DOI: 10.1007/s10535-017-0731-x BIOLOGIA PLANTARUM 61 (4): 741-750, 2017

Silicon enhances the tolerance of *Poa annua* to cadmium by inhibiting its absorption and oxidative stress

P. LI[1,2], C.Z. ZHAO[1], Y.Q. ZHAND[1], X.M. WANG[1], J.F. WANG[1], F. WANG[1], and Y.R. BI[1]*

Ministry of Education Key Laboratory of Cell Activities and Stress Adaptations, School of Life Sciences, Lanzhou University, Lanzhou 730000, P.R. China[1]
State Key Laboratory of Plateau Ecology and Agriculture, Qinhai University, Xining 810016, P.R. China[2]

Abstract

Silicon (Si) could enhance plant tolerance to heavy metals; however, the mechanism of Si-mediated alleviation of cadmium (Cd) toxicity in *Poa annua* was not clear. In this study, we found that 100 μM Cd significantly inhibited the growth of *Poa annua* seedlings. Furthermore, Cd enhanced the H_2O_2 and malondialdehyde content. The activities of superoxide dismutase and ascorbate peroxidase were enhanced, but the catalase and peroxidase activities were reduced by Cd treatment. Cd also altered the activity and expression of glucose-6-phosphate dehydrogenase (G6PDH) in *Poa annua* roots. Application of Na_3PO_4, an inhibitor of G6PDH, decreased the activity of G6PDH, the expression of *G6PDH*, and increased the Cd toxicity, suggesting that G6PDH is involved in the regulation of oxidative stress induced by Cd. Application of 1 mM Si alleviated the inhibition of Cd on the growth of *Poa annua* seedlings. Si application not only led to reduced oxidative injuries but also decreased the accumulation of Cd in *Poa annua* seedlings under Cd stress. Furthermore, Si decreased the activity of G6PDH and the expression of *G6PDH* under Cd stress, which demonstrated that Si attenuates the Cd toxicity in *Poa annua* probably through decreasing the expression of *G6PDH* under Cd stress. When G6PDH was inhibited, the alleviation impact of Si on Cd stress was abolished. Taken together, these results demonstrated that the Cd tolerance in *Poa annua* enhanced by Si is mainly due to the decrease of Cd uptake in roots and lowering the oxidative stress induced by Cd.

Additional key words: ascorbate proxidase, catalase, glucose-6-phosphate dehydrogenase, malondialdehyde, Na_3PO_4, peroxidase, superoxide dismutase.

Introduction

Heavy metal toxicity has become one of the very important limiting factors in the growth and yield of crop plants, which affect the sustainability of agricultural production and hence threaten the food security. Cadmium (Cd), which has high mobility and water solubility, is a common toxic heavy metal in environment and negatively impacts plant growth and development (Gallego *et al*. 2012). A number of studies have shown that Cd, even at trace concentrations, can cause serious problems on plants, such as unbalanced nutritional status (Wang *et al*. 2007), inhibition of photosynthesis and nitrate metabolism (Feng *et al*. 2010), and the excessive production of reactive oxygen species (ROS) (Sharma and Dietz 2009, Zhang *et al*. 2009, Shi *et al*. 2010, Song *et al*. 2011). The complex network of plant pathways that participates in Cd sensing and signal transduction has been recently reviewed (Dal Corso *et al*. 2010). Two different types of components should be considered in the response of plants to Cd:

Submitted 23 February 2016, *last revision* 4 January 2017, *accepted* 9 January 2017.
Abbreviations: APX - ascorbate peroxidase; CAT - catalase; EDTA - ethylene diamine tetraacetic acid; G6PDH - glucose-6-phosphate dehydrogenase; GR - glutathione reductase; GSH - glutathione; MDA - malondialdehyde; MS - Murashige and Skoog; NADPH - nicotinamide adenine dinucleotide phosphate; OPPP - oxidative pentose phosphate pathway; PAGE - polyacrylamide gel electrophoresis; PEPC - phospho*enol*pyruvate carboxylase; 6PGDH - 6-phosphogluconate dehydrogenase; PMSF - phenylmethanesulfonyl fluoride; POD - peroxidase; PVDF - polyvinylidene fluoride; PVP - polyvinyl pyrrolidone; ROS - reactive oxygen species; SOD - superoxide dismutase; TBA - thiobarbituric acid; TCA - trichloroacetic acid.
Acknowledgements: This work was supported by the National Natural Science Foundation of China (31671595; 31670244), the National Program on Key Basic Research Project (2012CB026105), the Foundation of Science and Technology Program of Gansu Province (1506RJZA209), the National High Technology Research and Development Program (2007AA021401), and the Foundation of Science and Technology Program of Lanzhou City (2015-3-53).
* Corresponding author; e-mail: yrbi@lzu.edu.cn

one is specifically triggered by Cd (Weber *et al*. 2006), whereas the other shares common features with other plant stresses caused by abiotic and biotic factors. These common stress responses include Cd-induced oxidative stress and enhancement of phytohormones ethylene and jasmonic acid (Rodríguez-Serrano *et al*. 2009). Contrastingly, in Cd hyper-accumulating species, a down-regulation of some defense pathways has been reported (Liu *et al*. 2015, Yan *et al*. 2016). Whether this process is controlled by ROS needs further research.

Silicon (Si) is the second most abundant element in soil (Exley 1998). Si exists within soils mainly in the form of inert quartz or crystalline silicate; however, monosilicic acids are soluble and thus available to plants and microbes (Balakhnina *et al.* 2012). Silicon has been found to be essential for some plant species growth and development (Ma and Yamaji 2006, Balakhnina *et al.* 2012). Numerous studies have revealed the ability of Si to restore various biotic (plant diseases and pests) and abiotic (heavy metals, drought, and salinity) stresses (Zhang *et al.* 2008, Khandekar *et al.* 2011, Rizwan *et al.* 2011, Li *et al.* 2012, Shetty *et al.* 2012). Its role on biotic stress resistance depends on the formation of a sub-cuticular double layer (Ma and Yamaji 2006). The contribution of Si to abiotic stress tolerance is not only due to its ability to reduce water loss through the leaf cuticle and enhancement of stem growth (Ma and Yamaji, 2006), but also its restriction of the uptake of toxic minerals through Si deposition in the roots as well as toxic metals chelation or complexation (Zhang *et al.* 2008). Furthermore, the involvement of Si in the stimulation of antioxidant systems (Shi *et al.* 2010, Khandekar *et al.* 2011, Song *et al.* 2011) and alleviation of inhibition to photosynthesis (Feng *et al.* 2010) protects plants from Cd stress. Si uptake and transport systems vary from species to species and three distinct Si uptake models have been proposed: active, passive, and rejective (Mitani and Ma 2005).

Glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), the first and rate-limiting enzyme in the pentose phosphate pathway, participates in the generation of NADPH and ribose-5-phosphate (Graeve *et al*. 1994). NADPH is necessary for the regeneration of reduced glutathione (GSH), the main reductant against reactive oxygen species (ROS) damage in cells (Sharma *et al.* 2009). Thus, G6PDH is important for the cellular antioxidant defense system (Salvemini *et al.* 1999). Many studies have reported that G6PDH plays a role in plant response to biotic and abiotic stresses. For example, the role of G6PDH under drought stress is confirmed in tobacco (Scharte *et al*. 2009). The transgenic tobacco containing *PsG6PDH* gene from *Populus suaveolens* shows enhanced tolerance to cold stress (Lin *et al*. 2013). Zhang *et al*. (2013) discovered the crucial role of G6PDH in rice suspension cells under salt stress, which is the result of the coordination of G6PDH and NADPH oxidase to maintain cell redox balance. On the basis of increased transcription of *ScG6PDH* and activity of G6PDH under $CdCl_2$, low temperature, NaCl, and drought treatments, Yang *et al*. (2014) suggested that ScG6PDH may play a positive role in sugarcane tolerance to environmental stresses. G6PDH activity could also be stimulated by some metals such as zinc, cadmium (Van Assche *et al*. 1988, Weber *et al*. 2006, Yang *et al*. 2014), and aluminum (Ślaski *et al*. 1996). Furthermore, some hormones or second messenger signals are involved in the increase of the G6PDH activity (Dal Santo *et al*. 2012, Wang *et al*. 2016). However, the contribution of G6PDH to Si-enhanced tolerance of *Poa annua* to Cd stress has not been found yet.

Poa annua plays significant roles in stabilizing sand dunes, as grassland in parks and playgrounds, and in supplying feed for animals. It usually suffers various stresses during growth. Many studies have reported that *P. annua* shows high tolerance to different abiotic stresses (Peng *et al*. 2013, Zhao *et al*. 2016). Moreover, there is almost no information available regarding physiological responses of *P. annua* under Cd stress. The objective of this work was to examine the effect of Si treatment of *P. annua* seedlings on their growth, oxidative stress, and cadmium uptake under Cd stress.

Materials and methods

Plants and culture conditions: The *Poa annua* L. seeds were soaked in water for 2 d and surface sterilized with 1.5 % (m/v) sodium hypochlorite for 15 min, washed five times with autoclaved water, placed at 4 °C for 3 d, and then planted on the Murashige and Skoog (MS) medium (pH 5.8) containing 3 % (m/v) sucrose and 0.8 % (m/v) agar in the growth room at a temperature of 23 ºC, a 16-h photoperiod, and an irradiance of 100 - 120 μmol(photons) m^{-2} s^{-1}. *P. annua* seedlings were grown for 5 d on MS medium and then different concentrations of $CdCl_2$, Si, Na_3PO_4 (an inhibitor of G6PDH) were added to the medium for indicated times. At least 20 seedlings were used for each treatment. After 7 d, fifty plants were harvested and the fresh masses of shoots and roots were measured immediately.

Determination of chlorophyll and element content: Chlorophyll was extracted and measured according to Porra *et al*. (1989). Fresh leaves (0.5 g) were ground to powder in liquid nitrogen and then placed in 10 cm^3 of acetone (80 %, v/v) and incubated at 4 °C in the dark until the leaf powder was colorless. Absorbance was measured at 647 and 664 nm after centrifugation at 10 000 *g* for 10 min, and total chlorophyll content was calculated.

Content of cadmium, calcium, potassium, sodium, magnesium, phosphorus, nitrogen, sulfur, silicon, and chlorine was determined using a scanning electron microscope (*Phillips Electronics*, Eindhalen, The Netherlands) fitted with an energy-dispersive X-ray detector (*Kevex*, Valencia, CA, USA) as described by Zhao *et al*. (2004). Briefly, following the treatments, the

roots were rinsed five times with the deionized water and then dried at 65 °C for 48 h. Tissues were crushed with a mortar and pestle, and then placed directly on the aluminum stage. At least five spots per sample were examined. The element content was expressed as a percentage of the total atomic number.

Hydrogen peroxide content and fluorescence assay: Hydrogen peroxide content was determined by the peroxidase-coupled assay according to Veljovic-Jovanovic *et al*. (2002). Roots or shoots were extracted with 1 M $HClO_4$ in the presence of 5 % (m/v) polyvinyl pyrrolidone at 4 °C. The homogenate was centrifuged at 12 000 *g* and 4 °C for 10 min. The supernatant was neutralized with 5 M K_2CO_3 to pH 5.6 in final 0.3 M phosphate buffer. The mixture was centrifuged at 12 000 *g* and 4 °C for 10 min and the supernatant was incubated with 1 U ascorbate oxidase (*Sigma*, St. Louis, MO, USA) for 10 min to oxidize the ascorbate prior to assay. The reaction mixture consisted of 0.1 M phosphate buffer (pH 6.5), 3.3 mM dimethylamine borane (DMAB; *Sigma*, St. Louis, USA), 0.07 mM 3-methyl-2-benzothiazolinone-hydrazone hydrochloride hydrate (MBTH; *Sigma*), and 0.3 U peroxidase (POX). The reaction was initiated by adding 0.2 cm^3 of the extracted sample. The absorbance at 590 nm was monitored. H_2O_2 content was calculated from a standard curve prepared by using 10 - 50 μM H_2O_2.

For H_2O_2 fluorescence measurement, roots were stained for 10 min with fluorecein diacetate (*Wako*, Japan) and then washed three times with deionized water. All samples were observed by using a microscope (*PROVIS AX70*, *Olympus*, Tokyo, Japan) equipped with a fluorescence module. Fluorescent images were captured separately using a CCD *Olympus* camera (monochrome images *CoolSNAP-HQ*, photometrics; colour images, *DP12*).

Lipid peroxidation assay: Lipid peroxidation was measured in terms of malondialdehyde (MDA) content following the method of Hodges *et al*. (1999) with some modifications. About 0.5 g of samples was homogenized in 5 cm^3 of 0.1 % (m/v) trichloroacetic acid (TCA) at 4 °C. After centrifugation at 3 000 *g* for 15 min, the supernatant was collected and incubated with an equal volume of 0.25 % (m/v) thiobarbituric acid (TBA; dissolved in 0.1 % TCA) at 100 °C for 30 min. The absorbance was measured at 440, 532, and 600 nm with a *DU640* (Beckman, USA) spectrophotometer.

Determination of antioxidant enzyme activities: Roots were homogenized in ice-cold 0.1 M Tris-HCl buffer (pH 7.8) containing 1 mM EDTA, 1 mM dithiothreitol and 4 % (m/v) polyvinyl pyrrolidone. The homogenate was centrifuged at 20 000 *g* and 4 °C for 10 min. The enzyme activities were measured at 25 °C as follows using the supernatant.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined as described by Prochazkova *et al*. (2001). Catalase (CAT; EC 1.11.1.6) activity was determined as described by Aebi (1984). Ascorbate peroxidase (APX; EC 1.11.1.11) activity was measured in the presence of 0.25 mM ascorbic acid, and 0.5 mM H_2O_2 by monitoring the decrease of absorbance at 290 nm (Janda *et al*. 1999). Peroxidase (POD; EC 1.11.1.7) activity was determined according to Ádám *et al*. (1995). All reactions were recorded in 3 cm^3 of reaction mixtures. Measurements were performed with a spectrophotometer (*Unico UV-2000*, China) with no lag period.

