 10.2% than Cd treatment, respectively.

Cd bioconcentration and translocation factors

At the silking stage, Cd+Si treatment increased Cd bioconcentration factor (BCF) in leaf in Zhongke11 by 200% than Cd treatment (Fig. 3c), but reduced BCF in root (Fig. 3a), stem (Fig. 3b) and leaf (Fig. 3c) in Shengrui999 by 35.2%, 37.1% and 18.2%, respectively. At the maturity, in Zhongke11, Cd+Si treatment increased root Cd BCF by 69.5% than Cd treatment (Fig. 3e), but reduced leaf Cd BCF by 26.2% (Fig. 3g); in Shengrui999, Cd+Si treatment significantly reduced Cd BCF values in root (Fig. 3e), stem (Fig. 3f), leaf (Fig. 3g) and grain (Fig. 3h) by

22.8–35.1% than Cd treatment, respectively. Roots had significantly higher Cd BCF values than other organs at both the silking stage and the maturity.

At the silking stage, Cd+Si treatment significantly increased leaf Cd translocation factor (TF) by 85.2% (Zhongke11) and 50.0% (Shengrui999) than Cd treatment, 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, in Zhongke11, Cd+Si treatment significantly increased root Cd TF by 69.5% than Cd treatment, reduced stem and leaf Cd TF by 39.3% and 55.9%, respectively (Fig. 4f, g). In Shengrui999, Cd+Si treatment reduced root Cd TF by 35.1%, and increased leaf Cd TF by 18.9% (Fig. 4e, g). Roots had significantly higher Cd TF than other organs at both the silking stage and the maturity (Fig. 4).

## Soil Cd concentration and pH

At the silking and maturity stages, soil Cd concentration was significantly affected by Cd,  $G \times Si$ and  $G \times Cd \times Si$  ( $P \le 0.01$ ; Fig. 5a, c); Si treatment significantly reduced soil Cd concentration in Zhongke11, but increased in Shengrui999. At the both growth stages, pH was significantly affected by Si and  $G \times Cd \times Si$  ( $P \le 0.05$ ; Fig. 5b, d). In Shengrui999, Cd + Si treatment significantly increased soil pH at both growth stages, but soil without Si application (i.e. Control and Cd treatments) had lower pH than Si treatments (i.e. Si, and Cd + Si treatments).

Plant Cd tolerance, daily intake and health risk assessment

At the silking stage, in Zhongke11, Cd treatment increased Cd tolerance by 6.0% than Control; no significant difference was found in Cd tolerance between Cd+Si and Si. In Shengrui999, Cd treatment increased Cd tolerance by 6.0% than Control, and Cd+Si treatment increased Cd tolerance by 5.6% than Si treatment (Fig. 6a). At maturity, in Zhongke11, Cd treatment reduced Cd tolerance index by 5.1% than Control, and Cd+Si treatment reduced Cd tolerance index by 6.0% than Si treatment; in Shengrui999, Cd treatment increased Cd tolerance index by 1.2% than Control, and Cd+Si treatment Fig. 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 (Si, 200 mg kg<sup>-1</sup>) and cadmium (Cd, 20 mg kg<sup>-1</sup>) 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  $(P \le 0.05)$ 



increased Cd tolerance index by 7.1% than Si treatment (Fig. 6c). Shengrui999 showed higher tolerant to Cd stress than Zhongke11 at both the silking stage and the maturity (Fig. 6a, c).

The application of Si significantly decreased daily intake of Cd (DIM) and health risk index (HRI) under Cd stress. The daily intake of Cd ranged from  $2.34E^{-05}$  (Cd+Si) to  $2.98E^{-05}$  (Cd) in Shen-grui999 (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 ( $P \le 0.05$ ). Root volume had significant positive correlation with root, stem and soil Cd concentration, Fig. 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 (Si, 200 mg kg<sup>-1</sup>) and cadmium (Cd, 20 mg kg<sup>-1</sup>) 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  $(P \le 0.05)$ 



root and stem bioaccumulation and translocation factor ( $P \le 0.05$ ). Average root diameter had significant positive correlation with root, stem and soil Cd concentration, root bioaccumulation and translocation factor ( $P \le 0.05$ ).

