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Selenium reduces cadmium uptake and mitigates cadmium toxicity in rice

Li Lin, Weihui Zhou, Huaxin Dai, Fangbin Cao, Guoping Zhang, Feibo Wu*

Department of Agronomy, College of Agriculture and Biotechnology, Zijingang Campus, Zhejiang University, Hangzhou, 310058, PR China

HIGHLIGHTS

- Se alleviated Cd-toxicity, reduced Cd content and $O_2^{\bullet -}$, H_2O_2, MDA in rice plants.
- Se counteracted Cd-induced alterations of antioxidant enzymes.
- Se suppressed Cd-induced increase in SOD, APX, but elevated depressed CAT activity.
- Se markedly increased H^+-ATPase, Ca^{2+}-ATPase activities in roots under Cd exposure.

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ABSTRACT

Hydroponic experiments were performed to investigate physiological mechanisms of selenium (Se) mitigation of Cd toxicity in rice. Exogenous Se markedly reduced Cd concentration in leaves, roots, and stems. Addition or pretreatment of 3 μM Se in 50 μM Cd solution significantly addressed Cd-induced growth inhibition, recovered root cell viability, and dramatically depressed $O_2^{\bullet -}$, H_2O_2, and malondialdehyde (MDA) accumulation. Supplemental Se counteracted 50 μM Cd-induced alterations of certain antioxidant enzymes, and uptake of nutrients, *e.g.* depressed Cd-induced increase in leaf and root superoxide dismutase (SOD) and leaf peroxidase (POD) activities, but elevated depressed catalase (CAT) activity; decreased Cd-induced high S and Cu concentrations in both leaves and roots. External Se counteracted the pattern of alterations in ATPase activities induced by Cd, *e.g.* significantly elevated the depressed root H^+- and Ca^{2+}-ATPase activities, but decreased the ascent root Na^+K^+-ATP activity. Results indicate that alleviated Cd toxicity by Se application is related to reduced Cd uptake and ROS accumulation, balanced nutrients, and increased H^+- and Ca^{2+}-ATPase activities in rice.

1. Introduction

Cadmium (Cd) is one of the most harmful and widespread pollutants in agricultural soils, and expresses a direct contact risk to both human and ecological receptors due to its relatively high toxicity and plant readily uptake [1,2]. Cereals especially rice, the staple food in East Asia, is a major source of Cd intake. For example, rice was estimated to represent 36–50% of the total oral intake of Cd for Japanese population during 1998–2001 [3]. In China, at least 13,330 ha of farmland in 11 provinces have been contaminated by Cd in varying degrees mainly due to anthropogenic activities, such as industrial emission, the application of sewage sludge and phosphate fertilizers, and municipal waste disposal containing Cd [1,4]. Thus, it is important to develop reliable approaches to prevent Cd accumulation in rice. The application of chemical regulators to alleviate Cd toxicity and reduce plant Cd uptake in medium or slightly contaminated farmlands might offer a cost-effective and practically acceptable strategy for the complete utilization of natural resource and safe food production.

Selenium (Se) is an essential element for humans and animals. The biochemical roles of Se have been extensively investigated [5,6]. The positive effect of Se on heavy metal stress was observed in different biological systems of animals and aquatic organisms. Beneficial effects of Se on Cd and silver exposure have been observed in mice and humans [7,8]. In plants, Se at low concentrations exerts positive effects, such as, growth improvement, increase of antioxidative capacity, reduction of reactive oxygen species (ROS) and lipid peroxidation, increased accumulation of starch and sugars [9,10], delay of senescence, and improved growth of ageing seedlings [10,11]. Gotsis [12] observed similar antagonism on the

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; Cd, cadmium; DW, dry weight; FDA, fluorescein diacetate; FW, fresh weight; GPX, glutathione peroxidase; H^+-ATPase, proton pump ATPase; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; Na_2-EDTA, disodium ethylenediaminetetraacetic acid; NBT, nitroblue tetrazolium; $O_2^{\bullet -}$, superoxide radical; OH, hydroxyl radical; PBK, potassium phosphate buffer; PBS, sodium phosphate buffer; POD, peroxidase; PI, propidium iodide; ROS, reactive oxygen species; Se, selenium; SOD, superoxide dismutase; SeCys, selenocysteine; SeMet, selenomethionine; SeMeSeCys, selenium methylselenocysteine; TBA, thiobarbituric acid.

