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Cadmium accumulation, sub-cellular distribution and chemical forms in rice seedling in the presence of sulfur

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ABSTRACT

Changes in cadmium (Cd) accumulation, distribution, and chemical form in rice seedling in the joint presence of different concentrations of sulfur (S) remain almost unknown. Therefore, the indoor experiments were performed to determine the accumulation, sub-cellular distribution and chemical forms of Cd under three S levels in rice seedling for the first time. The result showed that Cd accumulation in rice roots was more than in shoots. Sub-cellular distribution of Cd in rice roots and shoots indicated that the largest proportion of Cd accumulated in cell walls and soluble fractions. As S supply increased, the proportion of Cd in cell walls reduced, while it increased in the soluble fractions. The majority of Cd existed in inorganic form, and then gradually changed to organic forms that included pectates and proteins with increased S supply. The results showed that S supply significantly influenced Cd accumulation, distribution, and chemical forms, suggesting that S might provide the material for the synthesis of sulfhydryl protein and thereby affect Cd stress on plants. These observations provided a basic understanding of potential ecotoxicological effects of joint Cd and S exposure in the environment.

1. Introduction

Cadmium (Cd), one of the most hazardous heavy metals, has been released into agriculture soils through anthropogenic activities, such as leather processing, electroplating, application of metal-containing sewage sludge, and utilization of fertilizers (Nicholson et al., 1994; Satarug et al., 2003). Cd can be readily accumulated by agricultural crops and can inhibit plant growth and cause diseases in animals and human beings (Zhou and Song, 2004). Rice is one of the most important crops globally, and especially in Asia. The amount of Cd that enters the human diet from a crop depends on the amount of Cd accumulated in the plant, so the translocation of Cd in rice has a direct relationship to Cd intake in the diet.

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Sulfur (S) is one of the most important nutrient elements for plants. Some scholars reported that S could decrease Cd accumulation and alleviate its toxicity in plants due to the formation and precipitation of discernible CdS (Kashem and Singh, 2001; Hassan et al., 2005). However, there have been no report that describe the ways in which S affects the subcellular distribution and chemical form of Cd in plant.

The vacuole is the most important component in the soluble fraction of a cell (Ramos et al., 2002; Zhang et al., 2013). Regionalization of cell wall deposition and vacuolar compartmentation play a major role in heavy metal detoxification, tolerance, and hyperaccumulation in plants. Cell walls and vacuoles in plant roots are considered to have great potential for Cd accumulation (Vogeli-Lange and Wagner, 1990; Kupper et al., 2000; Boominathan and Doran, 2003). The different chemical forms in which Cd exists in plants could indicate one of the most important detoxification mechanisms, as demonstrated by a study of the chemical forms of Cd in *Bechmeria nivea* (L.) Gaud (Wang et al., 2008).

The objective of this study was to assess the accumulation, sub-cellular distribution, and chemical forms of Cd in rice roots and shoots under three concentrations of S and to explore the potential mechanisms behind these dynamics. At present, Cd pollution events in rice culture often appear in the world; therefore, the observations and related findings will provide some useful data to reduce Cd toxicity to rice in the presence of sulfur, which will establish a scientific basis for risk assessment of Cd in rice.

2. Materials and methods

2.1. Plant growth and treatments

Rice seeds (NO. 6 huaidao), supplied by Jiangsu Academy of Agricultural Sciences (JAAS). Seeds were sterilized with 1% NaClO for 20 min and germinated at 32 °C in the artificial climate incubator for 5–7 days, and then transferred into the hoagland nutrient solution contained: major elements: 221 mg L^{-1} NH_4NO_3, 150 mg L^{-1} $CaCl_2{\cdot}H_2O_2$, 171 mg L^{-1} KCl, 292 mg L^{-1} $MgCl_2{\cdot}6H_2O$, 113 mg L^{-1} $Al(NO_3)_3{\cdot}9H_2O$; trace elements: 310 mg L^{-1} NaH_2PO_4, 5 mg L^{-1} $ZnCl_2$, 117 mg L^{-1} $MnCl_2{\cdot}4H_2O$, 5 mg L^{-1} $Na_2MoO_4{\cdot}2H_2O$, 10 mg L^{-1} H_3BO_3; ferric salt: 7.45 g L^{-1} Na-EDTA, 4 g L^{-1} $FeCl_2{\cdot}4H_2O$. Until four-leaf stage, and then 30 uniform seedlings were selected and transplanted into 60 mL hydroponic solution with different treatments. Each treatment was conducted in three replicated runs.

After 7 days of growth, several S and Cd treatments were carried out, as follows: S ($MgSO_4$) 0, 48, 720 mg L^{-1} with Cd ($CdCl_2$) 10 mg L^{-1}, respectively. At the end of the experiment (after 12 d exposure to Cd and S), plants were separated into roots and shoots, then immediately frozen in liquid N_2 and kept frozen until use.

2.2. Determination of Cd

Roots and shoots of rice samples were carefully washed with deionized water for approximately 3 min (Liu et al., 2010). The samples were dried at 105 °C for 5 min, and then at 70 °C in an oven until completely dry. The dried plant samples were ground to powders. The Cd concentrations of the samples were determined with atomic absorption spectrometry (AAS) following HNO_3–$HClO_4$ (4:1) digestion procedures (Allen, 1989).

2.3. Tissue fractionation

Frozen materials were homogenized in precold extraction buffer containing 50 mM Tris–HCl (pH 7.5), 250 mM sucrose and 1.0 mM $C_4H_{10}O_2S_4$. Cells were separated into four fractions: cell wall, soluble fraction, organelle and membrane containing fraction using differential centrifugation technique (Weigel and Jager, 1980) with some modifications. The homogenate was sieved through a nylon cloth (100 mm mesh size) and the residue constituted the cell wall-containing fraction. The filtrate was centrifuge 10,000 × *g* for 30 min and the pellet retained was the organelle-rich fraction. The supernatant was then centrifuged at 100,000 × *g* for 30 min and the pellet designated as the membrane-containing fraction and the supernatant as the soluble fraction. The resultant pellets were resuspended in extraction buffer. All steps were performed at 4 °C. The fractions were dried and wet digested separately, and then Cd concentrations in the digests were determined by AAS.

2.4. Chemical forms extraction

Six chemical forms of Cd were extracted step by step using a sequence of designated extractants in the following order (Yang et al., 1995; Wu et al., 2005; Wang et al., 2008).

(1) 80% ethanol, extracting inorganic Cd, which included nitrate/nitrite, chloride, and aminophenol Cd (FE);
(2) Deionized water, extracting water-soluble Cd of organic acid complexes and $Cd(H_2PO_4)_2$ (FW);
(3) 1 mg L^{-1} NaCl, extracting Cd integrated with pectates and protein (FNaCl);
(4) 2% acetic acid (HAC), extracting insoluble $CdHPO_4$ and $Cd_3(PO_4)_2$ and other Cd-phosphate complexes (FHAC);
(5) 0.6 mg L^{-1} HCl, extracting oxalate acid bound Cd (FHCl);
(6) Cd in the residue (FR).

2.5. Statistical analysis

All experimental data, means of three replicates, were processed by OriginPro8.0. One-way ANOVA was applied to calculate statistical significance followed by Dunnett's test as a post hoc test to independently compare each exposure group. All statistical analyses were run separately by performed using SPSS 16.0 software (SPSS, Chicago, USA) and P value of less than 0.05 was considered to be statistically significant. The data were shown as mean ± standard error (SE).

3. Results

3.1. Cd content in rice roots and shoots

Cadmium content in roots generally declined with increasing S concentration following a dose–response relationship,