Glucose-6-phosphate dehydrogenase activity assay: G6PDH was extracted according to the method described by Esposito *et al*. (2001) with some modifications. Briefly, 0.5 g of roots were ground in liquid nitrogen and 1 cm^3 the extract buffer [50 mM Hepes-Tris (pH 7.8), 3 mM $MgCl_2$, 1 mM EDTA, 1 mM PMSF, 1 mM DTT] was added. The homogenate was centrifuged at 12 000 *g* and 4 °C for 20 min. 0.1 cm^3 aliquot of the extract was added to either the total dehydrogenase (G6PDH + 6PGDH) assay buffer [50 mM Hepes-Tris (pH7.8), 3.3 mM $MgCl_2$, 0.5 mM D-glucose-6-phosphate disodium salt, 0.5 mM 6-phospho-gluconate, 0.5 mM $NADPNa_2$] or the 6-phosphogluconate dehydrogenase (6PGD) assay buffer [50 mM Hepes-Tris (pH 7.8), 3.3 mM $MgCl_2$, 0.5 mM 6-phosphogluconate, 0.5 mM $NADPNa_2$]. The reduction of NADP to NADPH was measured as the change of absorbance at 340 nm for the initial 5 min. G6PDH activity was calculated as the total dehydrogenase activity minus the G6PGD activity (Tian *et al*. 1998).

Western-blot analysis: SDS-PAGE was performed as described by Laemmli (1970). About 50 μg of proteins were solubilized and separated on an 11.5 % (m/v) acrylamide gel containing 6 M urea. After electrophoresis, the separated proteins were transferred to a polyvinylidene difluoride membrane. The membrane was blocked for 2 h with 5 % (m/v) non-fat milk in 0.5 % (m/v) *Tween 20*, 10 mM Tris-HCl (pH 8.0), and 150 mM NaCl. The anti-G6PDH polyclonal antibody (*Sigma*, St. Louis, USA) was added and incubated with the membrane overnight. After washing, alkaline phosphatase-coupled secondary antibody was added and incubated for 2 h. The chemiluminescence assays were displayed according to the manufacturer's instruction. Protein was determined by Bradford (1976) method and bovine serum albumin was used as a standard.

Statistical analysis: Each experiment was repeated at least three times. Values are expressed as mean ± standard error. The data were statistically analyzed using *SPSS v. 17.0* and comparisons were performed using one-way *ANOVA* together with Duncan's test or Tukey's test for independent samples. In all cases, the confidence coefficient was set at $P < 0.05$.

Results

The fresh masses of shoots and roots were significantly decreased with the increase of Cd concentration (Fig. 1*A*,*B*): 100 μM Cd significantly inhibited the growth and the growth was almost completely arrested at 200 μM Cd. The root growth at 50 to 300 μM Cd was progressively reduced from 81.6 to 61.5 % of the control (Fig. 1*B*). Apart from these symptoms, the seedlings exposed to Cd stress also exhibited reduction in chlorophyll content after treatment with Cd for 3 to 10 d (Fig. 1*C*). The H_2O_2 and MDA content increased with elevated Cd concentration up to 100 μM and then decreased (Fig. 1*D*,*E*), and the H_2O_2 content in roots was higher than that in shoots. The changes in H_2O_2 and MDA content demonstrated that the cellular damage was induced by Cd.

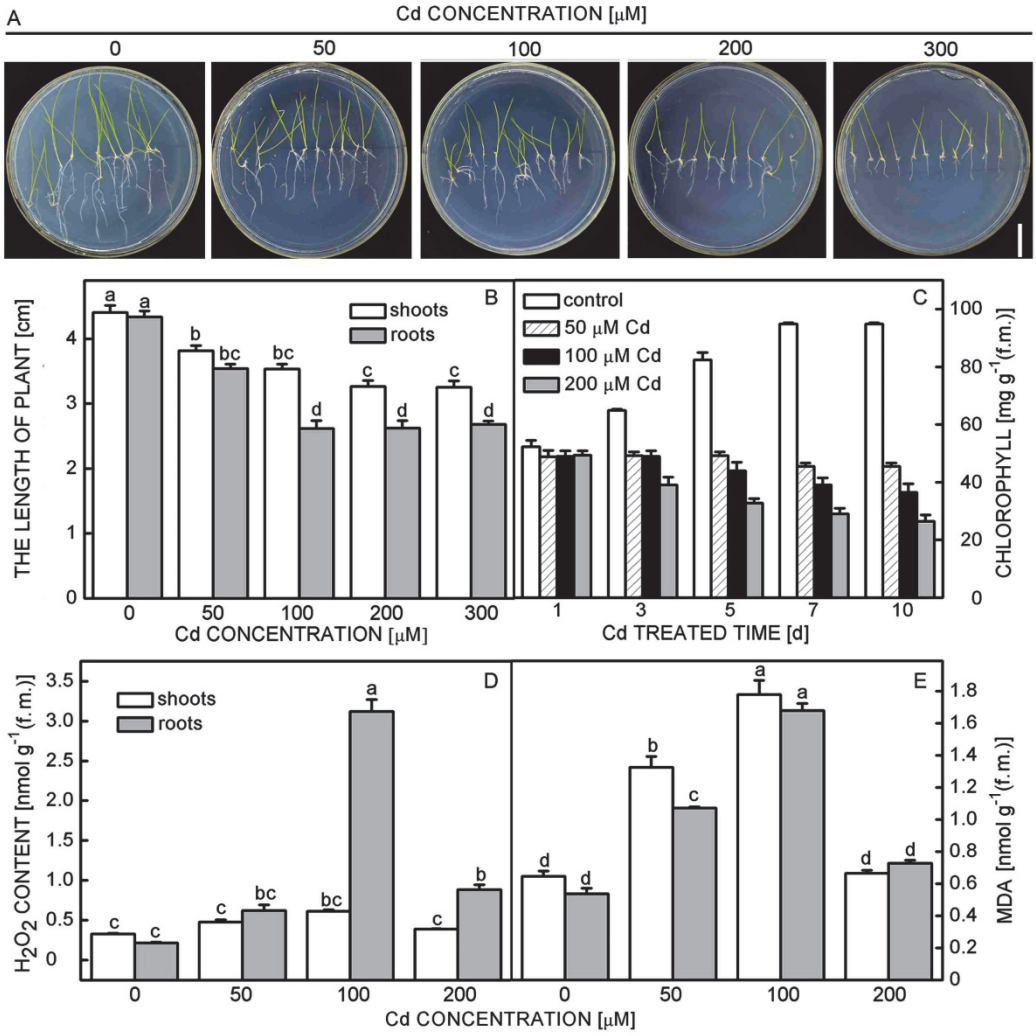

Fig. 1. Effects of Cd stress on *Poa annua* seedlings. The seedlings (5-d-old) were transferred to MS agar medium supplemented with different concentrations of Cd and grown for additional 7 d (*A*,*B*,*D*,*E*) or up to 10 d (*C*). Photographs of the seedlings (*A*, *bar* = 1 cm; representative of similar results in three independent experiments), the length of roots and shoots (*B*), chlorophyll content (*C*), H_2O_2 content (*D*), and MDA content (*E*). Means ± SEs, $n = 20$; different letters indicate significant differences at $P < 0.05$ (Duncan's multiple range test).

Poa annua seedlings (5-d-old) were transferred to solid medium containing both Si (0.5, 1, or 2 mM) and 100 μM Cd and the combined effects of Cd and Si on the growth of *P. annua* were investigated. Application of 0.5 mM Si partially alleviated the decrease of shoot growth caused by Cd, and 1 or 2 mM Si largely improved the growth of roots and shoots (Fig. 2*A*,*B*). We used 1 mM Si to investigate the role of Si in reducing Cd-induced damage of *P. annua* seedlings. Cd induced the decrease of chlorophyll *a* content, and Cd+Si exhibited significant alleviation of Cd effect on the chlorophyll content and enhanced the chlorophyll *a*/*b* ratio (Fig. 2*C*,*D*). Compared with the control, the MDA content was greatly increased by 178.1 % in shoots and 215.0 % in roots under 100 μM Cd, whereas it was restored to the control level in both shoots and roots under the treatment with Si+Cd (Fig. 2*E*).