At the maturity stage, the correlation among grain yield, ear parameters and yield components (ear rows, number of grains per row, 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 ( $P \le 0.05$ ); hundred 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 ( $P \le 0.05$ ). Hundred-seed weight and ear thickness had significant positive correlation with pH. Bare top length had significant negative correlation with pH ( $P \le 0.05$ ). Fig. 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 (Si, 200 mg kg<sup>-1</sup>) and cadmium (Cd, 20 mg kg<sup>-1</sup>) applications. For each attribute across genotypes, mean data ( $\pm$ SE, n=3) with different letters indicate significantly different ( $P \le 0.05$ ). ANOVA results for the main factors (genotype, G; silicon, Si; cadmium, Cd) and their interactions (G×Cd, G×Si,  $Cd \times Si$  and  $G \times Cd \times Si$ ) are given for each attribute if significantly different (\*,  $P \le 0.05; **, P \le 0.01)$ 

**Fig. 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 (Si, 200 mg kg<sup>-1</sup>) and cadmium (Cd, 20 mg kg<sup>-1</sup>) applications. For each attribute across genotypes, mean data ( $\pm$  SE, n=3) with different letters indicate significant difference ( $P \le 0.05$ )



Zhongkell Shengrui999



Principal component analysis of growth and physiological traits

At the silking stage, 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, PCA identified five PCs with Eigenvalue greater than one (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 and soil parameters

Root morphological traits have a positive correlation with root elongation and development (Qiao et al. 2019; Ur Rahman et al. 2021a). In our study, cadmium stress increased average root diameter considerably than Control in Zhongke11 (Fig. 1h). Cadmium stress increased root volume in Zhongke11 regardless Si application (Fig. 1g). These findings indicate that application of Si in maize growth media contaminated with low concentration of Cd are not toxic to plants, and even stimulate root growth (Romdhane et al. 2021; Ur Rahman et al. 2021a; Li et al. 2021). In the present study, application of Cd significantly increased root dry weight in Shengrui999 at both growth stages regardless Si status (Fig. 1a, Table 2). Si application significantly increased maize root dry weight under Cd (Rizwan et al. 2012), antimony (Vaculíková et al. 2014), and nickel stress (Vaculík et al. 2021). These findings showed that Si had significant effects on root dry weight under different heavy metals and Si deposited in leaf epidermal cells could enhance lightuse-efficiency by facilitating the transmission of light to the photosynthetic mesophyll tissue and photosynthetic products transportation through phloem under moderate stress of heavy metals (Rizwan et al. 2017b; Nwugo and Huerta 2008; Khan et al. 2021).

Silicon addition improved edaphic properties such as soil pH (Fig. 5b, d), and soil Cd concentration in Shengrui999 (Fig. 5a, c). Si application reduced the availability of Cd, increased Cd immobilization in the soil (Khan et al. 2021; Ma et al. 2021a). Various studies show that Si addition alters soil physical and biochemical properties (soil pH, soil type, clay minerals, cation exchange, organic matter, co-precipitation, soil particle size) leading to positive impacts such as enhanced root architectural traits, root respiration, nutrient uptake, root biomass, and metal reductase (Khan et al. 2021; Ma et al. 2021b; Liang et al. 2005) (Fig. 7).

In our study, correlation analysis indicated that root length, root surface area and root volume were positively correlated with root biomass; root volume and average root diameter were positively correlated with root Cd concentration, bioaccumulation and translocation factor under moderate Cd stress (Table S2). Root and soil parameters were significantly correlated with root Cd uptake and accumulation (Wang et al. 2016; Huang et al. 2015; Lu et al. 2013). PCA results demonstrated that the effects of Cd treatment were clearly separated by PC1, in which the key factors were root architectural traits, Cd concentration, content and bioaccumulation factor of organs at the silking stage (Fig S3, Table S4). Cd and Cd+Si treatments increased PC1 value of growth and physiological traits of maize genotypes from the silking stage to the maturity (Fig S3). These results suggested that Cd significantly affect plant growth at different growth stages (An et al. 2022; Zhang et al. 2019).

Si application affects Cd concentration,

bioaccumulation and translocation factors in different organs

Our results showed that roots had the highest Cd concentration and content among all organs of maize plant (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 (Fig. 7) (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 (Xu et al. 2017; 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).

