BRIEF COMMUNICATION

Root morphology and cadmium uptake kinetics of the cadmium-sensitive rice mutant

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Abstract

To understand the physiological mechanism that confers Cd sensitivity, root morphology and Cd uptake kinetics of the Cd-sensitive mutant and wild type rice were investigated. The root length, root surface area, and root number of mutant rice decreased more significantly with increasing Cd concentration in growth media compared with the wild type rice. The uptake kinetics for $^{109}Cd^{2+}$ in roots of both the mutant and wild type rice were characterized by a rapid linear phase during the first 6 h and a slower linear phase during the subsequent period. Concentration-dependent Cd^{2+} influx in both species could be characterized by the Michaelis-Menten equation, with similar apparent K_m values for mutant and wild type rice (2.54 and 2.37 μM, respectively). However, the V_{max} for Cd^{2+} influx in mutant root cells was nearly 2-fold higher than that for wild type rice, indicating that enhanced absorption into the root is one of the mechanisms involved in Cd sensitivity in mutant rice.

Additonal key words: *Oryza sativa,* Cd-accumulation, Cd-translocation.

Understanding heavy-metal tolerance very often first requires elucidation of the basic mechanisms of metal absorption into the plant. Available evidence indicates that plant species and genotypes of a given species show great variation in the uptake of Cd (Florjin and Van Beusichem 1993, Guo *et al*. 1995, Hart *et al*. 1998, Wójcik and Tukiendorf 2005). Little information is available regarding the relationship between root morphology, Cd accumulation and Cd tolerance (Schwarz and Grosch 2003, Linger *et al*. 2005, Dražić *et al*. 2006).

Uptake kinetics and translocation characteristics of Cd in plant species are very important to understand the mechanisms of metal absorption. Influx of Cd across the plasma membrane of root cells has been shown to occur *via* a concentration-dependent process exhibiting saturable kinetics in soybean (Cataldo *et al*. 1983) and maize (Mullins and Sommers 1986). These studies suggest that Cd is taken up *via* a carrier-mediated system. Recently, we have obtained a Cd-sensitive rice mutant (Lin *et al*. 2006). However, little is known regarding the mechanisms of Cd uptake and translocation that result in Cd sensivity in rice and on the relationship between Cd uptake and root morphology. Thus, the main objectives of the present study were: *1*) to compare root morphology in the mutant and wild type rice; and *2*) to characterize the root $^{109}Cd^{2+}$ uptake kinetics and its translocation to the shoot in the mutant and wild type rice.

The seeds of wild type rice (*Oryza sativa* L. cv. Zhonghua 11) and the Cd-sensitive rice mutant, which was obtained from the rice mutant populations constructed with T-DNA (Ac/Ds) insertion mediated by *Agrobacterium* using *japonica* rice as a receptor and identified as a homozygote after four generations of self progeny (Zhu *et al.* 2003, Lin *et al.* 2006) were surface sterilized in 0.5 % sodium hypochlorite for 20 min, rinsed, and germinated in the dark on moistened filter paper at 30 °C for 2 d, and then germinated on a plastic screen floating on distilled water at 28 °C for 4 d. Then,

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Abbreviations: Km - Michaelis constant; V_{max} - maximum influx rate.
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32 uniformly germinated seedlings were transferred to black polyethylene barrels containing 6 dm^3 complete nutrient solution [μM]: K_2SO_4 512, $CaCl_2$ 753, NH_4NO_3 1427, NaH_2PO_4 323, $MgSO_4$ 1643, Na_2SiO_4 820, H_3BO_3 18.88, $(NH_4)_6Mo_7O_{24}$ 0.08, $MnCl_2$ 9.47, $CuSO_4$ 0.16, $FeCl_3$ 36, $ZnSO_4$ 0.15, $C_6H_8O_7 \cdot H_2O$ 71; pH 5.0 - 5.1. Seedlings were grown for 16 d in a growth chamber with a photon flux density of 500 μmol s^{-1} m^{-2}, relative humidity about 65 % and day/night temperatures of 32/27 °C (14/10 h). During the growth period, the solution in each pot was renewed every 6 d. Then, 20-d-old seedlings of the mutant and wild type rice were exposed to different concentrations of Cd (0.1, 1, 5, 20, 50, and 100 μM Cd^{2+} applied as $CdCl_2$) while those without Cd^{2+} served as controls. Each treatment was carried out in triplicate. The nutrient solution was renewed every 4 d. Plants were harvested after exposure to Cd treatment for 12 d and root morphology parameters were determined with a root automatic scan apparatus (*MIN MAC*, *STD1600*, Epson, USA). At harvest, plant roots were soaked in 20 mM Na_2-EDTA for 15 min to remove metal ions adhering to the root surface (Yang *et al.* 1996). Harvested plants were separated into leaves, stems, and roots and oven dried at 65 °C. Dry masses were recorded. Cadmium concentration in roots and shoots of seedlings was determined by by a furnace atomic absorption spectrometry (*SolAAR-M6*, USA) after wet-ashing of dry matter in a $HNO_3/HClO_4$ mixture (4:1, v/v).

