

Effect of Silicon on Growth, Photosynthesis, Oxidative Status and Phenolic Compounds of Maize (*Zea mays* L.) Grown in Cadmium Excess

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Abstract Accumulation of heavy metals due to pollution of the environment, particularly in agricultural ecosystems, can cause serious deterioration of crop yield and quality. In this study, we assessed the effect of silicon on physiological, photosynthetic and stress-related aspects of cadmium toxicity in hydroponically grown maize plants (*Zea mays* L., hybrid Valentina). One concentration of silicon (5 mM) and two concentrations of cadmium (5 and 50 μ M) added to the cultivation medium were tested. Cadmium alone led to a significant growth inhibition and negatively affected the content of total chlorophylls and the efficiency of photosystem II. Especially in roots, application of cadmium resulted in the accumulation of reactive oxygen species and consequent membrane lipid peroxidation. The supplementation of silicon successfully ameliorated the toxic effect of cadmium on maize plants and enhanced growth, some of the photosynthetic parameters and reduced the level of oxidative stress. In plants exposed to higher concentrations of cadmium silicon also reduced its accumulation, especially in roots. Changes in the accumulation of phenolic compounds may indicate the influence of silicon on this aspect of secondary plant metabolism and its importance in the detoxification of heavy metals.

Keywords Cadmium · Heavy metals · Silicon · Oxidative stress · *Zea mays*

1 Introduction

Heavy metal pollution is a global problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. These elements may be introduced to the environment by many anthropogenic activities, such as mining, fertiliser use, metal-based pesticides and a wide range of industrial activities (Benavides et al. 2005; Sanità di Toppi and Gabrielli 1999). Cadmium (Cd) is one of the most dangerous metals because of its accumulation in the environment. It is widespread in soils, water and atmosphere. Due to its high mobility in the plant-soil system, Cd can enter the food chain by being taken up by crop plants used for feeding animals and humans (Azevedo et al. 2012). In plants, Cd has no known function as a nutrient and seems to be more or less toxic. Elevated content of Cd in the environment can lead to various toxicity symptoms in plants, including growth inhibition, severe disturbances in photosynthesis, water relations, mineral uptake, or stimulation of oxidative stress (Sanità di Toppi and Gabrielli 1999; Benavides et al. 2005; Azevedo et al. 2012).

Cd as a redox inactive metal does not directly participate in cellular redox reactions, since it does not take part in Fenton and Haber-Weiss reactions (Stohs and Bagchi 1995). However, it can indirectly activate NADPH oxidases in membranes, giving rise to an

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enhanced formation of superoxide radical and hydrogen peroxide and eventually, to an oxidative burst (Gallego et al. 2012). Cd may also induce expression of lipoxygenases in plant tissues and thus indirectly cause oxidation of polyunsaturated fatty acids (Skórzyńska-Polit et al. 2006). Impact of Cd has been studied in plants with various life forms, and it stimulates oxidative stress also in other monocotyledons (such as *Tillandsia* sp.) as confirmed by quantitative (Kováčik et al. 2012) and qualitative (Kováčik et al. 2014) techniques.

Plants produce various compounds which can reduce oxidative stress. Phenolic compounds are widely distributed and structurally diverse metabolites involved in many stress responses (Dixon and Paiva 1995). They have antioxidative properties and scavenge reactive oxygen species (ROS) directly or through enzymatic reactions (Sakihama et al. 2002). Moreover, they function as metal chelators (Vasconcelos et al. 1999) and may have important roles in Cd uptake and translocation (Kováčik et al. 2011).

Silicon (Si) has been the centre of attention in many plant research-related studies in recent years. Although it is the second most abundant element in the Earth's crust and soils, Si has not yet been listed among essential elements for higher plants. However, the beneficial effects of Si have been observed in a wide variety of plant species, especially in Si-accumulating plants under various abiotic and biotic stress conditions (Ma 2004). The roles of Si are often minimal under optimal conditions; therefore, its roles may be compared with those of organic secondary metabolites (Epstein 2009).

The alleviative effect of Si under various stresses, including various pests, diseases, as well as salt stress, drought stress, radiation stress, nutrient imbalance and metal toxicity, has been well documented (for reviews, see Ma 2004 and Liang et al. 2007). Despite the numerous studies about the positive effect of Si under heavy metal toxicity, mechanisms of Si-mediated tolerance remain poorly understood. The key mechanisms include the following: (1) stimulation of antioxidant systems in plants, (2) complexation and co-precipitation of toxic metal ions with Si, (3) immobilisation of toxic metal ions in growth media, (4) inhibition of root-to-shoot transport and (5) compartmentation of metal ions within plants (Liang et al. 2007). The ameliorative effect of Si on Cd-induced toxicity symptoms, including inhibition of growth, photosynthesis and enhanced oxidative stress, has been recently reported in different plant species: *Solanum nigrum* (Liu et al. 2013), cotton (Farooq

et al. 2013), cucumber (Feng et al. 2010), peanut (Shi et al. 2010) and maize (Lukačová et al. 2013; Vaculík et al. 2009).

