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Effect of selenium on the uptake kinetics and accumulation of and oxidative stress induced by cadmium in *Brassica chinensis*

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ABSTRACT

Pak choi can readily accumulate cadmium (Cd) into its edible parts; this can pose a threat to human health. Although not essential for higher plants, selenium (Se) can be favorable for plant growth and antioxidative defense under heavy metal stress conditions. A pak choi hydroponic experiment was conducted to investigate the effect of two forms of Se on the Cd uptake kinetics and accumulation and oxidative stress. The results showed that selenite and selenate remarkably enhanced Cd uptake kinetics in pak choi. The maximum Cd uptake rate increased by more than 100% after treatment with 5 μM of selenite and selenate, and it further increased after treatment with 20 μM of both Se forms. The effects of Se on Cd content depended on the Se form, exposure time, and Cd dosage. Selenite reduced the Cd content in shoots by 41% after 3 days of treatment with 10 μM Cd, whereas selenate increased this rate by 89%. Both forms of Se decreased Cd content in the shoots by 40% after 7 days of treatment with 10 μM Cd, but they increased the Cd content by approximately 30% after treatment with 50 μM Cd. Se enhanced Cd-induced oxidative stress in pak choi. Malondialdehyde (MDA) generation was promoted by more than 33% by selenite and selenate treatments in combination with 10 μM Cd, and it was further enhanced by 106% and 185% at 50 μM Cd, respectively. Selenite also increased the H_2O_2 content at both Cd doses, but selenate did not have significant effects on H_2O_2 production. The effects of Se on antioxidative enzyme activity also depended on the dose of Cd. Selenite and selenate inhibited catalase activity by 11% and 29%, respectively, at 10 μM Cd, and by 13% and 42%, respectively, at 50 μM Cd. Moreover, both forms of Se increased superoxide dismutase activity after treatment with 10 μM Cd but inhibited its activity at 50 μM Cd. Therefore, Se exhibits dual effects on Cd accumulation and oxidative stress in pak choi and might cause further stress when combined with higher doses of Cd.

1. Introduction

Cadmium (Cd) is a highly toxic heavy metal to both plants and animals without any known biological function (Rizwan et al., 2017). However, anthropogenic activities have resulted in Cd contamination of soil, thereby leading to its accumulation in the food chain; this is the main source of human Cd intake responsible for several diseases such as Itai-itai disease, kidney failure, and even cancer (Jarup, 2002). Moreover, Cd inhibits growth and photosynthesis in plants (Fargasova et al., 2006; Rizwan et al., 2017) and reduces the efficiency of the electron transfer chain, boosts reactive oxygen species (ROS), damages DNA, and promotes the dysfunction of some proteins in organisms (Liu et al., 2013). These adverse effects of Cd might induce oxidative stress in plants (Liu et al., 2013), thereby activating antioxidative defense (Clemens et al., 2013; Feng et al., 2013a; Rizwan et al., 2017). For example, Ding et al. (2014) found that, in rice, 3.53 μM of Cd enhanced the accumulation of malondialdehyde (MDA), which is an indicator of lipid peroxidation. Khan et al. (2015) observed that soil Cd content of 200 mg kg^{-1} significantly increased hydrogen peroxide (H_2O_2) by 186% in wheat.

There are two types of antioxidative defense in plants. One is non-enzymatic where metabolites such as glutathione (GSH) and ascorbic acid (AsA) act as electron donors to reduce ROS (Jimenez et al., 1998; Komives et al., 1997). The other is an enzymatic defense that functions through the reduction of ROS catalyzed by a set of antioxidative enzymes, including superoxide dismutase (SOD), catalase (CAT),

Abbreviations: GSH, glutathione; AsA, ascorbic acid; MDA, malondialdehyde; GR, glutathione reductase; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; MES, 2-(N-Morpholino) ethanesulfonic acid monohydrate

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peroxidase (POD), and glutathione reductase (GR; Feng et al., 2013a; Gill and Tuteja, 2010; Rizwan et al., 2017).

