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Remediation of cadmium toxicity in field peas (*Pisum sativum* L.) through exogenous silicon

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ABSTRACT

Cadmium (Cd) is an important phytotoxic element causing health hazards. This work investigates whether and how silicon (Si) influences the alleviation of Cd toxicity in field peas at biochemical and molecular level. The addition of Si in Cd-stressed plants noticeably increased growth and development as well as total protein and membrane stability of Cd-stressed plants, suggesting that Si does have critical roles in Cd detoxification in peas. Furthermore, Si supplementation in Cd-stressed plants showed simultaneous significant increase and decrease of Cd and Fe in roots and shoots, respectively, compared with Cd-stressed plants. At molecular level, *GSH1* (phytochelatin precursor) and MT_A (metallothionein) transcripts predominantly expressed in roots and strongly induced due to Si supplementation in Cd-stressed plants compared with Cd-free conditions, suggesting that these chelating agents may bind to Cd leading to vacuolar sequestration in roots. Furthermore, pea Fe transporter (*RIT1*) showed downregulation in shoots when plants were treated with Si along with Cd compared with Cd-treated conditions. It is consistent with the physiological observations and supports the conclusion that alleviation of Cd toxicity in pea plants might be associated with Cd sequestration in roots and reduced Cd translocation in shoots through the regulation of Fe transport. Furthermore, increased CAT, POD, SOD and GR activity along with elevated S-metabolites (cysteine, methionine, glutathione) implies the active involvement of ROS scavenging and plays, at least in part, to the Si-mediated alleviation of Cd toxicity in pea. The study provides first mechanistic evidence on the beneficial effect of Si on Cd toxicity in pea plants.

1. Introduction

Cadmium (Cd) is a toxic element causing plant yield loss and human health problems such as kidney and bone damage in human (Naeem et al., 2015; Järup and Åkesson, 2009). The Cd is frequently accumulated by crops due to anthropogenic contamination (Metwally et al., 2005). Cd stress decreases the uptake of essential nutrient elements (Sandalio et al., 2001), inhibits various enzyme activities (Obata et al., 1996), induces reactive oxygen species (Sandalio et al., 2001) and decreases the protein concentration (Manios et al., 2002) in field peas (*Pisum sativum* L.). Furthermore, Cd-rich field peas may increase Cd contamination in the adjacent crops grown in the cropping systems sharing nitrogen fixation products and phosphorus mobilization (Liu et al., 2012b). This high Cd uptake and translocation rate from roots to shoots is the largest source of Cd contamination in food (Greger and Löfstedt, 2004; Liu et al., 2007). Further, Cd inhibits seed germination and seedling growth in plants (Liu et al., 2012a; Jun-yu et al., 2008). Cd affects a broad range of cellular and metabolic activities including photosynthetic system (Burzyński and Zurek, 2007), nucleoli toxicity in apical meristems (Qin et al., 2010) and organization of the microtubular cytoskeleton in mitotic cells (Jiang et al., 2009).

Silicon (Si), is a beneficial element for higher plants but has been proved to reduce the adverse effects of some toxic metals (Song et al., 2011; Greger and Landberg, 2008; Ma et al., 2006). In general, Si reduces the translocation of Cd from roots to shoots or inhibits the uptake of Cd in the plants (Treder and Cieslinski, 2005; Greger and Landberg, 2008; Zhang et al., 2008). Si ties with root cell walls, traps Cd and prevents the cellular uptake of Cd (Liu et al., 2013). Further, Si regulates the stresses induced by heavy metals (HMs) through changes in root morphology (Kim et al., 2014). Recently, Greger et al. (2016) showed that Si minimizes Cd uptake in wheat plants at the cellular level. More precisely, Si affects the cell membranes, thereby preventing the cellular uptake of Cd in wheat. However, the mechanisms asso-

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ciated with Si-mediated alleviation of Cd toxicity in field peas are still not known.

The adaptive mechanisms of plants to HMs include an interrelated network of physiological and molecular mechanisms. One of them is the complexation of HM ions into the cells through various substances, for example, organic acids, phytochelatins (PC), metallothioneins (MT) and amino acids (Sanita di Toppi et al., 2002; Cho et al., 2003). PC is synthesized from glutathione or its precursor, gamma-glutamylcysteine in plants and it is functionally analogous to MT (Grill et al., 1987). Cd binds to cysteine-forming Cd-PC complexes and reduces the free Cd^{2+} in the cytosol. The PC-Cd complexes can then be transported into the vacuole or out of the cell by ATP-binding cassette transporters (Jasinski et al., 2003). A Cd^{2+}/H+-antiport mechanism can transport free Cd^{2+} in the cytosol into the vacuole. Further, an HMA3-ATPas has been shown to limit Cd concentration in the cytoplasm in rice (Salt and Wagner, 1993; Miyadate et al., 2011). MTs are sulfur-rich proteins that bind to metal ions and contribute to detoxifying the cytosolic environment from Cd toxicity (DalCorso et al., 2010; Cobbett and Goldsbrough, 2002). However, whether Si influences PC and MT formation have yet to be determined in peas.

The Cd is taken up into the cell via passive and active pathways mediated by carriers (Lindberg et al., 2004; Hart et al., 1998). Different transporter families contribute to Cd tolerance. The uptake of HMs into cells is influenced by iron-regulated transporters (*IRTs, NRAMP1*) in plants (Takahashi et al., 2011; Uraguchi et al., 2011). *IRT1* is essential for iron uptake in roots in iron deficiency, but it also accepts Cd as a substrate and is involved in the root-to-shoot transport of Cd (Rogers et al., 2000).