Maize (*Zea mays* L.) is an important agricultural crop, used in a number of foods, feed, pharmaceutical and industrial products. Due to its adaptability and productivity, it is the third most cultivated field crop after wheat and rice. The alleviative effect of Si on Cd-induced toxicity symptoms has been described in some papers (e.g. Vaculík et al. 2009); however, diverse responses among different maize hybrids were reported (Lukačová Kuliková and Lux 2010).

The aim of this study was to analyse the effect of Si supplementation on growth, photosynthesis, oxidative status and the content of phenolic compounds in young maize plants (*Z. mays* L., cv. Valentina) subjected to Cd excess. One concentration of Si (5 mM) and two concentrations of Cd (5 and 50 μ M) were tested. We assume that results of this study may contribute to the clarification of the mechanism of Si-mediated alleviation of heavy metal toxicity in maize as an agriculturally important crop and Si accumulator.

2 Material and Methods

2.1 Cultivation of Plants and Experimental Design

Uniform seedlings of maize (*Z. mays* L., hybrid Valentina) were cultivated hydroponically in dark plastic 3-L boxes (20 plants per box) with continual aeration of the solution. The experiment was performed in a growth chamber under controlled conditions: 12-h photoperiod (6.00 a.m. to 6.00 p.m.), 25/18 °C day/night temperature, 60 % relative humidity and a photon flux density of 210 μ mol m⁻² s⁻¹ PAR at leaf level supplied by cool white fluorescent tubes TLD 36 W/33 (Philips, France). Before germination, the seeds were sterilized for 10 min in 5 % Savo and washed with water. Thereafter, they were imbibed in distilled water for 5 h at 25 °C in a dark room and germinated in rolls of wet filter paper for 72 h under same conditions.

Seedlings were transferred to a half concentrated Hoagland solution (Hoagland and Arnon 1950) with or without Cd and/or Si. After 2 days, the cultivation medium was changed to full-strength Hoagland solution and the solutions were renewed every third day. The pH of each solution was adjusted to 6.2 using HCl or KOH.

The plants were cultivated in the growth chamber for 10 days. Six different treatments were studied:

1. Control (C)—Hoagland solution without Cd and Si
2. Silicon (Si)—Hoagland solution with 5 mM Si in the form of sodium silicate solution (Sigma, 27 % SiO₂ dissolved in 14 % NaOH)
3. 5 μM cadmium (Cd5)—Hoagland solution with addition of 5 μM Cd in the form of Cd(NO₃)₂·4H₂O
4. 5 μM cadmium + silicon (Cd5 + Si)—Hoagland solution with addition of 5 μM Cd and 5 mM Si
5. 50 μM cadmium (Cd50)—Hoagland solution with addition of 50 μM Cd
6. 50 μM cadmium + silicon (Cd50 + Si)—Hoagland solution with addition of 50 μM Cd and 5 mM Si

Plant material was harvested on the 13th day of the experiment (10th day of hydroponic cultivation). Uniform plants were selected, and whole roots and the second fully developed leaves were used for further analyses. Spectrometry was carried out using a spectrophotometer Uvi Light XTD 2 (Secomam, ALES Cedex, France).

Plants were divided into aboveground and belowground parts. Fresh and dry masses (dried at 95 °C to constant weight) were estimated to calculate the content of dry mass. The measured parameters were expressed per gram of dry weight (DW).

2.2 Quantification of Cd, Si and K

For the analysis of Si, Cd and K content, dried plant material (0.5 g DW) was mineralised in HNO₃ and HF in a closed vessel microwave-assisted digestion system ETHOS 1. The samples were analysed in a radial mode by inductively coupled plasma emission spectrometer ICPE 9000 Shimadzu with a charge-coupled device (CCD) detector. Analyses were performed by a certified laboratory (EKOLAB, s.r.o., Košice, Slovak Republic). Translocation factor (TF) and bioaccumulation factor (BAF) were calculated as mentioned in the footnote of Table 2.

2.3 Determination of Assimilation Pigments and Chlorophyll *a* Fluorescence

Total chlorophyll content was estimated according to the equations proposed by Wellburn (1994). Aliquot masses

of the second fully developed leaves were homogenised in methanol at laboratory temperature, and supernatants were analysed at wavelengths 666 nm (chlorophyll *a*), 653 nm (chlorophyll *b*), 470 nm (total carotenoids), 435/415 (chlorophyll *a* phaeophytinisation) and 750 nm to correct unspecific absorption.

Chlorophyll *a* fluorescence was measured using a FluorCam 800 MF (Photon Systems Instruments Ltd., Brno, Czech Republic). Whole plants were dark-adapted for 5 min prior to measurements. Results were expressed as F_v/F_m , determined as the maximal fluorescence (F_m) less the minimal fluorescence (F_o), divided by F_m of dark-adapted plants, i.e. $(F_m - F_o)/F_m = F_v/F_m$.

2.4 Analyses of Soluble Proteins, Oxidative Stress-Related Parameters and Total Phenols

Soluble proteins were estimated according to Bradford (1976) at 595 nm using 30 μl of supernatants (fresh mass was homogenised on ice bath in 50 mM potassium phosphate buffer, pH 7.0) and bovine serum albumin as standard.

