



Silicon mitigates cadmium toxicity in grain Amaranth (*Amaranthus cruentus* L.)

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Received: 29 July 2025 / Accepted: 12 December 2025
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Abstract

Cadmium (Cd) is a major environmental pollutant that is toxic to plants, animals, and humans. While many studies have demonstrated the ability of silicon (Si) to alleviate abiotic stress in plants, its role and potential mitigating mechanisms in pseudocereals are still underexplored. The aim of this study was to investigate the effect of exogenous Si (1 and 2.5 mM) on Cd (100 µM) toxicity in grain amaranth (*Amaranthus cruentus* L.) grown in hydroponics for 14 days. We evaluated various physiological traits such as plant growth, morphological and anatomical parameters of roots, leaf stomata and epidermal cell density, as well as nutrient uptake, expression of stress-related genes, antioxidant enzyme activities and photosynthetic pigment contents. The results showed that the addition of Si to Cd treatment increased all measured growth parameters and restored root tissue arrangement, including xylem area, compared to Cd-treated plants. We found that Cd accumulation significantly decreased under Si supplementation in a Si concentration-dependent manner, translocation of toxic Cd ions to the aerial parts was low, and Cd ions remained mainly stored in the roots. The application of Si had no obvious effect on the uptake of essential micro- and macroelements in amaranth roots, which was reduced by Cd. The effect of Si was more pronounced at the level of antioxidants and gene expression. Overall, our results showed that Si, at a concentration of 1 mM, effectively attenuated the negative effects of Cd on the vegetative growth of amaranth.

Keywords Cadmium stress · Gene expression · Grain amaranth · Ion homeostasis · Morphology · Silicon

Introduction

Soil contamination with heavy metals (HMs) is one of the most critical environmental issues today, posing a threat to natural biotopes, food production and human health (Angon

et al. 2024). The presence of HMs in cultivated areas significantly impairs harvests. Anthropogenic activities such as municipal waste disposal, smelting, mining, metal production, and the use of synthetic phosphate fertilizers are the primary causes of increased cadmium (Cd) levels in the environment (Haider et al. 2021).

Cadmium is harmful to plants and animals, and even at low concentrations, it can affect plant growth and interfere with various physiological and biochemical processes (Charagh et al. 2025). According to the available information, the most severe symptoms of Cd toxicity are typically observed in the root system, as the root is plant organ that first comes into contact with Cd in soil and is fully exposed to its harmful effects (Lux et al. 2011; Abbas et al. 2017). Morphological and anatomical changes associated with Cd stress, such as reduced root length, root surface area and number of root tips, can lead to a decrease in water uptake, absorption and translocation of nutrients (Maksimović et al. 2007; Zhang et al. 2022). The interference between nutrients and Cd leads to an imbalance in the uptake and translocation

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Communicated by Dezhi Wu

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of essential elements, resulting in disruption of their specific functions in plant metabolism and tissue depletion (Khan et al. 2016). The primary mechanisms of stress defence in plant cells include increased activity of antioxidant enzymes and non-enzymatic antioxidants (Nogueirol et al. 2016). Enhanced enzymatic activities can mitigate Cd-induced oxidative stress by inhibiting the production of reactive oxygen species (ROS) (Zeng et al. 2017). However, due to its high affinity for functional groups, Cd tends to inactivate the antioxidant enzymes and thus eliminate one of the fundamental plant defence processes against oxidative stress (Cuypers et al. 2011). Mitigating Cd toxicity and reducing Cd accumulation and translocation in plant tissues by environmentally friendly elements has garnered significant research interest in recent years (Gheshlaghpour et al. 2021; Kabir et al. 2021; Mišúthová et al. 2021; Zhou et al. 2023; Iwauala et al. 2024).

Metalloid silicon (Si), the second most abundant element in the Earth's crust, appears to have numerous benefits for plants. Its exogenous application helps plants to increase their tolerance to various biotic and abiotic stress factors, including HMs (Khan et al. 2020; Ur Rahman et al. 2020; Ngugi et al. 2021; Lakhneko et al. 2024). Silicon can mitigate the toxic effects of Cd in several ways, e.g., by precipitating toxic metals as cofactors, reducing bioavailability of metal in the soil, stimulating antioxidant enzymes, compartmentalising excess metals in cell vacuoles or cell walls, reducing metal uptake and translocation from roots to shoots, and influencing the expression of stress-related genes (Shao et al. 2017; Etesami and Jeong 2018; Bhat et al. 2019; Ngugi et al. 2021; Palmeros-Suárez et al. 2021). The different role of Si in attenuating Cd toxicity has been confirmed in various plant species (Thakur et al. 2023; Thakral et al. 2024).

As far as we know, there is little information on the role of Si in amaranth under stress conditions. This highly nutritious and non-allergenic plant, is one of the most representative species of pseudocereals. Current interest in amaranth is related to its exceptional adaptability to various stress conditions, such as salinity, drought, heat, and HMs (Lu et al. 2017; Adhikary et al. 2021; Cechin et al. 2022). Due to the resilience of amaranth, investigating its natural stress mechanisms provides insights important for the development of sustainable and resilient cropping systems to address global climatic challenges. The objective of the current study was to investigate the alleviating effect of Si at two selected concentrations on grain amaranth cultivar Pribina (*Amaranthus cruentus* L.) exposed to Cd for a duration of 14 days under hydroponic conditions. We focused on exploring the possible mechanisms by which Si alleviates Cd-induced stress, specifically in terms of plant growth, physiological parameters, morpho-anatomical characteristics of roots,

leaf stomata and epidermal cells, nutrient uptake, Cd and Si accumulation and translocation, and gene expression of stress-related genes. The results obtained may contribute to a better understanding of the responses of this pseudocereal to Cd stress, provide evidence for the beneficial effects of Si on *A. cruentus*, and outline hypotheses regarding the effect of Si under metal stress.

Materials and methods

Experimental design

In this study, grain amaranth cv. Pribina (*Amaranthus cruentus* L.) isolated and registered in Slovakia was used (Hricová et al. 2016). Cultivation was performed under controlled conditions (23 °C, 16/8 hours light/dark cycle, 50% humidity) in a plant growth chamber (KBWF 720, Binder, Germany). Plants were pre-cultivated in soil until the 4-leaf stage (approximately 4 weeks) and then hydroponically cultivated in Hoagland's nutrient solution for 7 days of acclimation (pH=7; Hoagland and Arnon 1950). Six different treatments were then applied in solutions: control (no Si and no Cd), Si (1 and 2.5 mM), Cd (100 µM), 1 Si+Cd and 2.5 Si+Cd; using cadmium chloride (CdCl₂, Sigma-Aldrich, Saint-Louis, MO, USA) and sodium silicate (Na₂SiO₃, Sigma-Aldrich, Saint-Louis, MO, USA). The Cd and Si concentrations were chosen based on the previously published data conducted on amaranth plants (Lancíková et al. 2020) and other cereals (Shafeeq-ur-Rahman et al. 2020; Mišúthová et al. 2021; Benslima et al. 2022). Plants were treated for 14 days (a total of 7 weeks of cultivation). The nutrient solutions were renewed after 7 days of cultivation. At least three biological replicates were established for each treatment and all samples were harvested at the same time of a day.

Plant growth parameters

Root and shoot fresh weight (RFW, SFW; g) and dry weight (RDW, SDW; g), total fresh weight (TFW; g) and total dry weight (TDW; g) were measured to determine the biomass at the time of harvest. Growth tolerance index (GTI; %) was calculated according to Lukačová et al. (2013) as follows: $GTI = (TFW \text{ of treated plants} / TFW \text{ of control plants}) \times 100$.

Root morphological traits, including total root length (cm), average root diameter (mm), total surface area (cm²), root volume (cm³), and number of root tips, were assessed using an automated scanner (EPSON Expression 11000XL, Reagent Instrument, Canada), equipped with 4.1 WinRHIZO™ image analysis software (Regent Instruments, 2000, Business, CA, USA).

Analysis of photosynthetic pigments

Content of total chlorophylls (Chl), chlorophyll *a* (Chl*a*), chlorophyll *b* (Chl*b*) and carotenoids (Car) was determined according to Lichtenthaler and Wellburn (1983). Fresh leaf tissues were powdered using the TissueLyzer II (Qiagen, Hilden, Germany) and immersed in 1 ml 96% (v/v) ethanol. The mixture was centrifuged (4000 rpm, 15 min, 20 °C). The supernatant was diluted with ethanol and measured spectrophotometrically at 665, 649 and 470 nm. The content of photosynthetic pigments was calculated using the following equations:

where *A* represents measured absorbance. The final values were expressed as $\mu\text{g g}^{-1}$ FW.

Estimation of enzymatic activities

Assay for the determination of ascorbate peroxidase (APX; EC 1.11.1.11), catalase (CAT; EC 1.11.1.6), and guaiacol peroxidase (GPX; EC 1.11.1.7) was performed according to El-Tayeb (2006) with some modifications. Leaf and root biomass were homogenised separately in a cold 100 mM potassium phosphate buffer (pH 7.4). The mixture was centrifuged (10 000 rpm, 10 min, 4 °C) and the supernatant was used as a crude extract. To determine GPX activity, a mixture of 10 mM phosphate buffer (pH 7.0), 40 mM H_2O_2 and 20 mM guaiacol was added to the extract. The assay mixture for APX included 50 mM phosphate buffer (pH 7.0), 250 mM H_2O_2 and 0.5 mM ascorbate. For the estimation of CAT activity, 50 mM phosphate buffer (pH 7.0) and 200 mM H_2O_2 were added to the enzyme extract. Enzyme activities were determined spectrophotometrically using the Synergy H1 Multimode Microplate Reader (BioTek Instruments, CA, USA) by measuring the increase in absorbance at 470 nm for GPX and the decrease in absorbance at 290 and 240 nm for APX and CAT, respectively, for 3 min and expressed as $\mu\text{mol min}^{-1} \text{g}^{-1}$ FW.

Multielement analysis

Plant samples were dried at 50 °C for 48 h, powdered and subjected to element (Ca, Fe, K, Mg, Na, Zn and Cd) analysis by inductively coupled plasma optical emission spectroscopy (ICP-EOS; ICP Thermo ICAP 7000 Dual, ThermoFisher Scientific, Waltham, MA, USA) according to the method described by Lancíková et al. (2020). For Si detection, the samples were analysed by flame atomic absorption spectrometry (FAAS; Perkin-Elmer 5000, Norwalk, CT, USA) in an acetylene- N_2O flame after digestion with HNO_3 , HF and H_2O_2 in a microwave digestion system (Multiwave Pro, Anton Paar GmbH, Graz, Austria). The concentrations of selected elements were determined in the

below- and above-ground parts of the plants and expressed in mg kg^{-1} DW. The translocation factor (TF) of Cd and Si was calculated as follows: $\text{TF} = \text{element concentration in shoots (mg kg}^{-1} \text{ DW)} / \text{element concentration in roots (mg kg}^{-1} \text{ DW)}$.

Histochemical localization of cadmium in roots and leaves

Dithizone method was used for the histochemical localization of Cd within both roots and the 5th leaves of amaranth plants according to Seregin and Kozhevnikova (2005) with some modifications. The 5th leaf of the amaranth plant was selected because it was fully mature and consistently present in plants across all treatment groups. The whole samples were stained for 90 min in the dark with a solution containing 50 mg of dithizone (Alfa Aesar, MA, USA) in 75 ml acetone, 25 ml distilled water and 15 drops of acetic acid glacial (Mikrochem Trade, Pezinok, Slovakia) and washed three times. A series of hand sections were then cut with a razor blade, placed on a microscope glass slide and photographed directly with the Zeiss Axioplan 2 light microscope equipped with the Sony DXC-S500 digital camera system (Carl Zeiss, Jena, Germany). Sections of primary roots were prepared at various distances from the root tip. The localization of Cd within the plant tissue was revealed by the reddish staining of the formed Cd-dithizone precipitates.

Toluidine blue staining and analysis of root cross sections

We analysed the anatomical structure of the primary roots by histochemical staining with toluidine blue. Root cross sections were prepared by hand sectioning with a razor blade approximately 20–40% from the root tip in the zone with fully developed endodermis. Sections were stained with 0.01% (w/v) toluidine blue (Sigma-Aldrich, Saint-Louis, MO, USA) in water for 5 min, washed and immediately viewed under a light microscope as described above (O'Brien et al. 1964; Ribeiro and Leitão 2020). For each biological replicate, three microscopic images and following anatomical traits of the primary roots were analysed using ImageJ software (version 1.43u, National Institutes of Health, Bethesda, MD, USA): root diameter (μm), stele diameter (mm), cross section (CS) area of root (μm^2), CS area of cortex including endodermis (μm^2), CS area of stele (μm^2) and CS area of xylem vessels (μm^2). The stele-to-root ratio (%) was calculated according to Lukačová et al. (2013) as follows: $(\text{CS area of stele} / \text{CS area of root}) \times 100$.

Analysis of stomatal and epidermal cell area and density

The epidermal tissue of the adaxial (AD) and abaxial (AB) side of leaf was imprinted using nail polish, adhesive tape and a glass slide, as described by Voleniková and Tichá (2001). The leaf imprints were examined with the Leica DM5500B light microscope equipped with a digital microscope camera (DFC450C, Leica Microsystems GmbH, Wetzlar, Germany). Microscopic images were taken from six randomly selected, middle-positioned areas on both sides of the leaves for each biological replicate and analysed with ImageJ. Following parameters were then calculated per same unit area (i.e., square millimetre of leaf surface) on both AD and AB sides of the leaves: stomatal density (mm^{-2}), stomatal area (mm^2), epidermal cell density (mm^{-2}) and epidermal cell area (mm^2). Stomatal index (SI; %), which calculates the ratio between the number of stomata and the number of epidermal cells on a given leaf area, was determined according to the definition of Salisbury (1927) as $\text{SI} = [\text{stomatal density}/(\text{stomatal density} + \text{epidermal cell density})] \times 100$.

RNA isolation and gene expression analysis by real-time quantitative PCR (qRT-PCR)

Total RNA was extracted from 100 to 200 mg of frozen root powder using TRI Reagent (Sigma-Aldrich, Saint-Louis, MO, USA), according to the protocol with modifications for amaranth plants (Lancíková et al. 2020). The quality and quantity of RNA was measured using the BioSpec-nano spectrophotometer (Shimadzu, Kyoto, Japan) and RNA integrity was checked on a 1.5% agarose gel. The cDNA was synthesized from 500 ng RNA using a Maxima First Strand cDNA Synthesis Kit for qRT-PCR (ThermoFisher Scientific, Waltham, MA, USA) with dsDNase treatment according to the manufacturer's instructions. Changes in the expression of abiotic stress-related genes were quantified using previously published primer sets for *Ah24* gene, chitinase 5 (*Chit 5*) gene, gene encoding a DUF642 protein (*AhDGR2*), early responsive to dehydration gene

(*AhERD*) and glutamate cystein ligase (*GSH1*) gene (Table S1). Amplifications were performed in the LightCycler[®] 96 system (Roche, Basel, Switzerland). The total volume of PCR reaction was 10 μL , including 5 μL 2x SsoAdvanced Universal SYBR[®] Green supermix (Bio-Rad, Hercules, CA, USA), 0.4 μL forward and reverse primers (10 μM), 1 μL cDNA (50 ng) and 3.2 μL nuclease-free water. Cycling conditions were initial denaturation at 95 °C for 30 s, followed by 45 cycles of denaturation at 95 °C for 15 s and annealing at 60 °C for 60 s. Melting curve analysis was then performed in the range of 60 to 97 °C at 0.1 °C/s to verify the specificity of the desired amplicon. The expression levels of genes were normalized in relation to their expression in roots of control plants, which was set to a value of 1.0. Relative gene expression was calculated using the $2^{-\Delta\Delta\text{CT}}$ comparative cycle threshold method (Livak and Schmittgen 2001). Two *Amaranthus hypochondriacus* housekeeping genes, actin (*AhACT*) and tubulin (*AhTUB*), were used as internal controls to normalize the data.

Statistical analysis

Data were analysed using GraphPad Prism 10.3.1 statistical software. The analysis of variance (ANOVA) and Tukey multiple comparison test were used to identify significant differences between the treatments at $p\text{-value} \leq 0.05$. In addition, the correlation analyses were assessed using Pearson's correlation at $p\text{-value} \leq 0.05$ and $p\text{-value} \leq 0.01$.