Compared with the control, the H_2O_2 content was greatly enhanced by 90.6 % in shoots and 1376.2 % in roots at Cd treatment. In the presence of Si, the H_2O_2 content was decreased by 12.5 % in shoots and 338.1 % in roots (Fig. 2*F*,*G*) in comparison with Cd alone. The results demonstrated that Si application reduced the oxidative stress induced by Cd. To investigate the mechanism of Si in attenuating the Cd-induced growth inhibition, we determined the relative content of endogenous Cd in *P. annua*. Cadmium dramatically accumulated in roots and shoots exposed to Cd for 7 d (Table 1). However, application of Si decreased the Cd content up to 24.0 % in shoots and 24.4 % in roots in comparison with Cd treatment alone. Meanwhile, Si content increased in shoots as well as in roots under Cd+Si treatment (by 102.1 % in shoots and 242.5 % in roots compared with Cd stress alone).

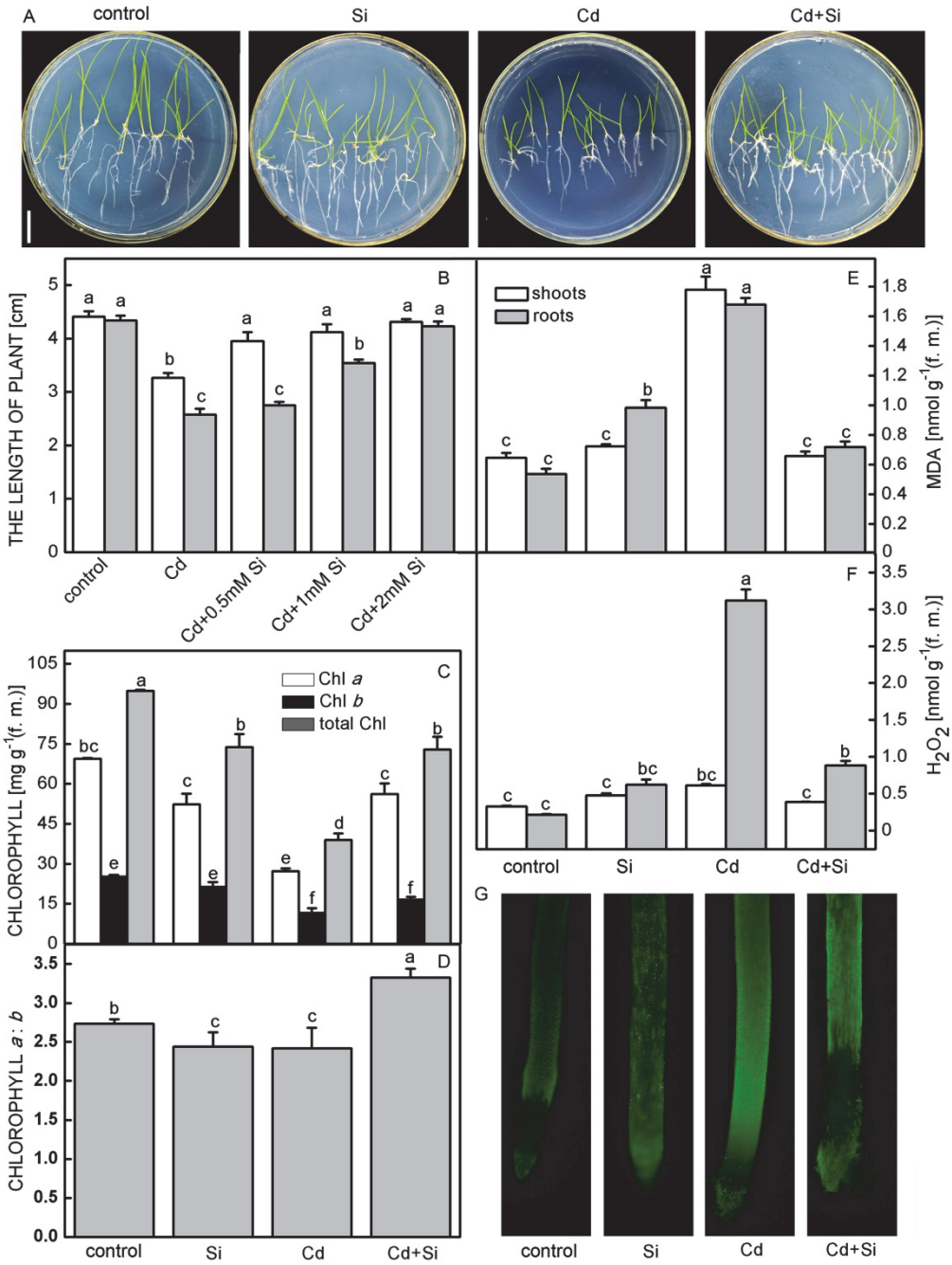

Fig. 2. Alleviation of Cd stress in *Poa annua* by Si. The seedlings (5-d-old) were treated with 100 μM Cd in the presence or absence of different concentration Si for 7 d. Photographs of the seedlings (*A*, *bar* = 1 cm; representative of similar results in three independent experiments), the length of root and shoot (*B*), chlorophyll content (*C*), chlorophyll *a*/*b* ratio (*D*), MDA content (*E*), H_2O_2 content (*F*), and H_2O_2 fluorescence (*G*). Means ± SEs, $n = 20$; different letters indicate significant differences at $P < 0.05$ (Duncan's multiple range test).

Table 1. Percentage of Cd in all elements in roots and leaves of *Poa annua* seedlings grown under 100 μM Cd or 100 μM Cd + 1 mM Si. Means ± SEs, $n = 5$; values followed by the same letters are not significantly different at $P < 0.05$ as determined by Duncan's multiple range test (n.d. - not detectable).

Treatments	Shoots	Roots
Control	n.d.	n.d.
Cd	10.16 ± 0.57a	7.85 ± 0.46a
Cd + Si	7.72 ± 0.22b	5.93 ± 0.24b

In order to further study the roles of Si in the possible alleviation of oxidative stress induced by Cd, we examined the activity of four key antioxidant enzymes in *P. annua* roots (Fig. 3). Compared with the control, the activities of SOD and CAT under Cd stress increased by 95.6 and 55.2 %, respectively, whereas the activities of POD and APX only slightly increased by 27.8 and 11.1 %, respectively. In the Cd+Si treatment, the activities of SOD and CAT were reduced by 37.5 and 32.7 %, respectively, but the activities of POD and APX were enhanced by 31.5 and 12.5 %, respectively. This result indicated that Cd stress dramatically affected antioxidant enzyme activities