The content of hydrogen peroxide and superoxide radical was measured in supernatants used for quantification of total soluble proteins. The accumulation of hydrogen peroxide was estimated by monitoring the titanium-peroxide complex at 410 nm as described by Jana and Choudhuri (1981). Superoxide radical was measured according to Elstner and Heupel (1976) by monitoring the formation of nitrite from hydroxylamine (530 nm). The amount of hydrogen peroxide and nitrite was calculated from standardised curve with known H₂O₂ and NaNO₂ concentrations, respectively. Level of membrane lipid peroxidation was estimated as the amount of thiobarbituric acid reactive species (TBARS) determined by the thiobarbituric acid reaction at 532 nm (Kováčik et al. 2006).

Soluble phenolics were determined by the Folin-Ciocalteu method as described by Kováčik and Bačkor (2007) in homogenates prepared with 80 % methanol and measured at 750 nm.

2.5 Statistical Analyses

Data were evaluated using ANOVA followed by a Tukey's test (MINITAB Release 16, Minitab Inc., State College, PA) at $P < 0.05$. Number of replications (n) in tables/figures denotes individual plants measured for each parameter. Error bars of graphs represent standard

deviations. Columns and lines sharing the same letter(s) are not significantly different.

3 Results

3.1 Growth Parameters and Content of Soluble Proteins

Increased Cd concentration in cultivation medium led to significant growth inhibition of maize plants. The fresh weight of shoots decreased with the increasing Cd concentration by 44.4 and 67.4 %; roots followed a similar but less evident pattern. The accumulation of dry weight was less affected by Cd excess; however, the dry mass content [%] significantly increased in Cd-treated plants, especially in shoots.

Si applied itself improved the growth of whole plants, increasing fresh weight (FW) and DW in both shoots and roots. In combined variants, Si supplementation successfully alleviated the negative effect of Cd, resulting in higher FW, DW and dry mass content in each tested treatment (Table 1).

The content of total soluble proteins in leaves decreased with the increasing Cd concentration (by 21.3 and 35.8 %, respectively), but it was not significantly affected by Si. Similarly to leaves, the protein content decreased in the roots of Cd-treated plants (by 8.8 and 36.2 % in Cd5 and Cd50 treatment, respectively); however, the application of Si led to a further decrease in

protein content in combined variants (by 31.7 % in Cd5 + Si and 12.6 % in Cd50 + Si variant; Table 1).

3.2 Content of Cd, Si and K

The content of Cd in Cd-treated plants increased with the increasing Cd concentration in cultivation medium (Fig. 1). Young maize plants retained much more Cd in their roots than was translocated to the shoots. In leaves, the content of Cd was 1.4-fold higher in Cd50 than in Cd5 treatment. The application of Si did not result in any significant changes; however, a slight (7.7 %) decrease of Cd content was observed in Cd50 + Si leaves when compared with the respective control without Si. The accumulation of Cd in roots followed a similar trend, being 2.4-fold higher in Cd50 than in Cd5 treatment. Si led to a significant decrease (by 15.5 %) of Cd content in Cd50 + Si when compared with Cd50 roots.

The uptake of Cd was also expressed as shoot BAF and TF (Table 2). Both factors significantly decreased with the increasing Cd concentration (7.2-fold and 1.75-fold decrease for BAF and TF, respectively). The application of Si only slightly increased the TF in both combined variants and led to a slight increase (in Cd5 + Si) or decrease (in Cd50 + Si) of BAF; however, none of the Si-mediated changes in the two above-mentioned parameters were statistically significant.

Maximum leaf Si was 21,672 mg kg⁻¹ DW in Si variant. Maximum root Si was detected in Cd5 + Si variant (14,516 mg kg⁻¹ DW). In leaves, higher

Table 1 Changes in fresh and dry weight (g), dry mass content (%) and the content of total soluble proteins (mg g⁻¹ DW) of shoots and roots of young maize plants grown with or without Cd, Si or Cd + Si, respectively

Treatment	C	Si	Cd5	Cd5 + Si	Cd50	Cd50 + Si
Shoots						
Fresh weight	2.059±0.018 b	2.535±0.238 a	1.145±0.05 c	1.924±0.27 b	0.671±0.058 d	1.286±0.128 c
Dry weight	0.123±0.011 b	0.171±0.014 a	0.084±0.004 c	0.149±0.021ab	0.059±0.005 c	0.122±0.01 b
Content of dry mass	5.97±0.10 f	6.73±0.07 e	7.30±0.06 d	7.75±0.14 c	8.74±0.07 b	9.51±0.17 a
Soluble protein content	240.95±5.02 a	209.52±14.20 b	189.59±14.80 b	183.99±1.37 b	154.64±10.28 c	154.71±4.66 c
Roots						
Fresh weight	0.593±0.038 bc	0.657±0.039 ab	0.485±0.020 cd	0.778±0.043 a	0.422±0.073 d	0.586±0.063 bc
Dry weight	0.030±0.004 b	0.034±0.005 ab	0.026±0.005 b	0.043±0.002 a	0.027±0.006 b	0.037±0.004 ab
Content of dry mass	5.06±0.44 c	5.22±0.51 bc	5.29±0.22 bc	5.55±0.26 abc	6.25±0.49 ab	6.37±0.30 a
Soluble protein content	107.40±6.80 a	84.79±6.56 bc	97.89±8.53 ab	66.83±6.37 cd	68.56±1.79 cd	59.91±8.40 d

