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Cadmium uptake dynamics and translocation in rice seedling: Influence of different forms of selenium

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ABSTRACT

Selenium (Se) can alleviate the toxicity of cadmium (Cd), but little is known about its mechanism in Cd uptake and translocation in plants. We investigated the effects of exogenous selenite, selenate, and selenomethionine (SeMet) on Cd uptake and translocation within rice (*Oryza sativa* L., Zhunliangyou 608) seedlings, and the concentration-dependent uptake kinetics of Cd into rice roots (with or without Se) were determined. The effect of the endogenous Se pool on Cd uptake was also investigated. Results of uptake kinetics showed that selenite slightly promoted Cd influx during 1 h of exposure, compared with no selenite addition; V_{max} of Cd uptake increased by 13.8% in 10 μM selenite treatment; while the presence of selenate had no effect on the influx of Cd. When exposed to Cd (5 μM) over 20 h (with selenite) or 30 h (with selenate or SeMet), Se addition (5 μM) decreased Cd uptake and root-to-shoot translocation; after 30 h selenite, selenate, or SeMet addition decreased Cd uptake by roots by 28.6%, 17.7% or 12.1%, respectively. Besides, as the selenite levels in the treatment solutions (1 μM Cd) increased (0, 0.1, 1, and 5 μM, Se), Cd uptake and translocation were both significantly reduced, while the inhibitive effect was more significant at lower levels of selenate. Pretreatment of selenite or selenate (5 μM) also decreased Cd uptake by 24.9% or 15.7%, and reduced the root-to-shoot transfer factor by 41.4% or 36.2% after 144 h of subjection to Cd (5 μM), respectively. The presence of selenite decreased Cd content more effectively than did selenate. Our results demonstrated that Se can effectively reduce the Cd translocation from roots to shoots in rice seedlings.

1. Introduction

Cadmium (Cd) is one of the most harmful and widespread heavy metals in agricultural soils. It enters from anthropogenic processes, then transfers to plants and accumulates in their edible parts, leading to yield reduction and a wide variety of acute and chronic toxic effects on human beings (Templeton and Liu, 2010). The recent joint report on the current status of soil contamination in China issued by the Ministry of Environmental Protection (MEP) and the Ministry of Land and Resources (MLR) of the People's Republic of China shows, among the heavy metals and metalloids, Cd ranks first in the percentage of soil samples (7.0%) exceeding the MEP limit (MEP and MLR, 2014). Paddy rice (*Oryza sativa* L.) is the staple food for Southeast Asian countries, and is a major source of Cd intake because of its great potential to accumulate Cd. In some areas of southern China, considerable proportions of rice grain exceed the limit of Cd in the Chinese food standard (0.2 mg kg^{-1}, GB2726-2012), causing rising public concern in recent years (Williams et al., 2009). China has limited land resources and large population, so it is very important to protect the soil resource from contamination and then protect human health against heavy metal toxicity.

Cd can be easily taken up by plants and transferred to the food chain, especially in acidic soils, and urgent measures are required to mitigate its accumulation in crops. In recent years, many strategies have been developed, such as reducing metal phytoavailability (Zhao and Saigusa, 2007 and Liu et al., 2013), breeding crop cultivars with low accumulations (Ueno et al., 2009), and water and fertilizer management (Hu et al., 2013). Cd availability in soil decreased after supplementation with porous hydrated calcium silicate (Zhao and Saigusa, 2007). It was also found that application of Zn or Fe could decrease Cd accumulation in plants because of the competition between them for uptake by roots (Hart et al., 2002 and Muneer et al., 2011).

