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Silicon Alleviates Cadmium Toxicity in *Triticum aestivum* L. Plants by Modulating Antioxidants, Nutrient Uptake, and Gene Expression

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SILICON (Si) is beneficial for plant growth and has the potential to alleviate the deleterious effects of heavy metals in plants grown on contaminated soils. This study aimed to evaluate the adaptive mechanisms induced by Si application (1mM sodium meta-silicate, $Na_2O_3Si.9H_2Ox$) in *Triticum aestivum* L. plants subjected to cadmium (Cd) stress (100 and 200µM $CdSO_4$). Under Cd stress, Si application significantly increased plant biomass, relative water content, nutrient uptake, and allocation as well as Si content while it decreased Cd accumulation compared to Cd-stressed plants. Si application also induced lignin content, mainly in roots, in the presence or absence of Cd in comparison to controls. Cd stress significantly increased the accumulation of oxalate, malate and citrate contents in the roots in comparison to control, whereas Si supplementation increased malate, and citrate in shoots. Additionally, Cd-induced oxidative stress designated by the increment of malondialdehyde, H_2O_2 contents and electrolyte leakage was diminished upon Si application. Concomitantly, Cd-stress markedly enhanced glutathione reductase (GR), glutathione peroxidase (GSHPx), and ascorbate peroxidase (APx) while GSH/GSSG and ASA/DHASA ratios decreased. Si application significantly induced all tested antioxidant enzymes and increased GSH/GSSG and ASA/DHASA ratios. Interestingly, low-affinity Cd transporter (LCT1), ATPase/heavy metal transporter (HMA2), and phytochelatine synthase (PCs) genes expression decreased in the shoots and roots of Si+ Cd-treated plants, while that of Si transporter (Si1) markedly increased, which may contribute to Cd uptake reduction and increased Si content. Taken together, the results highlight the role of Si in alleviating the adverse effect of Cd on wheat plants.

Keywords: Antioxidants, Cadmium stress, Lignin, Metal transporters, Organic acids, Silicon, *Triticum aestivum*.

Introduction

Environmental pollution with heavy metals has become one of the dominant abiotic stresses worldwide because of extensive urbanization, rapid industrialization, anthropogenic activities, and excessive use of sewage sludge and some fertilizers in agricultural practice (Xie et al., 2014). Heavy metals have notable detrimental effects on the human population, agriculture, and food security (Hussain et al., 2018). Among toxic heavy metals, cadmium (Cd) is considered a very toxic pollutant to nearly all life forms including human, animals, and plants, and it readily gets into the trophic chain primarily *via* plants (Rizwan et al., 2016; Bhuyan et al., 2020). This element is nonbiodegradable, rapidly absorbed by plants, and accumulates in various tissues owing to its very high mobility and hydrophilic nature, and it has been recently considered an important crop yield-limiting factor

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(Shoeva & Khlestkina, 2018).

Cadmium uptake by plant roots seems to occur *via* transpiration stream and/or various plasma membrane-associated transporters including low-affinity cation transporters (LCT1), the ATPase/heavy metal transporter (HMA2), and Fe-and Zn-transporters (Hart et al., 2006, Romè et al., 2016). After absorption, Cd ions may be retained in root cells *via* complexation with organic acids and phytochelatins (Lux et al., 2011) otherwise a modest amount can move toward the vascular cylinder through symplastic or apoplastic systems across cortical cells (Song et al., 2017). Transportation from roots to shoots through xylem elements is driven by transpiration (Huang et al., 2020) and through citrate transporters (Zorrig et al., 2010).

Ahmad et al. (2016) stated that the accumulation of Cd produces complicated changes in plants at physiological, biochemical, and genetic levels. Plants grown in Cd-polluted soils show stunted growth, chlorosis, epinasty, and browning of root tips (Lopez-Millan et al., 2009). At the physiological and metabolic levels, Cd causes disorder of photosynthetic machinery, imbalance of water and mineral absorption and allocation, and the modulation of enzymatic activities (Wang et al., 2017).

Although Cd is considered a non-redox metal, it induces the production of reactive oxygen species (ROS) that lead to severe damages of different cell organelles, ultimately suppressing the plant growth (Ahmad et al., 2016). Moreover, Cd stress can further bring about several modifications of gene expression (Farooq et al., 2016).

Silicon (Si) is the second most abundant element in the Earth's crust present in both liquid and solid phases (Cornelis et al., 2011). Nonetheless, the only bioavailable form for plants in natural soil solution is orthosilicic acid (Parveen & Ashraf, 2010). The Si uptake by plants occurs mainly through transpiration and/or H-ATPase-dependent mechanisms (Rains et al., 2006), and the uptake by root cells is further controlled by specific influx (LSi1 and LSi6) and efflux t (LSi2 and LSi3) transporters (Ma et al., 2015). Silicon is translocated *via* xylem elements from roots to shoots system as mono- and di-silicic acid, and then it is precipitated as amorphous silica under the leaf cuticle (photolith), forming physical barriers against various environmental stresses(Ma & Yamaji, 2006, Alzahrania et al., 2018).

Although the ratio of Si (dry weight) in plants ranges from 0.1% to 10.0% (Sommer et al., 2006), it is classified as a beneficial element rather than an essential element (Ma & Yamaji, 2006; Rizwan et al., 2016), and recently, numerous studies have demonstrated that it represents a substantial alternative to provide tolerance against the detrimental effects of different metals including Cd^{2+} (Shi et al., 2017) and Ni^{2+} (Abd-Allah et al., 2019). Nevertheless, the role of Si in sustaining plant growth under toxicants is still unclear. Recent studies reported that Si-mediated mitigation of Cd toxicity may include several external and internal mechanisms, such as the increase in nutrient uptake and the reduction in reducing metal uptake and translocation (Rizwan et al., 2016). Si may modify cell wall properties by promoting root lignification and suberinization and it binds to root cell walls trapping Cd and consequently resulting in the sequestration of metals in the cell walls (Alzahrania et al., 2018). In addition, the formation of Cd– Si co-complexation in the cell wall may prevent the entry of metal ions to plant cells (Ma et al., 2015). Further, Ribera-Fonseca et al. (2018) showed that the reduction in heavy metal uptake in the presence of Si could be related to the root exudation of various secondary metabolites such as phenolic compounds and organic acids, which chelate with metal cations and reduce their entering to the plant roots. Moreover, the downregulation of specific metal transporters in the presence of Si may manage the decline in metal uptake (Greger et al., 2016; Shao et al., 2017).

It has been documented that the ameliorative impact of Si on the inhibitory damage cause by various heavy metals in plant species may be associated with the activation of enzymatic antioxidant systems (Farooq et al., 2016; Ribera-Fonseca et al., 2018; Abd-Allah et al., 2019). Hussain et al. (2015) stated that Si application boosted CAT and GPx activity in Cd-treated wheat plants compared to the values observed in the absence of Si. Shi et al. (2017) reported that Si can decrease ROS generation in crops subjected to biotic stress by increasing antioxidant activities, particularly those involved in H_2O_2 eradication. Alzahrania et al. (2018) suggested that Si reduces ROS through the enhancement of SOD, peroxidases, and CAT, in addition to nonenzymatic antioxidants including GSH, ASA, and proline.

In terms of food security, wheat (*Triticum aestivum*) is a remarkable cereal crop worldwide,

and according to FAO publications, it is used as a staple food by almost 50% of the global population. Recently, it has attracted considerable attention from researchers and policymakers and, it is necessary to increase its production nationwide to cope with population growth. As wheat plants have a greater potential to accumulate Cd in their various parts as compared to other cereals (Naeem et al., 2016), it is crucial to minimize the absorption and transfer of Cd to aerial parts, to decrease the risks posed to humans and other living organisms consuming wheat. One of the most cost-efficient and, environment-friendly potential methods for preventing the adverse effects of Cd is to supply plants with beneficial elements (Rizwan et al., 2016). Accordingly, this investigation was conducted to obtain insights into the possible mechanisms involved in Si-mediated Cd detoxification. The role of Si in relation to the modulation of growth, mineral uptake, lignin, organic acid contents, and antioxidant defense of wheat plants grown under Cd-stress was examined. In addition, the changes in the expression of some transporter genes were assessed.