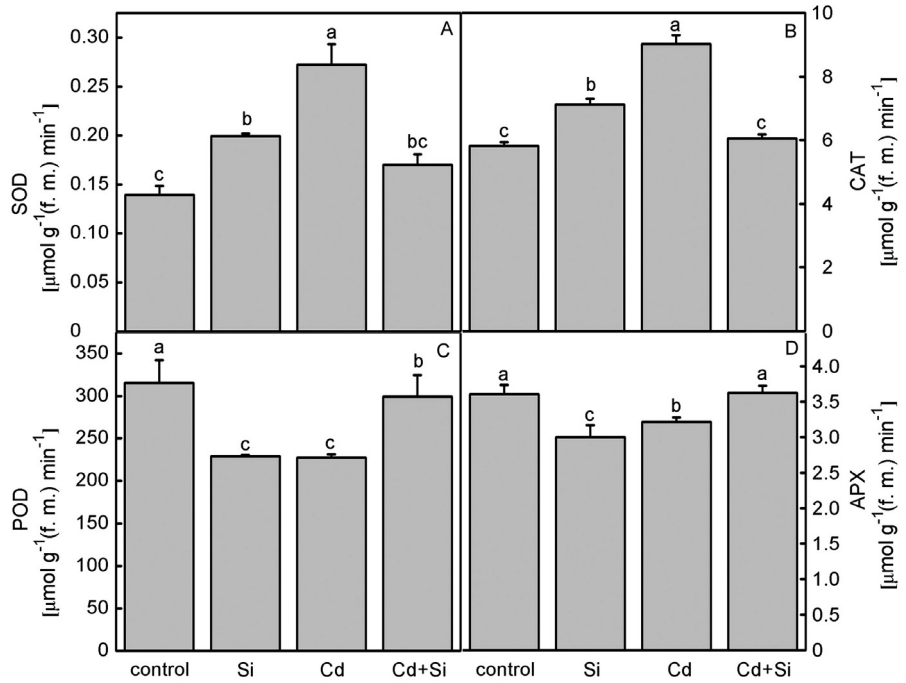

Fig. 3. Changes of antioxidative enzyme activities in roots of *Poa annua* seedlings under different treatments: control, 1 mM Si, 100 μM Cd, and 100 μM Cd + 1 mM Si. Means ± SEs, $n = 3$; different letters indicate significant differences at $P < 0.05$.

in roots and Si alleviated the Cd toxicity by maintaining the normal level of antioxidant system in plant tissues.

During Cd treatment for 7 d, the G6PDH activity significantly increased in *P. annua* roots to 169.9 % of control. Application of 2.5 mM Na_3PO_4, an inhibitor of G6PDH, significantly inhibited the growth of seedlings and the activity of G6PDH (to about 31.0 % of the control, Fig. 4*A*,*C* and 5*A*). Under Cd+Na_3PO_4 treatment, the growth of *P. annua* was also inhibited and the activity of G6PDH was decreased (about 77.1 % of the control). Meanwhile, Si stimulated the activity of G6PDH (increased by 40.8 %) but Cd+Si treatment decreased it by 28.0 % compared with Cd alone. Under Cd+Si+Na_3PO_4 treatment, the G6PDH activity was reduced by 65.9 % compared with Cd stress and by 52.8 % compared with Cd+Si treatment. Using Western blotting, we demonstrated that the change of *G6PDH* expression was in the similar pattern as the G6PDH activity under these conditions (Fig. 5*B*). H_2O_2 content and fluorescence analysis demonstrated that addition of Na_3PO_4 increased the accumulation of H_2O_2 in the roots (about 14 times of the control) (Fig. 4*B*, *D*). Si+Na_3PO_4 treatment also enhanced the H_2O_2 content (about 16 times of the control). Cd+Na_3PO_4 treatment markedly induced the H_2O_2 content (about 23 times of Cd stress). Cd+Si+Na_3PO_4 treatment also improved the H_2O_2 content (about 14 times of the control).

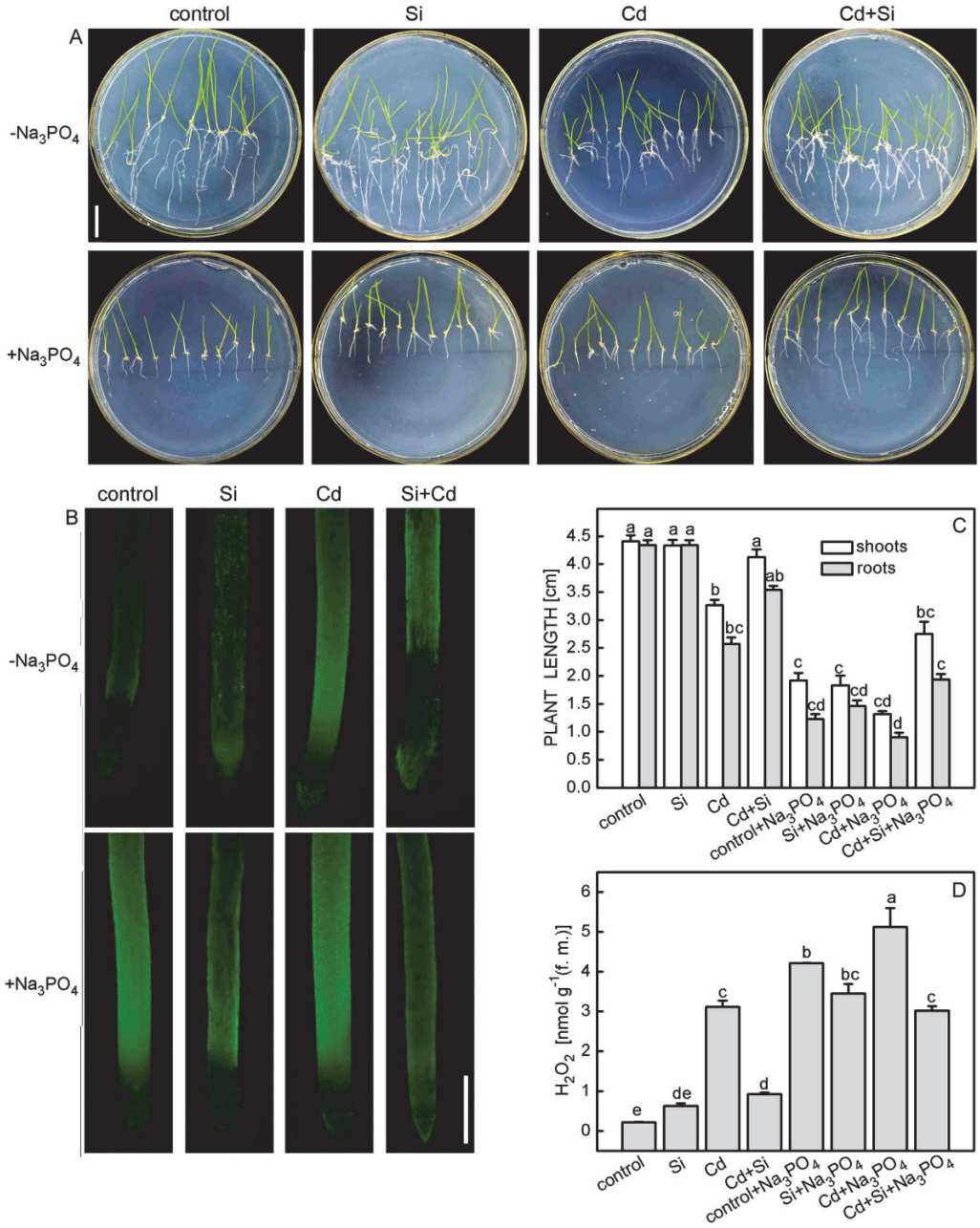

Fig. 4. Effects of 2.5 mM Na_3PO_4, 1 mM Si, and 100 µM Cd on growth and H_2O_2 content in *Poa annua*. The seedlings (5-d-old) were treated with 100 µM Cd in the presence or absence of Si and Na_3PO_4 for 7 d. Photographs of the seedlings (*A*, *bar* = 1 cm; representative of similar results in three independent experiments), the length of plants (*C*), H_2O_2 fluorescence (*B*, bar = 100 µm), and H_2O_2 content (*D*). Means ± SEs, $n = 20$; different letters indicate significant differences at $P < 0.05$ (Duncan's multiple range test).

