Environmental and Experimental Botany 107 (2014) 25–31

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Variations among rice cultivars in subcellular distribution of Cd: The relationship between translocation and grain accumulation

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ARTICLE INFO

Article history:
Received 21 January 2014
Received in revised form 13 May 2014
Accepted 14 May 2014
Available online 23 May 2014

Keywords:
Cadmium (Cd)
Rice (*Oryza sativa* L.)
Cultivar
Translocation factor
Subcellular distribution ratio

ABSTRACT

The variations of subcellular distribution of cadmium (Cd) among six rice (*Oryza sativa* L.) cultivars, and the relationships with Cd translocation in the plants and accumulation in the grain were studied. The results showed that the rice cultivars varied greatly in Cd translocation factors (TFs) from shoots to ears/grains and subcellular distribution ratios (SDRs) in cell wall fraction (F1) and soluble fraction (F4), particularly in Cd-contaminated soils. These variations resulted in different Cd levels in the rice grain. There were positive and highly significant ($P < 0.01$) correlations between grain Cd contents and the TF from shoots to ears/grains. The TF from shoots to ears/grains was negatively and significantly ($P < 0.05$, or 0.01) correlated with the SDR in F1 of shoots, but positively and significantly ($P < 0.05$, or 0.01) correlated with the SDR in F4 of shoots. These results indicate that the translocation of Cd from shoot to grain is a main factor responsible for Cd accumulation in the grain, and the soluble fraction of Cd in rice shoot is the main subcellular pool for transferring Cd into rice grain. The cell wall fraction plays a main role in Cd deposition in rice shoot, which restrains the translocation from shoot to the grain. So subcellular distribution of Cd in rice shoot is one of the main mechanisms that differentiate rice cultivars in governing the translocation and accumulation of Cd in rice grain.

1. Introduction

Cadmium (Cd) is one of the most detrimental pollutants in terms of food-chain contamination, because it can be readily absorbed by plants and translocated to different parts of plants, including edible parts (DalCorso et al., 2008; Mendoza-Cózatl et al., 2005). Cadmium can be found in low concentrations in the natural environments, but tends to be accumulated to a high or even toxic level in connection with mining, metal smelting, fuel burning, and excessive use of fertilization, wastewater and sewage sludge in agriculture (DalCorso et al., 2008; Toppi and Gabbrielli, 1999).

It was reported that people consuming Cd-contaminated rice developed 'itai-itai disease', which caused renal abnormalities and weak bones (Horiguchi et al., 2010). More than half of the world's population depends on rice as staple food. For these peoples, the major route of Cd exposure may be rice. Therefore, the rice grain contaminated with Cd represents a major risk to their health (Shimo et al., 2011).

There are three processes which are likely to determine Cd accumulation in plant shoots, and subsequently transporting into the seeds: (1) uptake of Cd by roots, (2) transport of Cd from roots to shoots, and (3) translocation of Cd from shoots to seeds.

Previous studies showed that rice cultivars varied greatly with regard to Cd uptake, distribution and accumulation (Ishikawa et al., 2005; Liu et al., 2003, 2005, 2007; Shi et al., 2009; Zeng et al., 2008). One effective approach for reducing Cd concentration in rice grain is to screen or breed rice cultivars that absorb or translocate less Cd to edible parts from Cd-contaminated soils, i.e. pollution-safe cultivars (PSCs) as proposed by Yu et al. (2006). However, the prerequisite of this is to understand the mechanisms of Cd uptake and transport in rice plants (Uraguchi et al., 2009). Although it has been reported that the gene OsNRAMP5 contributes to Mn, Cd, and Fe transport in rice plant, the mechanisms that control Cd uptake, translocation and accumulation into rice grains are not well understood (Ishimaru et al., 2012; Ishikawa et al., 2012).

In recent years, subcellular partitioning of metals within living cells has attracted great interest because of their importance in ecotoxicological and trophic transfer studies (Wu and Wang, 2011). Different distributions of Cd among tissue fractions can explain the difference in sensitivity to Cd between maize and pea. Maize was more tolerant to Cd than pea by incorporating more Cd into the cell wall, while pea showed severer damage caused by higher concentration of Cd in the soluble fraction (Lozano-Rodríguez et al., 1997). The retention of Cd in root cell walls, compartmentation

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http://dx.doi.org/10.1016/j.envexpbot.2014.05.004
0098-8472/

of Cd into vacuoles and the suppressed transportation of Cd from roots to shoots are the most important mechanisms involved in the detoxification of Cd in rice plants (Zhang et al., 2009). The subcellular distribution of Cd in rice plants may be responsible for the difference between two genotypes in Cd translocation from the roots to the aboveground parts (including stem, leaf and panicle) and thereby the sensitivity to Cd (He et al., 2008; Yu et al., 2012).

To our knowledge, few works have been conducted to determine the relationship between the subcellular distribution of Cd and the translocation in rice plant and accumulation in the grain. With the rice cultivars varying in grain Cd levels as the materials, the goal of this study was to test the hypotheses: (1) grain Cd concentrations may be related to the Cd translocation in rice plants, and (2) Cd translocation may be related to the Cd subcellular distribution. The result of this study will provide insights of the mechanisms that determine grain Cd accumulation and plant Cd translocation among differences rice cultivars. The information is also useful in the selection and breeding of rice cultivars for reducing Cd content in the diet in Cd-contaminated areas.

