

## Uptake, Subcellular Distribution, and Chemical Forms of Cadmium in Wild-Type and Mutant Rice\*<sup>1</sup>

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### ABSTRACT

Wild-type (Zhonghua 11) and mutant rice (*Oryza sativa* L.) plants were used to investigate the effect of cadmium (Cd) application on biomass production, to characterize the influx of Cd from roots to shoots, and to determine the form, content, and subcellular distribution of Cd in the roots, leaf sheaths, and leaves of the rice plants. Seedlings were cultivated in a nutrient solution and were treated with 0.5 mmol L<sup>-1</sup> of Cd<sup>2+</sup> for 14 d. The sensitivity of rice plants to Cd toxicity was tested by studying the changes in biomass production and by observing the onset of toxicity symptoms in the plants. Both the wild-type and mutant rice plants developed symptoms of Cd stress. In addition, Cd application significantly ( $P \leq 0.01$ ) decreased dry matter production of roots, leaf sheaths, and leaves of both types, especially the mutant. The Cd content in roots of the mutant was significantly ( $P \leq 0.05$ ) higher than that of the wild-type rice. However, there was no significant difference in the Cd content of roots, leaf sheaths, and leaves between the wild-type and mutant rice. Most of the Cd was bound to the cell wall of the roots, leaf sheaths, and leaves, and the mutant had greater Cd content in cell organelles than the wild type. The uneven subcellular distribution could be responsible for the Cd sensitivity of the mutant rice. Furthermore, different chemical forms of Cd were found to occur in the roots, leaf sheaths, and leaves of both types of rice plants. Ethanol-, water-, and NaCl-extractable Cd had greater toxicity than the other forms of Cd and induced stunted growth and chlorosis in the plants. The high Cd content of the toxic forms of Cd in the cell organelles could seriously damage the cells and the metabolic processes in mutant rice plants.

**Key Words:** cadmium, chemical form, rice, subcellular distribution, uptake

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### INTRODUCTION

Heavy metals are dispersed in natural and agricultural environments, principally through human activities, such as application of heavy metal-containing pesticides and fertilizers, irrigation with sewage sludge, mining, refining, application of municipal wastes, and combustion of fossil fuels (Wagner, 1993; McLaughlin *et al.*, 2000), as well as through natural rock mineralization processes (Sanità di Toppi and Gabrielli, 1999). In China, cadmium (Cd) is one of the most widespread contaminants in agricultural soils (Wang, 1997; Yang *et al.*, 2004). Owing to cadmium's high mobility and toxicity at low concentrations, considerable attention has been paid to its toxic effect on organisms. The ability to take up, transport, and accumulate Cd differs greatly among the plant species and even among genotypes, as can be seen in maize (Yang *et al.*, 1995), wheat (Hart *et al.*, 1998), barley (Wu and Zhang, 2002), and cotton (Wu *et al.*, 2004). The crop tolerance to Cd toxicity determines the potential of Cd accumulation

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and the physiological responses to Cd toxicity (Wu *et al.*, 2004). In most cases, plant roots have the highest Cd, and Cd content declines in the following order: roots > leaves > grains or seeds (Wagner, 1993). Also, the translocation of Cd from roots to shoots has been studied in several species, including tomato (Petit and Geijn, 1978), bean (Hardiman and Jacoby, 1984), maize (Yang *et al.*, 1995), and wheat (Hart *et al.*, 1998). The movement of Cd from roots to shoots is likely to occur *via* the xylem, driven by transpiration from the leaves (Salt *et al.*, 1995).

Cellular sequestration of Cd can greatly affect the level of free Cd in the cell and thus potentially influence the movement of Cd throughout the plant. One approach to detoxify Cd is to bind it to the cell wall (Nishizono *et al.*, 1987). In addition, ionic Cd<sup>2+</sup> concentrations in the cytosol can be regulated using at least two processes: Cd<sup>2+</sup> binding to phytochelatins (Grill *et al.*, 1985) and cellular compartmentalization, particularly in the vacuole (Cobbett, 2003). There is evidence that Cd can be transported across the tonoplast into the vacuole of oat root cells, both as free ions (Salt and Wagner, 1993) and as a complex with phytochelatins (Cobbett, 2003). In contrast, most of the Cd in the protoplasm is not in the form of free ionic Cd<sup>2+</sup> but is combined with ligands, and the formation of compounds with low biological activity reduces Cd toxicity.

Although numerous studies on the interaction between Cd and plants have been conducted over the past three decades, many aspects of Cd toxicity in plants remain unclear. The differing results may be attributed to the differences in the Cd concentrations applied, the kind of medium used, and the age of the plant when it was subjected to Cd treatments. Few scientists have reported the association of Cd subcellular distribution and the chemical forms with tolerance or sensitivity. Therefore, the objectives of this study were to investigate the effect of Cd on biomass production as well as the specific tolerance or sensitivity to and uptake mechanism of Cd, to characterize the influx of Cd from roots to shoots, and to determine the content, subcellular distribution, and chemical forms of Cd in wild-type and mutant rice (*Oryza sativa* L.) plants, which have the same genetic background.

## MATERIALS AND METHODS

The rice mutant was obtained by T-DNA insertion mediated *via Agrobacterium* using Zhonghua 11 (wild type) as the receptor. Healthy and equal-sized wild-type and mutant rice plants were chosen and grown for 2 weeks in a basic nutrient solution (Huang *et al.*, 2004). The nutrient solution was renewed every week, and the pH was adjusted to 5.0–5.1 every other day. The plants were then transferred to the fresh nutrient solution containing Cd<sup>2+</sup> (0 and 0.5 mmol L<sup>-1</sup>) supplied in the form of CdCl<sub>2</sub>. Each treatment consisted of 60 plants and was carried out in triplicate. The nutrient solution was renewed, and the solution pH was adjusted, as described above. Plants were harvested after 14 d treatment. At harvest, the root samples were immersed in 20 mmol L<sup>-1</sup> disodium ethylenediamine tetraacetic acid (Na<sub>2</sub>-EDTA) for 15 min and were then rinsed with deionized water.

The rice plant samples were divided into three groups. The first group was for the investigation of Cd subcellular distribution according to Weigel and Jäger (1980). The second group was for the examination of the chemical forms of Cd. The chemical forms of Cd were extracted step by step using a sequence of different extractants: 800 mL L<sup>-1</sup> ethanol for the inorganic Cd, giving priority to nitrate/nitrite, chloride, and aminophenol cadmium; deionized water for the water-soluble Cd of organic acids and metaphosphates); 1 mol L<sup>-1</sup> sodium chloride (NaCl) for the pectates and protein integrated Cd; 20 mL L<sup>-1</sup> acetic acid (HAc) for the undissolved cadmium phosphate including CdHPO<sub>4</sub> and Cd<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>; and 0.6 mol L<sup>-1</sup> hydrochloric acid (HCl) for the oxalic-acid bound Cd (Wu *et al.*, 2005). The third group for determining the dry weight was dried and then ground to pass through a 60 mesh. Relative growth rate (RGR) was calculated according to Peter *et al.* (2003). The samples (0.50 g dry weight) of roots, leaf sheaths, and leaves were weighed to determine the Cd content. To determine the Cd concentrations, the samples were digested in 15 mL concentrated HNO<sub>3</sub> and HClO<sub>4</sub> (v:v = 4:1) at 130–150 °C, diluted to 25 mL, and were then analyzed using atomic absorption spectrometry (SolAAR-M6).

The dry weights of the different plant parts were multiplied by the Cd content in the corresponding

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