To investigate the Cd uptake, roots of intact, 20-d-old seedlings were removed from nutrient solution, rinsed in deionized water for 2 min, and then placed in pre-treatment solution (2 mM Mes-Tris, pH 6.0, 0.5 mM $CaCl_2$) for 12 h prior to the uptake experiment to avoid the effect of other divalent cations on Cd uptake (Lasat *et al.* 1996). The uptake experiment started at 2 h after the light period began. Seedlings were immersed with roots in uptake solution containing 2 mM Mes-Tris (pH 6.0), 0.5 mM $CaCl_2$, and 5 μM $^{109}Cd^{2+}$ (17.5 kBq dm^{-3}). Following the appropriate incubation periods (0, 10, 20, 30, 60, 90, 120, 150, 180 and 210 min and 6, 12, 24, 48, 72 and 96 h), two plants from each species were harvested and the roots were desorbed for 20 min in a 5 mM Mes-Tris (pH 6.0), 5 mM $CaCl_2$, and 100 μM $CdCl_2$ ice-cold solution. Following desorption, roots were separated from shoots, blotted, and weighed, and ^{109}Cd was counted using a γ-detector (model *5530*, *Hewlett-Packard Instruments*, Downers Grove, IL, USA). During the experiment, fresh $^{109}Cd^{2+}$ solution was added as needed to maintain Cd concentration. Each treatment was replicated 4-fold.

For determination of concentration-dependent kinetics of $^{109}Cd^{2+}$ influx, roots of intact rice seedlings were immersed in the pretreatment solution (2 mM Mes-Tris, pH 6.0, and 0.5 mM $CaCl_2$) in uptake pots. Subsequently, Cd^{2+} was added as $CdCl_2$ to each uptake pot to yield a final Cd concentration (0.25, 0.5, 1, 2.5, 5, 10, 25, 50 μM) before the addition of 17.5 kBq dm^{-3} of $^{109}Cd^{2+}$. After a 30-min uptake period, uptake pots were refilled with ice-cold desorption solution (5 mM Mes-Tris pH 6.0, 5 mM $CaCl_2$, and 100 μM $CdCl_2$). Following a 20-min desorption period, seedlings were harvested and their roots excised, blotted, and weighed, and ^{109}Cd was quantified via γ-detection. Each treatment was replicated 4-fold.

Analysis of variance (*ANOVA*) was performed on data with *SAS* program. Significant differences are reported at $P < 0.05$.

The growth of the mutant rice was inhibited more significantly compared with the wild type rice when the Cd concentration was higher than 5 μM (Table 1). After 12 d growing in solutions containing 100 μM Cd^{2+}, the dry masses of the root and shoot of the mutant decreased by 70.9 and 72.9 %, respectively, whereas those of wild type rice decreased by 42.2 and 47.9 %, respectively, compared with control. Root length, root surface area, and root number of the mutant rice decreased significantly with increasing Cd concentrations and the difference was significant compared with control and wild type rice, except for the Cd concentration was 0.1 μM. After 12 d growth in solution containing 100 μM Cd^{2+}, root length, root surface area, and root number of the mutant rice were decreased by 67.9, 69.9, and 63.4 % compared with control, respectively, whereas those of the wild type decreased by 46.3, 54.6, and 46.3 %, respectively.

The Cd content in roots and shoots of both the mutant and wild type rice increased with an increase in the amount of Cd^{2+} supplied. After 12-d growth in solutions containing different Cd^{2+} concentrations (0.1 - 100 μM), the rice mutant accumulated more Cd in the roots, stems and new leaves, whereas the Cd concentration in old leaves in the mutant was the same as that in wild type rice. When the growth solution contained 100 μM Cd^{2+}, the roots, stems and new leaves of mutant accumulated 28.4, 22.6, and 46.7 % more Cd compared with the wild type (Fig. 1).