The treatments are described in Sect. 2. Data are means ± SD(s) (n=10). Values within rows, followed by the same letter(s), are not significantly different according to Tukey's test ($P < 0.05$)

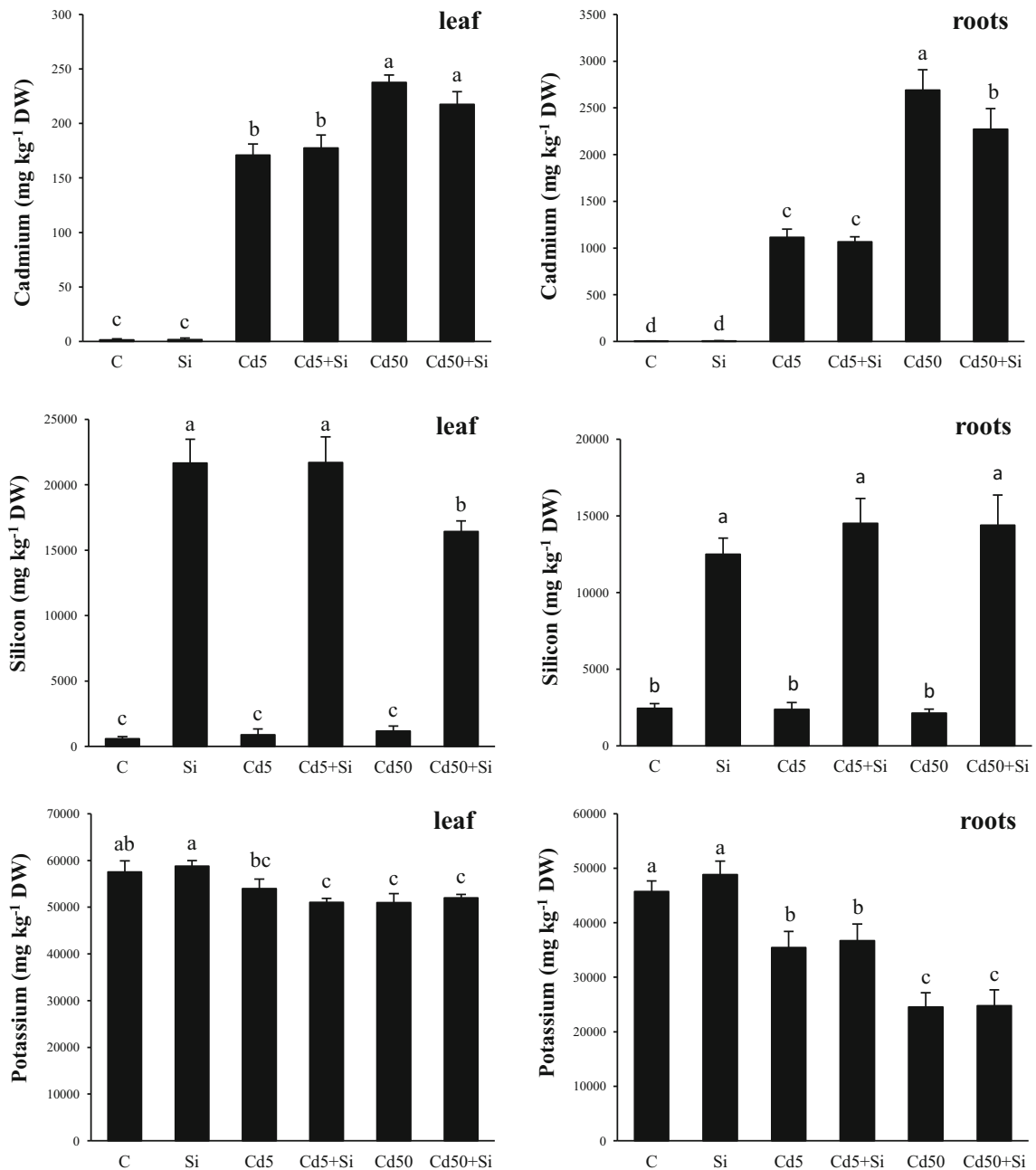


Fig. 1 Content of cadmium, silicon and potassium in the second leaves and roots of young maize plants grown with or without Cd, Si or Cd + Si, respectively. The treatments are described in Sect. 2. Data are means \pm SD(s) ($n=6$). Results of statistics are as in Table 1

concentration of Cd led to a significant decrease in Si content (by 24.28 %). No similar effect of Cd on Si content was observed in roots.

The content of potassium was affected by Cd excess in both leaves and roots; however, the negative effect of Cd was more evident in roots. K accumulation decreased by 22.5 and 46.4 % in Cd5 and Cd50 roots,

respectively. Si did not affect the K content in any of tested treatments.