2. Materials and methods

2.1. Soil preparation

The soil for the experiment was collected from uncontaminated fields (0–20 cm). After air-dried and passed through a 2-mm sieve, the soil samples were measured for following properties: particle size with hydrometer method, pH with pH meter (soil: distilled water = 1:2), organic matter with sequential extraction method, cation exchange capacity with ammonium acetate method, and Cd concentration with AAS following H_2O_2–HF–HNO_3–$HClO_4$ digestion (Amacher, 1996). The properties are shown in Table 1. The soil is a sandy loam with a high proportion of sand and neutral pH. It contains a moderate level of organic matter, CEC, and a low level of Cd.

Four kilogram of soil was placed in a pot (18 cm in diameter and 20 cm in height). Cadmium chloride ($CdCl_2$) was added to the soil to obtain Cd levels of 5 and 10 mg kg^{-1} (dry weight). The soil without additional Cd served as control.

$CdCl_2 \cdot 2.5H_2O$ was dissolved in deionized water and slowly poured into the soil while the soil was mixed manually. The thoroughly mixed soils were placed in pots and submerged in water (2–3 cm above the soil surface) for a month before rice seedlings were transplanted.

2.2. Rice plant materials

Six rice cultivars varying largely in Cd uptake, distribution and grain Cd levels were used in this experiment (Liu et al., 2003, 2005). The cultivars were Liangyoupeijiu (C01, *Hybrid Indica*) and Shanyou 63 (C02, *Hybrid Indica*), high Cd accumulators; CV6 (C03, *Indica*) and Yangdao 6 (C04, *Indica*), moderate Cd accumulators; Wuyunjing 7 (C05, *Japonica*) and Yu 44 (C06, *Japonica*), low Cd accumulators. Rice seeds were submerged in a water bath for about 48 h at room temperature (20–25 °C) and germinated under moist condition (seeds were covered with two layers of moist gauze cloth) at 32 °C for another 30 h and the germinated seeds were grown in uncontaminated soil (described in Section 2.1). After 30 days, uniform seedlings were selected and transplanted into the pots (three seedlings per pot). The pot soil was maintained under flooded condition (2–3 cm of water above soil surface) during the rice growth period.

2.3. Experimental design

The experiments were carried out under open-air conditions. There was a removable plastic roof to prevent precipitation. The pots were arranged in a randomized complete block design with six replicates. 230 mg of N, 171 mg of K and 68 mg of P were applied to each pot at following three time points, three days before seedling transplant, twenty days and seventy days after the transplant.

2.4. Determination of Cd concentrations in rice plants

Whole rice plants were sampled at tillering stage, panicle heading stage and at maturity. The rice plants were washed thoroughly with tap water and then with deionized water. The plants were divided into roots, shoots, and ears (at panicle heading stages) or grains (at maturity), and the plant parts were oven-dried at 70 °C to a constant weight. The oven-dried samples were ground with a stainless steel grinder to pass through a 100-mesh sieve. Cd concentrations were determined with AAS following HNO_3–$HClO_4$ (4:1, v/v) digestion procedures (Allen, 1989). For quality control, ten repeated measurements were carried out first with standard Cd solution provided by the Institute of Geophysical and Geochemical Exploration, China. Reagent blanks and certified plant reference material (GBW07602, GSV-3) (provided by the National Research Center for CRM's, China) were run simultaneously with the samples.

2.5. Subcellular fractionation and Cd analysis

Whole rice plants were sampled on the 40th day after seedling transplant and subjected to subcellular fractionation and Cd analysis according to Lozano-Rodríguez et al. (1997) and Wu et al. (2007).

Root and shoot tissues were weighed and immediately frozen in liquid N_2. The frozen shoot and root tissues were homogenized in pre-cold (4 °C) extraction buffer (50 mM Tris–HCl, 250 mM sucrose, 1.0 mM DTE, 5.0 mM ascorbic acid and 1.0% (w/v) Polyclar AT PVPP, pH 7.5) with a chilled mortar and a pestle. The homogenate was sieved through a nylon cloth (240 μm), and the residue was designated as cell wall-containing fraction (F1). The filtrate was centrifuged at 10,000 × *g* for 30 min, and the pellet retained was the organelle-rich fraction (F2). The supernatant was then centrifuged at 100,000 × *g* for 30 min. The pellet was designated as the membrane-containing fraction (F3), and the supernatant as the soluble fraction (F4). The resultant pellets were re-suspended in extraction buffer. All steps were performed at 4 °C.

Cadmium concentrations in the re-suspended F2 and F3, and in F4 were determined directly with AAS. F1 (cell wall fraction) was dried at 70 °C to constant weight, and then digested with acid

Table 1
Selected properties of the soil used in this experiment.

Soil type	Soil texture	Particle size (g kg^{-1})			pH	OM[a] (g kg^{-1})	CEC[b] (cmol kg^{-1})	Total Cd (mg kg^{-1})
		Sand	Silt	Clay				
Paddy soil	Sandy loam	587.5 ± 16.6	233.2 ± 3.1	179.3 ± 4.3	6.8 ± 0.2	27.6 ± 0.8	13.6 ± 0.3	0.12 ± 0.01

The values are mean ± SE (n = 3).
[a] Organic matter.
[b] Cation exchange capacity.