Materials and Methods

Plant material, growth conditions, and treatments

Wheat seeds (*Triticum aestivum* L. cv. Masri 2) obtained from the Agricultural Research Center, Giza, Egypt were surface-sterilized with 4% sodium hypochlorite for 10 min, rinsed with distilled water, soaked for 24 h at 25°C in aerated water and then transferred to weighed plastic pots filled with acid-washed quartz sand and clay (3:1). The pots were irrigated with half-strength modified Hoagland solution recommended by Epstein (1972), supplemented with 0, 100, and 200µM cadmium as ($CdSO_4.8H_2O$) in the absence or presence of 1mM sodium silicate as sodium meta-silicate ($Na_2O_3Si.9H_2O$). The pots were placed in an environmentally controlled growth chamber under a 14h photoperiod, 23/20°C±2°C light/dark temperature and light intensity of approximately 23µmol $m^{-2}s^{-1}$ (cool white fluorescent tubes). The pots were irrigated with the treatment solutions every two days throughout the whole experimental period. After 28 days, homologous plants were harvested, and washed thoroughly to remove adhering soil particles, then they were gently plotted, dissected to shoots and roots, and quickly stored for estimation of the various growth parameters and chemical analyses. Other samples were dried at 60°C to constant weight and were kept for determination of dry mass and element content.

Experimental methods

Growth parameters

The roots and shoots were separated and used for the determination of fresh (FM) and dry biomass (DM). Shoot height (SH) was measured in cm. Leaf relative water content (RWC) was determined as described in Silveira et al. (2003) based on the following equation: $[(FM - DM)/ (TM- DM)] \times 100$

where FM is the leaf fresh mass, DM is the leaf dry mass (after drying at 80°C for 48h) and TM is the turgid mass of leaves (after soaking in water for 4h at room temperature).

Estimation of elements by inductively coupled plasma optical emission spectroscopy (ICP-OES)

Dry plant material was ground and digested in a mixture of 3 mL of concentrated HNO_3 and 3mL of HCl. The Elements (K, Ca, Mg, P, Fe, Cd, Si, Zn, Cu, and Mn) were estimated by inductively coupled plasma optical emission spectroscopy (ICP-OES, Agilent 5100 VDV, USA) and expressed as µg g^{-1}DM.

Estimation of lipid peroxidation, H_2O_2 content, and electrolyte leakage (EL)

Lipid peroxidation was monitored spectrophotometrically as malondialdehyde (MDA) using thiobarbituric acid (TBA) as described in Valentovicˇ et al. (2006). MDA content was determined from the absorbance at 532nm, followed by correction for nonspecific absorbance at 600nm. The content of MDA was determined using the extinction coefficient of 155mM^{-1} cm^{-1}. Hydrogen peroxide content was determined according to the method of Velikova et al. (2000). The leaf electrolyte leakage (EL%) was measured based on the method described in Deshmukh et al. (1991), where leaf discs were washed with deionized water and were then placed in two sets of test tubes each containing 10mL of distilled deionized water. One set of tubes was incubated in a water bath at 40°C for 30min, and the other was incubated at 100°C in a boiling water bath for 15min. Then, their respective electrical conductivities, namely, L_1 and L_2 were measured using a conductivity meter. Electrolyte leakage was defined as:

$$EL (\%) = (L_1/L_2) \times 100.$$

Determination of lignin and organic acids

The oven-dried plant materials were digested with 2.6mL of 25% acetyl bromide in glacial acetic acid for 30min. at 70°C. Samples were cooled on ice, and 900μL of 2M NaOH with 100μL of 5M hydroxylamine-HCl was added to each. Subsequently, 4mL of glacial acetic acid was added to solubilize the lignin. The samples were then centrifuged and the absorbance of the supernatant was measured at 280nm (Moreira-Vilar et al., 2014). Lignin concentration was estimated using a standard curve generated with alkali lignin (Sigma-Aldrich, 370959).

Endogenous organic acid content was determined using a gas chromatography-mass spectrophotometer (Shimadzu GCMS-QP 2010 Plus) based on the method of Sharma et al. (2016). Organic acids were extracted from dried shoots and roots (0.1g) with 1mL of 0.5M HCl in methanol (1:1), followed by shaking for 3h. The resulting extract was then centrifuged for 10min at 12,000 × *g* at 4°C. An aliquot (400μL) of supernatant, 100μL of 50% H_2SO_4 and 300μL of methanol were added and the reaction mixture was heated overnight at 60°C. After cooling, 400μL of water and 800 μL of chloroform were added to the reaction vials, which were then vigorously shaken. The vials were left were allowed to settle until the aqueous layer separated from the chloroform layer. The bottom-settled chloroform layer was then injected into a GC column (DB-5, 30m × 0.025mm i.d.) at the following conditions: initial temperature of 50°C held for 1min, increased at 25°C min^{-1} to 125°C, then increased again at 10°C min^{-1} to 300°C, and held for 15min. The injection volume and temperature were 2μL and 250°C, respectively. Organic acid concentration was estimated from a standard curve, which was expressed as mg $100g^{-1}$ FM

Fluorometric estimation of cellular glutathione content

Reduced and oxidized glutathione (GSH and GSSG) contents were estimated fluorimetrically (Hissin & Hilf, 1976). The final assay mixture contained 100μL of dilute supernatant, 1.8mL phosphate EDTA buffer (pH 8.0), and 100μL of *o*-phthalaldehyde (OPT) containing 100μg of OPT. GSH content was calculated based on the fluorescence intensity at 425nm after excitation at 343nm. For the GSSG assay, 0.5mL of the tissue supernatant was incubated with 200μL of 0.04 M *N*-ethylmaleimide (NEM) to interact with GHS. Then, 4.3mL of 0.1N NaOH was added, to the mixture, and 100μL of this mixture was used for GSSG based on procedure outlined for GSH assay, except that 0.1N NaOH was used as the diluent rather than a phosphate-EDTA buffer.

High-performance liquid chromatographic (HPLC) method for the determination of ascorbate fractions

Ascorbate fractions were estimated by homogenizing of 1 g of frozen plant tissues and 5 mL of ice-cold 6% *m*-phosphoric acid (pH 2.8) containing 1mM EDTA (Gossett et al., 1994). The homogenate was centrifuged at 20,000 × *g* for 15min at 4°C. The supernatant was filtered through a 30-μm syringe filter, and 50μL of the filtrate was analyzed using an HPLC system (PerkinElmer series 200 LC and UV/VIS detector 200 LC, USA) equipped with a 5-μm column (Spheri-5 RP-18; 220 × 4.6mm; Brownlee). The solvent used was H_2O (pH 2.2 using H_3PO_4), which was run isocratically with a flow rate of 0.75mL/min (Gahler et al*.,* 2003). The detector was set at 260nm for the integration of peak areas after calibration with the external standard ASA and the results were expressed as μg g^{-1} FM.

Enzymes assay

To estimate the activity of antioxidant enzymes, frozen plant tissues were homogenized in ice-cold 0.1M potassium phosphate buffer (pH 6.8) containing 0.1mM EDTA. The homogenate was centrifuged at 15,000 × *g* for 20min at 4°C. After centrifugation, the supernatant was used for the determination of catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11), Glutathione peroxidase (GSH-Px, EC 1.11.1.9), and glutathione reductase (GR, EC 1.6.4.2) activities. **CAT** activity was determined based on Rios-Gonzalez et al. (2002) where the decomposition of H_2O_2 was followed at 240nm (1 EU= 1μmol H_2O_2 decomposed in 1min). **APX** activity was determined based on Nakano & Asada (1981), where the H_2O_2-dependent oxidation of ascorbic acid (AsA) was followed at 290nm, and enzyme activity was calculated using an extinction coefficient of 2.8mM^{-1} cm^{-1} and expressed as μmol H_2O_2 reduced g^{-1} FM min^{-1}. **GSH-Px** was assayed following the method described in Pagalia & Valentine (1967) and was expressed as U g^{-1} FM min^{-1}. **GR** was assayed following the oxidation of NADPH at 340nm, as described in Azevedo Neto et al. (2006). Enzyme activity was calculated using an extinction coefficient of 6.2mM^{-1} cm^{-1} and was expressed as

μmol $NADPH_2$ oxidized g^{-1} FM min^{-1}.