3.3 Changes in Photosynthesis-Related Parameters

The content of chlorophylls was negatively affected by the increasing concentration of Cd in the

Table 2 Effect of Si on Cd uptake and distribution expressed as shoot bioaccumulation factor (BAF) and translocation factor (TF)

Treatment	Cd5	Cd5 + Si	Cd50	Cd50 + Si
Shoot BAF ^a	305.4±18.3 a	317.1±21.2 a	42.4±1.2 b	38.8±2.1 b
TF ^b	0.154±0.019 a	0.166±0.005 a	0.088±0.005 b	0.096±0.010 b

The treatments are described in Sect. 2. Results of statistics are as in Table 1

^aRatio of shoot Cd content ($\mu\text{g g}^{-1}$ DW) to solution Cd content ($\mu\text{g ml}^{-1}$)

^bRatio of shoot Cd content ($\mu\text{g g}^{-1}$ DW) to root Cd content ($\mu\text{g g}^{-1}$ DW)

cultivation medium (Table 3). The sum of chlorophylls ($a+b$) decreased by 26.2 and 46.4 % in the Cd5 and Cd50 treatment, respectively. The application of Si resulted in a slight increment of total chlorophyll content in both tested variants (by 5.1 and 13.9 %, respectively); however, these changes were not statistically significant. Chlorophyll a seemed to be more influenced by the negative impact of Cd as well as the ameliorative effect of Si than chlorophyll b . The accumulation of carotenoids was not affected in any of the tested variants.

The level of chlorophyll a phaeophytinisation, expressed as a ratio A435/A415, did not change in any of the tested treatments. Chlorophyll a fluorescence decreased in Cd-treated plants, especially in the Cd50 variant (21 %), but Si ameliorated this negative effect (Table 3).

3.4 Accumulation of Reactive Oxygen Species, Level of Membrane Lipid Peroxidation and the Content of Phenolic Compounds

The metal-induced oxidative stress and consequent membrane damage was more evident in roots of

treated plants (Fig. 2). In leaves, the content of hydrogen peroxide was not significantly affected in metal-treated variants and Si caused only a minor decrease in the combined variants. The accumulation of superoxide radical and TBARS followed a similar trend and even decreased with the increasing Cd concentration (by 14.2 and 25.8 % for superoxide radical and by 8.1 and 20.6 % for TBARS). Only a non-significant decrease was observed in combined variants with Si, if compared with the respective controls without Si.

In roots, both Cd concentrations led to a similarity, approximately 1.8-fold rise in the content of hydrogen peroxide. In combined variants, Si significantly reduced the accumulation of hydrogen peroxide when compared with respective controls: by 46.1 % in Cd5 + Si and 38.2 % in Cd50 + Si treatment.

The accumulation of superoxide radical in maize roots followed a similar pattern as that of hydrogen peroxide (1.5-fold in Cd5 and 1.6-fold rise in Cd50 treatment). However, the effect of Si was not so evident (decrease by 22.9 % in Cd5 + Si and 12.4 % in Cd50 + Si treatment).

The increased content of Cd led to a 1.8- and 2.5-fold increase in the content of TBARS in Cd5 and Cd50

Table 3 Content of assimilation pigments (mg g^{-1} DW), chlorophyll a phaeophytinisation (A435/415) and chlorophyll a fluorescence in the second leaf of young maize plants grown with or without Cd, Si or Cd + Si, respectively

Treatment	C	Si	Cd5	Cd5 + Si	Cd50	Cd50 + Si
Chlorophyll a	26.91±1.64 a	24.24±0.93 ab	19.81±0.61 cd	21.15±1.19 bc	14.71±1.03 e	16.68±1.28 de
Chlorophyll b	11.96±0.18 a	10.33±0.79 b	8.86±0.32 b	8.97±0.71 b	6.13±0.05 c	7.06±0.83 c
Chlorophyll $a+b$	38.87±1.76 a	34.57±1.39 b	28.67±0.59 c	30.12±1.91 c	20.84±1.08 d	23.74±2.10 d
Carotenoids	2.10±0.27 a	2.19±0.19 a	2.37±0.09 a	2.33±0.17 a	2.14±0.16 a	1.99±0.20 a
A435/A415	1.212±0.019 a	1.208±0.004 a	1.217±0.004 a	1.212±0.014 a	1.215±0.004 a	1.217±0.005 a
Chlorophyll a fluorescence	0.776±0.009 a	0.771±0.013 a	0.751±0.006 ab	0.760±0.007 ab	0.613±0.026 c	0.722±0.027 b

The treatments are described in Sect. 2. Data are means ± SD(s) ($n=6$). Results of statistics are as in Table 1

treatments. In combined variants, Si alleviated the negative effect of Cd on membrane lipid peroxidation, leading to a decrease of TBARS by 32.2 % in Cd5 + Si and 41.7 % in Cd50 + Si treatment. The application of Si itself did not cause any notable changes in the oxidative status of maize roots.

The content of soluble phenolic compounds (Fig. 3) in leaves did not change significantly in any of the used treatments. In roots, only the lower Cd concentration resulted in a slight rise of soluble phenol content (by 3.2 %); however, Si application led to their decrease (by 22.7 % in Cd5 + Si and 9.01 % in Cd50 + Si).