Selenium (Se) is an essential element for humans and plays an important role in the synthesis of several essential proteins and enzymes, including glutathione peroxidases (GPX) and thioredoxin reductases (TrxR; Hatfield and Gladyshev, 2002). However, although there is no known evidence that Se is essential to plants (Terry et al., 2000), it can be beneficial when plants are under the stress of Cd exposure (Feng et al., 2013a). There are five valence states of Se, selenide (2-), elemental Se (0), thioselenate (2), selenite (4 +), and selenate (6 +; Terry et al., 2000). The form of Se species depends on the redox state and pH: selenate is the major form of Se under aerobic and neutral to alkaline conditions, whereas selenite is the dominant form in anaerobic environments. Many studies have shown that Se can promote growth, reduce Cd content, and improve photosynthesis in plants exposed to Cd. For example, Sun et al. (2016) found that 3 μM selenite alleviated Cd-induced inhibition of plant height, root length, and biomass in cucumber. Mozafariyan et al. (2014) reported that 7 μM selenite reduced Cd content in pepper fruits by 25% and 31% after exposure to 0.25 mM and 0.5 mM Cd, respectively. Haghighi and da Silva (2016) found that 50 μM selenite significantly increased the photosynthetic rate in cucumber, although it was severely inhibited by 7 μM Cd. Wan et al. (2016) reported that 5 μM selenate significantly reduced the Cd content by 30% in rice shoots exposed to 5 μM Cd. These protective effects can be attributed to the restored activities of antioxidative enzymes (Filek et al., 2008; He et al., 2004; Mozafariyan et al., 2014; Sun et al., 2016) and the induction of phytochelatins that prevent metals from translocating from the roots to shoots (Hawrylak-Nowak et al., 2014; Simmons and Emery, 2011). In addition, glutathione can function as both a chelating agent (Speiser et al., 1992) and a scavenger of ROS (Jimenez et al., 1998; Komives et al., 1997). Conversely, the enhancement of Cd toxicity in the presence of selenite has been reported (Ding et al., 2014; Feng et al., 2013b). Selenate might enhance the toxicity of Cd because both selenite and selenate consume antioxidants during their assimilation and then inhibit the biological functions of some enzymes (Feng et al., 2013a), especially when they are in excess (Paciolla et al., 2011). Shoot biomass of paddy rice exposed to 35.6 μM Cd was reported to be enhanced by about 30% after the addition of 1.27 and 12.7 μM selenite but was significantly reduced by 63.5 μM selenite. The toxic dose of Se was even lower when higher doses of Cd were applied (Feng et al., 2031b). Because of this dose-dependent relationship between Cd and Se in plants, considering how the dose of both the elements might directly affect the results of mediating Cd toxicity with Se treatments is important.

Pak choi (*Brassica chinensis* L.) is one of the most consumed leafy vegetables in China; however, it is also an accumulator of Cd (Bo and Chen, 2013). Yang et al. (2009) compared the Cd content in the edible parts of six plant species grown in pot experiments and field trials and found that pak choi contained the most Cd under both growth conditions. Liu et al. (2012) also found that Cd content in the edible parts of pak choi was the highest among four vegetables investigated. Thus, developing a method to decrease Cd accumulation in the edible parts of pak choi and reducing Cd toxicity risk to human health are urgently required. Since the Cd dose-dependent effect of Se on pak choi has been rarely investigated and the underlying mechanism is not yet clear, this study aimed to investigate the effects of selenite and selenate on the Cd uptake kinetics and accumulation and oxidative stress responses in pak choi to develop a method for reducing Cd uptake and toxicity in pak choi.