RNA isolation and quantitative real-time PCR

Expression analysis of the ATPase/heavy metal transporter HMA2, low-affinity cation transporter LCT1, Si transporter Si1, and phytochelatine synthase PCS genes was performed by real time - PCR for roots and shoots of 28-d old wheat plants. Total RNA was extracted with the TRI Reagent® RNA Isolation Reagent (Sigma-Aldrich corp., Cat. No. T9424) and was quantified using a SmartSpec™ UV–Vis spectrophotometer. The RNA was then used for first-strand cDNA synthesis using the Maxime RT PreMix Kit in a total reaction volume of 20 μL. The primers used for the amplification of target cDNA were designed based on wheat gene sequences available at the National Center for Biotechnology Information (NCBI) which were also used to design gene-specific Real-Time primers (Table 1).

Quantitative real-time PCR was then performed using a 7500 applied biosystem and the reaction consisted of 2μL of diluted cDNA as template, 10μL of RealMOD™ Green SF 2X qPCR mix, 1μL of 5μM forward primer (300 nM final concentration), 1μL of 5μM reverse primer (300nM final concentration), and 6μL of diethylpyrocarbonate treated water. The standard thermal cycling conditions were as follows: 95°C for 5min followed by 45 cycles of 95°C for 15s, 60°C for 30s and 72°C for 30s. The transcript levels of the target genes were expressed after normalization with β-actin using the Livak and Schmittgen method (Livak & Schmittgen, 2001).

Statistical analysis

Statistical analyses were performed using IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp**)**. The significance of the data was analyzed with one-and two-way analysis of variance (ANOVA). Data were described using the mean of three replicates ± standard deviation (SD). LSD was estimated at P≤0.05.

Results

Plant, growth parameters

The results clearly demonstrate that 1mM Si treatment enhanced the biomass accumulation of wheat seedlings, as well as shoot height and RWC. In addition, the results presented in Table 2 clearly showed that the exposure of wheat plants to cadmium stress exhibited a significant decline in the shoots and roots biomass, as well as leaf RWC. The reduction in the biomass was more pronounced in roots than in shoots. At 200μM Cd-treatment, the declines in shoot FM and DM were 75% and 59%, respectively, compared to the control, whereas the corresponding values in roots were 82% and 67%, respectively. In the presence of Si with Cd-contaminated medium, the biomass of roots and shoots also significantly decreased compared to the control; but, the values obtained were significantly higher than those in the absence of Si. The increases in FM in shoots and roots observed in 200μM Cd-treated plants in the presence of Si were 26% and 14%, respectively, compared to the values recorded in 200μM Cd-treated plants in the absence of Si. Parallel to the biomass reduction in roots and shoots in response to Cd stress, shoot length and leaf RWC were markedly depressed compared to those in the Cd-untreated control (Table 2). However, in Si + Cd-treatment, there was a marked increase in these two parameters compared to Cd-stressed plants in the absence of Si.

TABLE 1. Sequences of primers of target and reference genes used in qRT-PCR

Gene	Primer 5 '---------3' sequence	Tm (°C)	Gene bank Accession Number
HMA2	**F:**ACAGTTGGTGAGCGTGTTTC	58	HM021132
	R:AACTGTTTGACACGATGCCC	58	
PCS1	**F:** CTGGCCATTTCTCACCGATC	60	AF093752
	R:GCGCCTTGATACAAGCATGA	58	
LCT1	**F:** CGCCTCCAAGTGCCTATTTC	60	AF015523
	R:CGAGGTGATCTTTGATGCGG	60	
Si1	**F:** CGACTACTTCCCTCCTCACC	63	HM803114
	R:GGGGAAATGGCGGAATATGG	60	
Actin (reference)	**F:** TTGTGCTCGACTCTGGTGAT	58	AB181991
	R: GCTCATAATCAAGGGCCACG	60	

TABLE 2. Changes of fresh and dry biomass in the shoots and roots as well as shoot height and leaf relative water content (RWC) of wheat plant in response to cadmium stress in absence or presence of silicon

Treatment	Shoot		Root		Shoot height	RWC
	FM	DM	FM	DM		
	g plant^{-1}				(cm)	(%)
Control	$3.92^{ab} \pm 0.08$	$0.99^{ab} \pm 0.19$	$2.03^{b} \pm 0.06$	$0.58^{b} \pm 0.09$	$20.9^{b} \pm 1.21$	94.7
100µM Cd	$2.85^{c} \pm 0.10$	$0.71^{c} \pm 0.14$	$1.09^{c} \pm 0.11$	$0.37^{d} \pm 0.05$	$15.4^{c} \pm 2.07$	83
200µM Cd	$0.99^{e} \pm 0.27$	$0.41^{e} \pm 0.09$	$0.37^{e} \pm 0.05$	$0.19^{f} \pm 0.04$	$9.4^{e} \pm 0.41$	68.1
1mM Si	$4.44^{a} \pm 0.46$	$1.07^{a} \pm 0.20$	$2.46^{a} \pm 0.47$	$0.78^{a} \pm 0.05$	$23.3^{a} \pm 0.70$	96.1
100µM Cd+ 1mM Si	$3.96^{b} \pm 0.41$	$0.98^{b} \pm 0.03$	$1.39^{b} \pm 0.05$	$0.49^{c} \pm 0.08$	$19.9^{b} \pm 0.97$	89.6
200µM Cd+ 1mM Si	$1.25^{d} \pm 0.45$	$0.54^{d} \pm 0.04$	$0.42^{d} \pm 0.06$	$0.24^{e} \pm 0.04$	$12.7^{d} \pm 0.94$	79.3
F	43.477*	16.364*	50.866*	57.648*	62.285*	
P	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	

-Values are the means of 3 independent replicates ± SD.

- Means followed by different letters are significantly different at P≤ 0.05 according to the least significant difference (LSD).

In addition to the decrease in growth, the exposure of wheat seedlings to various Cd levels resulted in the appearance of the characteristic symptoms of Cd toxicity such as chlorosis and stunted root growth but these effects could be delayed, to some extent, by Si supplementation.

Changes in element content

Cadmium-stress resulted in a significant decline in macro- and micronutrients contents in the shoots and roots of wheat plants, most apparently in shoots. This reduction was accompanied by a significant increment of Cd concentration and a decline of Si content (Table 3). For example, the declines in K, Ca, Mn, and Si contents in the shoots of 200µM Cd-stressed plants were 89%, 81%, 44%, and 77%, respectively, compared to the control. The comparable values in roots were 72%, 65%, 50%, and 56%, respectively. Interestingly, the results demonstrate that Cd treatment resulted in a marked suppression of element allocation to the shoots and enhanced Cd transportation (Table 4). Under Cd stress, the amounts of Mg, P, Fe, and Si transported to the shoots were 48%, 31%, 28%, and 25% of the total element content, respectively. In contrast, these values amounted to 59%, 39%, 46%, and 38%, respectively in control. Supplementing the nutrient medium with the 1mM Si in the presence or absence of Cd markedly raised the element content in the roots and shoots of wheat plants, as well as transportation to the shoots, producing at the same time a decline in Cd content, compared to their controls (Tables 3, and 4). The increases in K, Ca, Zn, Cu, and Si content in shoots of Si+ Cd treated plants were 88%, 63%, 33%, 31%, and 119%, respectively, compared to the Cd-stressed ones, whereas, the decrease of Cd content in the shoots and roots reached 51% and 13%, respectively.