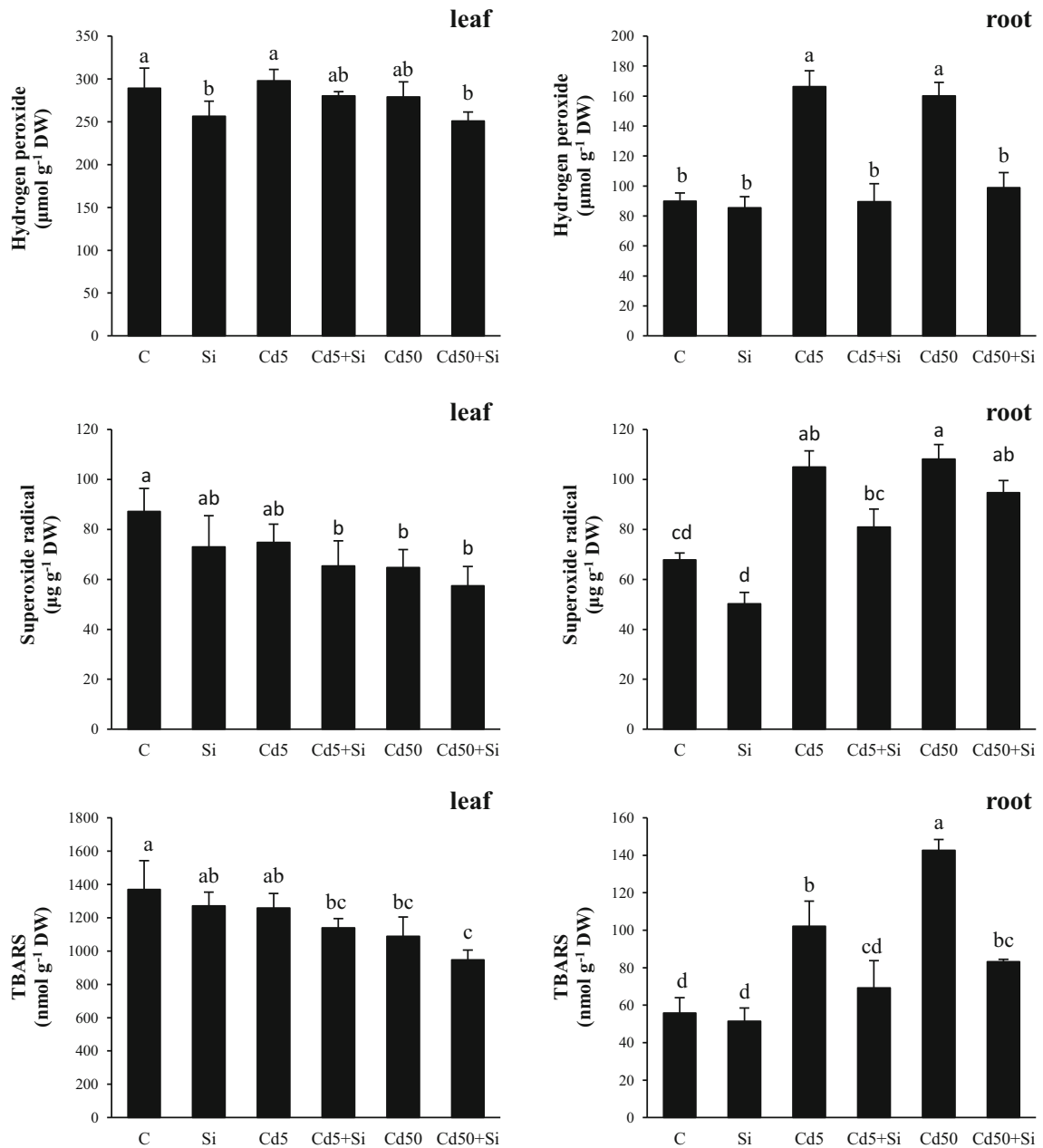


Fig. 2 Effect of silicon supplementation on cadmium-induced changes in the accumulation of reactive oxygen species, hydrogen peroxide and superoxide radical and thiobarbituric acid reactive

species (TBARS) in the second leaves and roots of young maize plants. The treatments are described in Sect. 2. Data are means ± SD(s) (n=6). Results of statistics are as in Table 1