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Fig. 7 Schematic model of the effects of silicon (Si) in soil, root, stem, leaf and grain under cadmium (Cd) stress at the maturity stage. Soil attributes include soil pH, soil type, clay minerals, cation exchange, organic matter, co-precipitation, and soil particle size. Under Cd stress, application of Si may contribute to the increase in root biomass, root architecture traits, nutrient uptake, root respiration, leaves erection, light

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;

interception and stem diameter. On the other hand, Si application may reduce Cd immobilization and accumulation in plant parts, Cd toxicity, Cd bioconcentration factor (BCF), Cd translocation factor (TF) and health risk index (HRI). Si and Cd can be transported to shoots via xylem by transpirational pull. Red arrows, increase; green arrows, reduce

Lukačová et al. 2013). Silicon application reduced stem, leaf and grain Cd concentration than combined Cd and Si treatment in Shengrui999 at the maturity (Fig. 2). From the silking to the maturity, Cd concentration, content, bioconcentration and translocation factor of root were reduced, but these Cd-related parameters were increased in stem, leaf and grain (Figs. 2, 3, and 4, S2). 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), while others reported maize treated with Si 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 and Do Nascimento 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 Cd bioaccumulation in stem leaf and grain tissues (Figs. 3 and 4). Similar results were reported by Liu et al. (2020) who found that Si application also reduced root, stem, leaf and grain Cd concentration under Cd stress. Plant organs differ in physiological and biochemical properties, which may result in variation in Cd uptake, accumulation and translocation under Si application (Lukačová et al. 2013; Yu et al. 2020; Ma et al. 2021b). Application of Si reduced grain Cd concentration, daily intake of metal (DIM) and health risk index (HRI) in maize (Fig. 6b, d), which is consistent with the results reported in rice (Zaman et al. 2021), wheat (Rizwan et al. 2017a) and maize (Liu et al. 2020). We observed a significant negative correlation between grain yield and DIM and HRI (Table S3). Therefore, exogeneous application of Si may be a feasible approach in these contexts.

Genotypic variation in response to Si application under moderate Cd stress

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. 2017b), in particular, differences in root to shoot translocation of Cd (Harris and Taylor 2013; Tavarez et al. 2015), and 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, Cd-tolerant Shengrui999 (shallow root system) had less root dry weight, root length, root surface area, and root volume than Cd-sensitive Zhongke11 (deep root system) (Fig. 1, Table 2). However, Cd concentration in roots was the highest among plant organs regardless of Si (Fig. 2). Shengrui999 had higher d concentration in stem and leaf, BCF and TF efficiency than Zhongke11 regardless of Si at the maturity (Figs. 2, 3, and 4). The capacity to translocate elements to shoots is an important factor involved in tolerance (Harris and Taylor 2013; Yan et al. 2018). Silicon application significantly reduced Cd concentration and BCF in root, stem, leaf and grain compared to non-Si treatment in Shengrui999 (Figs. 2 and 3), demonstrating that silicon is more effective in enhancing Cd tolerance in Cd-tolerant genotype than Cd-sensitive genotype, through suppressing Cd uptake and root to shoot Cd transport (Song et al. 2009).

# Conclusion

Maize plants accumulated more Cd in roots than in the aboveground parts with the least in grains. Application of Si had significant effects on regulating Cd uptake and bioconcentration factor in stem and leaf, and soil pH, and contributed to daily intake of Cd and Cd health risk index under Cd stress. Genotype Shengrui999 (shallow-rooted) was more effective in response to Si application under Cd stress than Zhongke11 (deep-rooted). Root morphology had significant correlations with root Cd concentration, bioaccumulation factor and translocation factor at the silking stage in response to silicon and Cd applications. Therefore, it can be considered as a strategy of maize plants to restrict the entry of Cd into the root systems. Future studies are required to reveal physiological and molecular mechanisms of the role of Si in alleviating plant tolerance to Cd stress involving different Si species and more genotypes under both controlled and field conditions.

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**Code availability (software application or custom code)** Not applicable.

#### Declarations

Conflicts of interest No conflicts of interest.

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