Electrolyte leakage, lipid peroxidation, and H_2O_2 content

There was a marked increment of electrolyte leakage in the leaves of Cd-stressed wheat plants grown in the absence or presence of Si, but the values obtained were considerably lower than those in the absence of Si. Increasing Cd levels led to a significant increase in MDA and H_2O_2 contents in the shoots and roots of wheat plants compared to control (Fig. 1). For example, at low and high Cd levels, the MDA contents in the shoots were 1.6- and 3.7-fold of the control, respectively. The corresponding values in the roots were 2.7- and 5.5- fold, respectively. The addition of 1 mM Si to the nutrient media in the presence of Cd resulted in a significant decrease in MDA and H_2O_2 contents compared to those of Cd-treated plants alone. The decrease of MDA and H_2O_2 content in the shoots of Si + 200µM Cd-treated plants was 29% and 33% respectively, compared to the values obtained in the of Si. The comparable values in the roots amounted to 23% and 17%, respectively.

Lignin and organic acid contents

Cadmium-stress significantly decreased lignin content in the roots of wheat plants compared to control and it had an insignificant effect in the shoots (Table 5). Notwithstanding that Si treatment induced lignin content, mainly in the roots in the presence or absence of Cd compared to the control. In the absence of Cd, the increase in lignin content in Si-treated roots was 23% compared to the control. At the same time, this increase in 200µM Cd-stressed roots in the presence of Si was 53% compared to that observed in the absence of Si.

TABLE 3. Change of elements concentrations in the shoots and roots of wheat plant in response to cadmium stress in absence or presence of silicon

Treatment		K	Ca	Mg	P	Fe	Zn	Cu	Mn	Si	Cd
		µg g^{-1} DM									
Shoot	Control	3471^{b} ± 30.0	2167^{b} ± 57.0	872^{b} ± 46.0	628^{b} ± 39.0	959^{a} ± 39.0	20^{d} ± 0.05	26^{c} ± 0.92	16^{c} ± 0.20	70^{d} ± 0.06	-
	200µm Cd	377^{c} ± 12.0	405^{d} ± 26.0	237^{d} ± 24.0	122^{d} ± 13.0	267^{c} ± 13.0	9^{a} ± 1.92	13^{a} ± 1.40	9^{a} ± 1.25	16^{c} ± 0.15	235^{a} ± 18.0
	1mM Si	4796^{a} ±423.0	2960^{a} ±170.0	1185^{a} ± 16.0	1164^{a} ± 42.0	983^{a} ± 18.0	26^{c} ± 0.08	28^{c} ± 0.18	19^{c} ± 1.17	119^{a} ± 1.0	-
	200µMCd +1mM Si	709^{c} ± 98.0	658^{c} ± 25.0	557^{c} ± 17.0	310^{c} ± 12.0	415^{b} ± 17.0	12^{b} ± 2.79	17^{b} ± 1.98	13^{b} ± 0.12	35^{b} ± 1.0	115^{b} ± 1.30
	F	291.694*	535.181*	616.592*	693.407*	708.884*	69.254*	157.859*	168.799*	9375.154*	16.124*
	P	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Root	Control	2815^{b} ± 17.0	2748^{b} ±263.0	594^{b} ± 10.0	1029^{b} ± 33.0	1135^{b} ± 6.0	11^{d} ± 2.0	29^{c} ± 2.0	22^{b} ± 1.0	112^{c} ± 2.0	-
	200µm Cd	783^{c} ± 18.0	974^{d} ± 7.0	260^{d} ± 11.0	276^{d} ± 11.0	671^{d} ± 30.0	22^{a} ± 3.0	24^{a} ± 3.0	11^{a} ± 2.0	49^{d} ± 2.0	337^{a}± 93.0
	1mM Si	3472^{a} ±244.0	3984^{a} ±128.0	845^{a} ± 24.0	1325^{a} ± 36.0	1277^{a} ± 8.0	32^{c} ± 2.0	31^{c} ± 1.0	21^{b} ± 2.0	149^{a} ± 2.0	-
	200µMCd +1mM Si	836^{c} ± 7.0	1306^{c} ± 95.0	426^{c} ± 28.0	551^{c} ± 9.0	831^{c} ± 51.0	18^{b} ± 3.0	18^{b} ± 2.0	16^{a} ± 2.0	89^{b} ± 2.0	294^{b}± 48.0
	F	376.410*	244.076*	382.035*	1027.526*	255.381*	49.346*	9.778*	25.538*	1729.500*	5.015*
	P	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.005*	<0.001*	<0.001*	0.007*

TABLE 4. Percent of allocation to shoot (%) in the shoots of wheat plant in response to cadmium stress in absence or presence of silicon

Treatment	K	Ca	Mg	P	Fe	Zn	Cu	Mn	Si	Cd
Control	55	44	59	39	46	53	47	48	38	-
200µM Cd	33	29	48	31	28	29	30	21	25	41
1mM Si	58	43	58	46	39	45	44	48	44	-
200µM Cd+ 1mM Si	46	34	57	36	20	40	34	43	28	28

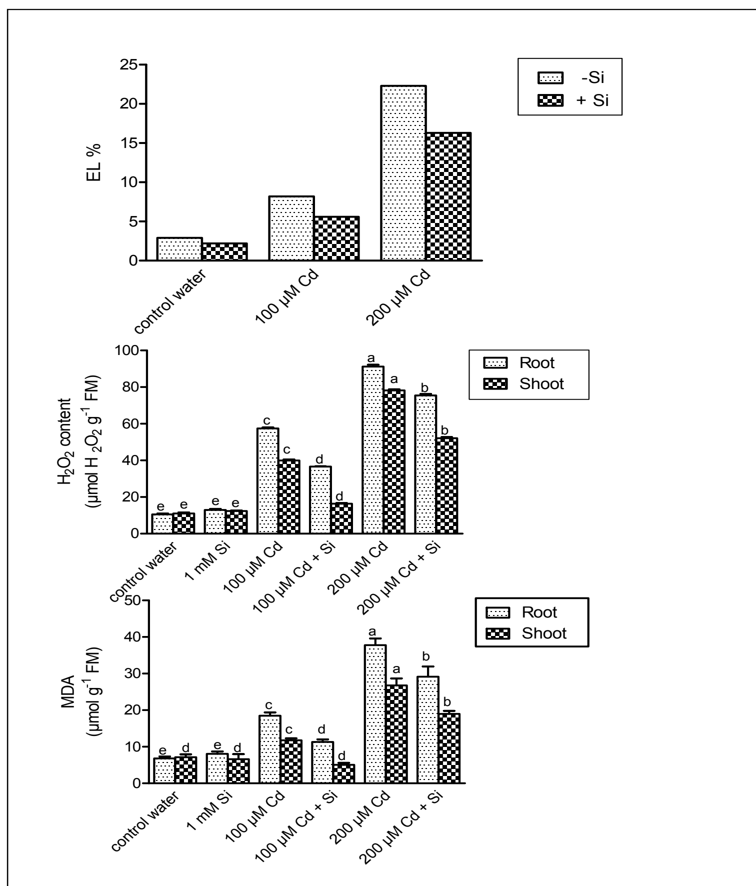

Fig. 1. Changes in leaf electrolyte leakage EL(%), hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) content in leaves and roots of 28-day-old wheat plants in response to Cd-stress in absence or presence of silicon [Values are the means of 3 independent replicates ± SE; means followed by different letters are significantly different at P≤ 0.05 according to the least significant difference (LSD)]

TABLE 5. Changes of lignin and organic acids content in the shoots and roots of wheat plant in response to cadmium stress in absence or presence of silicon