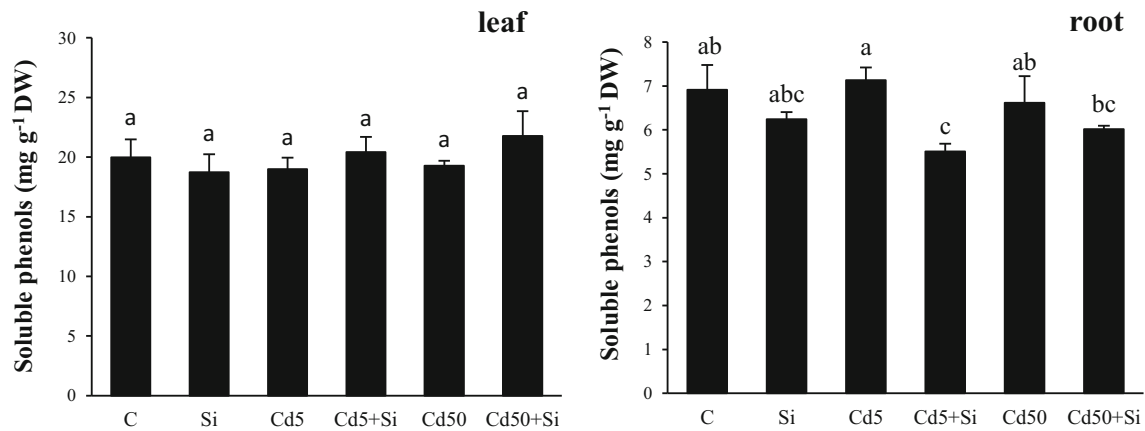


Fig. 3 Content of soluble phenols in the second leaves and roots of young maize plants grown with or without Cd, Si or Cd + Si, respectively. The treatments are described in Sect. 2. Data are means \pm SD(s) ($n=6$). Results of statistics are as in Table 1

4 Discussion

Numerous experiments have been carried out to study the potential effect of Si supplementation on heavy metal-stressed plants. The Si-mediated alleviation of growth inhibition, which is one of the negative aspects of heavy metal toxicity, has been reported in various plant species, e.g. *Solanum nigrum* (Liu et al. 2013), peanut (Shi et al. 2010), cucumber (Feng et al. 2010) or cotton (Farooq et al. 2013). In accordance with our results, Si also improved the growth of maize plants subjected to Cd excess (Vaculík et al. 2009; Lukačová et al. 2013). However, slight discrepancies were observed in the content of dry mass found in our study and the results by Lukačová Kuliková and Lux (2010). The mentioned study gives a detailed overview of the effect of Cd and Si on 30 maize hybrids, including the hybrid Valentina used in our experiments. The increased content of dry mass in Cd + Si variants (when compared with the respective Cd variants) was observed only in roots of hybrid Valentina, while under our experimental conditions, shoots followed the same pattern as roots. These differences could be ascribed to different cultivation conditions as well as higher Cd concentration (100 μ M) than in our experiment.

A positive correlation between Cd concentration in cultivation medium and in the shoots and roots has been reported in many plant species, including maize (e.g. Vaculík et al. 2012). The ability of uptake, accumulation and translocation of Cd by maize plants is not only hybrid-dependent (Lukačová Kuliková and Lux 2010) but also concentration-dependent. In our study, the TF decreased with the increasing Cd concentration,

suggesting that less Cd was translocated to the shoots. High shoot BAF for hybrid Valentina used in our study indicates its potential for accumulating considerable amounts of Cd in its aboveground parts.

The addition of Si resulted in a decreased content of Cd in peanut (Shi et al. 2010), cucumber (Feng et al. 2010), pakchoi (Song et al. 2009) or cotton (Farooq et al. 2013). Lukačová Kuliková and Lux (2010) found a Si-induced decrease in Cd concentration in shoots and roots of various maize hybrids. Contrary to these results, Vaculík et al. (2012) reported an increase in root and shoot Cd concentration in maize plants (hybrid Jozefina) treated with 5 μ M Cd and 5 mM Si simultaneously than in Cd treatment alone. However, at higher Cd concentrations (50 μ M), addition of Si did not affect or decreased the content of Cd in shoots and roots, respectively (Vaculík et al. 2012). Lower (5 or 10 μ M) Cd and 0.08 mM Si concentration used by Lukačová et al. (2013) led to increased accumulation of Cd in both shoots and roots of maize (hybrid Szegedi). In our results, Si significantly decreased the content of Cd only in the roots of Cd50 + Si variant; however, a similar tendency was observed in leaves of the same variant. These results are consistent with the data for maize hybrid Valentina in the study of Lukačová Kuliková and Lux (2010). The content of Si was similar in all Si-treated variants, except for the Cd50 + Si variant in leaves. Cd-induced decrease in Si accumulation, probably due to reduced uptake and/or translocation of Si, was also observed by Vaculík et al. (2012) in maize hybrid Jozefina and other hybrids in the study of Lukačová Kuliková and Lux (2010). It can be concluded that concentration-dependent differences exist in reaction

to Cd and Si + Cd not only between species but also intraspecifically, what can be used in agronomical praxis (Lukačová Kuliková and Lux 2010).

The negative effect of Cd on soluble protein accumulation, which was observed in our study, has been also reported in chamomile plants and indicates active metabolism and probably oxidation of proteins (Kováčik and Bačkor 2007). Contrary to our results, Si increased the content of soluble proteins in cotton exposed to low (1 and 5 μM) concentrations of Cd (Farooq et al. 2013). Si-induced changes in protein content may be explained by the probable function of proteins in Si deposition in cell walls; however, the nature of silicon's association with cell wall components is not fully understood (Currie and Perry 2007).

Accumulation of Cd in leaf tissues leads to inhibition of photosynthesis and reduced the content of chlorophylls. Photosystem II (PSII) is sensitive to Cd, and its function is inhibited to much greater extent than that of PSI (Ekmekçi et al. 2008). No changes in the level of chlorophyll *a* pheophytinisation indicate that rather inhibition of chlorophyll biosynthesis than its degradation occurred in our experiment. The impact of elevated Cd concentrations on the efficiency of PSII was well documented by da Silva et al. (2012). The positive effect of Si on photosynthesis-related parameters under heavy metal excess was observed in cotton (Farooq et al. 2013), cucumber (Feng et al. 2010) and barley (Ali et al. 2013). However, in our experiment, Si caused only a slight non-significant increase in the content of chlorophylls. Similarly to our results, Lukačová et al. (2013) did not find any effect of Si on the chlorophyll and carotenoid content in Cd-treated maize plants (hybrid Szegedi).