Results

Effect of silicon on physiological properties of Amaranth under cadmium stress

The effect of Cd treatment and different Si concentrations on the growth parameters of amaranth plants is shown in Table 1; Fig. 1A. Cadmium toxicity was clearly reflected by significant reduction of TFW (55%), RFW (56%) and SFW (54%) compared to control. Under Si supplementation, especially with 1 mM Si, RFW, TFW and RDW

Table 1 Effect of Si on Amaranth growth parameters under Cd stress

Treatment	RFW (g)	SFW (g)	TFW (g)	RDW (g)	SDW (g)	TDW (g)
Control	2.65 ± 0.56 ^{ab}	3.23 ± 0.61 ^{ab}	5.87 ± 0.96 ^a	0.14 ± 0.04 ^{ab}	0.42 ± 0.04 ^a	0.55 ± 0.07 ^a
1 Si	2.87 ± 0.29 ^a	3.12 ± 0.21 ^{ab}	5.99 ± 0.48 ^a	0.13 ± 0.04 ^{ab}	0.46 ± 0.16 ^a	0.59 ± 0.18 ^a
2.5 Si	2.32 ± 0.47 ^{abc}	3.50 ± 0.71 ^a	5.82 ± 1.16 ^{ab}	0.13 ± 0.04 ^{ab}	0.35 ± 0.08 ^{ab}	0.48 ± 0.11 ^{ab}
Cd	1.17 ± 0.28 ^d	1.48 ± 0.55 ^c	2.66 ± 0.72 ^d	0.10 ± 0.02 ^b	0.22 ± 0.14 ^b	0.32 ± 0.15 ^b
1 Si + Cd	1.91 ± 0.27 ^{bc}	2.38 ± 0.27 ^{bc}	4.23 ± 0.25 ^{bc}	0.18 ± 0.06 ^a	0.40 ± 0.05 ^{ab}	0.58 ± 0.05 ^a
2.5 Si + Cd	1.71 ± 0.14 ^{cd}	1.95 ± 0.37 ^c	3.66 ± 0.50 ^{cd}	0.16 ± 0.03 ^{ab}	0.31 ± 0.08 ^{ab}	0.46 ± 0.10 ^{ab}

Data are the means of four replicates ± SD. Different letters indicate significant differences between the treatments at $p\text{-value} \leq 0.05$ according to Tukey test. RFW – root fresh weight; SFW – shoot fresh weight; TFW – total fresh weight; RDW – root dry weight; SDW – shoot dry weight; TDW – total dry weight

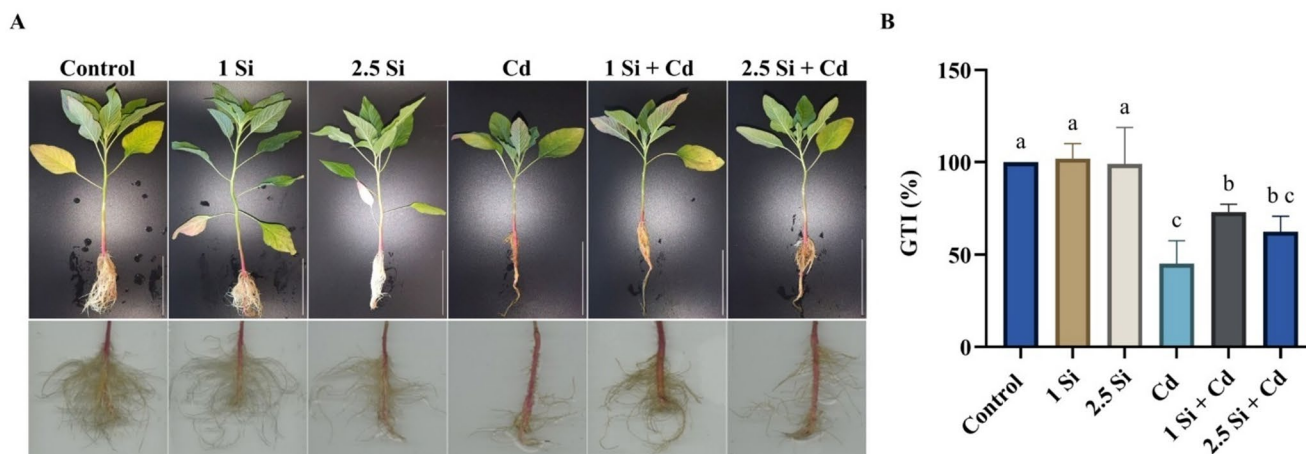


Fig. 1 Phenotype, root morphology (A) and growth tolerant index (GTI, %; B) of hydroponically cultivated amaranth plants (*Amaranthus cruentus* L., cv. Pribina) exposed to different Cd and Si treatments applied during 14-days. Each image represents a typical appearance of

four plants per treatment. Scale bar = 10 cm. Data are the means of at least four replicates $\pm$ SD. Different letters indicate significant differences between the treatments at p -value ≤ 0.05 according to Tukey test

Table 2 Effect of Si and Cd on root characteristics of Amaranth determined by scanning root system

Data are the means of at least four replicates $\pm$ SD. Different letters indicate significant differences between the treatments at p -value ≤ 0.05 according to Tukey test

Treatment	Total root length (cm)	Total root surface area (cm ²)	Average root diameter (mm)	Root volume (cm ³)	Number of root tips
Control	141.91 $\pm$ 16.19 ^{ab}	10.04 $\pm$ 1.91 ^a	1.21 $\pm$ 0.60 ^a	2.83 $\pm$ 1.80 ^{ab}	1640.67 $\pm$ 839.79 ^a
1 Si	169.94 $\pm$ 32.42 ^a	12.59 $\pm$ 1.76 ^a	1.43 $\pm$ 0.64 ^a	4.98 $\pm$ 2.14 ^a	1571.75 $\pm$ 132.91 ^a
2.5 Si	138.29 $\pm$ 37.17 ^{ab}	9.63 $\pm$ 0.72 ^a	0.74 $\pm$ 0.21 ^a	1.12 $\pm$ 0.54 ^{bc}	1103.25 $\pm$ 397.92 ^{ab}
Cd	83.58 $\pm$ 15.52 ^c	10.41 $\pm$ 1.33 ^a	0.78 $\pm$ 0.18 ^a	0.58 $\pm$ 0.31 ^c	429.00 $\pm$ 75.55 ^b
1 Si + Cd	143.70 $\pm$ 34.28 ^{ab}	12.55 $\pm$ 2.12 ^a	1.24 $\pm$ 0.56 ^a	1.63 $\pm$ 0.93 ^{bc}	647.25 $\pm$ 147.39 ^b
2.5 Si + Cd	97.27 $\pm$ 1.89 ^{bc}	9.36 $\pm$ 0.40 ^a	0.66 $\pm$ 0.14 ^a	0.50 $\pm$ 0.26 ^{bc}	571.75 $\pm$ 200.99 ^b

increased significantly by 63%, 59% and 80%, respectively. The higher concentration of Si (2.5 mM) added to Cd only slightly improved plant growth indices and the changes were not significant. When Si was supplied alone, no expressive effect on plant growth was detected compared to control plants.

In general, GTI shows the potential tolerance of plant genotypes to different environmental stressors. Here, the GTI calculations (Fig. 1B) supported the biomass data presented in Table 1 and showed the negative effect of Cd on amaranth growth parameters. Cadmium significantly reduced the total GTI of the amaranth plants by 55% compared to control plants. Under both Si supplementation (1 Si + Cd and 2.5 Si + Cd) the growth tolerance improved significantly by about 30%, with a slightly better response observed when 1 Si was applied. According to these results, the tolerance of amaranth plants to Cd could be aligned as 1 Si + Cd > 2.5 Si + Cd > Cd.

Morphological and anatomical response of roots to cadmium stress under silicon supplementation

Visual symptoms of damage caused by Cd treatment were observed in root system of amaranth plants (Fig. 1A). Several root morphology data such as total length, total surface area, average diameter, volume and number of root tips are presented in Table 2. As shown, Cd treatment reduced the total root length by 41%, root volume by 80% and number of root tips by 74% compared to control (Fig. 1A; Table 2). The addition of Si (1 mM) attenuated the negative effects of Cd on root parameters as follows: total root length increased by 72%, root volume by 181% and number of root tips by 51%. Higher Si supplementation (2.5 mM) had no such a significant effect. Total surface area and average diameter of amaranth roots were not significantly affected by any of the treatments tested.

Toluidine blue staining and analysis of primary root cross sections were performed to better assess the anatomical response of amaranth roots to the applied treatments (Fig. 2; Table 3). Cadmium stress decreased root diameter, CS area of root, stele and stele-to-root ratio compared to control. Under both Si + Cd treatments, root diameter and stele

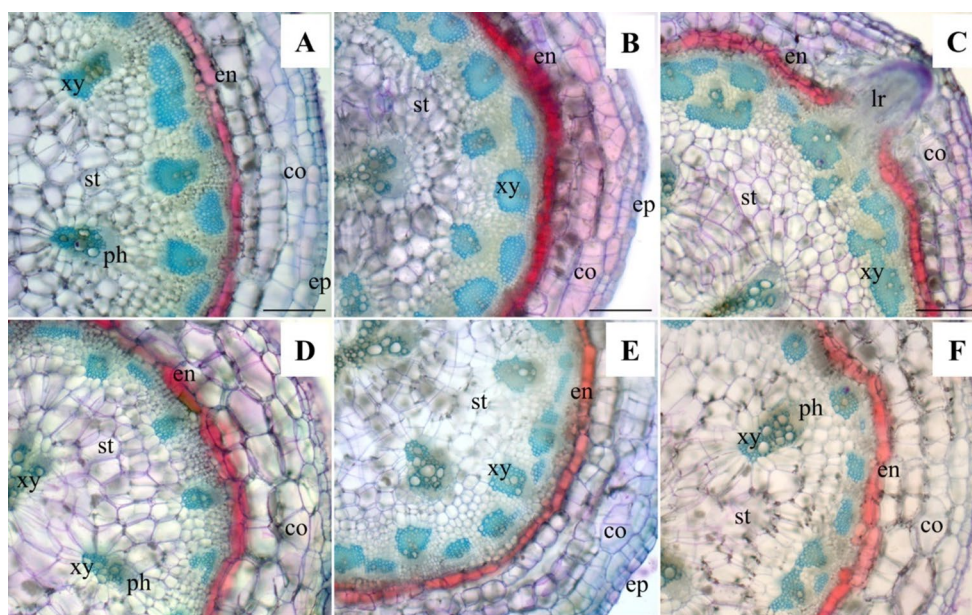


Fig. 2 Primary root anatomy of *Amaranthus cruentus* L. plants after staining with toluidine blue. Cross-sections of control (A), 1 Si (B), 2.5 Si (C), Cd (D), 1 Si+Cd (E) and 2.5 Si+Cd (F) treated plants in area of 40% from the root apex. This staining differentiates the bluish-green colour of lignified cell walls in xylem vessels, while the phloem vessels remain almost unstained. Reddish to purple-coloured staining

reveals primary cell wall types. The endodermis, as the innermost layer of the root cortex, is naturally pink in this cultivar. The intensity of the staining is directly proportional to the thickness of the hand-made root section. Co – cortex; en – endodermis; ep – epidermis; lr – lateral root; ph – phloem; st – stele; xy – xylem. Scale bar = 500 μ m

Table 3 The anatomical parameters of primary roots of *Amaranthus cruentus* L. plants exposed to different treatments

Treatment	Root diameter (mm)	Stele diameter (mm)	CS area root (mm ²)	CS area cortex (mm ²)	CS area stele (mm ²)	CS area xylem vessels (mm ²)	CS area stele/CS area root (%)
Control	5.29 $\pm$ 0.21 ^{abc}	3.30 $\pm$ 0.16 ^{bc}	21.89 $\pm$ 1.20 ^{bc}	11.47 $\pm$ 0.83 ^{ab}	10.42 $\pm$ 0.59 ^b	1.33 $\pm$ 0.21 ^b	47.61 $\pm$ 1.67 ^{ba}
1 Si	5.06 $\pm$ 0.15 ^c	3.40 $\pm$ 0.15 ^d	19.98 $\pm$ 0.81 ^d	10.95 $\pm$ 0.47 ^{bc}	9.03 $\pm$ 0.55 ^c	1.59 $\pm$ 0.43 ^a	45.16 $\pm$ 1.63 ^d
2.5 Si	5.34 $\pm$ 0.36 ^{ab}	3.80 $\pm$ 0.32 ^{ab}	22.29 $\pm$ 2.25 ^b	10.59 $\pm$ 0.54 ^c	11.71 $\pm$ 1.84 ^a	1.75 $\pm$ 0.33 ^a	52.27 $\pm$ 3.00 ^a
Cd	5.25 $\pm$ 0.30 ^{bc}	3.50 $\pm$ 0.29 ^{cd}	21.22 $\pm$ 1.33 ^c	11.65 $\pm$ 1.12 ^a	9.57 $\pm$ 1.39 ^c	0.82 $\pm$ 0.31 ^c	45.02 $\pm$ 5.06 ^d
1 Si+Cd	5.42 $\pm$ 0.10 ^{ab}	3.70 $\pm$ 0.10 ^{ab}	22.34 $\pm$ 0.80 ^b	11.83 $\pm$ 0.70 ^a	10.51 $\pm$ 0.35 ^b	1.62 $\pm$ 0.46 ^a	47.08 $\pm$ 1.69 ^{cd}
2.5 Si+Cd	5.54 $\pm$ 0.18 ^a	3.90 $\pm$ 0.15 ^a	23.48 $\pm$ 0.75 ^a	11.86 $\pm$ 0.43 ^a	11.62 $\pm$ 0.79 ^a	1.14 $\pm$ 0.24 ^b	49.46 $\pm$ 2.16 ^b

Data are the means of three microscopic images of roots per sample $\times$ three biological replicates per treatment $\pm$ SD. Different letters indicate significant differences between the treatments at p -value ≤ 0.05 according to Tukey test. CS area – cross section area

diameter and CS arrangements of individual root tissues increased compared to Cd treatment, being highest when 2.5 mM Si was used. Silicon action at this concentration prompted a significant increase in root and stele diameter by about 6% and 11%, respectively, and an increase in CS area of root, stele and stele-to-root ratio by about 11%, 21% and 10%, respectively. No significant differences were observed in CS area of cortex between Cd and Si+Cd treatments. The CS area of xylem vessels differed most between the applied treatments in the examined area of primary roots. Cadmium significantly reduced the xylem area by about 38% compared to control. Compared to Cd-treated plants, the addition of 1 and 2.5 mM Si to Cd progressively increased CS area of xylem vessels by almost 98% and 39%, respectively.

Response of photosynthetic pigments and antioxidant enzymes to cadmium stress under silicon supplementation

As presented in Fig. 3, our results showed a similar trend in the levels of photosynthetic pigments (Chla, Chlb and Car) among all tested treatments. Under Cd, a significant increase in chlorophylls and carotenoids was observed by about 70% and 65%, respectively, compared to control plants. Both Si concentrations (1 and 2.5 mM) significantly reversed this effect, but the impact of 1 mM Si as a supplement to Cd treatment was the strongest. In fact, 1 Si was able to mitigate Cd toxicity by reducing the photosynthetic pigments content to the level of control. A higher Si concentration (2.5 mM) applied in the absence of the metal resulted in

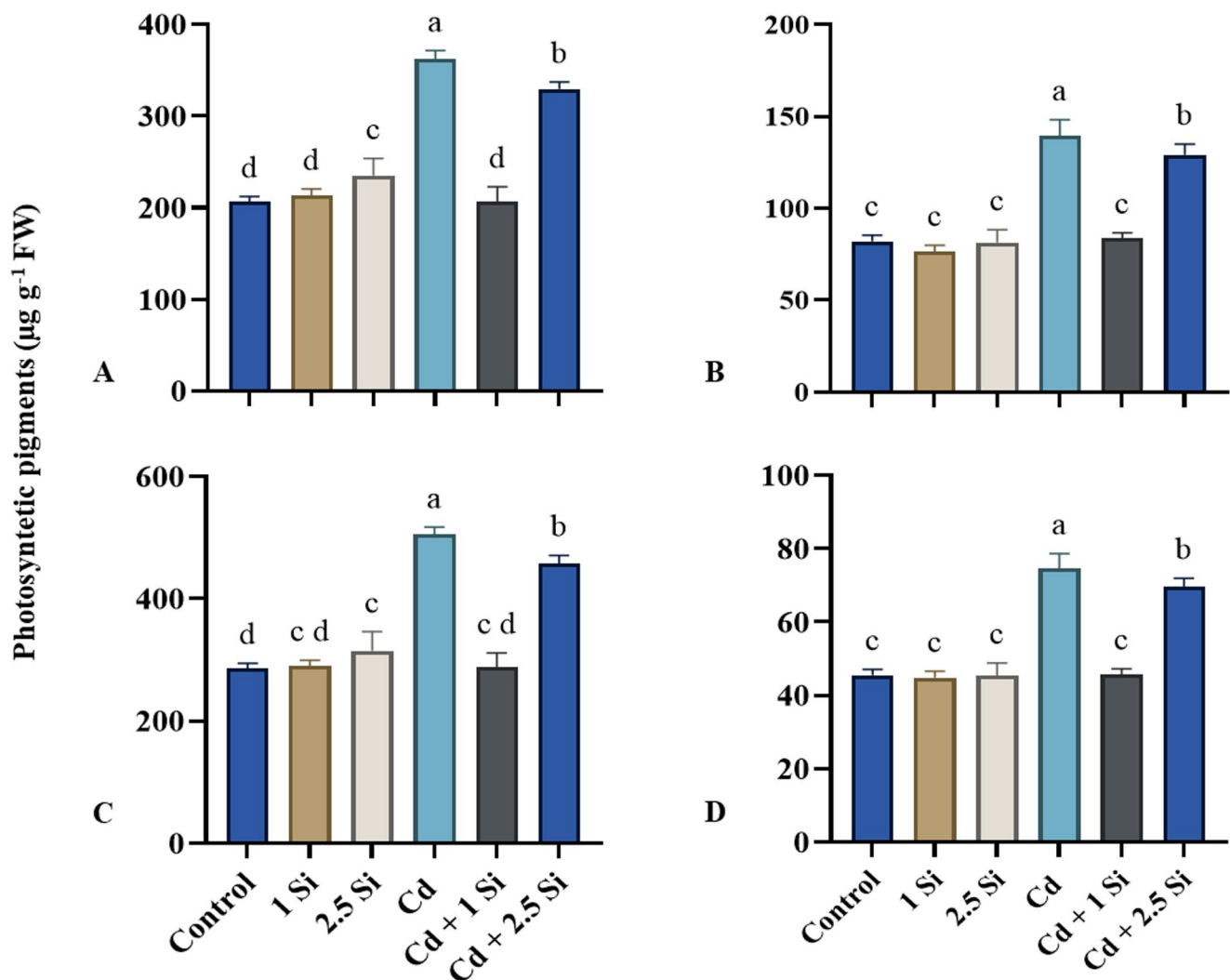


Fig. 3 Effect of Si and Cd on the content of photosynthetic pigments in amaranth leaves. (A) chlorophyll *a*, (B) chlorophyll *b*, (C) total chlorophylls, (D) carotenoids. Data are the means of at least four rep-

licates $\pm$ SD. Different letters indicate significant differences between treatments at p -value ≤ 0.05 according to Tukey test

a significant increase in Chl *a* (13%) and total chlorophylls (9%) compared to control.