Treatment	Shoot	Root	Shoot			Root		
	Lignin (mg g^{-1} DM)		mg 100g^{-1} FM					
			Oxalic	Malic	Citric	Oxalic	Malic	Citric
Control	12.5^{a} ± 0.33	15.57^{b} ± 0.55	4.3^{a} ± 0.70	5.68^{a} ± 0.02	4.27^{a} ± 0.33	8.54^{c} ± 0.76	2.04^{c} ± 0.26	3.03^{c} ± 0.05
200µM Cd	10.95^{a} ± 0.28	7.76^{d} ± 0.22	4.91^{a} ± 0.89	7.73^{a} ± 0.07	5.08^{a} ± 0.48	19.81^{a} ± 0.21	12.67^{a} ± 0.24	11.89^{a} ± 0.41
1mM Si	12.71^{a} ± 0.19	19.19^{a} ± 0.51	5.83^{a} ± 0.25	4.12^{a} ± 0.58	3.65^{a} ± 0.21	7.14^{c} ± 0.26	3.19^{c} ± 0.81	3.79^{c} ± 0.39
200µM Cd+ 1mM Si	11.56^{a} ± 0.23	11.88^{c} ± 0.30	6.79^{a} ± 1.24	14.45^{b} ± 0.89	10.48^{b} ± 0.55	11.67^{b} ± 0.14	8.41^{b} ± 1.39	8.64^{b} ± 0.26
F	12.09^{a} ± 0.41	17.18^{ab} ± 0.50	52.722*	138.833*	121.852*	104.160*	131.371*	320.718*
P	11.56^{a} ± 0.23	11.88^{c} ± 0.30	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

- Values are the means of 3 independent replicates ± SD.
- Means followed by different letters are significantly different at P≤ 0.05 according to the least significant difference (LSD).

Cd stress had an insignificant effect on the accumulation of organic acids in the shoots, whereas in the roots, oxalate, malate, and citrate were significantly increased compared to the control. Si supplementation to Cd-containing media significantly decreased the accumulation of oxalate, malate, and citrate contents in the roots compared to the values obtained in the absence of Si. The decreases of oxalate, malate, and citrate in Si + Cd-treated plants were 41%, 34%, and 28%, respectively, compared to Cd-stressed ones. In the shoots, only malate and citrate contents significantly increased in Si + Cd-treated plants versus Cd-treated ones (Table 5).

Cellular glutathione and ascorbate contents

The exposure of wheat plants to various Cd levels significantly reduced TG, GSH, and glutathione redox potential (GSH/GSSG) in the shoots and roots of wheat plants. Simultaneously a significant increment in GSSG content was recorded (Table 6). The decreases in TG and GSH contents in the shoots of 200µM Cd-stressed plants were 37% and 59%, respectively, compared to the control. The corresponding values in the roots were 39% and 61%, respectively. Nonetheless, TG and GSH contents, and the GSH/GSSG ratio were significantly reduced in the shoots and roots of Si + Cd-treated plants compared to the control, but the values obtained were significantly higher than those observed in the absence of Si. The increases in TG and GSH contents in the shoots of 200µM Cd-stressed plants in the presence of Si were 32% and 73%, respectively, compared to the values recorded in the 200µM Cd-stressed ones. In the roots, the increase values amounted to 16% and 56%, respectively. The GSH/GSSG ratio in the shoots and roots decreased from 4.33 and 3.63, respectively, in the control to 1.13 and 1.03, respectively, in 200µM Cd-treated plants.

As observed for the glutathione trend, there was a significant decline in total and reduced ascorbate content (ASA), as well as ASA/DHA ratio, under Cd stress ,and the content of the oxidized form (DHASA) was enhanced (Table 7). Supplementation of Si significantly raised the total ASA and ASA/DHASA ratio compared to the values obtained in Cd-stressed plants. In the presence of Si, the ASA content reached 2.6- and 6.7-fold of 200µM Cd-stressed shoots and roots, respectively. In addition, the decrease in DHASA content in the shoots and roots amounted to 21% and 31%, respectively.

TABLE 6. Changes of glutathione fractions in the shoots and roots of wheat plant in response to cadmium stress in absence or presence of silicon

Treatment	Shoot				Root			
	GSH	GSSG	TG	GSH / GSSG	GSH	GSSG	TG	GSH / GSSG
	µg g^{-1} FM				µg g^{-1} FM			
Control	176.9^{a} ± 3.10	40.8^{e} ± 1.20	217.7^{a} ± 7.30	4.33^{a} ± 0.34	143.6^{b} ± 3.30	39.6^{b} ± 1.70	183.2^{b} ± 3.80	3.63^{b} ± 0.17
100µM Cd	134.5^{d} ± 3.50	60.9^{b} ± 2.10	195.4^{b} ± 5.60	2.21^{d} ± 0.09	105^{c} ± 3.90	42.9^{b} ± 1.90	137.9^{d} ± 2.10	2.45^{cd} ± 0.35
200µM Cd	72.8^{f} ± 2.20	64.5^{a} ± 2.70	137.1^{d} ± 4.0	1.13^{e} ± 0.13	56.3^{e} ± 2.70	54.9^{a} ± 3.70	111.2^{f} ± 1.80	1.03^{e} ± 0.05
1mM Si	168.4^{b} ± 1.60	42.8^{e} ± 2.10	211.2^{a} ± 2.80	3.93^{b} ± 0.07	164.8^{a} ± 4.80	34.2^{c} ± 1.80	199^{a} ± 3.80	4.82^{a} ± 0.20
100µM Cd+ 1mM Si	156.3^{c} ± 3.70	49.7^{d} ± 2.30	178^{c} ± 4.0	3.14^{c} ± 0.16	109.3^{c} ± 1.60	39.3^{b} ± 1.70	148.6^{c} ± 2.60	2.78^{c} ± 0.17
200µM Cd+ 1mM Si	125.9^{e} ± 3.90	54.6^{c} ± 1.20	180.5^{c} ± 2.90	2.31^{d} ± 0.29	87.9^{d} ± 2.30	41.4^{b} ± 2.40	129.3^{e} ± 2.10	2.12^{d} ± 0.18
F	444.073*	67.805*	114.048*	100.158*	421.250*	27.096*	420.486*	119.728*
P	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

- Values are the means of 3 independent replicates ± SD.
- Means followed by different letters are significantly different at P≤ 0.05 according to the least significant difference (LSD).

TABLE 7. Changes of ascorbate fractions in the shoots and roots of wheat plant in response to cadmium stress in absence or presence of silicon

Treatment	Shoot				Root			
	ASA	DHASA	Total	ASA/ DHASA	ASA	DHASA	Total	ASA/ DHASA
	µg g^{-1} FM				µg g^{-1} FM			
Control	5.1[a] ± 1.10	1.15[c] ± 0.40	6.25[a] ± 0.70	3.52[a] ± 0.43	3.77[a] ± 0.93	0.51[d] ± 0.15	4.28[a] ± 0.22	2.54[c] ± 0.26
100µM Cd	3.07[c] ± 0.93	1.85[ab] ± 0.20	4.92[b] ± 1.13	1.12[d] ± 0.13	1.61[c] ± 0.31	0.82[b] ± 0.17	2.43[c] ± 0.12	1.96[d] ± 0.08
200µM Cd	0.73[e] ± 0.08	2.04[a] ± 0.06	2.77[d] ± 0.14	0.36[e] ± 0.14	0.21[d] ± 0.09	0.99[a] ± 0.08	1.2[e] ± 0.30	0.65[e] ± 0.09
1mM Si	5.32[a] ± 1.04	1.19[c] ± 0.23	6.51[a] ± 0.81	3.09[b] ± 0.12	3.19[b] ± 0.09	0.49[d] ± 0.19	3.88[ab] ± 0.17	4.62[a] ± 0.39
100µM Cd+ 1mM Si	4.76[b] ± 0.74	1.29[c] ± 0.37	6.05[ab] ± 0.37	2.38[c] ± 0.22	2.88[b] ± 0.17	0.69[c] ± 0.16	3.57[bc] ± 0.27	4.17[a] ± 0.33
200µM Cd+ 1mM Si	1.87[d] ± 0.13	1.61[b] ± 0.41	3.48[c] ± 0.54	1.16[d] ± 0.08	1.4[c] ± 0.30	0.68[c] ± 0.08	2.08[cd] ± 0.06	3.33[b] ± 0.25
F	14.956*	1.450	16.378*	96.500*	18.867*	8.372*	94.305*	96.116*
P	<0.001*	0.276	<0.001*	<0.001*	<0.001*	0.001*	<0.001*	<0.001*

- Values are the means of 3 independent replicates ± SD.
- Means followed by different letters are significantly different at P≤ 0.05 according to the least significant difference (LSD).