2. Materials and methods

2.1. Pak choi culture

Pak choi (*B. chinensis* L., Suzhouqinggen) was hydroponically cultured in a greenhouse under the following growth conditions: 25/20 °C (day/night) during a 14 h d^{-1} photoperiod, with 70% relative humidity and 240 − 350 μmol $(m^2\ s)^{-1}$ of light. Pak choi seeds were thoroughly rinsed in deionized water and surface-sterilized with 10% H_2O_2 for 30 min. Sterilized seeds were then incubated with saturated $CaSO_4$ for 4 h and germinated in vermiculite for 4 days. Vermiculite had a pH of 7.0 and a Cd content of 0.02 mg kg^{-1}, but Se was not detected, as revealed by inductively coupled plasma mass spectrometry (ICP-MS 7700; Agilent Technologies, USA). Twelve days after germination, seedlings with three leaves were transplanted to 2.5 L plastic pots containing 1/5-strength Hoagland solution (Hoagland and Arnon, 1941). Its composition was as follows: 1.0 mM KNO_3, 0.4 mM $MgSO_4$, 0.5 mM $NH_4H_2PO_4$, 1.0 mM $Ca(NO_3)_2$, 0.03 mM EDTA-Fe, 3×10^{-3} mM H_3BO_3, 1×10^{-3} mM $MnSO_4$, 1×10^{-3} mM $ZnSO_4$, 2×10^{-4} mM $CuSO_4$, and 2×10^{-4} mM Na_2MoO_4. The nutrient solution (pH 5.8) was buffered with 1 mM 2-(*N*-morpholino) ethanesulfonic acid monohydrate (MES) and it was renewed every 3 days except during Cd exposure.

2.2. Cd uptake kinetics

Twenty-five days after transplantation from vermiculite to nutrient solution (i.e., 37 days after germination), pak choi plants were harvested and washed in deionized water. Roots were then cut off and soaked in 200 mL uptake solution that contained different concentrations of Cd ($Cd(NO_3)_2{\cdot}4H_2O$) and Se as either selenite (Na_2SeO_3) or selenate (Na_2SeO_4) buffered with 1 mM MES and $Ca(NO_3)_2$ (pH 5.8). Cd was added to obtain seven concentrations: 1, 10, 20, 40, 80, 160, and 320 μM. Selenite or selenate was applied to obtain three concentrations: 0, 5, and 20 μM. Each treatment had three biological replicates with one plant per replicate. The uptake incubation lasted 1 h, after which pak choi roots were thoroughly rinsed in deionized water, followed by 15 min incubation in 150 mL of ice-cold eluent (1 mM $CaSO_4$ and MES, pH 5.8). Root samples were oven-dried at 105 °C for 30 min and then at 75 °C for 48 h.

2.3. Three- and seven-day exposure to Cd

Twenty-five days after transplantation from vermiculite to nutrient solution (i.e., 37 days after germination), pak choi plants were exposed to nutrient solution containing 10 or 50 μM Cd with 10 μM Se as either selenite or selenate for 3 or 7 days (i.e., 28 or 32 days after transplantation). As per the findings of our and other previous studies (Ding et al., 2014; Feng et al., 2013b), Se at this dose is not significantly toxic to the growth and antioxidative systems of plants when applied alone. Each treatment had three biological replicates with two plants per replicate. After exposure to Cd and Se, pak choi plants were harvested, divided into shoots and roots, and washed in deionized water. The same procedure was used for root wash-off as mentioned above. Shoots and roots were then frozen in liquid nitrogen and pulverized. The pulverized samples were stored at −25 °C before use for the determination of Cd and enzyme activities.

2.4. Determination of Cd and Se contents in pak choi

Approximately 0.25 g dried root sample was used for the measurement of Cd uptake kinetics; 0.60 g fresh shoot samples and 0.35 g fresh root samples were digested in a microwave oven (Mars 5, CEM, USA) in 8 mL 65% HNO_3 (guaranteed reagent) at 180 °C for the exposure experiments. The concentration of elements was determined using inductively coupled plasma optical emission spectrometry (Optima 7300 DV; PerkinElmer, USA) for the uptake kinetics experiment and using inductively coupled plasma mass spectrometry (ICP-MS 7700; Agilent Technologies, USA) for the exposure experiment. The stock standards for calibration of ^{111}Cd and ^{78}Se (1000 mg L^{-1} in 2% HNO_3) were purchased from the National Institute of Metrology of China. The Cd and Se concentrations were determined at 228.80 and 196.02 nm,