To evaluate the effects of Cd stress and Si supplementation on the antioxidant defence mechanisms of amaranth, the activities of GPX, APX and CAT enzymes were analysed (Fig. 4). As shown, Cd stress significantly increased GPX and APX activities in the roots by 25% and 76%, respectively, compared to control. The application of Si to Cd attenuated Cd toxicity by affecting GPX and APX activity in the roots with similar values as in the control plants. Although CAT activity under Cd treatment was not significantly different from that of control plants, the addition of Si enhanced the activity of this enzyme in both roots and shoots.

Cadmium did not significantly increase the activities of all tested enzymes in the amaranth leaves, while the lowest APX activity was observed in 1 Si treated plants. On the

other hand, 1 Si + Cd led to a 30% increase in APX activity in the leaves compared to Cd alone.

Cadmium and silicon accumulation and translocation in Amaranth

To investigate the effect of Si application on the Cd accumulation and distribution within the plant, the concentrations of Cd and Si in the roots and shoots of amaranth (Fig. 5A) and corresponding translocation factors (Fig. 5B) were estimated. Under Cd treatment, amaranth accumulated a large amount of Cd mainly in the roots ($1480.01 \text{ mg kg}^{-1} \text{ DW}$) than in the shoots ($322.64 \text{ mg kg}^{-1} \text{ DW}$). The Cd concentration in plants decreased significantly in direct proportion to the increasing concentration of Si in the growth solution. The maximum reduction in Cd uptake was observed under 2.5 Si + Cd treatment, when the Cd concentration

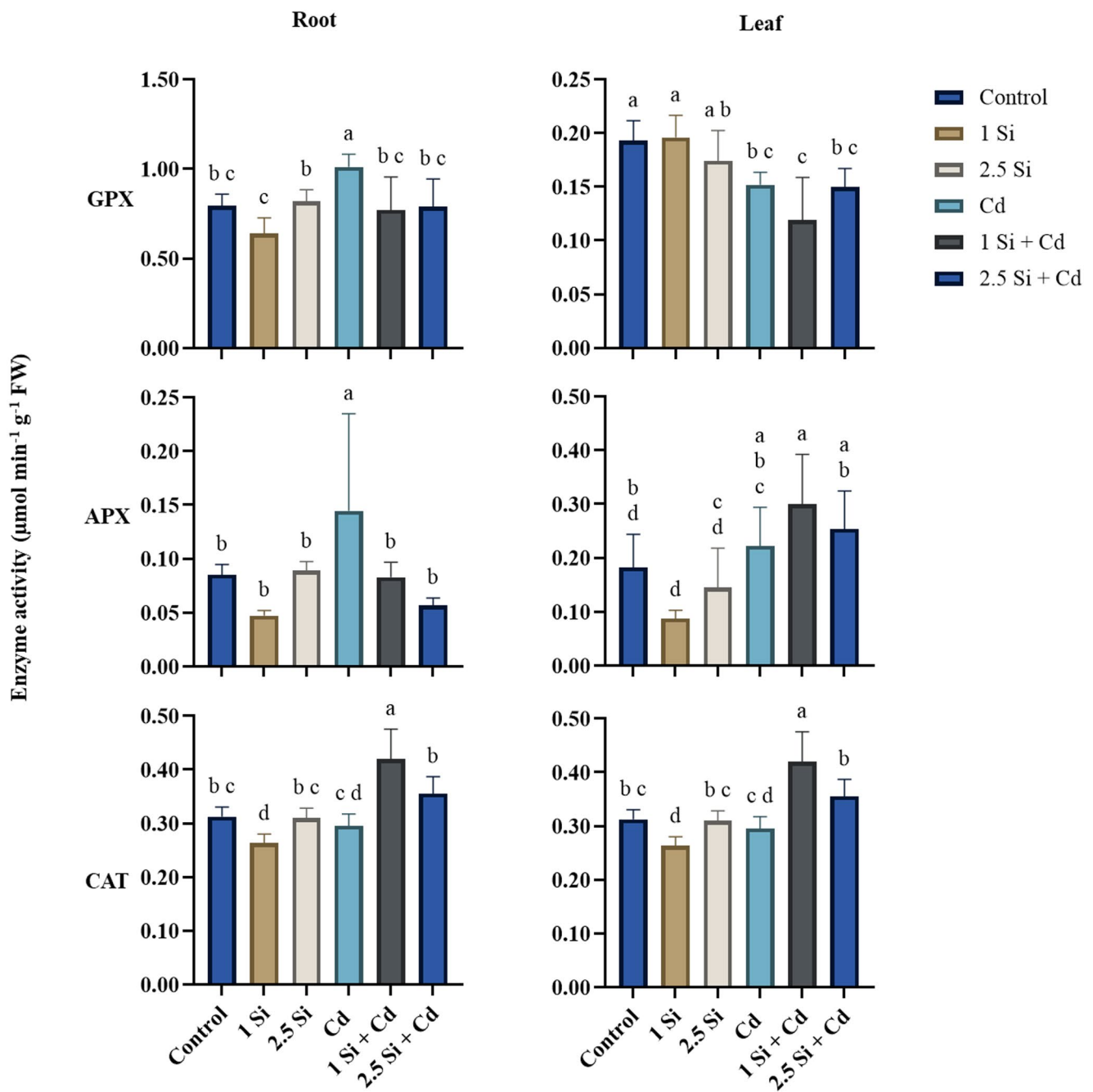


Fig. 4 Effect of Si and Cd treatment on the activity of guaiacol peroxidase (GPX), ascorbate peroxidase (APX) and catalase (CAT) in amaranth roots and leaves. Data are the means of at least four rep-

licates $\pm$ SD. Different letters indicate significant differences between the treatments at p -value ≤ 0.05 according to Tukey test

was reduced by 49% in roots and 80% in shoots relative to Cd-treated plants. Regarding Si, the significantly highest Si content was found in root of plants treated with 1 Si ($5075.00 \text{ mg kg}^{-1} \text{ DW}$) compared to other treatments supplemented with Si. The simultaneous application of Si and Cd resulted in a lower accumulation of Si in the roots. On the other hand, Cd did not reduce the translocation of Si from amaranth root to shoot.

Translocation factor of Cd, which indicates the ability of a plant to translocate an element from roots to shoots, was less than 0.3. The addition of 1 mM Si and 2.5 mM Si to Cd significantly reduced transport of Cd from roots to the upper part of plant by 28% and 49%, respectively (Fig. 5B). In the case of Si translocation, plants cultivated in the presence of Si (both 1 and 2.5 mM) exhibited TF of less than 1, while Si transport within the plants was increased under Cd stress.

The significantly highest TF for Si was calculated under 1 Si + Cd treatment with a value of about 2.5.

Cadmium distribution in root and leaf tissue

Accumulation of Cd was visualised histochemically by the presence of reddish-stained Cd-dithizone complexes in the roots and leaves of amaranth plants treated with Cd (Fig. 6A, B, E, F, I, J) 1 Si + Cd (Fig. 6C, D, G, H, K-M) and 2.5 Si + Cd (Fig. 6D, H, J, M). As shown, the Cd distribution in the plant tissues varied considerably. In Cd treated plants, the metal ions were present in the root epidermis and some cells of the cortex of the primary roots (Fig. 6A). Cadmium was detected both in the apoplast space and in the cytosol of cortical cells. In the stele, the xylem vessels were the main sites of Cd accumulation (Fig. 6E, F, I). The adjacent parenchyma cells and most cells in the stele showed low Cd content. No Cd was observed in the phloem vessels or in the young lateral root primordia located within the cortex of the primary roots. However, numerous Cd precipitates were found in the older lateral roots emerging from the primary roots (Fig. 6B, I). In the leaves of Cd-treated plants, Cd was observed in the epidermis, trichomes, vascular bundles and surrounding parenchyma cells (Fig. 6J).

Both Si supplementations to Cd treatment resulted in a notable reduction in Cd accumulation in the roots and leaves of amaranth plants (Fig. 6C, D, G, H, K-M). Less or no Cd was detected in the epidermal cells, cortex, endodermis and stele of the primary roots (Fig. 6C, D, G, H). When Cd was present, the older lateral roots and the xylem vessels were the main sites of Cd localization (Fig. 6D, G). Similarly, less Cd was found in the leaves of plants treated with Si + Cd than in those treated with Cd alone (Fig. 6K-M). No staining was observed in control (no Si, no Cd) and Si (1 and 2.5 mM Si) samples (Fig. S1).

Nutrient content under silicon and cadmium exposure

To show the effects of Cd treatment and different Si concentrations applied as a mitigating agent on the distribution and content of nutrients in amaranth tissue, Ca, Fe, K, Mg, Na and Zn were analysed in roots and shoots (Fig. 7). In the roots of Cd-treated plants, the concentrations of Fe, K, Mg and Zn were significantly reduced by 57%, 20%, 47% and 58%, respectively, compared to control. Cadmium also reduced the content of Ca and Na in roots, but not significantly. In shoots, Cd exposure significantly reduced Ca (34%), Fe (62%), K (36%) and Zn (44%) accumulation compared to untreated plants. The addition of Si to Cd treatment did not significantly restore the concentrations of Ca, Fe, K, and Zn decreased by Cd. A slight increase of Ca and

K concentrations (23% and 11%, respectively) was observed under 1 Si + Cd treatment in shoots, and even higher increase (37% and 39%, respectively) with 2.5 Si supplementation. Under non-stressed conditions, 1 Si treatment resulted in a significant decrease in K and Mg content in roots and Zn content in shoots of amaranth plants by 17%, 7% and 41%, respectively. In addition, both Si treatments (1 and 2.5 mM) increased the Na accumulation in roots (70% and 286%) and in shoots (50% and 86%) compared to control.

Correlation analysis between selected elements and Amaranth growth parameters

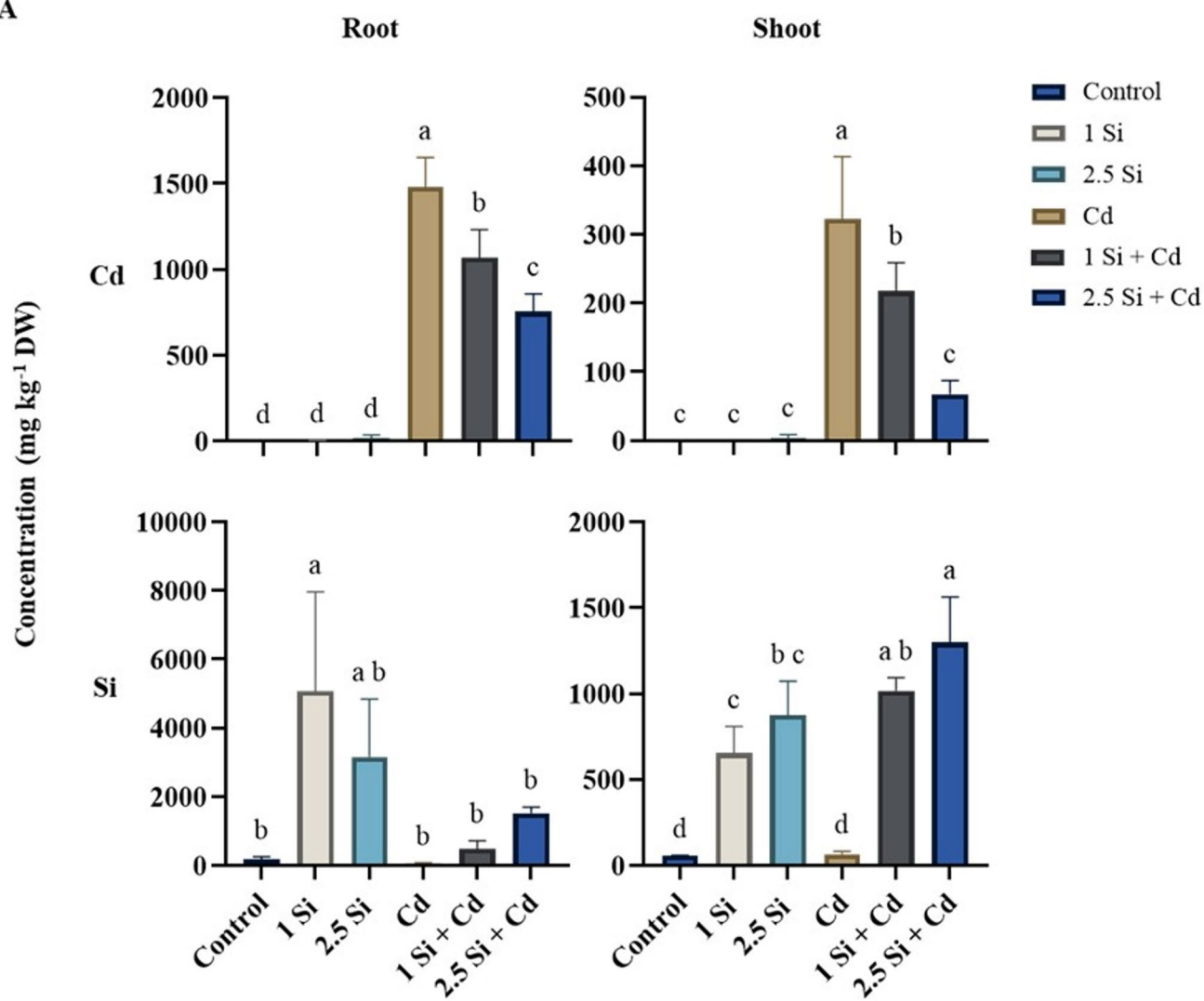
To better understand the relationships between the studied elements (including Si and Cd) and the growth parameters of the amaranth plants, as well as between the elements themselves in all studied treatments, a Pearson correlation analysis was performed (Fig. 8). As expected, Cd in amaranth tissues had a highly significant negative correlation with RFW, SFW and TFW. On the other hand, RFW showed a positive correlation with Ca in shoots and Fe in both roots and shoots. In addition, SFW and TFW were positively correlated with Ca and K content in shoots. Cadmium in roots showed a significant negative correlation with Ca, Fe and Zn in both roots and shoots, as well as with K in shoots and Mg in the roots. In addition, Cd in roots showed a highly significant positive correlation with Cd in shoot tissue. Silicon found in the roots was only significantly positively correlated with Na in shoot and, conversely, Si in the shoots was only positively correlated with Na in roots. Surprisingly, Si in the shoots showed a significant negative correlation with Fe and K in the roots.

Among the analysed elements, there was a highly significant positive correlation between Ca, Fe and Zn in amaranth roots. The Ca content in shoots also showed a highly significant positive correlation with Fe and K in shoots and Mg in both tissues. In addition, the presence of Zn in shoots correlated positively with K and Mg in roots and with Fe in both roots and shoots.