Antioxidant enzyme activity

Prolonged exposure to Cd stress produced a significant increase in CAT, APx, GSHPx, and GR activities in the shoots and roots of wheat plants compared to control (Fig. 2). Moreover, Si supplementation further enhanced the activity of all antioxidant enzymes tested.

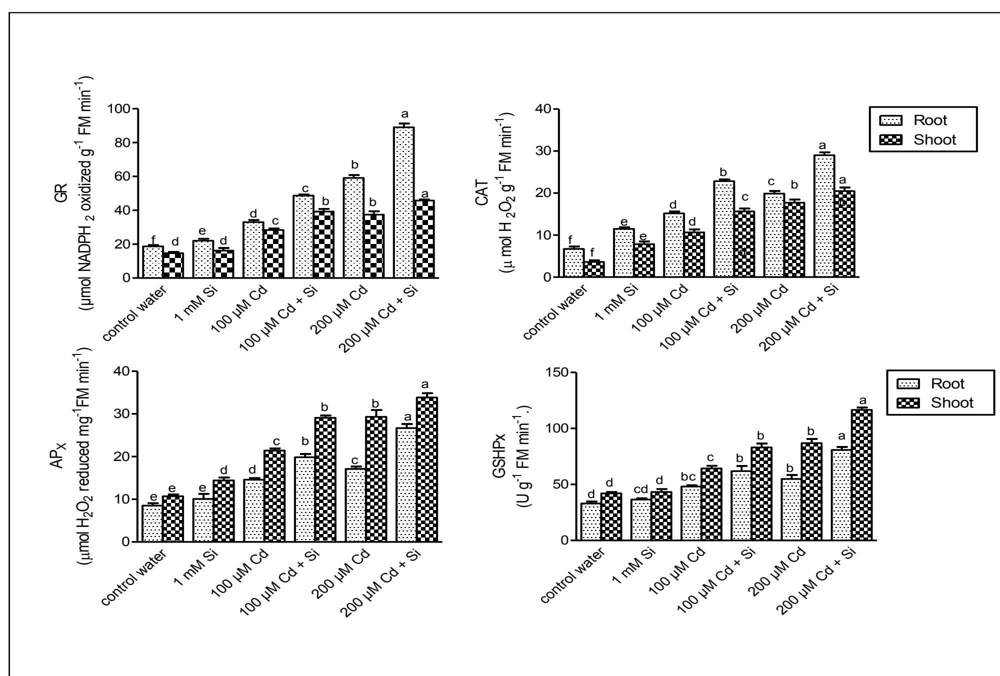

Fig. 2. Changes in CAT, APX, GSHPX and GR, activities in roots and leaves of 28-day-old wheat plants in response to Cd-stress in absence or presence of silicon [Values are the means of 3 independent replicates ± SE; means followed by different letters are significantly different at P≤ 0.05 according to the least significant difference (LSD)]

RNA expression of transporter and phytochelatin synthase genes

Prolonged exposure to Cd stress resulted in downregulation of Si1 expression, while LCT1, HMA2, and PCS expressions were upregulated in the shoots and roots of wheat plants (Fig. 3). The expression level was highly noticeable in roots, reaching 6.3-fold changes for LCT1, 2.2-fold changes for HMA2, and 2.06-fold changes for PCs. By contrast, Si supplementation with Cd resulted in downregulation of LCT1, HMA2, and PCS expressions, and that of Si1 was upregulated compare to Cd-treated ones.

Interestingly, the results showed that Si application in the absence of Cd markedly upregulated the expression of Si1 reaching 2.45- and 1.31-fold changes in the shoots and roots, respectively whereas LCT1, HMA2, and PCS were downregulated.

Discussion

Growth biomarkers, lignin, Cd, and organic acid contents

Worldwide, agricultural soil is polluted by Cd which causes alterations in numerous biochemical and physiological processes leading to a pronounced reduction of plant growth and productivity (Wang et al., 2017; Rehman et al., 2020). The damaging effects of Cd on the morphological and growth attributes of wheat observed in the current study were similar to those described in several reports on various crops subjected to under Cd stress such as pea (Rahman et al., 2017) and rice (Bhuyan et al., 2020). The suppression of wheat biomass in response to Cd stress observed in the present study might be due to the increased uptake of Cd and its cellular accumulation, resulting in an enhancement of ROS generation and oxidative stress in various cellular components. Cadmium led to a significant increment of leaf EL%, as well as H_2O_2 and MDA contents in the shoots and roots, revealing the disturbance of plasma membrane integrity and imbalance of nutrient uptake, which subsequently reduced the plant growth. Numerous studies have shown that Cd toxicity causes a marked accumulation of Cd in different plant tissues, leading to increased ROS production. These ROS have been shown to instantaneously cause a marked degradation of proteins, lipids, pigments, nucleic acids, peroxidation of polyunsaturated fatty acids of plasma membranes, and inactivation of several enzymes, thus inhibiting the plant growth (Tauqeer et al., 2016). It has also been reported that Cd toxicity provokes a marked accumulation of Cd in the roots of several plants including soybean (Holubek et al., 2020) and wheat plants (Rehman et al., 2020), and that was accompanied with a great reduction in growth. In accordance with these findings, the results of the present study showed a higher accumulation of Cd in the roots and lesser allocation to the shoots. At the same time, greater inhibition of root growth compared to shoot growth was observed, along with a marked decrease in root lignin content. The reduction in root growth in response to Cd stress might be attributed to the disturbance of cortical cells and stellar structure (Koren et al., 2013), inhibition of cell division in root apex (Zhang et al., 2013), and peroxidation of polyunsaturated fatty acids in the plasma membranes of root cells leading to accumulation of Cd and therefore increasing its toxicity. In addition, the decline of lignin content in Cd-stressed roots compared to the control ones might indicate the decrease of several functional groups in the secondary walls and xylem elements resulting in a depressing of Cd-binding capacity with them, therefore increasing the cytosolic Cd concentration and inducing Cd toxicity on wheat growth. Yang et al. (2011) concluded that the binding between Cd and functional groups of lignin could lead to a reduction in Cd levels in the cytoplasm, and therefore counteracting the toxicity of this element.

In recent years, accumulating evidence has shown that Si plays an important role in alleviating biotic and abiotic stresses on plants (Abdelaal et al., 2020). Herein, Si-mediated mitigation of Cd-induced toxicity in wheat plants was clearly demonstrated by suppressed Cd accumulation and oxidative damage, as well as by enhanced plant biomass, leaf RWC, and nutrient uptake and allocation in plants grown under Si + Cd treatment compared to those grown under Cd-stress only. These findings suggest that Si might alleviate Cd toxicity by strengthening plasma membrane integrity against oxidative damage, resulting in the maximization of water and nutrient uptake, and allocation from roots to shoots along with decreasing Cd uptake. Cadmium detoxification through the suppression of Cd uptake and ROS generation has been elucidated in several plant species (Ma et al., 2015; Shao et al., 2017). Shi et al. (2017) showed that Si supply with Cd- containing media prompted a marked decline in H_2O_2 and MDA content in the leaves of wheat plants compared to those under Cd alone.