Discussion

The present study was performed to determine the role of Si in enhancing tolerance of *P. annua* seedlings to Cd stress. We demonstrated that Si restored the growth of seedlings inhibited by Cd stress. The symptoms of Cd toxicity in *P. annua* seedlings were visibly attenuated by Si supplementation associated with a reduction in Cd content in both roots and shoots. Rizwan *et al*. (2011) suggested that a dilution effect (the same uptake but larger biomass) or a reduction of Cd uptake may be responsible for Si-mediated alleviation. Furthermore, since improvement of plant growth under stressed condition is critical for the optimal performance of phytoremediation (Sun *et al*. 2009), Si-promoted plant growth would be useful for *P. annua* to remediate Cd-contaminated soils. Exogenous

Si markedly inhibited the Cd uptake in roots, the percentage of Cd in roots obviously decreased under Cd+Si than in those treated with Cd alone (Table 1). However, various effects of Si addition on the Cd translocation were reported previously in different plant species. In rice, Si supply greatly enhanced Cd retention in roots and reduced its translocation to shoots (Zhang *et al*. 2008). Similar results were also observed in durum wheat (Rizwan *et al*. 2011). Application of Si reduced the Cd content in roots and shoots as well as translocation factor in both Cd-sensitive and Cd-tolerant cultivars of peanut (Shi *et al*. 2010). In maize, Si exerted little effects on Cd translocation from roots to shoots at low Cd concentration (5 mM), but decreased it at 50 mM Cd (Vaculík *et al*. 2012). Whether Si supply have roles in the translocation of Cd from roots to shoots, we need further proves. The differences between our study and reports of Zhang *et al*. (2008) and Rizwan*et al*. (2011) could be explained in several ways. First of all, rice, cucumber, or durum wheat is non-accumulators for metals. Secondly, the translon-cation factor was reported to change with extended time (Zhang *et al*. 2008), but the treatment time was just 7 d in this study.

Cadmium belongs to non-redox heavy metals and

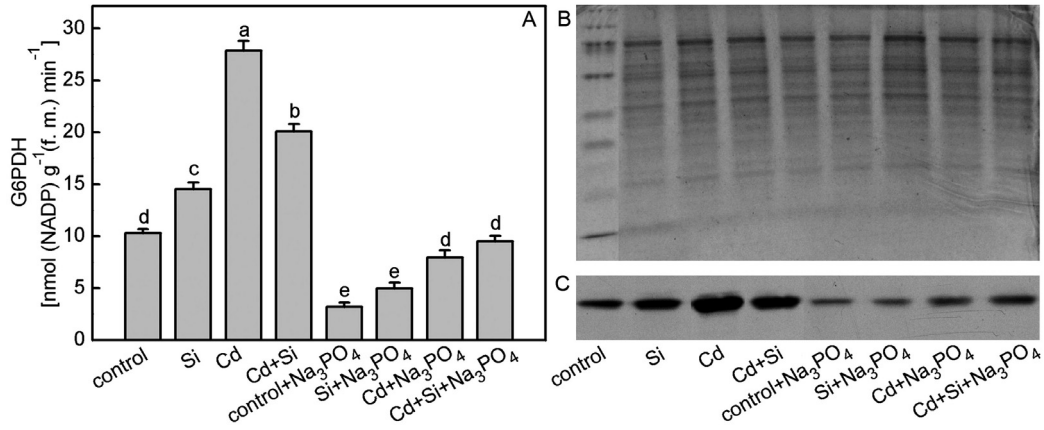

Fig. 5. Modulation of the G6PDH activity in roots of *Poa annua* by 100 μM Cd, 1 mM Si, 2.5 mM Na_3PO_4 in different combinations (*A*). Means ± SEs, $n = 3$; different letters indicate significant differences at $P < 0.05$. The Coomassie Brilliant Blue-stained gel was used to show that an equal amount of proteins were loaded in all lanes (*B*). Western-blot analysis of the G6PDH expression at various treatments (*C*).

cannot directly activate the generation of ROS. However, it can bind to and interfere with targets or compete for binding sites (such as thiols, carboxylic acids, and histidyl residues), thereby altering the functions of target proteins, which in turn involves the production of ROS (Mittler *et al*. 2004, Sharma and Dietz 2009, Zhang *et al*. 2009). ROS are produced during the electron transport in chloroplasts and mitochondria and oxidative metabolism in the peroxisomes (Sharma and Dietz 2009). However, excess ROS accumulation causes cellular redox imbalances and disturbances leading to growth inhibition and cell damage (Sharma and Dietz 2009, Zhang *et al*. 2009). It is clear that the content of ROS in cells needs to be tightly regulated by efficient ROS-scavenging mechanisms, including enzymatic and non-enzymatic components (Mittler *et al*. 2004). SOD was responsible for scavenging O_2^-, while APX, POD, and CAT are responsible for H_2O_2 decomposition (Zhang *et al*. 2009). Cd stress greatly enhanced the H_2O_2 and MDA content in *Poa annua* roots (Fig. 2*E*-*G*) and significantly stimulated the activity of SOD and CAT, but reduced the activity of POD and APX (Fig. 3). Maintaining a high activity of antioxidant enzymes avoid oxidative damage, which is considered to be a hallmark of heavy metal tolerant plants (Wang *et al*. 2008b). Heavy metal stress dramatically stimulates antioxidant enzyme activities in many plants. Si stimulated *Cu/Zn-SOD* and *metallothionein* (MT) genes expression and SOD activity in leaves of *Arabidopsis thaliana* upon Cu exposure (Khandekar *et al*. 2011). Activities of SOD, CAT, and APX were notably increased in roots of Si-supplied plants in both Zn-sensitive and Zn-resistant rice cultivars when exposed to excess Zn (Song *et al*. 2011). Si application also influences content of non-enzymatic antioxidant glutathione, non-protein thiols, and ascorbic acid in rice under high Mn stress (Li *et al*. 2012). The changes in the activities of antioxidant enzymes are generally dependent on plant species, age, duration of treatment, and experimental conditions (Sharma and Dietz 2009). Under Cd+Si treatment, POD and APX activities were higher than under Cd stress alone and reached the level of the control, whereas, SOD and CAT activities were reduced (Fig. 3). Meanwhile, H_2O_2 and MDA content was also lower at Cd+Si treatment than at Cd stress alone (Fig. 2*E*-G). This indicated that Si maintained the ROS level and improved seedling growth by altering the activities of antioxidant enzymes.