Expression of stress-related genes in Amaranth exposed to silicon and cadmium treatment

Since the root is the first organ to come into contact with Cd, we focused on analysing the expression of selected stress-related genes in amaranth roots using qRT-PCR. To determine whether Si can regulate the expression of genes potentially involved in Cd-induced defence strategies, the expression of *Ah24*, *Chit5*, *AhDGR2*, *AhERD*, and *GSH1* genes was examined. The results presented in Table 4 show that Cd treatment significantly upregulated the expression of *Ah24*, *Chit5*, *AhERD* and *GSH1* compared to control,

A



B

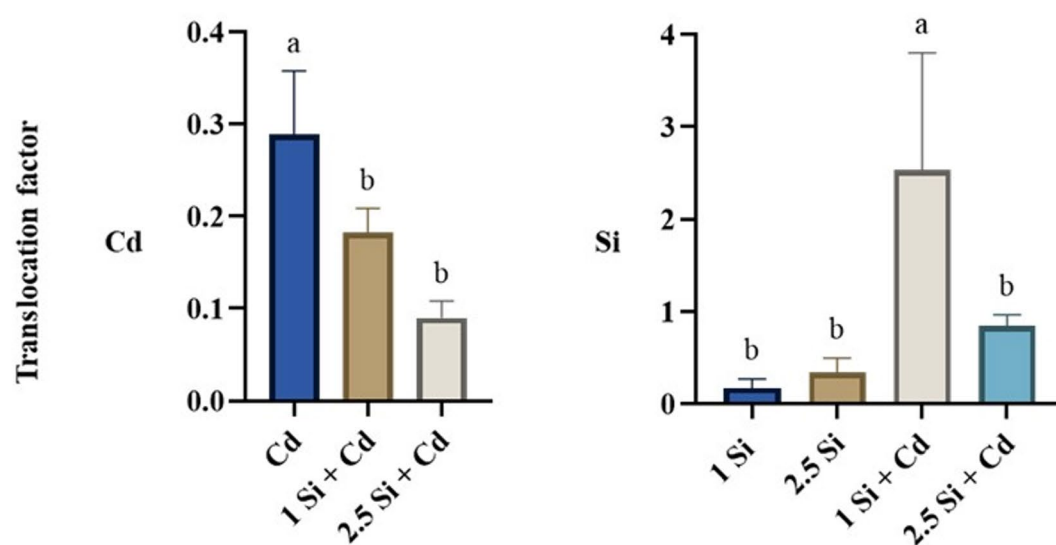


Fig. 5 Concentration of Cd and Si in roots and shoots of amaranth plants treated with Si and Cd (A) and translocation factor of Cd and Si (B). Data are the means of at least four replicates $\pm$ SD. Different letters indicate significant differences between treatments at a p -value ≤ 0.05 according to Tukey test

with the highest increase in *Ah24* (9.22-fold) and *AhERD* (11.76-fold). The addition of Si at both concentrations to Cd treatment led to a downregulation in the expression of these genes, although the results were more pronounced with 2.5 Si supplementation. For example, the *Ah24* gene was downregulated 6.66-fold and *Chit5*, *AhERD* and *GSH1* were significantly downregulated 1.96-fold, 10.28-fold and 2.28-fold, respectively, in response to 2.5 Si + Cd treatment. Neither Cd nor Si + Cd had a significant effect on expression of *AhDGR2* in amaranth roots.

Effect of silicon and cadmium on stomata and epidermal cells

To further investigate the effect of Si in response to Cd stress in the leaves, the stomatal and epidermal cell densities on the adaxial side (AD) and abaxial side (AB) of fully expanded amaranth leaves were analysed (Table 5). The representative image of the leaf surfaces reflecting the applied treatments is shown in Fig. S2. Compared to control, Cd stress significantly increased the number of stomata and epidermal cells on AD side by about 34% and 37%, respectively, and on AB side by 15% (non-significantly) and 19% (significantly), respectively (Table 5). The addition of Si to Cd treatment had a contradictory effect on density of stomata and epidermal cells in leaves compared to that observed under Cd stress. On AD side, stomatal and epidermal cell densities were reduced when both 1 and 2.5 mM Si supplemented Cd treatment, although only 1 mM Si caused a significant decrease in epidermal cell density of about 16% (Table 5). In contrast, the number of stomata was increased under both Si + Cd treatments on AB side, while the number of epidermal cells was only increased under the action of 2.5 mM Si (Table 5). At the same time, stomatal density on AD side under both Si + Cd treatments was comparable to that observed on AB side. The area of stomata increased significantly by about 27% under Cd stress compared to control on AD side. On the other hand, only treatment with 2.5 Si + Cd resulted in a significantly comparable stomatal area as in non-stressed plants. The area of the epidermal cells remained unchanged on AD side under all treatments tested, although it increased significantly by 9% compared to Cd treatment on AB side under 2.5 Si + Cd action.

Stomatal index indicates the ratio of stomata to the number of epidermal cells on a given leaf surface. The observed SI values were higher on AB side compared to AD side of the leaves (Table 5). Concurrently, the SI values remained

unchanged between control and Cd treatment; however, the addition of 1 mM Si to Cd treatment significantly increased SI on AD and AB leaves surfaces by about 8% and 9%, respectively, compared to Cd-induced stress.

Discussion

Although silicon is not strictly essential for plant growth, it can play an important role in mitigating various biotic and abiotic stresses through mechanisms such as up-regulation of plant antioxidant defence systems, complexation, co-precipitation, immobilization and compartmentalization of toxic metal ions within plants, regulation of plant hormone biosynthesis, and escalation of active plant components associated with stress resistance (Liang et al. 2007; Thakral et al. 2021, 2024; Verma et al. 2024). Exogenous addition of Si has been found to reverse Cd toxicity in many plants (Ahmed et al. 2023; Khan et al. 2023; Zhao et al. 2023), and the mechanisms of amelioration of Cd stress could be cultivar and tissue dependent (Lux et al. 2011; Imtiaz et al. 2016; Yadav et al. 2021). To date, only one study has examined the impact of Si on Cd toxicity in amaranth plants in a pot experiment (Lu et al. 2017). The plants of *Amaranthus hypochondriacus* showed improvements in biomass and pigment levels, as well as reduced Cd concentrations, following calcium silicate supplementation. In contrast to the pot experiments by Lu et al. (2017), our study conducted in hydroponics with *Amaranthus cruentus* provided a strictly controlled environment, where nutrient composition, pH and Cd concentration were precisely managed. Moreover, the high solubility of sodium silicate ensured immediate Si availability with direct physiological effects on Cd uptake and plant responses, without interference from soil interactions or slow Si release.

Herein, the inhibitory effect of Cd treatment was reflected in a significant reduction in the growth of amaranth plants (Table 1; Fig. 1A), especially the growth of root system (Table 2), decreased anatomical traits of primary roots and the arrangement of individual inner root tissues, with the CS area of stele and xylem vessels being most affected (Fig. 2; Table 3). The application of Si significantly restored normal morphological characteristics of plants and improved the GTI of Cd-treated plants (Fig. 1B), mainly at a concentration of 1 mM Si. Similar positive trends reflecting the effect of Si on attenuating Cd stress on growth parameters were observed in rice, maize, wheat and tomato (Rizwan et al. 2012; Bari et al. 2020; Liu et al. 2020; Wei et al. 2021a). Moreover, both 1 Si and 2.5 Si supplementation not only increased the anatomical parameters of primary roots, but these roots also exhibited a more complex internal root architecture compared to the roots of Cd-treated plants,

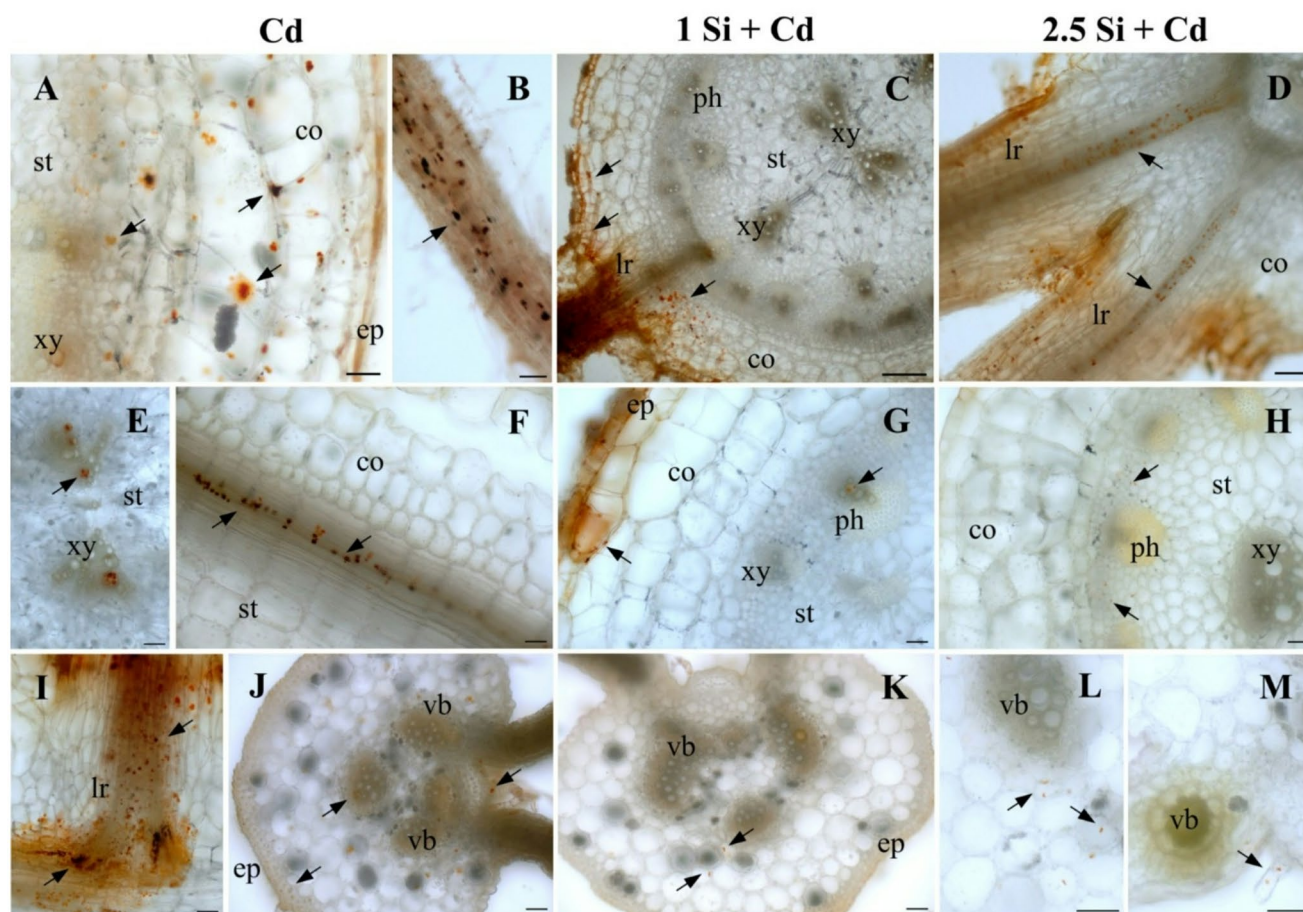


Fig. 6 Histochemical detection of Cd in Cd (A, B, E, F, I, J), 1 Si + Cd (C, G, K) and 2.5 Si + Cd (D, H, L, M)-treated amaranth plants. Arrows show reddish-stained Cd-dithizone complexes in cross (A, C-E, G-I) and transverse (F) sections of primary roots, lateral roots (B) and cross

sections of leaves (J-M). Co – cortex; ep – epidermis; lr – lateral root; ph – phloem; st – stele; vb – vascular bundle; xy – xylem. Scale bar = 100 μ m in A, B, E, F, J, 200 μ m in I and 500 μ m in C, D, G, H and K-M

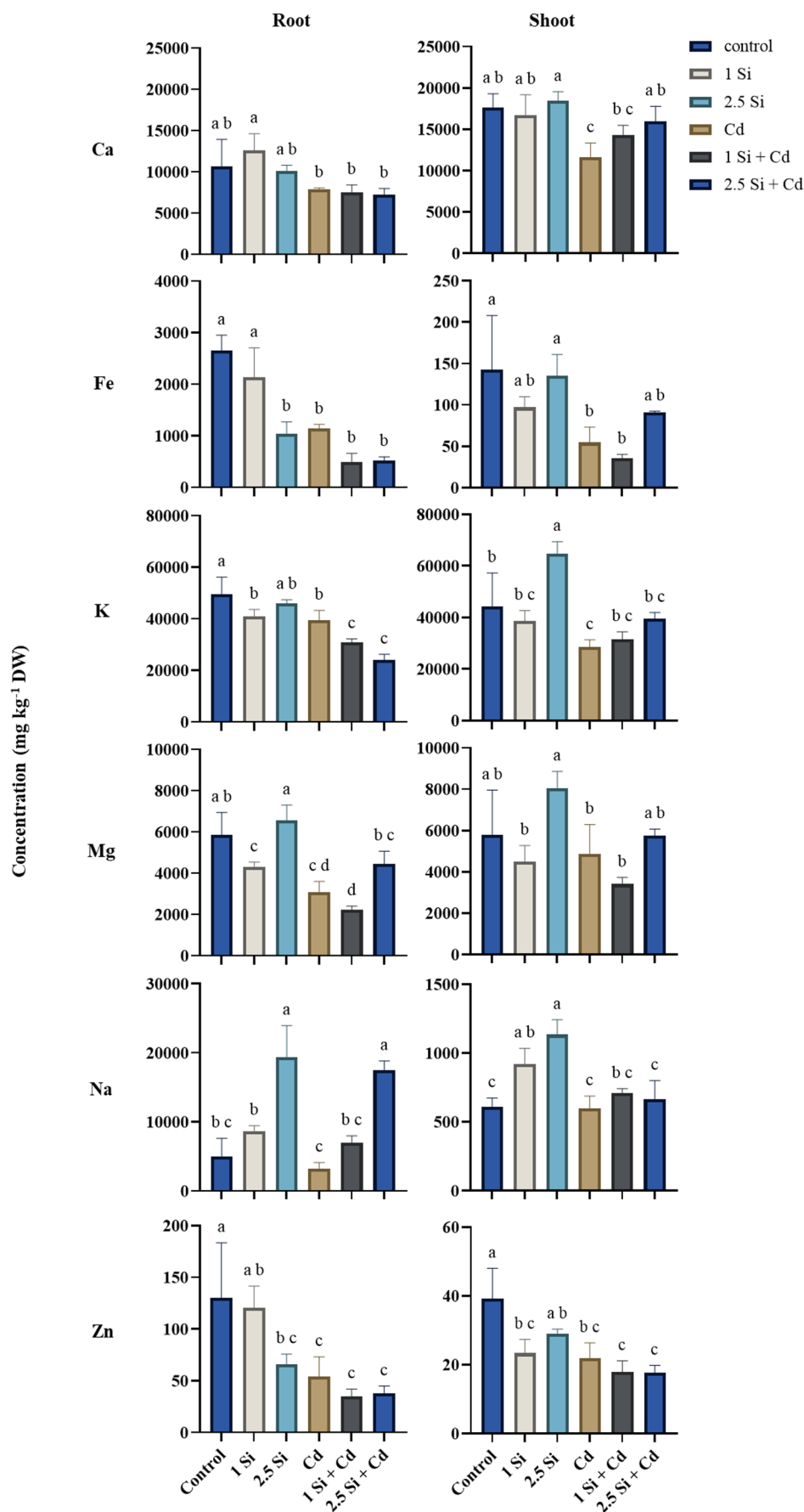
as evidenced by the stele-to-root ratio (Fig. 2; Table 3). In particular, the area of xylem vessels was almost twice as large under 1 mM Si supplementation. Few studies have addressed the reverse effect of Si on root tissue re-arrangement in rice (Fan et al. 2016) and pea plants (El-Okkiah et al. 2022) when it complements Cd treatment. As reported by Lukačová et al. (2013), these changes could even be dose-dependent, both for Si and Cd, as high Si concentrations under Cd stress had no effect in maize on root tissue parameters.

The ability to accumulate Cd varies among plant species (Lux et al. 2011) and also among amaranth cultivars (Fan and Zhou 2009; Chi et al. 2019; Lanciková et al. 2020). Amaranth (cv. Pribina) roots accumulated a higher amount of Cd compared to the aerial parts of the plant, mainly due to direct Cd exposure. Histochemical staining of the roots of Cd-treated plants showed that Cd entered the roots via both symplasmic and apoplasmic pathways (Fig. 6). In fact, several studies suggest that Cd is mainly transported and accumulated through the intercellular spaces and cell walls,

but small quantities of Cd ions could enter the root cytoplasm and vacuoles via selective cation channels (Seregin and Kozhevnikova 2011; Lux et al. 2011; Chi et al. 2019). In this study, we observed a non-uniform distribution of numerous Cd precipitates within the cells of root tissues and intracellular spaces. Cadmium was detected in xylem vessels of primary and lateral roots. As a result, Cd was translocated to leaves, although in a significantly less amount (Fig. 6). These observations indicate that most of the Cd from the nutrient solution reached the stele via root apices and also by passing the endodermal barriers developed at a greater distance (Kabata-Pendias 2010; Van der Ent et al. 2013).