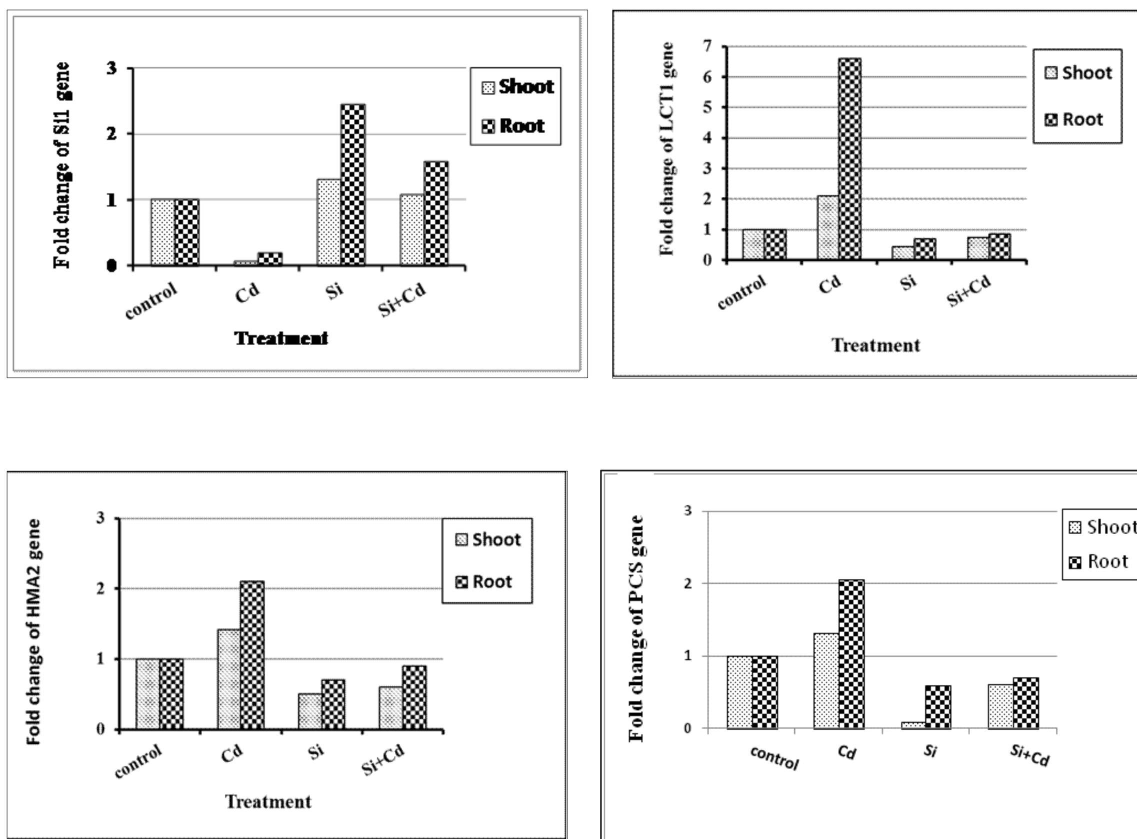

Fig. 3. Fold change for m-RNA expression analysis of Si1, LCT1, HMA2, and PCS gene using qRT-PCR in the roots and shoots of wheat plant in response to Cd-stress in presence or absence of Si

It has been suggested that the decline in Cd uptake and transport in the presence of Si might be attributed to an increase in Cd binding to the lignin of secondary walls (Lux et al., 2011; Feng et al., 2017), formation of Cd–Si complexes with the cell walls (Ma et al., 2015), and/or modulation of the gene expression of specific plasma membrane-associated Cd transporters (Greger et al., 2016). Silicon application significantly stimulated lignin biosynthesis, particularly in Cd-stressed roots, and it was associated with a greater decrease in Cd content and its transportation to the shoots compared to Cd-stressed plants. These findings might reveal the role of Si in regulating Cd uptake by the root system. Moreover, the increase in Si content observed in Si + Cd-treated plants compared to that in Cd-stressed ones could restrain apoplastic Cd transportation from roots to shoots. Shi et al. (2017) and Farooq et al. (2016) concluded that Si uptake is an active process that strongly binds and conjugates to the cross-linkage of cell wall materials.

Organic acids are renowned to alleviate the toxic effects of Cd stress (Sidhu et al., 2019). Zhu et al. (2011) mentioned that *Lycopersicon esulentum* roots mainly exudate malate and maize roots preferably exudate citrate to hinder the Cd uptake and its toxicity. The current investigation showed that Cd stress resulted in significant increments of oxalate, malate, and citrate contents in wheat roots with increasing Cd allocation to the shoots compared to the Cd-untreated control. On the other hand, Si supply with Cd significantly decreased the organic acids contents in wheat roots, whereas only malate and citrate concentrations were significantly increased in the shoots compared to the Cd-stressed ones. Rizwan et al. (2016) stated that the increase in Cd content in Cd-treated wheat shoots might be related to an increase in Cd translocation mediated by organic complexes. Accordingly, the significant increments of oxalate, malate, and citrate contents in wheat roots, observed in this study, indicate that these compounds might bind with Cd in the roots and facilitate its translocation to the shoots as an organic ligand. In addition, Adrees et al. (2015) reported that Si-mediated

chelation between metals and organic acids in plants or in surrounding growth media resulted in the decrease in metal toxicity. Thus, the decline in organic acids in wheat roots under Si + Cd treatment might be attributed to root exudation and chelation with external Cd, which therefore hindered Cd uptake by roots and its transport to the shoots. Moreover, the increase of malate and citrate contents in the shoots might bind with Cd causing a decrease in cytosolic Cd levels and facilitating its translocation to the metabolically inactive sites of the cells (vacuoles), resulting in the mitigation of Cd toxicity and maintenance of wheat growth.

Nutrient uptake and expression of transporter genes

Many evidences revealed that the uptake of macronutrients and Si is an active absorption process mediated by H-ATPases (Greger et al., 2016). In the present study, the decrease in nutrient uptake in response to Cd stress might be attributed to the generation of ROS and dysfunction of plasma membrane-associated enzymes (as H-ATPases) and protein carriers, which could affect element transportation (Satoh-Nagasawa et al., 2012). It is well shown that Cd is first taken up apoplastically and is then translocated through plasma membranes by means of specific cation transporters including LCT1, HMA2, zinc/iron transporter, and/or Nromp5 (Ma et al., 2015; Shi et al., 2017; Abedi & Mojiri, 2020). In this study, Cd treatment markedly upregulated LCT1 and HMA2 gene expression in the shoots and roots of wheat plants revealing an increment of Cd uptake and transportation in conjunction with the decline in essential element content. These results might suggest that the decrease in elements and Si contents might be attributed to competition between Cd and these cations for the specific transporters (Romè et al., 2016). Moreover, Cd stress downregulated the expression of the LSi1 transporter, which is specific for Si-translocation, resulting in a decrease in Si content. On the other hand, in the presence of Si, element content was significantly increased with decreasing Cd content both in the shoots and roots compared to those exposed to Cd alone. It is also noteworthy that Si supply downregulated the expression of the LCT1 and HMA2 genes in Cd-treated plants, denoting the contribution of Si to the decreased Cd accumulation in wheat plants. In accordance with these findings, many researchers mentioned that Si treatment markedly downregulates LCT1 and HMA2, leading to a decrease in heavy metal transportation in plant cells (Shi et al., 2017). As previously mentioned, Greger et al. (2016) reported that Si downregulated LCT1 and HMA2 expression and this could alleviate Cd toxicity in wheat plants. In addition, Romè et al. (2016) reported that several membrane transporters including AtHMA2 and AtHMA3 can load heavy metals, such as Cd, to the inactive sites of cells (apoplast, xylem, and vacuoles). Thus in the present study, the decrease in Cd uptake observed after Si application might be due to downregulation of LCT1 and HMA2, and induction of Cd compartmentalization in the inactive cell sites (vacuoles). This is considered one feature of the defense mechanism(s) induced by Si to alleviate Cd toxicity in wheat plants.