In recent years, antagonism by selenium (Se) on Cd stress has been observed in many different plants. Lin et al. (2012) demonstrated that exogenous Se markedly reduced Cd concentration in

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rice plants under hydroponic conditions. The addition of Se significantly decreased the accumulation of Cd in grains of paddy rice subjected to Cd-contaminated soil (Hu et al., 2014). Other evidences have suggested that exposure to Se alleviates Cd absorption and toxicity in maize (Shanker et al., 1996), pepper (Mozafariyan et al., 2014), garlic (Sun et al., 2010) and sunflower (Saidi et al., 2014). Se is beneficial to plants in low dosages, and can be used to alleviate both abiotic (e.g. salt and heavy metal toxicity) and biotic (e.g. plant diseases, pests) stresses in plants (Feng et al., 2013). It may be used to reduce concentrations of heavy metals in crops and also enhance Se content in the edible parts to satisfy the needs of people for Se as well.

Until recently, most of the reports about the interaction between Se and Cd focused on the protective mechanism of Se on Cd toxicity at high levels. Se could preserve the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), decrease the ascorbate peroxidase (APX) and malondialdehyde (MDA) concentration in the plants (Pedrero et al., 2008; Filek et al., 2008 and Malik et al., 2012), rebuild chloroplasts and recover cell membrane integrity (Filek et al., 2010). The relevant Cd detoxification mechanisms by Se might be attributed to the inhibition of uptake and translocation of Cd from the roots to above-ground parts and/or the speciation transformation to non-toxic species (Saidi, 2014). Cd^{2+} can enter the root cell through ZIP transporters or via other cation channels (Lux et al., 2011), and thus there is competitions between Cd^{2+} and other cations which share same pathways with Cd (such as Zn^{2+}) for uptake by roots (Hart et al., 2002). However, selenate or selenite is taken up by plant roots via sulfate transporters or silicon influx transporters, respectively (Sors et al., 2005 and Qiang et al., 2010), and selenite can also be taken up by phosphate transporters (Li et al., 2008 and Zhang et al., 2013). Cd and Se are taken up by plants through different channels or transporter, so it seems they may not present competition on the root surface. It is clearly important to examine the interaction between Se and Cd on root surfaces and in plants. Se can interact with Cd to provide antagonism or significant reduction in toxicity; however, almost all studies have been carried out using exogenous Se at acute levels. Further research is needed on endogenous Se with toxic metals at relatively lower concentrations. In this study we investigated the effect of endogenous and exogenous Se on Cd uptake and transportation by rice plants grown in different conditions.

2. Materials and methods

2.1. Plant culture

After being surface sterilized by 30% (v/v) H_2O_2 for 15 min, rice seeds (*Oryza sativa* L., Zhunliangyou 608) were thoroughly rinsed with distilled water and soaked in a saturated $CaSO_4$ solution overnight at 25 ± 2 °C in the dark. The seeds were then germinated on a pre-sterilized plastic net floating in deionized water at 25 °C. After 7 days, healthy and uniform seedlings were transferred to 2.5 L pots (four plants per pot) with ½-strength Kimuar nutrient solution for another 35 days. The composition of the nutrient was (mM): KNO_3 0.091, $Ca(NO_3)_2 \cdot 4H_2O$ 0.183, $MgSO_4 \cdot 7H_2O$ 0.274, KH_2PO_4 0.1, $(NH_4)_2SO_4$ 0.183, $MnSO_4 \cdot H_2O$ 1×10^{-3}, H_3BO_3 3×10^{-3}, $(NH_4)_6Mo_7O_{24} \cdot 5\,h_2O$ 1×10^{-3}, $ZnSO_4 \cdot 7H_2O$ 1×10^{-3}, $CuSO_4 \cdot 5H_2O$ 2×10^{-4}, and Fe(III)-EDTA 6×10^{-2}. The pH of this solution was adjusted to 5.5 with diluted NaOH or HCl and the solution was renewed every 3 days. Plants were grown in a greenhouse with controlled conditions as follows: 25 ± 4 °C/20 ± 2 °C day/night temperatures, 14 h photoperiod with a light intensity of 240–350 μmol $(m^2\,s)^{-1}$, and relative humidity of 60–70%.