The alleviating effect of Si was demonstrated by a reduced Cd uptake in roots and its translocation to shoots of amaranth (Figs. 5 and 6). Cadmium concentration in the roots decreased with increasing Si concentration in the growth medium, suggesting an external interaction between Si and toxic Cd ions. Similar pattern was observed in another study on *A. hypochondriacus*, where Cd

Fig. 7 Concentration of Ca, Fe, K and Mg, Na and Zn in roots and shoots of amaranth plants treated with Si and Cd. Data are the means of at least four replicates $\pm$ SD. Different letters indicate significant differences between treatments at a p -value ≤ 0.05 according to Tukey test



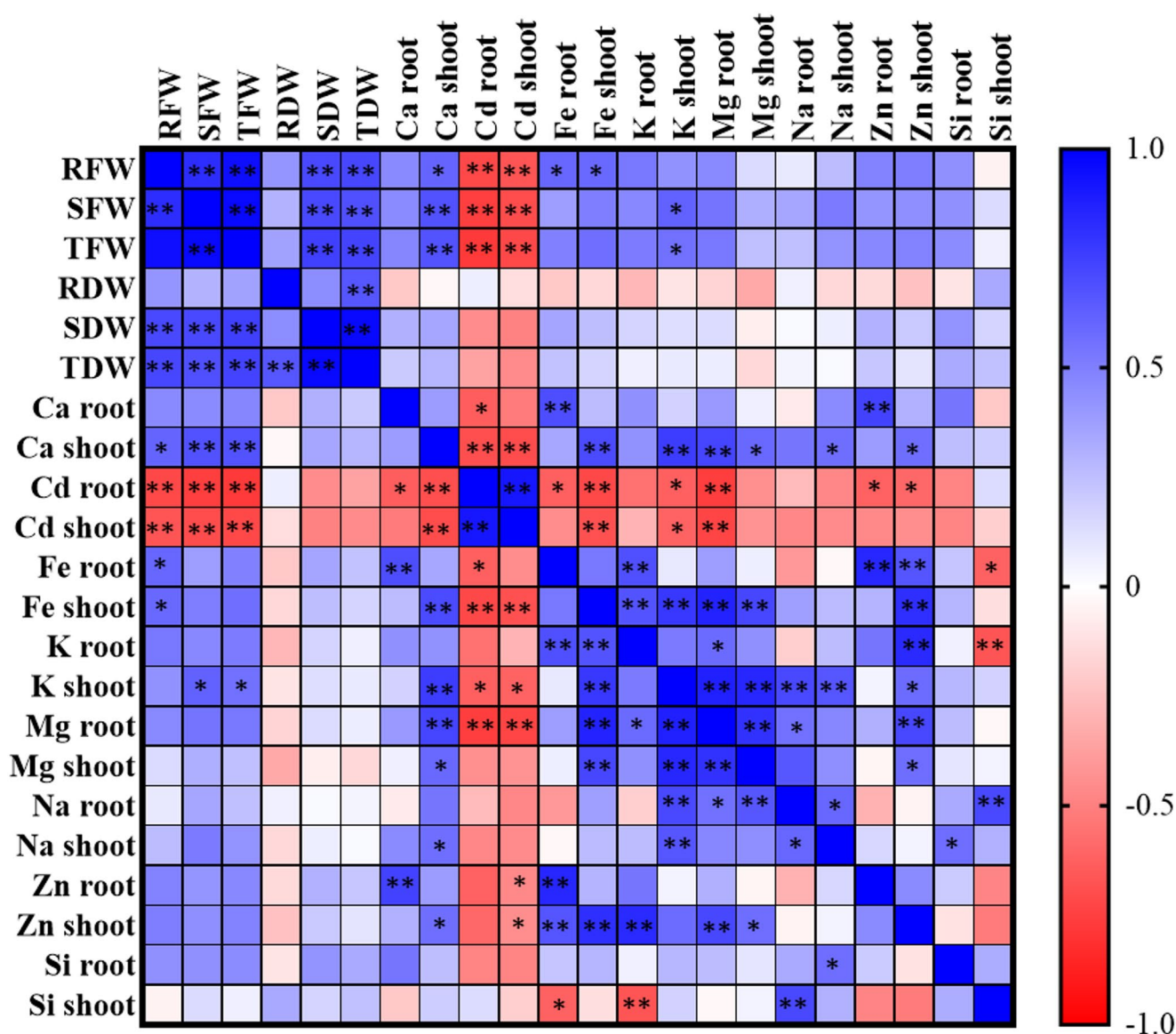


Fig. 8 Correlations among Ca, Fe, K, Mg, Na, Zn, Cd and Si themselves, and among the elements and root fresh weight (RFW), root dry weight (RDW), shoot fresh weight (SFW), and shoot dry weight (SDW)

in amaranth tissues. Significant positive correlations are shown in blue colour and negative correlations are shown in red colour. Significant differences are indicated at p-value ≤ 0.05 (*) and p-value ≤ 0.01 (**)

Table 4 Expression of selected stress-related genes of Amaranth in response to Si and Cd treatment

Treatment	Ah24	Chit5	AhDGR2	AhERD	GSH1
Control	1.00 ± 0.84 ^b	1.00 ± 0.51 ^c	1.00 ± 0.44 ^{ab}	1.00 ± 0.41 ^b	1.00 ± 0.18 ^b
1 Si	0.32 ± 0.10 ^b	0.93 ± 0.41 ^c	0.62 ± 0.12 ^{ab}	2.07 ± 0.76 ^b	1.89 ± 0.46 ^{ab}
2.5 Si	0.24 ± 0.12 ^b	0.43 ± 0.10 ^c	0.50 ± 0.10 ^b	2.38 ± 1.16 ^b	1.40 ± 0.30 ^b
Cd	9.22 ± 6.81 ^a	3.43 ± 1.09 ^a	1.13 ± 0.21 ^a	11.76 ± 8.43 ^a	3.79 ± 1.94 ^a
1 Si + Cd	4.31 ± 1.09 ^{ab}	2.80 ± 0.50 ^{ab}	0.91 ± 0.08 ^{ab}	2.43 ± 0.27 ^b	1.82 ± 0.15 ^{ab}
2.5 Si + Cd	2.56 ± 0.54 ^{ab}	1.47 ± 0.17 ^{bc}	0.72 ± 0.12 ^{ab}	1.48 ± 0.17 ^b	1.51 ± 0.11 ^b

Data are the means of three replicates ± SD. Different letters indicate significant differences between the treatments at p-value ≤ 0.05 according to Tukey test

concentration decreased with increasing doses of calcium silicate (Lu et al. 2017), despite different concentrations and growth systems used. Our findings are supported by previous reports indicating that Si application reduces Cd content

in various plants (Naeem et al. 2018; Khan et al. 2020; Ur Rahman et al. 2021). It should be assumed that Si deposition into cell walls can at least partially minimize the penetration of toxic HM ions into the roots by strengthening

Table 5 Response of stomata and epidermal cells to Si and Cd treatment on adaxial and abaxial sides of leaf epidermis in *Amaranthus cruentus* L.

Stomatal and epidermal cell densities were calculated from six identical, randomly selected areas in the middle part of the adaxial and abaxial side of the 5th leaf. Data are the means of three replicates per treatment $\pm$ SD. Different letters indicate significant differences between the treatments at p -value ≤ 0.05 according to Tukey test. SI – stomatal index

Treatment	Stomatal density (mm ²)	Stomatal area (mm ²)	Epidermal cell density (mm ²)	Epidermal cell area (mm ²)	SI (%)
Adaxial					
Control	86.56 $\pm$ 21.88 ^c	0.01 $\pm$ 0.00 ^b	439.72 $\pm$ 87.93 ^c	0.98 $\pm$ 0.02 ^a	16.28 $\pm$ 1.18 ^{bc}
1 Si	122.40 $\pm$ 27.30 ^a	0.01 $\pm$ 0.00 ^a	647.78 $\pm$ 137.94 ^a	0.99 $\pm$ 0.00 ^a	15.89 $\pm$ 0.76 ^{bc}
2.5 Si	110.30 $\pm$ 14.89 ^{ab}	0.01 $\pm$ 0.00 ^a	539.56 $\pm$ 76.70 ^{cd}	0.99 $\pm$ 0.00 ^a	17.06 $\pm$ 1.98 ^{ab}
Cd	116.10 $\pm$ 24.56 ^{ab}	0.01 $\pm$ 0.01 ^a	603.17 $\pm$ 143.21 ^{ab}	0.99 $\pm$ 0.01 ^a	16.28 $\pm$ 1.78 ^{bc}
1 Si + Cd	108.40 $\pm$ 17.65 ^b	0.01 $\pm$ 0.00 ^a	507.78 $\pm$ 72.22 ^d	0.99 $\pm$ 0.00 ^a	17.56 $\pm$ 1.15 ^a
2.5 Si + Cd	107.20 $\pm$ 17.07 ^b	0.01 $\pm$ 0.00 ^b	573.61 $\pm$ 88.72 ^{bc}	0.99 $\pm$ 0.00 ^a	15.78 $\pm$ 1.06 ^c
Abaxial					
Control	89.67 $\pm$ 4.13 ^c	0.02 $\pm$ 0.00 ^{ab}	337.56 $\pm$ 33.82 ^c	0.98 $\pm$ 0.00 ^c	20.94 $\pm$ 2.92 ^{ab}
1 Si	119.11 $\pm$ 7.23 ^a	0.03 $\pm$ 0.00 ^a	444.00 $\pm$ 72.69 ^a	0.97 $\pm$ 0.00 ^c	21.22 $\pm$ 1.59 ^{ab}
2.5 Si	109.67 $\pm$ 14.21 ^{ab}	0.02 $\pm$ 0.01 ^a	401.44 $\pm$ 102.22 ^{ab}	0.98 $\pm$ 0.01 ^c	21.61 $\pm$ 2.43 ^{ab}
Cd	103.17 $\pm$ 11.37 ^{bc}	0.02 $\pm$ 0.00 ^{ab}	400.17 $\pm$ 54.72 ^{ab}	0.98 $\pm$ 0.00 ^{bc}	20.44 $\pm$ 1.82 ^b
1 Si + Cd	108.28 $\pm$ 4.97 ^{ab}	0.02 $\pm$ 0.00 ^b	368.00 $\pm$ 67.38 ^{bc}	0.98 $\pm$ 0.00 ^{ab}	22.17 $\pm$ 1.92 ^a
2.5 Si + Cd	108.83 $\pm$ 6.65 ^{ab}	0.02 $\pm$ 0.01 ^{ab}	414.67 $\pm$ 54.83 ^{ab}	0.98 $\pm$ 0.00 ^a	20.78 $\pm$ 1.48 ^{ab}

their mechanical barrier properties after HM-induced stress (Liang et al. 2007; Lukacova et al. 2021), thus further slowing the apoplasmic bypass flow of Cd within the inner root tissues and from the roots to aerial tissues (Ma and Yamaji 2006). Additionally, Si can reduce Cd uptake by downregulating genes involved in Cd uptake (Khelifi et al. 2024). As far as we know, this is the first study showing the spatial histochemical detection of Si-mitigated Cd accumulation in grain amaranth *A. cruentus* L. (Fig. 6). Similar to Cd treatment, Cd precipitates were detected in the xylem vessels of lateral roots both under 1 Si and 2.5 Si supplementation. Nevertheless, Cd remained mainly stored in the primary roots and distributed almost exclusively in the apoplasm and only minimally in cortical parenchyma cells or stele in the roots and leaves. These findings correlated with data from the ICP-EOS analysis (Figs. 5 and 6) and confirm that only a small amount of Cd was translocated via phloem and reached the shoots and leaves. In contrast to our results, Lu et al. (2017) observed a decrease of Cd in the apoplasm and an increase of Cd in the symplasm in subcellular fractions of leaves under Cd stress, but no differences in the subcellular distribution of Cd in the roots between Cd and Si + Cd treatments in *A. hypochondriacus* L. under calcium silicate supplementation. However, it has been shown that Si could reduce both symplasmic and apoplasmic Cd concentrations, and at the same time the ratio of Cd bound to cell walls could be higher compared to intracellular Cd concentrations in roots of various plant species such as maize (Vaculik et al. 2015; Lukačová et al. 2013), rice (Ma et al. 2016; Wei et al. 2021b), and also in subcellular leaf fractions of spinach (Waheed et al. 2021). This could be a plant defence strategy to avoid damage to photosynthetically active tissues under HM stress (Vaculik et al. 2009; Júnior et al. 2014; Yadav et al. 2021). Notably, our results suggest that the application of Si (at both concentrations) reduced, but did not completely

block, the risk of Cd accumulation in edible above-ground organs of amaranth plants.

As reported, the accumulation of Si varies greatly among plant species and depends on the mechanisms involved in its uptake, aggregation and transportation (Ma et al. 2006, 2007; Imtiaz et al. 2016). According to Ma and Yamaji (2015), the Si accumulation also varies among varieties within a species. Different Si transport proteins need to be identified as they not only show species-dependent expression patterns but are moreover tissue- and cell-dependent. Silicon can be taken up by the roots either passively or actively and stored in different parts of the plant at various concentrations (Ma and Yamaji 2015; Lux et al. 2020; Mandlik et al. 2020). In the current work, Si was mainly stored in the roots of *A. cruentus* cv. Pribina grown in the presence of Si (Fig. 7). On the other hand, the Si content in the shoots was significantly increased by the presence of Cd in growth media. Moreover, a TF of Si above 1 reflected an effective translocation of Si into shoots in the presence of Cd (Fig. 8). On the one hand, the higher Si deposition under Cd treatment in amaranth shoots could be the result of increased area of xylem traits, especially under 1 Si supplementation (Table 4; Fig. 6) and transpiration stream. On the other hand, it could be related to the expression of Si transporter genes as a possible internal mechanism for the detoxification of Cd by Si (Chaiwong and Prom-u-thai 2022). As far as we know, Si deposits have not yet been detected in amaranth.

Although the negative effects of Cd on plant development and its role in photosynthetic performance are well known, the changes induced in plants may vary depending on the plant species and the amount of Cd applied (Song et al. 2019). Our previous work has shown that photosynthetic pigments tend to increase in some amaranth cultivars after exposure to other metals (Pb, Zn, and Mn), possibly to compensate for the reduced photochemistry (Hunková et al. 2024). In agreement with these results, Cd increased the

content of Chla, Chlb, total chlorophylls and carotenoids in amaranth cv. Pribina in this study (Fig. 3). A possible explanation could be that the plants increase chlorophyll content above the homeostatic level as a stress coping mechanism (Agathokleous et al. 2020). This can be confirmed by the study of Hu et al. (2014), in which Cd at low concentrations increased the chlorophyll content and the light energy captured and transferred to photosystem II reaction centre in soybean seedlings. Silicon is assumed to protect the integrity of chloroplasts by reducing oxidative stress, and its influence could be more direct via higher quantum efficiency in the photosystems. However, this mechanism is not yet well understood (Cao et al. 2015; Thorne et al. 2020). In the current study, Si significantly reversed the negative effect of Cd and adjusted the chlorophyll and carotenoid levels of control plants (Fig. 3). Although studies suggest that Si increases content of photosynthetic pigments in plants exposed to stress (Sienkiewicz-Cholewa et al. 2018; Dar et al. 2022), we hypothesize that Si has the opposite effect in Cd-treated amaranth, as an indirect effect of reduced Cd. Furthermore, we showed that pigment content returned to the homeostatic level of control plants when 1 Si was applied, indicating an optimal Si concentration for the growth of *A. cruentus* in hydroponic experiment. In contrast to our results, calcium silicate increased chlorophyll content of leaves of *A. hypochondriacus* cultivated in Cd-containing soil (Lu et al. 2017).