Although Cd does not generate ROS directly, increased production of ROS is one of the basic mechanisms underlying Cd-induced toxicity (Sanità di Toppi and Gabrielli 1999). In this study, a tissue-dependent reaction to Cd excess was observed. In leaves, the content of ROS and TBARS slightly decreased in the presence of Cd. Similar results were found in the time-course study of Li et al. (2012). The content of malondialdehyde in *Artemisia annua* decreased with the increasing Cd concentration after 216 h. It can be concluded, that time-dependent changes in the oxidative status of plants occurred also under our experimental conditions and should be studied more carefully in further experiments. The impact of Cd on the oxidative status was more evident in maize roots. This can be

explained by the fact that roots are in the direct contact with Cd in cultivation medium. Shi et al. (2010) found that accumulation of TBARS was more enhanced in the roots of Cd-treated peanut seedlings, whereas the leaves were not affected. Similarly to our observations, Si application alleviated the negative impact of ROS accumulation on membrane lipid peroxidation in peanut roots. In cotton, the content of hydrogen peroxide, the level of electrolyte leakage as well as that of membrane lipid peroxidation increased in both leaves and roots of Cd-exposed plants; however, all of these negative aspects of Cd toxicity were successfully alleviated by Si. The restoration of redox balance in plant tissues was explained by the Si-mediated enhancement of the activities of all the studied antioxidative enzymes (Farooq et al. 2013). Similarly, Song et al. (2009) reported the Si-mediated enhancement of antioxidative system in pakchoi (*Brassica chinensis*) exposed to Cd excess. Si addition resulted in increased antioxidative enzyme activities and reduced the lipid peroxidation and accumulation of hydrogen peroxide. The stimulation of antioxidative systems in plants may be one of the Si-mediated alleviative mechanisms (Liang et al. 2007). On the other hand, Liu et al. (2013) reported decreased enzyme activities in leaves of *Solanum nigrum* grown in Cd- and Si-treated medium. However, Si application decreased the content of hydrogen peroxide as well as the level of electrolyte leakage. It is suggested that although Cd concentration in leaves was not decreased significantly, the active Cd chemical forms in leaves were reduced (Liu et al. 2013). In our study, Si-induced reduction of Cd content in roots could contribute to the decreased content of ROS only in the case of higher Cd concentration (50 μM). Si successfully alleviated the Cd-caused oxidative stress in both tested concentrations; therefore, either the enhancement of antioxidative systems or reduction of active Cd forms could occur. Decrease in potassium content is one of the symptoms of Cd toxicity (e.g. in chamomile reported by Kováčik et al. 2006), due to the increased membrane peroxidation and electrolyte leakage. In our study, the Si-mediated protection of membranes was apparently not sufficient enough to mitigate the negative effect of Cd on K content in maize.

Phenolic compounds are involved in many stress responses and accumulate in plant tissues under various stress conditions. In terms of heavy metal stress, they function as metal chelators (Vasconcelos et al. 1999) and participate in ROS scavenging (Sakihama et al. 2002).

Cd-induced accumulation of phenolic metabolites is well documented in chamomile subjected to high (up to 120 μM) concentrations of Cd (Kováčik and Bačkor 2007). However, only few studies are focused on the effect of Si on metal-induced changes in phenolic metabolism. According to Fang et al. (2003), the deposition of Si in higher plants is associated with co-precipitation of Si and lignin that can be induced by the polymerisation of phenols, and therefore, formation of these complexes may affect the pool of free phenols in plant tissues. Dragašić Maksimović et al. (2007) also reported a lower concentration of phenolic compounds, which are involved in lignin biosynthesis (coniferyl alcohol, ferulic and coumaric acid), in the leaves of Si-supplied cucumber plants grown in Mn excess. Moreover, the key mechanisms of Si-mediated tolerance to heavy metals include co-precipitation of metal ions with Si, suggesting a possible role of phenolic compounds in this process (Liang et al. 2007). Decreased content of soluble phenols in Si-treated maize roots in our study is consistent with these findings. Changes in phenolic metabolites were also found to be related to alteration of Cd amount in other species (Kováčik et al. 2009). However, the role of phenolic compounds in Si-mediated tolerance to Cd excess will be the objective of further study.

In conclusion, we found that the application of Cd excess resulted in growth inhibition, reduced content of chlorophylls and decreased efficiency of photosynthesis. Especially in roots, higher concentrations of Cd led to the overproduction of ROS and consequent membrane lipid peroxidation. Most of these symptoms of Cd toxicity were successfully ameliorated by the application of Si. However, the effect of Si on Cd accumulation was evident only in the case of higher Cd concentration (50 μM), mainly in roots. Based on these observations, it can be concluded that Si application can improve Cd tolerance in maize plants, particularly in the selected hybrid Valentina.

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