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Silicate reduces cadmium uptake into cells of wheat☆

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A B S T R A C T

Cadmium (Cd) is a health threat all over the world and high Cd content in wheat causes high Cd intake. Silicon (Si) decreases cadmium content in wheat grains and shoot. This work investigates whether and how silicate (Si) influences cadmium (Cd) uptake at the cellular level in wheat. Wheat seedlings were grown in the presence or absence of Si with or without Cd. Cadmium, Si, and iron (Fe) accumulation in roots and shoots was analysed. Leaf protoplasts from plants grown without Cd were investigated for Cd uptake in the presence or absence of Si using the fluorescent dye, Leadmium Green AM. Roots and shoots of plants subjected to all four treatments were investigated regarding the expression of genes involved in the Cd uptake across the plasma membrane (i.e. *LCT1*) and efflux of Cd into apoplasm or vacuole from the cytosol (i.e. *HMA2*). In addition, phytochelatin (PC) content and PC gene (*PCS1*) expression were analysed. Expression of iron and metal transporter genes (*IRT1* and *NRAMP1*) were also analysed. Results indicated that Si reduced Cd accumulation in plants, especially in shoot. Si reduced Cd transport into the cytoplasm when Si was added both directly during the uptake measurements and to the growth medium. Silicate downregulated *LCT1* and *HMA2* and upregulated *PCS1*. In addition, Si enhanced PC formation when Cd was present. The *IRT1* gene, which was downregulated by Cd was upregulated by Si in root and shoot facilitating Fe transport in wheat. *NRAMP1* was similarly expressed, though the effect was limited to roots. This work is the first to show how Si influences Cd uptake on the cellular level.

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1. Introduction

Cadmium (Cd) is a toxic element causing detrimental human health effects such as kidney and bone damage (Järup and Åkesson, 2009). Cadmium can be naturally high in agricultural soils but is also elevated due to anthropogenic contamination. The correlation between soil concentration and the concentration in wheat grains is poor (Mench et al., 1997). Wheat takes up Cd and translocates it into its grains to different degrees depending on the wheat types and cultivars (Greger and Löfstedt, 2004). Wheat grains and products are widely consumed, and in Sweden 43% of the daily Cd intake originates from wheat flour (Hellstrand and Landner, 1998). It is thus important to find ways to reduce the Cd concentration in wheat grains.

We recently demonstrated that silicon (Si), added as silicate and silica, reduces the Cd content by up to 10% in the grains of field-grown wheat (Greger and Landberg, 2015) and, depending on the wheat cultivar, up to 50% of the Cd content was reduced in grains of wheat grown hydroponically (Greger and Landberg, 2008). One reason for this effect is that Si reduces the translocation of Cd from roots to shoots, as has been demonstrated in wheat and some other plant species (Greger and Landberg, 2008; Zhang et al., 2008). Si is beneficial for higher plants: it promotes structural stability, protects plants by improving resistance to external abiotic and biotic stresses, and increases biomass production (Ma, 2004; Shi et al., 2005; Epstein, 2009). Several monocots such as wheat, bamboo, sugar cane, and rice are Si accumulators and high Si levels can be found in them (Rafi and Epstein, 1999).

Cadmium stress is an abiotic stress mitigated by Si (Malčovská et al., 2014), and one mechanism underlying such mitigation is decreased Cd uptake into the plant (Treder and Cieslinski, 2005). Another mechanism preventing Cd stress is decreased translocation from roots to shoots, preventing the inhibitory effect of Cd on photosynthesis (Landberg and Greger, 1996). Si promotes the formation of the suberized barrier in the endodermal cell walls near the root tip, preventing Cd transport into the stele and, thereby, farther up to the shoot (Vaculik et al., 2012). Si associates with cell wall components including polysaccharides, lignin, and

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proteins (Perry and Lu, 1992). Si binds to root cell walls, trapping Cd and thereby preventing the cellular uptake of Cd (Liu et al., 2013). However, whether Si affects Cd transport across the plasma membrane is not yet known.

Cadmium is taken up into the cell via passive and active pathways, mediated by carriers, and via channels permeable to both calcium and potassium transport in the plasma membrane (Costa and Morel, 1993, 1994; Hart et al., 1998; Lindberg et al., 2004). The uptake of heavy metals into cells is influenced by metal-transporting transmembrane proteins, including low-affinity cation transporters (LCTs), and iron-regulated transporters (IRTs, NRAMP1) in plants (Takahashi et al., 2011; Uraguchi et al., 2011). In addition, heavy metal ATPases (HMAs) e.g. HMA2, predominantly localised to the plasma membrane, accounts for the cellular efflux of heavy metals and is thought to regulate the translocation of Cd and Zn from root to shoot (Wong and Cobbett, 2009; Nocito et al., 2011; Mills et al., 2012; Satoh-Nagasawa et al., 2012).

Inside the cytosol, Cd triggers the formation of phytochelatin (PC), cysteine-rich peptides that alleviate heavy metal stress, and are formed from glutathione and enzymatically synthesized by PC synthase after induced expression of the PC synthase gene (PCS1) (Steffens, 1990; Cobbett, 2000; Semane et al., 2007). Cadmium binds to the sulphur group of cysteine-forming Cd-PC complexes, reducing 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 (Salt and Rauser, 1995; Jasinski et al., 2003). Free Cd^{2+} in the cytosol may also be transported into the vacuole by the HMA3-ATPas in a Cd^{2+}/H^{+}-antiport mechanism, and may reduce the Cd concentration in the cytoplasm in rice (Salt and Wagner, 1993; Miyadate et al., 2011). Certain ions, such as Cd, Cu, and Zn ions, are known to induce PC formation, although not all these elements bind to PCs (Steffens, 1990). However, whether Si influences PC formation has yet to be determined.