The first and rate-limiting enzyme in the pentose phosphate pathway is G6PDH. However, the number of reports concerning the role of the *G6PDH* gene expression in response to metal stress is limited. Cd stimulated the activity of G6PDH, which is associated with the oxidative stress (Van Assche *et al*. 1988, Yang *et al*. 2014). When

the Zn or Cd content of bean leaves exceeds a threshold value, an increase in the activity of the G6PDH is observed (Van Assche *et al*. 1988). Moreover, a rapid increase in G6PDH activity in aluminum-resistant wheat cultivars is observed during the first 10 h of treatment with 100 μM aluminum, while no change is detected in sensitive cultivars during 24 h exposure, suggesting that G6PDH may be associated with the mechanism of aluminum tolerance (Ślaski *et al*. 1996). In addition, changes in *G6PDH* transcription shows a 43-fold increase after 12 h exposure to $CdCl_2$ treatment compared with the control; these high expression is maintained until 24 h. The G6PDH activity also shows an increase and reaches a maximum after 12 h (Yang *et al*. 2014). Further, the high transcription is maintained for the duration of the investigation period, implying possible involvement of G6PDH in response to metal stress in sugarcane, which is similar to previous research (Ślaski *et al*. 1996, Van *et al*, 1988). Under stress conditions, G6PDH improved the tolerance of plants to stress through generating NADPH for ascorbate-glutathione cycle, thus inhibiting the production of ROS (Wang *et al*. 2008a). As shown in Fig. 5, the activity and the expression of G6PDH in *P. annua* roots were significantly increased by Cd stress. The activity and the expression of G6PDH were markedly inhibited by the application of Na_3PO_4. H_2O_2 accumulation in *P. annua* roots increased when G6PDH was inhibited by Na_3PO_4, suggesting that G6PDH is important for plant responses to Cd stress. The inhibition of G6PDH restricted the Cd stress alleviation done by Si. Si significantly restored the growth of *P. annua* seedlings under Cd stress by inhibiting the accumulation of H_2O_2, and the G6PDH activity and expression, indicating that Si improves the environment of plant growth and does not need to stimulate the expression of G6PDH. However, application of Na_3PO_4 completely inhibited the growth of *Poa annua* seedlings and the expression of G6PDH under Cd or Cd+Si treatment, which suggest that G6PDH truly plays an important role and is involved in the process of Si alleviation of Cd toxicity.

Taken together, these results demonstrated that the Si-enhanced Cd tolerance in *P. annua* seedlings mainly due to the decrease of Cd uptake by roots and by lowering the oxidative stress and by decreasing the activity and expression of G6PDH induced by Cd stress. This information provides a new evidence for alleviation of metal stress in plants by Si application.

References

Ádám, A.L., Bestwick, C.S., Barna, B., Mansfield, J.W.: Enzymes regulating the accumulation of active oxygen species during the hypersensitive reaction of bean to *Pseudomonas syringae* pv. *phaseolicola*. - Planta **197**: 240-249, 1995.

Aebi, H.: Catalase *in vitro*. - Methods Enzymol. **105**: 121-126, 1984.

Balakhnina, T.I., Matichenkov, V.V., Wlodarczyk, T., Borkowska, A., Nosalewicz, M. Fomina, I.R.: Effects of silicon on growth processes and adaptive potential of barley plants under optimal soil watering and flooding. - Plant Growth Regul. **67**: 35-43, 2012.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Dal Corso, G., Farinati, S., Furini, A.: Regulatory networks of cadmium stress in plants. - Plant Signal Behav. **5**: 663-667, 2010.

Dal Santo, S., Stampfl, H., Krasensky, J., Kempa, S., Gibon, Y., Petutschnig, E., Rozhon, W., Heuck, A., Clausen, T., Jonak, C.: Stress-induced GSK3 regulates the redox stress response by phosphorylating glucose-6-phosphate dehydrogenase in *Arabidopsis*. - Plant Cell **24**: 3380-3392, 2012.

Esposito, S., Carfagna, S., Massaro, G., Vona, V., Di, M.R.V.: Glucose-6-phosphate dehydrogenase in barley roots: kinetic properties and localization of the isoforms. - Planta **212**: 627-634, 2001.

Exley, C.: Silicon in life: a bioinorganic solution to bioorganic essentiality. - J. Inorg. Biochem. **69**: 139-144, 1998.

Feng, J.G., Shi, Q.H., Wang, X.F., Wei, M., Yang, F.J., Xu, H.N.: Silicon supplementation ameliorated the inhibition of photosynthesis and nitrate metabolism by cadmium (Cd) toxicity in *Cucumis sativus* L. - Sci. Hort. **123**: 521-530, 2010.

Gallego, S.M., Pena, L.B., Barcia, R.A., Azpilicueta, C.E., Iannone, M.F., Rosales, E.P., Zawoznik, M.S., Groppa, M.D., Benavides, M.P.: Unravelling cadmium toxicity and tolerance in plants: insights into regulatory mechanisms. - Environ. exp. Bot. **83**: 33-46, 2012.

Graeve, K., Von Schaewen, A., Scheibe, R.: Purification, characterization, and cDNA sequence of glucose-6-phosphate dehydrogenase from potato (*Solanum tuberosum* L.). - Plant J. **5**: 353-361, 1994.

Hodges, D.M., Delong, J.M., Forney, C.F., Prange, R.K.: Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. - Planta **207**: 604-711, 1999.

Janda, T., Szalai, G., Tari, I., Páldi, E.: Hydroponic treatment with salicylic acid decreases the effects of chilling in maize (*Zea mays* L) plants. - Planta **208**: 175-180, 1999.

Khandekar, S., Leisner, S.: Soluble silicon modulates expression of *Arabidopsis thaliana* genes involved in copper stress. - J Plant Physiol. **168**: 699-705, 2011.

Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. - Nature **227**: 680-685, 1970.

Li, P., Song, A., Li, Z.J., Fan, F.L., Liang, Y.C.: Silicon ameliorates manganese toxicity by regulating manganese transport and antioxidant reactions in rice (*Oryza sativa* L.). - Plant Soil **354**: 407-419, 2012.

Lin, Y.Z., Lin, S.Z., Guo, H., Zhang, Z.Y., Chen, X.Y.: Functional analysis of PsG6PDH, a cytosolic glucose-6-phosphate dehydrogenase from *Populus suaveolens*, and its contribution to cold tolerance improvement in tobacco plants. - Biotechnol. Lett. **35**: 1509-1518, 2013.

Liu, S., Yang, R., Pan, Y., Ma, M., Pan, J., Zhao, Y., Cheng, Q., Wu, M., Wang, M., Zhang, L.: Nitric oxide contributes to minerals absorption, proton pumps and hormone equilibrium under cadmium excess in *Trifolium repens* L. plants. - Ecotoxicol. environ. Safety **119**: 35-46, 2015.