Accumulation of Cd^{2+} was significantly different in desorbed intact roots of the mutant and wild type rice. For the roots of both mutant and wild type rice, $^{109}Cd^{2+}$ uptake kinetics in roots could be showed initial rapid linear phase during the first 10 min followed by a second, slower linear phase over the subsequent 200 min (Fig. 2*A*). We interpreted the initial rapid accumulation as cell wall-associated $^{109}Cd^{2+}$ not removed by the desorption treatment, but the slower linear phase was primarily due to $^{109}Cd^{2+}$ transport into the cytoplasm, with a only minor component of undesorbed cell wall $^{109}Cd^{2+}$. These results are consistent with those performed in *Thlapsi caerulescens* (Lasat *et al.* 1996). After a 210-min absorption period, 1.8-fold more $^{109}Cd^{2+}$ was accumulated in the roots and 1.2-fold more $^{109}Cd^{2+}$ was

translocated in the shoots of the mutant compared with wild type rice (Fig. 2). At the end of a 96-h uptake period, 38 % more $^{109}Cd^{2+}$ accumulated in the roots of the mutant rice compared with the wild type rice, whereas, Cd translocation to the shoot was approximately 1.9-fold greater in the mutant compared with the wild type rice (Fig. 2). Studies of the accumulation of several different cations in roots have demonstrated that the time-dependent cation uptake kinetics is biphasic, with an initial rapid component followed by a slower phase of uptake (Veltrup 1978, Hart *et al.* 1992, Lasat *et al*. 1998).

Concentration-dependent uptake kinetics for $^{109}Cd^{2+}$ influx in the roots of the mutant rice were characterized by a smooth, nonsaturating curve that approached linearity at Cd concentrations above 5 μM while that for the wild type was linear above 10 μM (Fig. 3). In studies of Zn^{2+} (Lasat *et al.* 1996) and paraquat (Hart *et al.* 1992) influx into maize roots similar nonsaturating transport kinetics was observed. The Cd uptake kinetics could be characterized by the Michaelis-Menten equation, with apparent Km values of 2.54 and 2.37 μM for the mutant and wild type rice, respectively. The V_{max} values were 77.92 and 43.99 nmol(Cd^{2+}) g^{-1}(f.m.) h^{-1} for the mutant and wild type rice, respectively, indicating that Cd^{2+} influx in the mutant roots was more than 1.8-fold higher compared with influx into the wild type rice roots. A

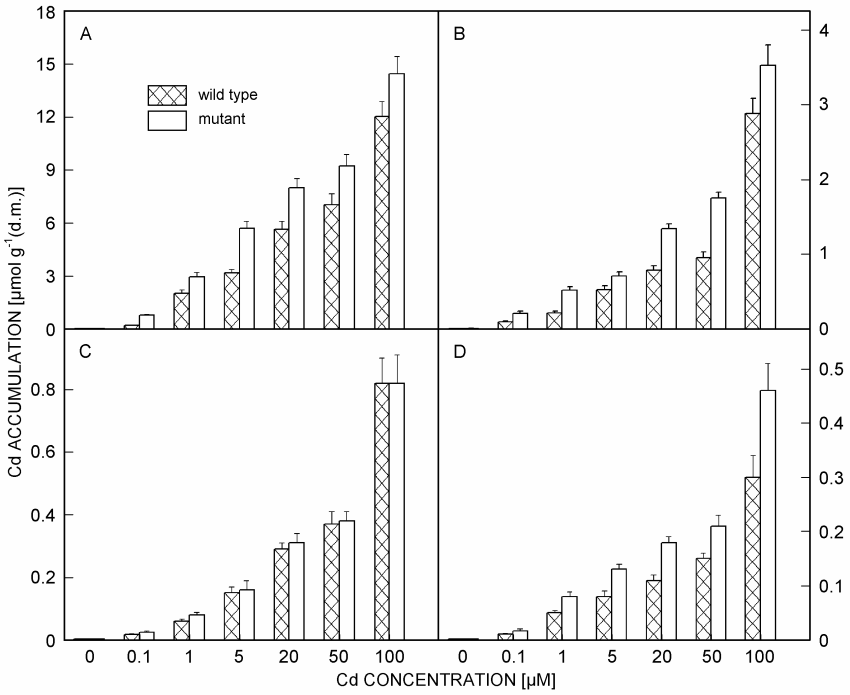

Fig. 1. Cd accumulation in the mutant and wild type rice roots (*A*), stems (*B*), old leaves (*C*) and new leaves (*D*) exposed to different concentrations of Cd^{2+} in nutrient solution for 12 d. Means ± SE of three replicates.

Table 1. The effect of Cd (0, 0.1, 1, 5, 20, 50, 100 μM) on root and shoot dry mass [mg plant^{-1}], root length [cm plant^{-1}], root surface area [cm^2 plant^{-1}] and root number [plant^{-1}] of the mutant and wild type rice. Means ± SE, n = 3, means in a row followed by a different letter are significantly different ($P < 0.05$) according to Duncan test.