Experiment 1. Effect of selenite or selenate on Cd uptake kinetics.

The first experiment was carried out to investigate the interaction between Cd and Se on root surfaces. At 42 days, seedlings were totally soaked in a series of uptake solutions (1 L, one plant per pot) containing 0, 1, 5, 10, 20, 40, 80, and 100 μM Cd ($3CdSO_4 \cdot 8H_2O$), with or without 5 or 10 μM Se (Na_2SeO_3 or Na_2SeO_4)) for 1 h. The pH of these uptake solutions was adjusted to 5.5 with HCl or NaOH. Each treatment was replicated in three pots.

Experiment 2. Effect of selenite or selenate on Cd uptake in different time.

This experiment was conducted to investigate the interaction between Cd and Se in plants by setting up the time course of Cd uptake influenced by Se addition. At 42 days, seedlings were transferred to pots (two plant per pot) containing 2.5 L of nutrient solution, in which Se (5 μM, Na_2SeO_3 or Na_2SeO_4) or Cd (5 μM, $3CdSO_4 \cdot 8H_2O$) was added to form two treatments: (1) Cd; and (2) Cd+Se (selenite or selenate). The composition of other nutrients was the same as that in the normal nutrition solution (pH 5.5). The plants were sampled at 1, 3, 6, 20, 30, 48 and 72 (120 for selenate) h after treatment, and each treatment was replicated in three pots.

Experiment 3. Effect of levels of selenite or selenate on Cd uptake.

This experiment was set up to investigate Cd uptake by rice seedlings exposed to different levels of selenite or selenate. At 42 days, seedlings were transferred to pots (two plant per pot) containing 2.5 L of nutrient solution, in which Se (0, 0.1, 1 or 5 μM of Na_2SeO_3 or Na_2SeO_4) and Cd (1 μM, $3CdSO_4 \cdot 8H_2O$) was added to form 4 treatments: (1) Cd; (2) Cd+Se 0.1; (3) Cd+Se 1; (4) Cd+Se 5 (selenite or selenate). The composition of other nutrients was the same as that in the normal nutrition solution (pH 5.5). The plants were sampled at 20 and 120 h after treatment, and each treatment was replicated in three pots.

Experiment 4. Effect of Se pretreatment on Cd uptake.

The effect of endogenous Se conversion in plants on Cd uptake and translocation was investigated in this experiment. At 42 days, seedlings were transferred to the corresponding containers (two plant per pot) with 2.5 L nutrient solution, in which Se (5 μM, Na_2SeO_3, or Na_2SeO_4) or Cd (5 μM, $3CdSO_4 \cdot 8H_2O$) was added to form five treatments: (1) Cd; (2) Cd + SeIV; (3) Cd + SeVI; (4) pre-SeIV + Cd; and (5) pre-SeVI + Cd (48 h pretreatment before Cd exposure), and the composition of other nutrients was the same as in the normal nutrition solution (pH 5.5). The plants were sampled at 48, 96, and 144 h after treatment with three replicates.

Experiment 5. Effect of selenomethionine on Cd uptake.

Selenate and selenite taken up by plants can be converted to organic forms, so in this experiment we investigated the effect of selenomethionine (SeMet) on Cd translocation. At 42 days, seedlings were transferred to pots (two plant per pot) containing 2.5 L of nutrient solution, in which Se (5 μM, SeMet) or Cd (5 μM, $3CdSO_4 \cdot 8H_2O$) was added, and these treatments were identified as Cd and Cd+SeMet. The composition of other nutrients was the same as in the normal nutrition solution (pH 5.5). The plants were sampled at 20 and 30 h after treatment, and each treatment was replicated in three pots.

2.2. Determination of total Se and Cd contents

The root of each harvested plant was rinsed with deionized water three times and then soaked in an ice-cold desorption solution (1 mM $CaSO_4$) for 15 min to remove Se and Cd on the root

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