Enzymatic and non-enzymatic antioxidants

It is well documented that the generation of ROS in plants is regulated by various enzymatic and non-enzymatic antioxidants defense mechanisms (Biyani et al., 2019). In the present study, Cd stress produced a significant increment of enzymatic antioxidant activities (GR, APX, and GSHPx) in the shoots and roots of wheat plants compared to the control, while the GSH and ASA contents as well as cellular redox potentials (GSH/GSSG and ASA/DHASA), were markedly decreased. These results might reflect the imbalance between the rate of ROS production and their elimination by enzymatic antioxidants and/or through the Hallwell–Asada cycle, despite the increase recorded in GR and APX activities, hence resulting in wheat growth reduction. Similarly, Cuypers et al. (2011) and Hasanuzzaman et al. (2017) reported a marked increase in GR and APx activities with a decline in the GSH/GSSG value in Cd-stressed *Arabidopsis thaliana* and *Brassica napus* plants, respectively. Phytochelatins are formed from glutathione and are enzymatically synthesized by PC synthase after induced expression of the PCs1 gene (Yadav, 2010). In the present investigation, Cd stress markedly induced GSHPx and upregulated PC gene expression. These observations are related to the decreased GSH content in comparisons to controls, revealing the formation of a PC–Cd complex that facilitated the entry of Cd into vacuoles as a defense mechanism against Cd stress. On the other hand, the decline of ASA content may be attributed to the decrease in GSH as a reductant of DHASA to ASA through the ASA-GSH cycle. Farooq et al. (2016) mentioned

that Cd stress decreased GSH content in rice plants which was involved with phytochelatin synthesis. Similarly, numerous studies showed a marked increase in PC synthase expression under various heavy metal treatments (Rahman et al., 2017).

Multiple studies have demonstrated that Si supplementation can alleviate the phytotoxicity of heavy metals through the induction of non-enzymatic and enzymatic antioxidant capacity (Abd-Allah et al., 2019). In accordance with these researches, the present study showed that Si supply significantly increased GSHPx, GR, and APX activities and both GSH and ASA contents in the shoots and roots of Cd-stressed wheat plants compared to those in the absence of Si. In conjunction with these observations, a significant decline in H_2O_2 and MDA accumulation was detected. Balakhnina & Borkowska (2013) reported that the induction of GR activity and GSH pool with an increase in ASA and APx activity can scavenge the generated H_2O_2 *via* the ASA–GSH cycle. Therefore, it can be concluded that Si supply with Cd might alleviate Cd phytotoxicity in wheat plants through the enhancement of the ASA−GSH cycle and the cellular redox homeostasis, revealing the role of Si in eliminating Cd-induced oxidative damage and improving the growth.

Farooq et al. (2016) suggested that the lower Cd concentrations in the cytoplasm of Cd-stressed rice roots in the presence of Si can reduce PC synthase activity and involve GSH in phytochelatin synthesis. In the present study, there was a significant decrease in Cd content in Cd + Si-treated wheat plants. Thus, the downregulation of PCS gene expression in Cd + Si -treated wheat plants might be attributed to the suppression of Cd absorption and cytosolic Cd level. This may increase the drainage of GSH into the ASA–GSH cycle instead of involvement into phytochelatin synthesis, to scavenge the generated ROS and alleviate the effect of Cd phytotoxicity on wheat plants.

Conclusion

The results of this study pointed out that Cd stress induced the generation of ROS and oxidative damage of cellular components and consequently suppressed the growth of wheat plants. The application of Si with Cd-containing media was beneficial in alleviating the adverse effect of Cd by altering its availability and reducing its uptake and accumulation. This alteration might occur *via*: **i**, immobilizing Cd in soil by secretion of organic acids (chelators) from the roots to form Cd-organic acid complexes; **ii**, binding of Cd with the secondary wall components such as lignin; and **iii**, down-regulating the LCT1 and HMA2 transporters gene expression, responsible for Cd transportation. Moreover, the induction of antioxidant systems in Si + Cd-treated plants could improve the plasma membranes integrity, and thus facilitate the water and macro elements transportation ultimately protect the physiological and biochemical processes.

Conflicts of interest: No conflicts of interest have been declared.

Author contribution statement: The authors confirm contribution to the paper as follows: study conception and design: Saber N.E., Ismail G.S.M., and Abdel-Rahman M.M., following lab experiments: Eldebawy M.M., Saber N.E., Ismail G.S., and Mabrouk M. E. Data analysis and interpretation: Saber N.E., Ismail G.S.M., Eldebawy M.M., and Abdel-Rahman M.M, draft manuscript preparation: Saber N.E., Ismail G.S.M., and Eldebawy M.M., critical revision of the article and final approval of the version to be published: Saber N.E. and Ismail G.S.M.

Ethical approval: Not applicable

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السيليكون يخفف من سميه الكادميوم في نباتات القمح عن طريق تعديل مضادات الاكسده وامتصاص المغذيات والتعبير الجيني

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يعد السيليكون (Si) مفيدا لنمو النبات ولديه القدرة على تخفيف الآثار الضارة للمعادن الثقيلة في النباتات التي تزرع في الأراضي الملوثة. تهدف هذه الدراسة إلى تقييم عمليات المواءمة أو التكيف الناتجه من استخدام السيليكون (1 ملي مول ميتا سيليكات الصوديوم) في نبات القمح النامي تحت ضغط الكادميوم (100 و200 ميكرومول كبريتات الكادميوم). وقد وجد ان معاملة الوسط الغذائي بالسيليكون استطاعت أن تحسن بشكل ملحوظ الكتله الحيوية للنبات، والمحتوى النسبي للماء (RWC)، وامتصاص المغذيات وتوزيعها، وكذلك محتوى السيليكون كما ساعد ايضاعلي انخفاض تراكم الكادميوم مقارنة بالنباتات المجهدة بالكادميوم. كما ادى استخدام السيليكون إلى تحسين محتوى اللجنين، بشكل رئيسي في الجذور، في وجود أو عدم وجود الكادميوم مقارنة بضوابط التجربة. تسببت زياده الكادميوم في حدوث زياده معنويه في محتويات أحماض الأكساليك والماليك والسيتريك في الجذور مقارنة بضوابط التجربة بينما ادى استخدام السيليكون إلى حدوث زيادة معنويه في محتوي الماليك والستريك في المجموع الخضري. كما ادى استخدام السيليكون إلى تقليل الأضرار التأكسدية الناجمة عن الكادميوم كما هو مبين من انخفاض محتوي فوق اكسيد الهيدروجين، اكسدة اللييبيدات، وتسرب الأيونات. وبالتزامن ادى اجهاد الكادميوم إلى تحفيز نشاط انزيمات الجلوتاثيون ريدكتيز، الجلوتاثيون البيروكسيديز، وأسكوربات البيروكسيديز، في حين انخفضت نسبة الجلوتاثيون المختزل إلى المؤكسد، ونسبة الأسكوربات المؤكسد إلى المختزل. ادى استخدام السيليكون إلى انتاج زياده معنويه في نشاط كل انزيمات مضادات الأكسدة المختبرة ونسبة الجلوتاثيون المختزل إلى المؤكسد، وكذلك نسبة الأسكوربات المؤكسد إلى المختزل. ومن المثير للاهتمام أن التعبير الجيني لناقل الكادميوم (LCT1) وناقل ايتيبيز / ناقل المعادن الثقيلة (HMA2) والفيتوكيلاتين سينثيز (PCs) انخفض في المجموع الخضري وكذلك جذور النباتات المعالجة بالسيليكون في وجود الكادميوم، بينما زاد التعبير الجيني لناقل السيليكون (Si1) بشكل ملحوظ مما قد يساهم في تقليل امتصاص الكادميوم وزيادة محتوى السيليكون. واجمالا القت الدراسة الضوء على دور السيليكون في التخفيف من التأثير السلبي للكادميوم على نباتات القمح.