The leaves of *A. cruentus* have stomata distributed across both leaf surfaces as an adaptation to increased stomatal conductance and higher photosynthetic rates (Wei et al. 2022; Padmini and Rao 1993). Both processes are associated with short-term physiological responses in the leaves, such as opening and closing of stomata and minimization of water loss to avoid osmotic stress during leaf transpiration. In long term, these processes should be associated with changes in the density and size of stomata and epidermal cells and may represent an auxiliary response of plants to unfavourable conditions (Haworth et al. 2016; Hasanuzzaman et al. 2023). In the present study, the significantly increased number of stomata and epidermal cells and the unchanged area of epidermal cells on both sides of the leaves of Cd-treated plants, along with the increased stomatal area on AD side were consistent with the increased chlorophyll content under Cd stress compared to control (Fig. 5; Table 5, Fig. S2). As reported, higher stomatal density can increase stomatal conductance, CO₂ assimilation and consequently have a positive effect on photosynthetic capacity (Tanaka et al. 2013). The application of Si increased stomatal density regardless of whether it was applied alone (as 1 Si or 2.5 Si) or in combination with Cd treatment compared to control (Tables 6 and 7). However, the variations of stomatal and epidermal cell density on both sides of the

amaranth leaves revealed different responses to Cd stress under Si supplementation compared to Cd treatment. Stomatal density, epidermal cell density and SI decreased on AD side under both Si + Cd treatments. In contrast, stomatal density and SI increased under both Si supplementations on AB side; moreover, stomatal area and epidermal cell density were increased under 2.5 Si supplementation. These data can be correlated with the measurement of chlorophyll contents and more pronounced effect of higher Si concentration (Fig. 3). A higher density of stomata was found after Si foliar spraying of pea plants grown under Cd stress (El-Okkiah et al. 2022), and also in cotton seedlings grown under salt and low-temperature dual stress after the application of Si nanoparticles (Liang et al. 2023). As presented in the current study, the strategy of *A. cruentus* leaves how to cope with Cd stress is characterized by variations in the long-term morphological response (via changes in stomatal density), even under Si supplementation, at the applied concentrations.

It is known that non-redox Cd promotes excessive formation of ROS and suppresses the antioxidant system (Abbas et al. 2017). The increased activities of enzymes such as CAT, APX, GPX, superoxide dismutase (SOD) and glutathione reductase (GR) can attenuate oxidative stress caused by Cd toxicity by scavenging ROS (Benavides et al. 2005; Zeng et al. 2017). The response of antioxidant enzymes to HM stress varies greatly depending on the species, cultivar, plant tissue, metal used and exposure concentration of HM (Shi et al. 2010; Hunková et al. 2024). Exogenous application of Si modulates the antioxidant system, both enzymes and non-enzymatic antioxidants. The ameliorative effect of Si can be further enhanced by promoting the expression of key genes related to hormone metabolism and attenuation of oxidative stress (Ashkavand et al. 2016; Mostofa et al. 2021). Here we show that GPX and APX activities increased significantly under Cd treatment in the amaranth roots, while CAT activity remained unchanged compared to control plants (Fig. 6). Although no significant increase in GPX, APX and CAT activities was observed in the leaves of Cd-treated plants, the enhancing effect of Si was evident in the increased activity of GPX, APX and CAT in the leaves as well as in the increased CAT activity in the roots. In amaranth, Si supplementation increased activity of antioxidant enzymes especially in the leaves, particularly at 1 mM concentration, which minimized Cd-induced stress. The increased and improved activity of antioxidant enzymes under Si treatment is an alternative approach of plants to alleviate Cd toxicity (Iwuala et al. 2024). In contrast to our results, Cd significantly increased POD, SOD and CAT activities in rice (Jalil et al. 2023). Farooq et al. (2013) and Shafeeq-ur-Rahman et al. (2020) reported that Si supplementation attenuated Cd toxicity by further increasing antioxidants in wheat and

cotton. Since we did not observe increased activities of all monitored enzymes under Cd stress in both roots and leaves of amaranth plants, the time period of treatment, or even possible partial resistance of amaranth to stress caused by HMs could be the reason. The Si-induced inhibited uptake of Cd and the effective Si translocation into amaranth shoots could be indirectly responsible for the increased activities of the antioxidant enzymes.

Monitoring the uptake of nutrients by plants is important with regard to HM pollution, as they are essential for growth and developmental processes of plants (Mišúthová et al. 2021). Cadmium has been reported to affect the absorption and distribution of various macro- and microelements and its effects on nutrient uptake are highly dependent on dose, time of exposure, culture media, plant species, cultivars and genotypes (Das et al. 1997; Naeem et al. 2019; Zulfiqar et al. 2022). In amaranth, Cd toxicity was reflected in impaired nutrient transport, reduced plant growth and metabolism (Figs. 7 and 8) as further confirmed by a decreased proportion of xylem traits (Fig. 4; Table 4), which are responsible for the effectiveness of nutrient and water transport from roots to shoots (Ramachandran et al. 2020; Cornelis and Hazak 2022; Li et al. 2024). Cadmium decreased the concentrations of Ca, Fe, K, Mg and Zn in the roots and Ca, Fe, K and Zn in the shoots. The effect of Si on mineral status is plant-specific and needs further investigation (Greger et al. 2018; Pavlovic et al. 2021). In our experiments, the addition of Si to Cd-treated plants did not alter the toxic effect of Cd on the ionic imbalance. In addition, Si applied individually (1 mM) decreased K and Mg uptake in the roots and Zn content in the shoots. Moreover, Si treatment increased Ca and Na concentrations in amaranth plant. As far as we are aware, there are no previous studies addressing the effect of Si on the amaranth ionome. However, the results presented in this paper are consistent with those of Mišúthová et al. (2021), who found that Si had no enhancing effect on the uptake of macro- and microelements in maize plants exposed to arsenic.

Overall, a lower concentration of Si (1 mM) was more effective at mitigating Cd phytotoxicity than a higher (2.5 mM) Si concentration in amaranth. Although Si was supplied as sodium silicate, which could induce mild salt stress from Na ions in Si-treated plants (Fig. 7), we assume this process did not take place in view of the biomass values, growth parameters, and the total absence of typical visual salt stress responses. A possible explanation is that the effect of Si dosage was not linear, lower Si levels better stimulated amaranth growth and antioxidant defence, whereas higher levels did not increase these responses and may have even suppressed them. Conversely, the combination of higher levels of both Si and Na, and Cd toxicity might override the beneficial effects of Si.

Investigating the activity and regulation of stress-related genes can help to understand adaptation mechanisms of plants to unfavourable environmental conditions. In this study, the role of Si in modulating the expression of five genes previously described as involved in abiotic stress responses in amaranth was examined (Lancíková et al. 2020; Reyes-Rosales et al. 2023). The highest expression in a response to Cd treatment was observed in *AhERD* and *Ah24* genes in amaranth roots (Table 4). The expression of *ERD* genes has been associated with heat-induced damage, drought, light, temperature and salt stress (Wu et al. 2022; Reyes-Rosales et al. 2023). Sixteen *ERD* genes with different functions have been identified in *Arabidopsis thaliana* (Wu et al. 2022). Transgenic plants of *A. thaliana* constitutively expressing the maize *ZmERD4* gene showed enhanced tolerance to drought and high salinity. While these results show that *ERD* genes play an important role in the adaptation of plants to stress conditions, there is no evidence in the literature for their involvement in responses to HM-induced stress. However, in this study, the significantly increased expression of *AhERD* under Cd treatment in amaranth roots suggests a broader protective and adaptive role of *AhERD* in attenuating Cd-induced stress. As specific functional studies on *AhERD* are very limited, its role can be interpreted on the basis of *ERD* gene family, particularly encoding the dehydrins *ERD10* and *ERD14*. These proteins accumulate in response to abiotic stress and play a protective role in protein denaturation, ROS damage and membrane binding and stabilization (Kovacs et al. 2008; Maskowska et al. 2017). The members of the *ERD15*-like subfamily serve as multiple stress-induced regulators of senescence and drought responses in soybean (Fraga et al. 2024). The addition of Si to Cd resulted in a decreased *AhERD* expression to the level of control plants. Considering the data on activities of antioxidant enzymes, low *AhERD* expression in Si + Cd-treated plants could reflect the indirect mitigating effect of Si reducing oxidative stress in Cd-treated plants.

The *Ah24* gene plays a multifactorial role in growth regulation, intracellular communication and recovery after wounding or defoliation (Cabrales-Orona and Délano-Frier 2021). The possible role of this gene has been studied in transgenic *Ah24* overexpressing *Arabidopsis thaliana* and *Nicotiana tabacum* resulting in enhanced growth and increased leaf number, and increased herbivore resistance, respectively (Massange-Sanchez et al. 2015). As far as we know, there is limited information available regarding the *Ah24* gene, and not known homologue in plants associated with HM-induced stress response. However, its role in HM stress resistance was also demonstrated in our previous works by the strong induction in the roots of *A. hypochondriacus* cv. Plainsman under Cd stress (Lancíková et al. 2020), as well as in the roots of cv. Pribina after Pb exposure

(Hunková et al. 2024). In the current study, a lower expression level of *Ah24* under the supplementation of Si to Cd treatment than in Cd-treated plants could be a sign of effective stress mitigation.

In addition to *Ah24* gene, the gene encoding a DUF642 protein (*DGR2*) is also associated with growth and stress tolerance of grain amaranth (Délano-Frier et al. 2011). Expression of *DGR2* was significantly enhanced in young *A. hypochondriacus* plants exposed to salt and water deficit, indicating its role in grain amaranth stress tolerance (Palmeros-Suárez et al. 2017). Our previous analyses showed that the *DGR2* gene was downregulated in amaranth tissues and its involvement in HM stress was unclear (Lancíková et al. 2020). However, according to data presented in this work, *DGR2* was slightly upregulated under Cd treatment. Similarly, Reshma and Meenal (2024) found an increased *DGR2* expression in *A. cruentus* plants under Zn stress. Herein, Si supply had no significant effect on the relative expression of the *DGR2* gene in amaranth roots.

The *GSH1* gene encodes γ -glutamylcysteine synthetase, the key enzyme controlling the GSH synthesis pathway. Plants with a higher *GSH1* expression level are characterized by a higher content of the non-enzymatic antioxidant GSH (Noctor et al. 2011; Jiang et al. 2016; Song et al. 2017). In amaranth, the increase in *GSH1* expression could indicate increased GSH synthesis and consequently more efficient chelation of Cd in amaranth cells. The addition of Si to Cd led to a downregulation of *GSH1* expression. It could therefore be speculated whether a Cd-chelating GSH was displaced by the presence of Si, which can also chelate HMs.

Plant chitinases are hydrolytic enzymes that exhibit antimicrobial and antifungal activities (Kumudini et al. 2018). In recent decades, there has been substantial evidence that chitinases are substantially involved in HM stress mechanisms. The increased expression of chitinase genes was found in barley (Cao et al. 2014), sunflower (Keulen et al. 2008), pea, faba beans, soybean (Mészáros et al. 2014), maize (Békésiová et al. 2008), and mangrove (Zhou et al. 2023) under metal stress. Similar to the results of the present study, our previous work also showed upregulation of *Chit5* in different amaranth cultivars exposed to Cd, Pb, Zn and Mn (Lancíková et al. 2020; Hunková et al. 2024). Here, we demonstrated that after Si application, the expression of *Chit5* decreased in a Si concentration-dependent manner. In summary, Si supply significantly contributed to the regulation of *Ah24*, *Chit5*, *AhERD* and *GSH1* gene expression, and the higher the Si concentration used, the stronger the effect. Although the specific roles of *Ah24*, *DGR2*, *AhERD*, and *GSH1* in Cd accumulation and detoxification in amaranth are not yet fully established, their altered expression patterns under Cd and Si treatments in our study suggest that these genes participate in general stress-responses and

Si-mediated metal tolerance mechanisms. We therefore propose that molecular-level regulation may be one of the important mechanisms by which Si alleviates Cd-induced stress in amaranth roots.

Conclusion

The current study showed the beneficial effects of Si on grain amaranth (*A. cruentus* L.) grown in the presence of toxic Cd. In particular, 1 mM Si improved plant biomass as well as physio-morphological and biochemical parameters. Silicon effectively reduced the accumulation of toxic Cd in the roots, but especially in the aerial plant tissues, which was confirmed by spectrometric analysis. Microscopic observations complemented the results and provided the spatial information on the decreased symplasmic localization of Cd in root and leaf tissues under Si supplementation. Moreover, the internal root architecture of Cd-treated plants was more complex under Si supplementation, as shown by restored area of xylem traits. Surprisingly, Si had an opposite effect on the density of stomatal and epidermal cells on adaxial and abaxial side of the leaves under Cd stress. For the first time, we address the nutrient response of amaranth plants under Si+Cd treatment.

We have outlined the several possible processes by which Si mitigates Cd toxicity in amaranth, such as exclusion of Cd with reduced translocation and accumulation, attenuation of oxidative stress conditions, restoration of xylem vessels in primary roots, and involvement in the regulation of stress-related genes.

The results of current work offer perspectives for the application of an appropriate Si concentration to reduce the accumulation of toxic Cd in amaranth plants, and possibly in other pseudocereals, and to minimize the transfer of this metal from the contaminated environment into the plant. However, given the complexity of the soil in the field, the findings obtained should be validated before broader agronomic application.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10725-025-01412-5>.

Acknowledgements The authors gratefully acknowledge Dr. Marián Tomka (Institute of Biotechnology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Nitra, Slovakia) for his contribution to the ICP-EOS analysis.

Author contributions Monika Szabóová: Writing – review & editing, Writing – original draft, Visualization, Software, Data curation. Veronika Mistriková: Writing – review & editing, Writing – original draft, Visualization, Software. Jana Kačírová: Formal analysis, Visualization, Software, Data curation. Monika Lisinovičová: Writing – original draft, Formal analysis. Marek Bujdoš: Formal analysis, Data curation.

Andrea Hricová: Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Funding Open access funding provided by The Ministry of Education, Science, Research and Sport of the Slovak Republic in cooperation with Centre for Scientific and Technical Information of the Slovak Republic. This research was financially supported by COST Action CA20133, Scientific Grant Agency VEGA grant number 2/0013/22 and the Operational program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, cofinanced by the European Regional Development Fund.

Data availability All data analysed during this study are included in this published article and its supplementary information files.

Declarations

Competing interests The authors declare no competing interests.

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References

- Abbas MS, Akmal M, Ullah S, Hassan MU, Farooq S (2017) Effectiveness of zinc and gypsum application against cadmium toxicity and accumulation in wheat (*Triticum aestivum* L.). Commun. Soil Sci Plant Anal 48:1659–1668. <https://doi.org/10.1080/00103624.2017.1373798>
- Adhikary D, Deyholos MK, Délano-Frier JP (2021) The Amaranth genome. Springer International Publishing, Switzerland. <https://doi.org/10.1007/978-3-030-72365-1>
- Agathokleous E, Feng ZZ, Peñuelas J (2020) Chlorophyll hormones: are chlorophylls major components of stress biology in higher plants? Sci Total Environ 726:138637. <https://doi.org/10.1016/j.scitotenv.2020.138637>
- Ahmed T, Masood HA, Noman M, Al-Huqail AA, Alghanem SM, Khan MM, Muhammad S, Manzoor N, Rizwan M, Qi X, Abeer AHA, Li B (2023) Biogenic silicon nanoparticles mitigate cadmium (Cd) toxicity in rapeseed (*Brassica Napus* L.) by modulating the cellular oxidative stress metabolism and reducing cd translocation. J Hazard Mater 459:132070. <https://doi.org/10.1016/j.jhazmat.2023.132070>
- Angon PB, Islam MS, Shreejana KC, Das A, Anjum N, Poudel A, Suchi SA (2024) Sources, effects and present perspectives of heavy metals contamination: Soil, plants and human food chain. Heliyon 10:e28357. <https://doi.org/10.1016/j.heliyon.2024.e28357>
- Ashkavand P, Tabari M, Zarafshar M, Tomášová I, Struve D (2016) Effect of SiO₂ nanoparticles on drought resistance in Hawthorn seedlings. Leśne Prace Badawcze 76:350–359. <https://open.icm.edu.pl/handle/123456789/10837>
- Bari MA, Prity SA, Das U, Akther MS, Sajib SA, Reza MA, Kabir AH (2020) Silicon induces phytochelatin and ROS scavengers facilitating cadmium detoxification in rice. Plant Biol 22:472–479. <https://doi.org/10.1111/plb.13090>
- Békésiová B, Hraška S, Libantová J, Moravčíková J, Matušíková I (2008) Heavy-metal stress induced accumulation of chitinase isoforms in plants. Mol Biol Rep 35:579–588. <https://doi.org/10.1007/s11033-007-9127-x>
- Benavides MP, Gallego SM, Tomaro ML (2005) Cadmium toxicity in plants. Braz J Plant Physiol 17:21–34. <https://doi.org/10.1590/S1677-04202005000100003>
- Benslimam W, Ellouzi H, Zorrig W, Abdelly C, Hafsi C (2022) Beneficial effects of silicon on growth, nutrient dynamics, and antioxidant response in barley (*Hordeum vulgare* L.) plants under potassium deficiency. J Soil Sci Plant Nutr 22:2633–2646. <https://doi.org/10.1007/s42729-022-00832-w>
- Bhat JA, Shivaraj SM, Singh P, Navadagi DB, Tripathi DK, Dash PK, Solanke AU, Sonah H, Deshmukh R (2019) Role of silicon in mitigation of heavy metal stresses in crop plants. Plants 8:71. <https://doi.org/10.3390/plants8030071>
- Cabrera-Olona G, Délano-Frier JP (2021) Searching for an identity: functional characterization of taxonomically restricted genes in grain Amaranth. In: Adhikary D, Deyholos MK, Délano-Frier JP (eds) The Amaranth genome. Springer, Cham, pp 167–181. https://doi.org/10.1007/978-3-030-72365-1_7
- Cao F, Chen F, Sun H, Zhang G, Chen Z, Wu F (2014) Genome-wide transcriptome and functional analysis of two contrasting genotypes reveals key genes for cadmium tolerance in barley. BMC Genom 15:1–14. <https://doi.org/10.1186/1471-2164-15-611>
- Cao BL, Ma Q, Zha Q, Wang L, Xu K (2015) Effects of silicon on absorbed light allocation, antioxidant enzymes and ultrastructure of chloroplasts in tomato leaves under simulated drought stress. Sci Hortic 194:53–62. <https://doi.org/10.1016/j.scienta.2015.07.037>
- Cechin I, Da Silva LP, Ferreira ET, Barrochelo SC, de Melo FPD, Dokkedal AL, Saldanha LL (2022) Physiological responses of *Amaranthus cruentus* L. to drought stress under sufficient- and deficient-nitrogen conditions. PLoS One 17:0270849. <https://doi.org/10.1371/journal.pone.0270849>
- Chaiwong N, Prom-u-thai C (2022) Significant roles of silicon for improving crop productivity and factors affecting silicon uptake and accumulation in rice: a review. J Soil Sci Plant Nutr 22:1970–1982. <https://doi.org/10.1007/s42729-022-00787-y>
- Charagh S, Wang J, Hui S, Raza A, Cao R, Zhou L, Yang L, Xu B, Zhang Y, Mawia AM, Sheng Z, Tang S, Hu S, Hu P (2025) Smart reprogramming of plants against cadmium toxicity using membrane transporters and modern tools. Plant Physiol Biochem 224:109919. <https://doi.org/10.1016/j.plaphy.2025.109919>
- Chi K, Zou R, Huo W, Fan H (2019) Cellular distribution of cadmium in two Amaranth (*Amaranthus Mangostanus* L.) cultivars differing in cadmium accumulation. Environ Sci Pollut Res 26:22147–22158. <https://doi.org/10.1007/s11356-019-05390-w>
- Cornelis S, Hazak O (2022) Understanding the root xylem plasticity for designing resilient crops. Plant Cell Environ 45:664–676. <https://doi.org/10.1111/pce.14245>
- Cuyppers A, Keunen E, Bohler S, Jozefczak M, Opdenakker K, Gielen H, Vercamp H, Bielen A, Schellingen K, Vangronsveld J, Remans T (2011) Cadmium and copper stress induce a cellular oxidative challenge leading to damage versus signalling. In: Gupta DK, Sandalio LM (eds) Metal toxicity in plants: Perception, signaling and remediation. Springer, Berlin, pp 65–90. https://doi.org/10.1007/978-3-642-22081-4_4
- Dar FA, Tahir I, Hakeem KR, Rehman RU (2022) Silicon application enhances the photosynthetic pigments and Phenolic/Flavonoid