Silicon is taken up as silicic acid and the same Si form is found in the xylem sap (Casey et al., 2004). The cellular uptake of Si occurs via transporters, and a low-affinity transporter (Lsi1), a homologue of aquaporins, takes up Si into cortical cells and into the distal cells of the exo- and endodermis (Ma, 2010). This transporter varies in affinity and density, accounting for the variation in Si uptake among plant species. In rice, a second transporter (Lsi2) is responsible for xylem loading, which occurs by passive diffusion in non-accumulator plants (Ma, 2010). A third transporter (Lsi6) is involved in unloading Si from the xylem to the surrounding cells (Ma, 2010).

Si influences the uptake, translocation, and accumulation of Cd in plants at the tissue level, but the effect of Si on Cd uptake across the cell membrane has yet to be investigated. Our aim was therefore to investigate whether and how Si influences the cellular uptake of Cd at the plasma membrane level. This was done by investigating the effect of Si on Cd uptake into the cytosol of cell-wall-free protoplasts, both with and without prior treatment with Si during seedling cultivation. To understand how Si influences the cellular uptake of Cd, kinetics studies were performed and the gene expression of plasma membrane transporters was analysed. Moreover, we investigated whether Si influences the formation of PCs for binding free Cd, when Cd is entering the cytosol.

2. Materials and methods

2.1. Plant cultivation

Caryopses of spring durum wheat (*Triticum durum* L. cv. Grandur) and spring bread wheat (*Triticum aestivum* L. cv. Vinjett) were surface sterilized with 0.5% Na-hypochlorite for 15 min. The seeds were then rinsed carefully with tap water and soaked in 5 mM $CaSO_4$ for 3 h. Thereafter, seeds were transferred to nets covered with double layers of Miracloth (LIC, Stockholm, Sweden) for germination. The nets were placed on top of 1-L bowls containing complete nutrient medium formulated according to Lindberg and Strid (1997) as follows: 2 mM KNO_3, 1 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 1 mM KH_2PO_4, 0.5 mM Na_2HPO_4, 1.5 μM H_3BO_3, 0.5 μM $MnSO_4$, and 0.5 mM Fe-EDTA, pH 6.0. After 2–4 days for germination, when roots had reached the nutrient medium, the medium was renewed and treated with 0 or 1 μM $CdCl_2$ and 0 or 1 mM K_2SiO_3. After four days of treatment, the plants were harvested and used for protoplast experiments or analysed to determine their element and PC concentrations. Plants were grown at 20 ± 1 °C in a climate chamber equipped with 400 W HQI-BT lamps (Osram, Munich, Germany) emitting 200–250 μmol m^{-2} s^{-1} in a 16 h:8 h, light:dark regime and at a relative humidity of 50–60%.

2.2. Analysis of Cd, Fe, and Si concentrations in roots and shoots

Plants of spring bread wheat grown for four days in the four treatments were harvested and their roots rinsed in distilled water. Plants were divided into root and shoot fractions and dried at 90 °C for two days. These fractions were then digested with 65% HNO_3 and H_2O_2 (5:3, v:v) in a Speedwave microwave oven (Berghof Products + Instruments, Eningen, Germany) and thereafter analysed to determine the Cd, Fe, and Si contents using an atomic absorption spectrophotometer (SpectrAA 55B, Varian, Belrose, Australia) with a furnace (GTA 100, Varian). Standards were added to the samples to eliminate the interaction of the sample matrix.

2.3. Protoplast isolation

Protoplasts were isolated from seedlings pre-treated or not pretreated with 1 μM $CdCl_2$ and/or 1 mM K_2SiO_3. The leaf protoplasts were isolated from 5–7-day-old durum wheat seedlings as described by Lindberg (1995), with some modifications. Leaves were cut into *c.* 1 mm pieces and 1 g of the leaf material was incubated for 2.5 h at 30 °C in 10 ml of enzyme solution containing 1% cellulase from *Trichoderma resei* (Sigma–Aldrich, St. Louis, MO, USA) and 0.3% Macerozyme R-10 macerase (Serva, Heidelberg, Germany), 0.5 M sorbitol, 0.2% bovine albumin (BSA), 0.05% (w/v) polyvinylpyrrolidone (PVP; Sigma–Aldrich), 1 mM $CaCl_2$, and 20 mM MES (Sigma–Aldrich) buffer, pH 5.5. After incubation, the suspension was strained off and the leaf pieces were rinsed twice, each time for 30 s, with the same solution as described above but without the enzymes (Medium 1). All leaf-containing suspensions were pooled and strained through a nylon net with 100-μM pores. The filtrate was centrifuged at 42×*g* for 6 min. The pellet was resuspended in 4 ml of medium containing 0.5 M sucrose, 1 mM $MgCl_2$, and 5 mM TRIS buffer, pH 7.0 (Medium 2). The pooled leaf suspension was topped with a layer of 0.5 ml of a suspension containing 0.4 M sucrose, 0.1 M sorbitol, 1 mM $MgCl_2$, and 5 mM TRIS buffer, pH 7.0 (Medium 3). Finally, Medium 3 was topped with a layer of 500 μL of Medium 1. After centrifugation at 240×*g* for 5 min, the protoplasts were collected from the intermediate (green) layer (i.e. Medium 3) and centrifuged at 60×*g* for 5 min. The pellet was then resuspended in 1 ml of loading medium (described below).

2.4. Dye loading

One ml of the loading medium, i.e. 0.5 M sorbitol, 0.1 mM $CaCl_2$, 0.05% PVP (w/v), 0.2% BSA (w/v), and 5 mM MES buffer, pH 5.5, was added to the protoplast suspension. The protoplasts were loaded with Leadmium Green AM dye (Molecular Probes–Life

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