One of the significant consequences of HM accumulation is the production of ROS (reactive oxygen species), which causes cell damage in plants. Cd produces ROS directly via the Fenton and Haber–Weiss reactions, and indirectly by inhibiting antioxidant enzymes (Romero-Puertas et al., 2007). To counteract this oxidative stress, plants have evolved antioxidant enzymes and antioxidant non-enzyme molecules that are termed as antioxidant system. These antioxidant enzymes include catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), etc. SOD is the primary defense against ROS, dismutating O_2 to an oxygen molecule and H_2O_2 (Irfan et al., 2014). HMs, in particular, Cd activates antioxidant enzymes such as SOD and GR (Romero-Puertas et al., 2007). Another strategy of plants to counter HM stress is the activation or modification of plant metabolism to allow adequate functioning of metabolic pathways and rapid repair of damaged cell structures. Glutathione (GSH) is the major intracellular antioxidant metabolite inside the cell and is the precursor of PCs (Cobbett, 2000). It can also form complexes with metal ions, particularly Cd (Wójcik and Tukiendorf, 2011) and is activated in response to HM stress. In contrast to these studies, experiments on Si and Cd toxicity in pea plants are scarce.

Cd contamination in field peas is a serious threat for health hazards as it is a favorite legume crop primarily used for human consumption or as a livestock feed worldwide. This study examined the effect of Si on morpho-physiological features, protein content, electrolyte leakage, Cd uptake, and translocation, both with and without prior treatment with Si. To understand how Si influences the cellular uptake of Cd, gene expression analysis was performed in roots and leaves. Moreover, we investigated whether Si influences the induction of antioxidant enzymes and S-containing amino acids associated with Cd detoxification in peas.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of *Pisum sativum* L. var. BARI-1 were disinfected by superficial treatment with 95% (v/v) ethanol for 10 min and washed in deionized water. For germination, seeds were placed in Petri dishes in wet tissue paper for 2 days in the dark at room temperature. Afterward, 2-d old uniform seedlings were transplanted to solution culture (Hoagland and Arnon, 1950) supplemented with the nutrients (μM): KNO_3 (16,000), $Ca(NO_3)_2{\cdot}4H_2O$ (6000), $NH_4H_2PO_4$ (1000), $MgSO_4{\cdot}7H_2O$ (2000), KCl (50), H_3BO_3 (25), Fe-EDTA (25), $MnSO_4{\cdot}4H_2O$ (2), $ZnSO_4$ (2), $Na_2MoO_4{\cdot}2H_2O$ (0.5) and $CuSO_4{\cdot}5H_2O$ (0.5). Plants were grown in 2 L of the aerated solution in a growth chamber (25 °C temperature and 70% humidity) under 10 h light and 14 h dark (550–560 μmol s-1 per μA). The pH was adjusted to 6.0 by using NaOH or HCl. The medium was treated with 0 or 20 μM $CdSO_4$ and 0 or 1.8 mM H_4O_4Si, which were found to be optimum for Cd study in peas (Rahman, 2015). The solution was replaced every 3 days. All control and stressed plants were grown concurrently for 1-week after treatment was imposed and harvested at the same time.

2.2. Measurement of morphological features

Root length and shoot height of each plant was measured. Further, separated root tissue was washed with deionized water and blotted in tissue paper. Afterward, both root and leaf tissues were dried at 80 °C for 2 days before measuring the dry weight in digital balance.

2.3. Determination of chlorophyll content

For chlorophyll (a and b) determination, freshly harvested shoots were weighed and ground with 2 mL methanol in mortal and pestle. The homogenate was then centrifuged at 12,000*g* for 10 min and the clear supernatant was placed in a microcentrifuge tube. The absorbance was read at 662 (chlorophyll a) and 646 (chlorophyll b) on a spectrophotometer (UV-1650PC, Shimadzu) and calculated as previously described (Lichtenthaler and Wellburn, 1985).

2.4. Determination of Cd and Fe in plant tissue by AAS (atomic absorption spectroscopy)

Briefly, harvested tissues were washed with $CaSO_4$ (1 mM) and deionized water before drying in an oven at 80 °C for 3 days (Kabir et al., 2015; Kabir, 2016). Once tissues were dried, 3 mL HNO_3 and 1 mL of H_2O_2 were mixed with samples before heating at 75 °C for 10 min. The concentration of Cd and Fe was then analyzed by Flame Atomic Absorption Spectroscopy (AAS) outfitted with an ASC-6100 autosampler and air-acetylene atomization gas mixture system (Model No. AA-6800, Shimadzu). Standard solutions of Cd and Fe were separately prepared from their respective concentration of stock solutions (Shimadzu).

2.5. Determination of total soluble proteins

Total soluble proteins in both roots and shoots were measured using the calibration curve of different concentration of bovine serum albumin (BSA) as previously described (Guy et al., 1992). Briefly, roots and shoots were harvested and washed with deionized water before homogenization with a chilled mortar-pestle using a buffer containing ice-cold 50 mM Tris-HCl, pH 7.5; 2 mM EDTA and 0.04% (v/v) 2-mercaptoethanol. The homogenate was then centrifuged at 12,000*g* for 10 min at room temperature, and clear supernatant (100 μl) was transferred to glass cuvette containing 1 mL Coomassie Brilliant Blue. Finally, absorbance was read at 595 nm in a spectrophotometer, and the concentration of total soluble proteins was calculated using the calibration curve of BSA.

2.6. Measurement of electrolyte leakage

Electrolyte leakage (EL) was of roots and shoots was measured by an electrical conductivity meter (Lutts et al., 1996). Briefly, harvested