* Corresponding author. Tel.: +86 571 88982827; fax: +86 571 88982827.
E-mail address: wufeibo@zju.edu.cn (F.B. Wu).

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cell growth of the planktonic *alga Dunaliella minuta* Lerche when combinations of Se/Hg and Se/Cu were used for cultures with and without prior exposure to either metal. Zembala et al. [13] reported that Se shows the tendency to counterbalance the Cd-induced changes in nutrients and reduces the lipid peroxidation in rape and wheat seedlings, and the protective role of Se was most evident in rape. The stimulatory effect on yield of foliar spraying of exogenous Se was observed on pumpkin fruits of UV-stressed plants [14]. Similar results were observed on ryegrass [11], lettuce [10], and potato [9]. Recently, Zahedi et al. [15] and Pazoki et al. [16] found that Se, as foliar application improves yield of rapeseed under drought stress condition. However, apart from the results on UV- or drought-stressed plants and Cd-stressed rape and wheat mentioned above, no information is available on Cd–Se interaction in rice. It is of considerable interest to know whether external Se could act as a regulator or capable of initiating antioxidant intervention strategy to respond to oxidative stress brought about by Cd. Moreover, better understanding of how plants adjust to adverse environment should be achieved.

The present study was conducted *via* hydroponic experiment to investigate the potential role of external Se in modulating Cd-induced oxidative stress, Cd uptake/translocation, ATPase, photosynthetic performance, and growth in rice plants. We aim to provide a basis for developing strategies to reduce risks associated with Cd toxicity and maintaining sustainable plant production.

2. Materials and methods

2.1. Plant material and experimental designs

Hydroponic experiment was carried out on Huajiachi Campus, Zhejiang University, Hangzhou, China. Rice seeds (cv Xiushui 63, *Japonica* unwaxy rice, *Oryza sativa* L.) were surface-sterilized in 2% H_2O_2 solution for 10 min and fully rinsed by deionized water. Sterilized seeds were soaked in deionized water in the dark at 25 °C for 2 d. The seeds were germinated on a plastic net floating on deionized water at 35 °C for 1 d, and sown into sand in a controlled chamber with photoperiod of 16 h day/8 h night at 30 °C/25 °C, RH 85%, and light intensity of $225 \pm 25\ \mu mol\ m^{-2}\ s^{-1}$. Ten-day-old rice seedlings with similar size were selected and transplanted into a 5 L plastic container containing 4.5 L nutrient solution. The nutrient solution was prepared according to Zeng et al. [17]. The composition of the BNS ($mg\ L^{-1}$) was: NH_4NO_3 114.3, $NaH_2PO_4{\cdot}2H_2O$ 50.4, K_2SO_4 89.3, $CaCl_2$ 110.8, $MgSO_4{\cdot}7H_2O$ 405, $MnCl_2{\cdot}4H_2O$ 1.88, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.09, H_3BO_3 1.17, $ZnSO_4{\cdot}7H_2O$ 0.05, $CuSO_4{\cdot}5H_2O$ 0.04, Fe-citrate 14.88. The solution pH was adjusted to 5.1 using 1 M HCl or NaOH. Half-strength nutrient solution was applied for the first 3 d and changed to full strength solution for another 4 d. On the 7th day after transplanting, Cd (as 50 μM $CdCl_2$ [18,19]) and Se (3 μM as Na_2SeO_3 [13]) were added to the corresponding containers to form 6 treatments: (1) control, basal nutrient solution (BNS); (2) Se, BNS+ Na_2SeO_3; (3) Cd, BNS+ $CdCl_2$; (4) Cd + Se, BNS+ $CdCl_2$+ Na_2SeO_3; (5) *pre*-Se, BNS+ Na_2SeO_3 (24 h pretreatment); and (6) *pre*-Se + Cd, BNS+ Na_2SeO_3 (24 h pretreatment before Cd exposure), and then BNS + $CdCl_2$. The experiment was laid in a completely randomized design with three replicates, and the culture solutions were renewed every 4 d. Plant samples were collected after 5, 10 and 15 d of treatment at seedling stage, immediately immersed in liquid nitrogen and stored frozen at −80 °C for further analyses or directly used for various biochemical assays.