- content by modulating the phenylpropanoid pathway in common buckwheat under aluminium stress. *Silicon* 14:323–334. <https://link.springer.com/article/https://doi.org/10.1007/s12633-021-01501-w>
- Das P, Samantaray S, Rout GR (1997) Studies on cadmium toxicity in plants: a review. *Environ Pollut* 98:29–36. [https://doi.org/10.1016/S0269-7491\(97\)00110-3](https://doi.org/10.1016/S0269-7491(97)00110-3)
- Délano-Frier JP, Avilés-Arnaut H, Casarrubias-Castillo K, Casique-Arroyo G, Castrillón-Arbeláez PA, Herrera-Estrella L, Massange-Sánchez J, Martínez-Gallardo NA, Parra-Cotta FI, Vargas-Ortiz E, Estada-Hernández MG (2011) Transcriptomic analysis of grain *Amaranth* (*Amaranthus hypochondriacus*) using 454 pyrosequencing: comparison with *A. tuberculatus*, expression profiling in stems and in response to biotic and abiotic stress. *BMC Genomics* 12:363. <https://doi.org/10.1186/1471-2164-12-363>
- El-Okkiah SAF, El-Tahan AM, Ibrahim OM, Taha MA, Korany SM, Alsharif EA, Abdelgawad H, Abo Sen EZF, Sharaf-Eldin MA (2022) Under cadmium stress, silicon has a defensive effect on the morphology, physiology, and anatomy of pea (*Pisum sativum* L.) plants. *Front Plant Sci* 13:997475. <https://doi.org/10.3389/fpls.2022.997475>
- El-Tayeb MA (2006) Differential response of two *Vicia faba* cultivars to drought: growth, pigments, lipid peroxidation, organic solutes, catalase and peroxidase activity. *Acta Agron Hung* 54:25–37. <https://doi.org/10.1556/AAgr.54.2006.1.3>
- Etesami H, Jeong BR (2018) Silicon (Si): review and future prospects on the action mechanisms in alleviating biotic and abiotic stresses in plants. *Ecotoxicol Environ Saf* 147:881–896. <https://doi.org/10.1016/j.ecoenv.2017.09.063>
- Fan HL, Zhou W (2009) Screening of amaranth cultivars (*Amaranthus Mangostanus* L.) for cadmium hyperaccumulation. *Agr Sci China* 8:342–351. [https://doi.org/10.1016/S1671-2927\(08\)60218-7](https://doi.org/10.1016/S1671-2927(08)60218-7)
- Fan X, Wen X, Huang F, Cai Y, Cai K (2016) Effects of silicon on morphology, ultrastructure and exudates of rice root under heavy metal stress. *Acta Physiol Plant* 38:197. <https://doi.org/10.1007/s11738-016-2221-8>
- Farooq MA, Ali S, Hameed A, Ishaque W, Mahmood K, Iqbal Z (2013) Alleviation of cadmium toxicity by silicon is related to elevated photosynthesis, antioxidant enzymes; suppressed cadmium uptake and oxidative stress in cotton. *Ecotoxicol Environ Saf* 96:242–249. <https://doi.org/10.1016/j.ecoenv.2013.07.006>
- Fraga OT, Silva LAC, Silva JCF, Bevilori R, Silva FDA, Pereira WA, Reis PAB, Fontes EPB (2024) Expansion and diversification of the *Glycine max* (Gm) ERD15-like subfamily of the PAM2-like superfamily. *Planta* 260:108. <https://doi.org/10.1007/s00425-024-04538-4>
- Gheshlaghpour J, Asghari B, Khademian R, Sedaghati B (2021) Silicon alleviates cadmium stress in basil (*Ocimum Basilicum* L.) through alteration of phytochemical and physiological characteristics. *Ind Crops Prod* 163:1–12. <https://doi.org/10.1016/j.indcrop.2021.113338>
- Greger M, Landberg T, Vaculík M (2018) Silicon influences soil availability and accumulation of mineral nutrients in various plant species. *Plants* 7:41. <https://doi.org/10.3390/plants7020041>
- Haider FU, Liqun C, Coulter JA, Cheema SA, Wu J, Zhang R, Wenjun M, Farooq M (2021) Cadmium toxicity in plants: impacts and remediation strategies. *Ecotoxicol Environ Saf* 211:111887. <https://doi.org/10.1016/j.ecoenv.2020.111887>
- Hasanuzzaman M, Zhou M, Shabala S (2023) How does stomatal density and residual transpiration contribute to osmotic stress tolerance? *Plants* 12:494. <https://doi.org/10.3390/plants12030494>
- Haworth M, Killi D, Materassi A, Raschi A, Centritto M (2016) Impaired stomatal control is associated with reduced photosynthetic physiology in crop species grown at elevated [CO₂]. *Front Plant Sci* 7:1568. <https://doi.org/10.3389/fpls.2016.01568>
- Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. *Calif Agric Exp Stn Circ* 347:1–39
- Hricová A, Fejér J, Libiaková G, Szabóová M, Gažo J, Gajdošová A (2016) Characterization of phenotypic and nutritional properties of valuable *Amaranthus cruentus* L. mutants. *Turk J Agric For* 40:761–771. <https://doi.org/10.3906/tar-1511-31>
- Hu H, Wang L, Wang Q, Jiao L, Hua W, Zhou Q, Huang X (2014) Photosynthesis, chlorophyll fluorescence characteristics, and chlorophyll content of soybean seedlings under combined stress of bisphenol A and cadmium. *Environ Toxicol Chem* 33:2455–2462. <https://doi.org/10.1002/etc.2720>
- Hunková J, Lisinovičová M, Lancíková V, Szabóová M, Kačírová J, Mistríková V, Hricová A (2024) A comparative analysis of heavy metal stress responses in different grain amaranth cultivars. *Plant Stress* 14:100619. <https://doi.org/10.1016/j.stress.2024.100619>
- Imtiaz M, Rizwan MS, Mushtaq MA, Ashraf M, Shahzad SM, Yousaf B, Saeed DA, Rizwan M, Nawaz MA, Mehmood S, Tu S (2016) Silicon occurrence, uptake, transport and mechanisms of heavy metals, minerals and salinity enhanced tolerance in plants with future prospects: a review. *J Environ Manage* 183:521–529. <https://doi.org/10.1016/j.jenvman.2016.09.009>
- Iwuala E, Olajide O, Abiodun I, Odjegba V, Utoblo O, Ajewole T, Oluwajobi A, Uzochukwu S (2024) Silicon ameliorates cadmium (Cd) toxicity in pearl millet by inducing antioxidant defense system. *Heliyon* 10:e25514. <https://doi.org/10.1016/j.heliyon.2024.e25514>
- Jalil S, Nazir MM, Al-Huqail AA, Ali B, Al-Qthanin RN, Asad MAU, Eweda MA, Zulfiqar F, Onursal N, Masood HA, Yong JWH, Jin X (2023) Silicon nanoparticles alleviate cadmium toxicity in rice (*Oryza sativa* L.) by modulating the nutritional profile and triggering stress-responsive genetic mechanisms. *Ecotoxicol Environ Saf* 268:115699. <https://doi.org/10.1016/j.ecoenv.2023.115699>
- Jiang L, Chen Z, Gao Q, Ci L, Cao S, Han Y, Wang W (2016) Loss-of-function mutations in the APX1 gene result in enhanced selenium tolerance in *Arabidopsis thaliana*. *Plant Cell Environ* 39:2133–2144. <https://doi.org/10.1111/pce.12762>
- Júnior CAL, Mazzafera P, Arruda MAZ (2014) A comparative ionomic approach focusing on cadmium effects in sunflowers (*Helianthus annuus* L.). *Environ Exp Bot* 107:180–186. <https://doi.org/10.1016/j.envexpbot.2014.06.002>
- Kabata-Pendias A (2010) Trace elements in soils and plants, fourth edn. CRC, Boca Raton, USA. <https://doi.org/10.1201/b10158>
- Kabir AH, Das U, Rahman MA, Lee KW (2021) Silicon induces metallochaperone-driven cadmium binding to the cell wall and restore redox status through elevated glutathione in Cd-stressed sugar beet. *Physiol Plant* 173:352–369. <https://doi.org/10.1111/pl.13424>
- Keulen HV, Wei R, Cutright TJ (2008) Arsenate-induced expression of a class III chitinase in the Dwarf sunflower *Helianthus annuus*. *Environ Exp Bot* 63:281–288. <https://doi.org/10.1016/j.envexpbot.2007.11.012>
- Khan MA, Gemenet D, Villordon A (2016) Root system architecture and abiotic stress tolerance: current knowledge in root and tuber crops. *Front Plant Sci* 7:1584. <https://doi.org/10.3389/fpls.2016.01584>
- Khan A, Bilal S, Khan AL, Imran M, Al-Harrasi A (2020) Silicon-mediated alleviation of combined salinity and cadmium stress in date palm (*Phoenix dactylifera* L.) by regulating physio-hormonal alteration. *Ecotoxicol Environ Saf* 188:1–11. <https://doi.org/10.1016/j.ecoenv.2019.109885>
- Khan I, Awan SA, Rizwan M, Brestic M, Xie W (2023) Silicon: an essential element for plant nutrition and phytohormones signaling mechanism under stressful conditions. *Plant Growth Regul* 100:301–319. <https://doi.org/10.1007/s10725-022-00872-3>
- Khlifi N, Ghabriche R, Ayachi I, Zorrig W, Ghnaya T (2024) How does silicon alleviate Cd-induced phytotoxicity in barley. *Hordeum*

- Vulgare L ? Chemosphere 362:142739. <https://doi.org/10.1016/j.chemosphere.2024.142739>
- Kovacs D, Kalmar E, Torok Z, Tompa P (2008) Chaperone activity of ERD10 and ERD14, two disordered stress-related plant proteins. *Plant Physiol* 147:381–390. <https://pmc.ncbi.nlm.nih.gov/articles/PMC2330285/pdf/pp1470381.pdf>
- Kumudini BS, Jayamohan NS, Patil SV, Govardhana M (2018) Primary plant metabolism during plant. In: Ahmad P, Ahanger MA, Singh VP, Tripathi DK, Alam P, Alyemeni MN (eds) *Pathogen interactions and its role in defense*. Elsevier, Academic, pp 215–229. <https://doi.org/10.1016/C2016-0-03727-0>
- Lakhneko O, Fialová I, Fiala R, Kopáčová M, Kováč A, Danchenko M (2024) Silicon might mitigate nickel toxicity in maize roots via chelation, detoxification, and membrane transport. *Ecotoxicol Environ Saf* 288:117334. <https://doi.org/10.1016/j.ecoenv.2024.117334>
- Lancíková V, Tomka M, Žiarovská J, Gažo J, Hricová A (2020) Morphological responses and gene expression of grain Amaranth (*Amaranthus* spp.) growing under cd. *Plants* 9:575. <https://doi.org/10.3390/plants9050572>
- Li S, Li H, Wang J, Lu S, Liu Z, Jia H, Wei T, Guo J (2024) The response of physiological and xylem anatomical traits under cadmium stress in *Pinus thunbergii* seedlings. *Tree Physiol* 44:tpae046. <https://doi.org/10.1093/treephys/tpae046>
- Liang Y, Sun W, Zhu YG, Christie P (2007) Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. *Environ Pollut* 147:422–428. <https://doi.org/10.1016/j.envpol.2006.06.008>
- Liang Y, Liu H, Fu Y, Li P, Li S, Gao Y (2023) Regulatory effects of silicon nanoparticles on the growth and photosynthesis of cotton seedlings under salt and low-temperature dual stress. *BMC Plant Biol* 23:504. <https://doi.org/10.1186/s12870-023-04509-z>
- Lichtenhaler HK, Wellburn AR (1983) Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem Soc Trans* 11:591–592. <https://doi.org/10.1042/bst0110591>
- Liu XX, Yin LN, Deng XP, Gong D, Du S, Wang SW, Zhang ZY (2020) Combined application of silicon and nitric oxide jointly alleviated cadmium accumulation and toxicity in maize. *J Hazard Mater* 395:122679. <https://doi.org/10.1016/j.jhazmat.2020.122679>
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2[−]ΔΔCT method. *Methods* 25:402–408. <https://doi.org/10.1006/meth.2001.1262>
- Lu H, Li Z, Wu J, Shen Y, Li Y, Zou B, Tang Y, Zhuang P (2017) Influences of calcium silicate on chemical forms and subcellular distribution of cadmium in *Amaranthus hypochondriacus* L. *Sci Rep* 7:40583. <https://doi.org/10.1038/srep40583>
- Lukacova Z, Liska D, Bokor B, Svubova R, Lux A (2021) Silicon and cadmium interaction of maize (*Zea mays* L.) plants cultivated in vitro. *Biologia* 76:2721–2733. <https://doi.org/10.1007/s11756-021-00799-6>
- Lukačová Z, Švubová R, Kohanová J, Lux A (2013) Silicon mitigates the Cd toxicity in maize in relation to cadmium translocation, cell distribution, antioxidant enzymes stimulation and enhanced endodermal apoplasmic barrier development. *Plant Growth Regul* 70:89–103. <https://doi.org/10.1007/s10725-012-9781-4>
- Lux A, Martinka M, Vaculík M, White PJ (2011) Root responses to cadmium in the rhizosphere: a review. *J Exp Bot* 62:21–37. <https://doi.org/10.1093/jxb/erq281>
- Lux A, Lukačová Z, Vaculík M, Švubová R, Kohanová J, Soukup M, Martinka M, Bokor B (2020) Silicification of root tissues. *Plants* 9:111. <https://doi.org/10.3390/plants9010111>
- Ma JF, Yamaji N (2006) Silicon uptake and accumulation in higher plants. *Trends Plant Sci* 11:392–397. <https://doi.org/10.1016/j.tplants.2006.06.007>
- Ma JF, Yamaji N (2015) A cooperative system of silicon transport in plants. *Trends Plant Sci* 20:435–442. <https://doi.org/10.1016/j.tplants.2015.04.007>
- Ma JF, Tamai K, Yamaji N, Mitani N, Konishi S, Katsuhara M, Ishiguro M, Murata Y, Yano M (2006) A silicon transporter in rice. *Nature* 440:688–691. <https://doi.org/10.1038/nature04590>
- Ma JF, Yamaji N, Mitani N, Tamai K, Konishi S, Fujiwara T, Katsuhara M, Yano M (2007) An efflux transporter of silicon in rice. *Nature* 448:209–212. <https://doi.org/10.1038/nature0596>
- Ma J, Sheng H, Li X, Wang L (2016) iTRAQ-based proteomic analysis reveals the mechanisms of silicon-mediated cadmium tolerance in rice (*Oryza sativa*) cells. *Plant Physiol Biochem* 104:71–80. <https://doi.org/10.1016/j.plaphy.2016.03.024>
- Maksimović I, Kastori R, Krstić L, Liković J (2007) Steady presence of cadmium and nickel affects root anatomy, accumulation and distribution of essential ions in maize seedlings. *Biol Plant* 51:589–592. <https://doi.org/10.1007/s10535-007-0129-2>
- Mandlik R, Thakral V, Raturi G, Shinde S, Nikolić M, Tripathi DK, Sonah H, Deshmukh R (2020) Significance of silicon uptake, transport, and deposition in plants. *J Exp Bot* 71:6703–6718. <https://doi.org/10.1093/jxb/eraa301>
- Massange-Sanchez JA, Palmeros-Suarez PA, Martinez-Gallardo NA, Castrillon-Arbelaiz PA, Avilés-Arnaut H, Alatorre-Cobos F, Tiessen A, Délano-Friér JP (2015) The novel and taxonomically restricted Ah24 gene from grain Amaranth (*Amaranthus hypochondriacus*) has a dual role in development and defense. *Front Plant Sci* 6:602. <https://doi.org/10.3389/fpls.2015.00602>
- Mészáros P, Rybanský L, Spieß N, Socha P, Kuna R, Libantová J, Moravčíková J, Piršelová B, Hauptvogel P, Matušíková I (2014) Plant chitinase responses to different metal-type stresses reveal specificity. *Plant Cell Rep* 33:1789–1799. <https://doi.org/10.1007/s00299-014-1657-9>
- Mišúthová A, Slovákova L, Kollárová K, Vaculík M (2021) Effect of silicon on root growth, ionomics and antioxidant performance of maize roots exposed to as toxicity. *Plant Physiol Biochem* 168:155–166. <https://doi.org/10.1016/j.plaphy.2021.10.012>
- Mostofa MG, Rahman MM, Ansary MMU, Keya SS, Abdelrahman M, Miah MG, Phan Tran LS (2021) Silicon in mitigation of abiotic stress-induced oxidative damage in plants. *Crit Rev Biotechnol* 41:918–934. <https://doi.org/10.1080/07388551.2021.1892582>
- Naeem A, Zia-ur-Rehman M, Akhtar T, Zia MH, Aslam M (2018) Silicon nutrition lowers cadmium content of wheat cultivars by regulating transpiration rate and activity of antioxidant enzymes. *Environ Pollut* 242:126–135. <https://doi.org/10.1016/j.envpol.2018.06.069>
- Naeem A, Zafar M, Khalid H, Zia-Ur-Rehman M, Ahmad Z, Ayub MA, Qayyum MF (2019) Cadmium-induced imbalance in nutrient and water uptake by plants. In: Hasanuzzaman M, Prasad MNV, Fujita M (eds) *Cadmium toxicity and tolerance in plants*. Academic, Elsevier, pp 299–326. <https://doi.org/10.1016/B978-0-12-814864-8.00012-7>
- Ngugi MM, Gitari HI, Muiri C, Gweyi-Onyango P (2021) Cadmium mobility, uptake, and accumulation in spinach, kale, and amaranth vegetables as influenced by silicon fertilization. *Bioremediat J* 26:113–127. <https://doi.org/10.1080/10889868.2021.1924111>
- Noctor G, Queval G, Mhamdi A, Chaouch S, Foyer CH (2011) Glutathione Arabidopsis Book 9:1–32. <https://doi.org/10.1199/tab.0142>
- Nogueiro RC, Monteiro FA, Gratão PL, da Alcantara Silva BK, Azevedo RA (2016) Cadmium application in tomato: nutritional imbalance and oxidative stress. *Water Air Soil Pollut* 227:210. <https://doi.org/10.1007/s11270-016-2895-y>
- O'Brien TP, Feder N, McCully ME (1964) Polychromatic staining of plant cell walls by toluidine blue o. *Protoplasma* 59:368–373. <https://doi.org/10.1007/BF01248568>