Parameters		0	0.1	1	5	20	50	100
Root d.m.	mutant	43.6 ± 2.1^a	44.8 ± 2.1^a	40.1 ± 1.7^a	34.8 ± 1.5^b	27.9 ± 1.2^c	19.1 ± 1.5^d	12.7 ± 1.4^e
	wild type	43.1 ± 1.7^a	43.9 ± 1.6^a	42.5 ± 1.4^a	39.4 ± 1.2^b	34.3 ± 1.3^c	28.1 ± 1.5^d	24.9 ± 1.7^e
Shoot d.m.	mutant	103.8 ± 4.7^a	110.0 ± 5.2^a	97.1 ± 5.5^a	82.2 ± 3.8^b	66.0 ± 3.5^c	44.2 ± 3.2^d	28.1 ± 2.9^e
	wild type	100.3 ± 4.3^a	105.8 ± 5.4^a	98.2 ± 4.2^a	92.6 ± 3.9^b	79.8 ± 4.5^c	63.5 ± 3.3^d	52.2 ± 3.1^e
Root length	mutant	356.0 ± 17.0^a	367.0 ± 18.0^a	321.0 ± 14.0^b	269.0 ± 10.0^c	201.0 ± 11.0^d	159.0 ± 13.0^e	114.0 ± 12.0^f
	wild type	350.0 ± 19.0^a	357.0 ± 17.0^a	342.0 ± 15.0^a	312.0 ± 11.0^b	271.0 ± 13.0^c	225.0 ± 11.0^d	188.0 ± 12.0^e
Root surface	mutant	59.1 ± 3.9^a	61.8 ± 3.4^a	50.9 ± 3.5^b	37.2 ± 2.6^c	29.1 ± 1.4^d	22.2 ± 1.5^e	17.8 ± 1.3^f
area	wild type	57.3 ± 4.1^a	59.1 ± 3.7^a	52.2 ± 3.5^a	44.1 ± 2.8^b	36.9 ± 2.4^c	30.1 ± 1.7^d	25.9 ± 1.6^e
Root number	mutant	891.0 ± 39.0^a	923.0 ± 45.0^a	774.0 ± 33.0^b	605.0 ± 33.0^c	487.0 ± 25.0^d	403.0 ± 26.0^e	326.0 ± 24.0^f
	wild type	880.0 ± 41.0^a	907.0 ± 39.0^a	822.0 ± 31.0^b	693.0 ± 36.0^c	601.0 ± 31.0^d	534.0 ± 29.0^e	472.0 ± 23.0^f

significantly higher V_{max} value suggested that there might be a higher density of Cd transporters per unit membrane area in roots of the mutant. The kinetic parameters obtained for the wild type rice (Fig. 3) were similar to the values reported by Homma and Hirata (1984). Both the time-course (Fig. 2) and concentration-dependent Cd uptake (Fig. 3) indicated greater Cd influx into the mutant roots compared with wild type and consequently higher Cd content in shoot of the mutant than in shoot and of wild type rice.

These results indicate that increased Cd influx into the root contributes to the increase in translocation of Cd to the shoot, Cd accumulation and Cd sensitivity.

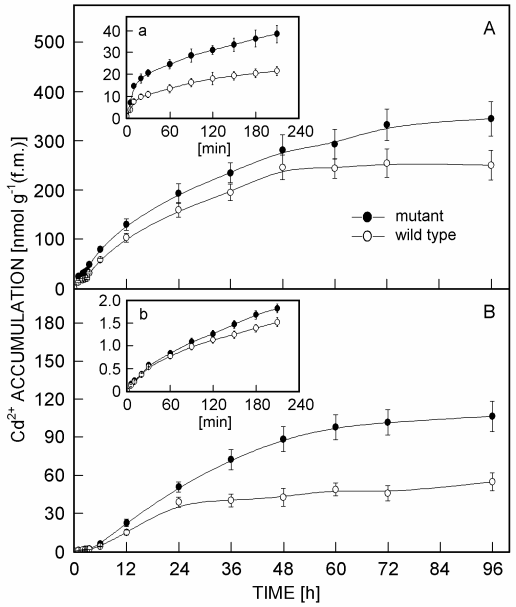

Fig. 2. Time course of Cd^{2+} accumulation in roots (*A*) and shoots (*B*) of the mutant and wild type rice roots. The insets were derived from the corresponding main data. Means ± SE of four replicates.

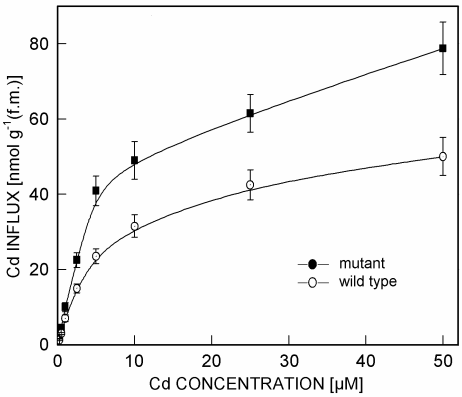

Fig. 3. Concentration-dependent Cd^{2+} uptake in roots of intact mutant and wild type rice seedlings. Means ± SE of four replicates.

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