2.2. Plant growth and biomass, and element determination

After 5, 10 and 15 d of treatment, plant height and root length were measured before determining shoot and root dry weights after oven-drying at 75 °C for 2 d. For elemental detection, plants were sampled after 10 d treatment; roots were soaked in 20 mM Na_2-EDTA for 2 h and rinsed in deionized water to eliminate possible chemical contamination on the surface.

Dried leaf, stem, and root samples were ground and digested in a mixture of HNO_3–$HClO_4$ (4:1). Concentrations of Cd, Se, and other elements such as S, K, Fe, Mn, Cu, and Zn were determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (SPS 1200 VR, Seiko Co., Ltd., Japan).

2.3. Cd fluorescence localization

Visualization of Cd distribution in root tips was performed after 10-d Cd exposure. According to Xiong et al. [20], roots were immersed in 20 mM Na_2-EDTA for 15 min and rinsed 3 times with deionized water. Subsequently, roots were stained with the reagent from the LEADMIUM™ kit (Molecular Probes, Invitrogen, USA) for 30 min in the dark, and washed 3 times with 50 mM PBS (pH 7.8) for 5 min. Root sections were observed using a confocal laser scanning microscope (LSM 510, Zeiss, Germany) with excitation and emission wavelengths of 488 and 515 nm. Relative fluorescence density was calculated using Image J software.

2.4. Evaluation of cell viability by fluorescein diacetate-propidium iodide (FDA-PI) assay

To determine cell viability, root tips treated with Cd for 10 d were rinsed 3 times with deionized water, and blotted dry gently. Samples were loaded with FDA-PI double assay for 40 min and washed 3 times with deionized water for 5 min. Red and green fluorescence and concurrent differential interference contrast images were obtained with a Zeiss LSM 510 fluorescent microscope (LSM 510, Zeiss, Germany) with excitation at 485 nm and emission at 530 nm. The non-fluorescent esterase substrate fluorescein di-acetate (FDA) is cleaved by esterases in viable cells, releasing fluorescein which stains the cells green. While, the characteristics of PI are totally opposite with FDA, which may interact with DNA/RNA in cells, leaving the red fluorescence of dead cells [21].

2.5. Histochemical staining of $O_2^{\bullet-}$, and determination of H_2O_2, $O_2^{\bullet-}$ and lipid peroxidation

Histochemical staining of $O_2^{\bullet-}$ was performed as described by Chen et al. [22] with minor modification. After 10 d treatment, leaves were infiltrated with 0.1% NBT in 50 mM PBK (pH 6.4) containing 10 mM Na-azide and illuminated until dark spots appeared. Leaves were bleached in boiling ethanol to visualize the spots after incubation. H_2O_2 and $O_2^{\bullet-}$ contents were determined as described by Hu et al. [23]. The level of lipid peroxidation was quantitated as the amount of malondialdehyde (MDA) as determined by the TBA (thiobarbituric acid) reaction in all treatments [24].

2.6. Assay for chlorophyll content and enzyme activities

Chlorophyll content (SPAD value) of the first fully expanded leaves was measured after 5, 10, and 15 d of Cd exposure using a chlorophyll meter (Minolta SPAD-502, Japan).

To determine enzyme activity after 5, 10, and 15 d treatments, fresh plant samples were homogenized in 8 ml 50 mM PBS (pH 7.8) using pre-chilled mortar and pestle, and subsequently centrifuged at $10{,}000 \times g$ for 15 min at 4 °C. The supernatant was used for the assays of all enzyme activity. Superoxide dismutase (SOD; EC 1.15.1.1), peroxidase (POD; EC 1.11.1.7), and catalase (CAT; EC 1.11.1.6) activities were determined as described by Wu et al. [24].