Ma, F.J., Yamaji, N.: Silicon uptake and accumulation in higher

plants. - Trends Plant Sci. **11**: 392-397, 2006.
Mitani, N., Ma, J.F.: Uptake system of silicon in different plant species. - J. exp. Bot. **56**: 1255-1261, 2005.
Mittler, R., Vanderauwera, S., Gollery, M., Breusegem, F.V.: Reactive oxygen gene network of plants. - Trends Plant Sci. **9**: 490-498, 2004.
Peng, Y., Huang, B.R., Xu, L.X., Li, Z.: Heat stress effects on osmotic potential, membrane fatty acid composition and lipid peroxidation content of two Kentucky bluegrass cultivars differing in drought tolerance. - Acta horticult. sin. **40**: 971-980, 2013.
Porra, R.J., Thompson, W.A., Kriedman, P.A.: Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. - Biochim. biophys. Acta **975**: 384-394, 1989.
Prochazkova, D., Sairam, R.K., Srivastava, G.C., Singh, D.V.: Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. - Plant Sci. **161**: 765-771, 2001.
Rizwan, M., Meunier, J.D., Miche, H., Keller, C.: 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. **209-210**: 326-334, 2011.
Rodríguez-Serrano, M., Romero-Puertas, M.C., Pazmiño, D.M., Testillano, P.S., Risueño, M.C., Del Rio, L.A.,Sandalio, L.M.: Cellular response of pea plants to cadmium toxicity: cross talk between reactive oxygen species, nitric oxide, and calcium. - Plant Physiol. **150**: 229-243, 2009.
Salvemini, F., Franze, A., Iervolino, A., Filosa, S., Salzano, S., Ursini, V.: Enhanced glutathione levels and oxido resistance mediated by increased glucose-6-phosphate dehydrogenase expression. - J. biol. Chem. **274**: 2750-2757, 1999.
Scharte, J., Schön, H., Tiaden, Z., Weis, E., Von Schaewen, A.: Isoenzyme replacement of glucose-6-phosphate dehydrogenase in the cytosol improves stress tolerance in plants. - PNAS **106**: 8061-8066, 2009.
Sharma, S.S., Dietz, K.J.: The relationship between metal toxicity and cellular redox imbalance. - Trends Plant Sci. **14**: 43-50, 2009.
Shetty, R., Jensen, B., Shetty, N.P., Hansen, M., Hansen, C.W., Starkey, K.R., Jørgensen, H.J.L.: Silicon induced resistance against powdery mildew of roses caused by *Podosphaera pannosa*. - Plant Pathol. **61**: 120-131, 2012.
Shi, G.R., Cai, Q.S., Liu, C.F., Wu, L.: Silicon alleviates cadmium toxicity in peanut plants in relation to cadmium distribution and stimulation of antioxidative enzymes. - Plant Growth Regul. **61**: 45-52, 2010.
Ślaski, J.J., Zhang, G.C., Basu, U., Stephens, J.L., Taylor, G.J.: Aluminum resistance in wheat (*Triticum aestivum*) is associated with rapid, Al-induced changes in activities of glucose-6-phosphate dehydrogenase and 6-phospho-gluconate dehydrogenase in root apices. - Physiol. Plant. **98**: 477-484, 1996.
Song, A., Li, P., Li, Z.J., Fan, F.L., Nikolic, M., Liang, Y.C.: The alleviation of zinc toxicity by silicon is related to zinc transport and antioxidative reactions in rice. - Plant Soil **344**: 319-333, 2011.
Sun, Y.B., Zhou, Q.X., Wang, L., Liu, W.T.: The influence of different growth stages and dosage of EDTA on Cd uptake and accumulation in Cd hyperaccumulator (*Solanum nigrum* L.). - Bull. Environ. Contam. Toxicol. **82**: 348-353, 2009.
Tian, W.N., Braunstein, L.D., Pang, J., Stuhlmeier, K.M., Xi, Q.C., Tian, X., Stanton, R.C.: Importance of glucose-6-phosphate dehydrogenase activity for cell growth. - J. biol. Chem. **273**: 10609-10617, 1998.
Vaculík, M., Landberg, T., Greger, M., Luxová, M., Stoláriková, M., Lux, A.: Silicon modifies root anatomy, and uptake and subcellular distribution of cadmium in young maize plants. - Ann. Bot. **110**: 433-443, 2012.
Van Assche, F., Cardinaels, C., Clijsters, H.: Induction of enzyme capacity in plants as a result of heavy metal toxicity: dose-response relations in *Phaseolus vulgaris* L., treated with zinc and cadmium. - Environ. Pollut. **52**: 103-115, 1988.
Veljovic-Jovanovic, S.D., Noctor, G., Foyer, C.H.: Are leaf hydrogen peroxide concentrations commonly overestimated? The potential influence of artefactual interference by tissue phenolics and ascorbate. - Plant Physiol. Biochem. **40**: 501-507, 2002.
Wang, H.H., Yang, L.D., Li, Y., Hou, J.J., Huang, J.J., Liang, W.H.: Involvement of ABA- and H_2O_2-dependent cytosolic glucose-6-phosphate dehydrogenase in maintaining redox homeostasis in soybean roots under drought stress. - Plant Physiol. Biochem. **107**: 126-136, 2016.
Wang, M., Zou, J.H., Duan, X.C., Jiang, W.S., Liu, D.H.: Cadmium accumulation and its effects on metal uptake in maize (*Zea mays* L.). - Bioresour. Technol. **98**: 82-88, 2007.
Wang, X.M., Ma, Y.Y., Huang, C.H., Wan, Q., Li, N., Bi, Y.R.: Glucose-6-phosphate dehydrogenase plays a central role in modulating reduced glutathione levels in reed callus under salt stress. - Planta **227**: 611-623, 2008a.
Wang, Z., Zhang, Y.X., Huang, Z.B., Huang, L.: Antioxidative response of metal accumulator and non-accumulator plants under cadmium stress. - Plant Soil **310**: 137-149, 2008b.
Weber, M., Trampczynska, A., Clemens, S.: Comparative transcriptome analysis of toxic metal responses in *Arabidopsis thaliana* and the Cd^{2+}-hypertolerant facultative metallophyte *Arabidopsis halleri*. - Plant Cell Environ. **29**: 950-963, 2006.
Yan, H., Filardo, F., Hu, X., Zhao, X., Fu, D.: Cadmium stress alters the redox reaction and hormone balance in oilseed rape (*Brassica napus* L.) leaves. - Environ. Sci. Pollut. Res. Int. **23**: 3758-69, 2016.
Yang, Y.T., Fu, Z.W., Su, Y.C., Zhang, X., Li, G.Y., Guo, J.L., Que, Y.X., Xu, L.P.: A cytosolic glucose-6-phosphate dehydrogenase gene, *ScG6PDH*, plays a positive role in response to various abiotic stresses in sugarcane. - Sci. Rep. **4**: 7090, 2014.
Zhang, C.C., Wang, L.J., Nie, Q., Zhang, W.X., Zhang, F.S.: Long-term effects of exogenous silicon on cadmium translocation and toxicity in rice (*Oryza sativa* L.). - Environ. exp. Bot. **62**: 300-307, 2008.
Zhang, F.Q., Zhang, H.X., Wang, G.P., Xu, L.L., Shen, Z.G.: Cadmium-induced accumulation of hydrogen peroxide in the leaf apoplast of *Phaseolus aureus* and *Vicia sativa* and the roles of different antioxidant enzymes. - J. Hazard Mater. **168**: 76-84, 2009.
Zhang, L., Liu, J., Wang, X.M., Bi, Y.R.: Glucose-6-phosphate dehydrogenase acts as a regulator of cell redox balance in rice suspension cells under salt stress. - Plant Growth Regul. **69**: 139-148, 2013.
Zhao, C.Z, Li, P., Wang, X.M., Li, P., Wang, X.Y., Wang, F., Wang, J.F., Chang, N., Bi Y.R.: Nitrogen deprivation induces cross-tolerance of *Poa annua* callus to salt stress. - Biol. Plant. **60**: 543-554, 2016.
Zhao, L.Q., Zhang, F., Guo, J.K., Yang, Y.L., Li, B.B., Zhang, L.X.: Nitric oxide functions as a signal in salt resistance in the calluses from two ecotypes of reed. - Plant Physiol. **134**: 849-857, 2004.