- Padmini S, Rao RS (1993) Foliar stomatal distribution in relation to taxonomy: Amaranthaceae. Feddes Rept 104:481–484. <https://doi.org/10.1002/fedr.19931040711>
- Palmeros-Suárez PA, Massange-Sánchez JA, Sánchez-Segura L, Martínez-Gallardo NA, Rangel EE, Gómez-Leyva JF, Délano-Frier JP (2017) *AhDGR2*, an amaranth abiotic stress-induced DUF642 protein gene, modifies cell wall structure and composition and causes salt and ABA hyper-sensitivity in transgenic Arabidopsis. *Planta* 245:623–640. <https://doi.org/10.1007/s00425-016-2635-y>
- Palmeros-Suárez PA, Casarrubias-Castillo K, Massange-Sánchez JA (2021) Amaranth transcription factors in response to biotic and abiotic stresses. In: Adhikary D, Deyholos MK, Délano-Frier JP (eds) *The Amaranth genome*. Springer, Cham, pp 167–181. https://doi.org/10.1007/978-3-030-72365-1_10
- Pavlovic J, Kostic L, Bosnic P, Kirkby EA, Nikolic M (2021) Interactions of silicon with essential and beneficial elements in plants. *Front Plant Sci* 12:697592. <https://doi.org/10.3389/fpls.2021.697592>
- Ramachandran P, Augstein F, Nguyen V, Carlsbecker A (2020) Coping with water limitation: hormones that modify plant root xylem development. *Front Plant Sci* 11:570. <https://doi.org/10.3389/fpls.2020.00570>
- Reshma Z, Meenal K (2024) Zinc biofortification and implications on growth, nutrient efficiency, and stress response in *Amaranthus cruentus* through soil application of biosynthesized nanoparticles. *Applied Food Research* 4:100385. <https://doi.org/10.1016/j.afres.2023.100385>
- Reyes-Rosales A, Cabrales-Orona G, Martínez-Gallardo NA, Sánchez-Segura L, Padilla-Escamilla JP, Palmeros-Suárez PA, Délano-Frier J (2023) Identification of genetic and biochemical mechanisms associated with heat shock and heat stress adaptation in grain amaranths. *Front Plant Sci* 14:1101375. <https://doi.org/10.3389/fpls.2023.1101375>
- Ribeiro VC, Leitão CAE (2020) Utilisation of toluidine blue O pH 4.0 and histochemical inferences in plant sections obtained by free-hand. *Protoplasma* 257:993–1008. <https://doi.org/10.1007/s00709-019-01473-0>
- Rizwan M, Meunier JD, Miche H, Keller C (2012) Effect of silicon on reducing cadmium toxicity in durum wheat (*Triticum turgidum* L. cv. Claudio W.) grown in a soil with aged contamination. *J Hazard Mater* 209:326–334. <https://doi.org/10.1016/j.jhazmat.2012.01.033>
- Salisbury EJ (1927) On the cause and ecological significance of stomatal frequency with special reference to the woodland flora. *Philos Trans R Soc Lond B Biol Sci* 216:1–65. <https://doi.org/10.1098/rstb.1928.0001>
- Seregin IV, Kozhevnikova AD (2005) Distribution of cadmium, lead, nickel, and strontium in imbibing maize caryopses. *Russ J Plant Physiol* 52:565–569. <https://doi.org/10.1007/s11183-005-0084-8>
- Seregin IV, Kozhevnikova AD (2011) Histochemical methods for detection of HMs and Sr in the tissues of higher plants. *Russ J Plant Physiol* 58:721–727. <https://doi.org/10.1134/S1021443711040133>
- Shafeeq-ur-Rahman, Xuebin Q, Yatao X, Ahzad MI, Shehzad M, Zain M (2020) Silicon and its application methods improve physiological traits and antioxidants in *Triticum aestivum* (L.) under cadmium stress. *J Soil Sci Plant Nutr* 20:1110–1121. <https://doi.org/10.1007/s42729-020-00197-y>
- Shao JF, Che J, Yamaki N, Shen RF, Ma JF (2017) Silicon reduces cadmium accumulation by suppressing expression of transporter genes involved in cadmium uptake and translocation in rice. *J Exp Bot* 68:5641–5651. <https://doi.org/10.1093/jxb/erx364>
- Shi G, Cai Q, Liu C, Wu L (2010) Silicon alleviates cadmium toxicity in peanut plants in relation to cadmium distribution and stimulation of antioxidative enzymes. *Plant Growth Regul* 61:45–52. <https://doi.org/10.1007/s10725-010-9447-z>
- Sienkiewicz-Cholewa U, Sumisławska J, Sacala E, Dziągwa-Becker M, Kieloch R (2018) Influence of silicon on spring wheat seedlings under salt stress. *Acta Physiol Plant* 40:54. <https://doi.org/10.1007/s11738-018-2630-y>
- Song J, Feng SJ, Chen J, Zhao WT, Yang ZM (2017) A cadmium stress-responsive gene AtFC1 confers plant tolerance to cadmium toxicity. *BMC Plant Biol* 17:187. <https://doi.org/10.1186/s12870-017-1141-0>
- Song X, Yue X, Chen W, Jiang H, Han Y, Li X (2019) Detection of cadmium risk to the photosynthetic performance of hybrid *Pen-nisetum*. *Front Plant Sci* 10:798. <https://doi.org/10.3389/fpls.2019.00798>
- Tanaka Y, Sugano SS, Shimada T, Hara-Nishimura I (2013) Enhancement of leaf photosynthetic capacity through increased stomatal density in Arabidopsis. *New Phytol* 198:757–764. <https://doi.org/10.1111/nph.12186>
- Thakral V, Bhat JA, Kumar N, Myaka B, Sudhakaran S, Patil G, Sonah H, Shivaraj SM, Deshmukh R (2021) Role of silicon under contrasting biotic and abiotic stress conditions provides benefits for climate smart cropping. *Environ Exp Bot* 189:104545. <https://doi.org/10.1016/j.envexpbot.2021.104545>
- Thakral V, Raturi G, Sudhakaran S, Mandlik R, Sharma Y, Shivaraj SM, Tripathi DK, Sonah H, Deshmukh R (2024) Silicon, a quasi-essential element: availability in soil, fertilizer regime, optimum dosage, and uptake in plants. *Plant Physiol Biochem* 208:108459. <https://doi.org/10.1016/j.plaphy.2024.108459>
- Thakur A, Singh A, Tandon A, Sharma V (2023) Insights into the molecular mechanisms of uptake, phytohormone interactions and stress alleviation by silicon: a beneficial but non-essential nutrient for plants. *Plant Growth Regul* 101:1–13. <https://doi.org/10.1007/s10725-023-01002-3>
- Thorne SJ, Hartley SE, Maathius FJ (2020) Is silicon a panacea for alleviating drought and salt stress in crops? *Front Plant Sci* 11:1221. <https://doi.org/10.3389/fpls.2020.01221>
- Ur Rahman S, Xuebin Q, Yatao X, Ahmad MI, Shehzad M, Zain M (2020) Silicon and its application methods improve physiological traits and antioxidants in *Triticum aestivum* (L.) under cadmium stress. *J Soil Sci Plant Nutr* 20:1110–1121. <https://doi.org/10.1007/s42729-020-00197-y>
- Ur Rahman S, Xuebin Q, Zhao Z, Du Z, Imtiaz M, Mehmood F, Hongfeil L, Hussain B, Ashraf MN (2021) Alleviatory effects of silicon on the morphology, physiology, and antioxidative mechanisms of wheat (*Triticum aestivum* L.) roots under cadmium stress in acidic nutrient solutions. *Sci Rep* 11:1958. <https://doi.org/10.1038/s41598-020-80808-x>
- Vaculik M, Lux A, Luxová M, Tanimoto E, Lichtscheidl I (2009) Silicon mitigates cadmium inhibitory effects in young maize plants. *Environ Exp Bot* 67:52–58. <https://doi.org/10.1016/j.envexpbot.2009.06.012>
- Vaculik M, Pavlovič A, Lux A (2015) Silicon alleviates cadmium toxicity by enhanced photosynthetic rate and modified bundle sheath's cell chloroplasts ultrastructure in maize. *Ecotoxicol Environ Saf* 120:66–73. <https://doi.org/10.1016/j.ecoenv.2015.05.026>
- Van der Ent A, Baker AJM, Reeves RD, Pollard AJ, Schat H (2013) Hyperaccumulators of metal and metalloid trace elements: facts and fiction. *Plant Soil* 362:319–334. <https://doi.org/10.1007/s11104-012-1287-3>
- Verma KK, Song XP, Liang Q, Huang HR, Bhatt R, Xu L, Chen GL, Li YR (2024) Unlocking the role of silicon against biotic stress in plants. *Front Plant Sci* 15:1430804. <https://doi.org/10.3389/fpls.2024.1430804>
- Voleniková M, Tichá I (2001) Insertion profiles in stomatal density and sizes in *Nicotiana tabacum* L. plantlets. *Biol Plant* 44:161–165. <https://doi.org/10.1023/A:1017982619635>
- Waheed S, Ahmad R, Irshad M, Khan SA, Mahmood Q, Shahzad M (2021) Ca²⁺-SiO₄⁴⁻

- Subscript> chemigation reduces cadmium localization in the subcellular leaf fractions of spinach (*Spinacia oleracea* L.) under cadmium stress. *Ecotoxicol Environ Saf* 207:111230. <https://doi.org/10.1016/j.ecoenv.2020.111230>
- Wei T, Li X, Li H, Sun Y, Hua L, Ren X, Guo J (2021a) Effect of exogenous silicon and methyl jasmonate on the alleviation of cadmium-induced phytotoxicity in tomato plants. *Environ Sci Pollut Res* 28:51854–51864. <https://doi.org/10.1007/s11356-021-14252-3>
- Wei W, Peng H, Xie Y, Wang X, Huang R, Chen H, Ji X (2021b) The role of silicon in cadmium alleviation by rice root cell wall retention and vacuole compartmentalization under different durations of Cd exposure. *Ecotoxicol Environ Saf* 226:112810. <https://doi.org/10.1016/j.ecoenv.2021.112810>
- Wei Z, Abdelhakim LOA, Fang L, Peng X, Liu J, Liu F (2022) Elevated CO₂ effect on the response of stomatal control and water use efficiency in amaranth and maize plants to progressive drought stress. *Agric Water Manage* 266:107609. <https://doi.org/10.1016/j.agwat.2022.107609>
- Wu G, Tian N, She F, Cao A, Wu W, Zheng S, Yan N (2022) Characteristics analysis of early responsive to dehydration genes in *Arabidopsis thaliana* (AtERD). *Plant Signal Behav* 18:e2105021. <https://doi.org/10.1080/15592324.2022.2105021>
- Yadav V, Arif N, Kováč J, Singh VP, Tripathi DK, Chauhan DK, Vaculik M (2021) Structural modifications of plant organs and tissues by metals and metalloids in the environment: a review. *Plant Physiol Biochem* 159:100–112. <https://doi.org/10.1016/j.plaphy.2020.11.047>
- Zeng J, Dong Z, Wu H, Tian Z, Zhao Z (2017) Redox regulation of plant stem cell fate. *EMBO J* 36:2844–2855. <https://doi.org/10.15252/embj.201695955>
- Zhang H, Zhu J, Gong Z, Zhu JK (2022) Abiotic stress responses in plants. *Nat Rev Genet* 23:104–119. <https://doi.org/10.1038/s41576-021-00413-0>
- Zhao J, Yu B, Wang X, Chen L, Akhtar K, Tang S, Lu H, He J, Wen R, He B (2023) Differences in the response mechanism of cadmium uptake, transfer, and accumulation of different rice varieties after foliar silicon spraying under cadmium stressed soil. *Front Plant Sci* 13:1064359. <https://doi.org/10.3389/fpls.2022.1064359>
- Zhou YY, Wang YS, Sun CC, Fei J (2023) Cloning and expression of class I chitinase genes from four Mangrove species under heavy metal stress. *Plants* 12:2772. <https://doi.org/10.3390/plants12152772>
- Zulfiqar U, Ayub A, Hussain S, Waraich EA, El-Esawi MA, Ishfaq M, Ahmad M, Ali N, Maqsood MF (2022) Cadmium toxicity in plants: recent progress on morpho-physiological effects and remediation strategies. *J Soil Sci Plant Nutr* 22:212–269. <https://doi.org/10.1007/s42729-021-00645-3>

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