Rice Science, 2007, 14(3): 217-222

Cadmium Accumulation and Its Toxicity in *Brittle Culm 1 (bc1)*, a Fragile Rice Mutant

SHAO Guo-sheng[1, 2], CHEN Ming-xue[1], ZHANG Xiu-fu[1], XU Chun-mei[1], WANG Dan-ying[1], QIAN Qian[1], ZHANG Guo-ping[2]

([1] *China National Rice Research Institute, Hangzhou 310006, China;* [2] *College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310029, China*)

Abstract: Cadmium (Cd) accumulation and toxicity in rice plants were characterized and identified by using *brittle culm 1* (*bc1*), a fragile rice mutant and its wild type (Shuangkezao, an indica rice) as materials by hydroponics. The low Cd level didn't obviously affect the growth parameters in both rice genotypes, but under high Cd levels (1.0 and 5.0 μmol/L), the growth of both rice plants were substantially inhibited. Moreover, *bc1* tended to suffer more seriously from Cd toxicity than Shuangkezao. Cd accumulation in both rice plants increased with the increase of Cd levels. There was a significant difference in Cd accumulation between the two rice genotypes with constantly higher Cd concentration in *bc1*, which also accumulated more Cd at 0, 0.1, and 1.0 μmol/L Cd levels. The same case was found in the two rice plants grown on Cd-contaminated soil. This suggested that cell wall might play an important role in Cd accumulation in rice plants by the physiological mechanisms. The malondialdehyde (MDA) content, superoxide dismutase (SOD) and peroxidase (POD) activities in rice plants were affected differently under Cd treatments, and which implied that POD might play the main role in detoxifying active oxygen free radical. A significant difference in antioxidative system between the two rice genotypes was found with constantly higher MDA content, SOD and POD activities in *bc1*. In summary, *bc1* accumulated more Cd and appeared to be more sensitive to Cd stress compared with its wild type.

Key words: rice (*Oryza sativa*); cadmium; brittle culm mutant; malondialdehyde; oxidative stress; toxicity; superoxide dismutase; peroxidase; active oxygen free radical; enzyme activity

Cadmium (Cd) is a widespread heavy metal, which causes serious environmental and human health problems due to its high mobility in the soil-root-shoot (grain) system [1]. Cd can be taken up into plant root system through some other nutrient metabolic pathways such as zinc, iron, and calcium [2-8] after it reaches root cell membrane via the apoplast, including cell wall continuum and intercellular space. Once Cd ion enters cell, it may disturb many physiological and metabolic processes in plants [9-11]. Therefore, Cd concentration in cytosol of plant cells must be tightly controlled to maintain the level of Cd below critical limits for plant growth.

Rice, like other higher plants, e.g. *Arabidopsis*, has developed many strategies to reduce uptake and accumulation of cadmium in plants and subsequent toxicity, including reduction of Cd phyto-availability in rhizosphere, chelation with phytochelatins (PCs) and compartmentation into cytosol [12]. However, a first barrier against Cd stress, operating mainly at the root level, may be retention of Cd in the cell wall [13] and/or damming back Cd in soil solution out of cell wall. Nishizono et al [13] had demonstrated that cell wall could accumulate high concentration of Cd. Recently, Saúl et al [1] suggested that root cell wall not only accumulated high level of Cd, but had, to a certain extent, impacts on Cd tolerance in white lupin.

Cell wall is a strong inerratic fibrillar network consisting of cellulose, hemicelluloses, glycoproteins and pectins and so on, and any change of these components and structure will result in severe impacts on its function [14] and response to abiotic stress [15]. There are dramatic differences in root Cd concentration among plant species or the genotypes within a species. Some species or genotypes contain very high Cd concentrations in root without obvious toxic symptom, indicating high tolerance to Cd toxicity. It may be assumed that some root properties are attributed to Cd

Received: 8 June 2007; **Accepted:** 20 August 2007
Corresponding author: ZHANG Guo-ping (zhanggp@zju.edu.cn)

tolerance. Recently, a rice mutant *bc1*, which is characterized by brittle culm due to deficiency in expression of the gene *BC1* encoding a COBRA-like protein in developing sclerenchyma cells and in basal vascular bundles was isolated from an indica rice Shuangkezao. There are many differences between the mutant (*bc1*) and its wild type Shuangkezao, in cell wall structure and components. In comparison with the wild type, cellulose and Klason lignin contents in the cell wall of the mutant (*bc1*) were decreased by 30% respectively, and correspondingly the components of cell walls were also greatly changed [14]. In the current study, we characterized and identified the role of cell wall in Cd uptake and accumulation in rice plants by using *bc1* and its wild type Shuangkezao.

MATERIALS AND METHODS

Plant materials and treatments

The seeds of *bc1* and its wild type (*Oryza sativa* L. cv. Shuangkezao) were sterilized with 0.1% H_2O_2 for 2 h, soaked in de-ionized water for 2 d, germinated at 30-35℃ for 1 d and then sown on sandy bed, which was previously washed with dilute sulfuric acid. The three-leaf seedlings were transplanted into 4.5-L plastic pots, which were filled with nutrient solution according to International Rice Research Institute with some modifications [16]. The levels of macro-elements including N, P and K in solution were reduced by half at the first ten days after transplanting and then increased up to a normal level. The solution was changed every ten days, and adjusted to pH 5.5 with KOH or HCl as required. Cadmium treatment was done at the 7th day after transplanting. There were four Cd levels, 0 (control), 0.1, 1.0 and 5.0 μmol/L, with $CdSO_4$ as Cd source. The experiment was arranged in a completely random block design with six replications.

In addition, the five-leaf seedlings were transplanted to a Cd-contaminated paddy field at Fuyang City, Zhejiang Province, China. The total Cd content and DTPA-extractable Cd content in soil were 1.596 and 0.329 mg/kg, respectively.

Sampling and measurements

When the plants were at the eight-leaf stage, chlorophyll content (SPAD value) was measured in the second uppermost fully expanded leaf with a chlorophyll meter (Minolata SPAD-502, Japan), and then the whole plants were sampled from both hydroponic culture and field. After measuring the plant height, the sampled plants were prepared for measurement of Cd and physiological parameters. Roots were immerged in a solution containing 1.0 mmol/L ethylene-diamine-tetra acetic acid (EDTA) for 2 h, and then rinsed with de-ionized water twice. Plants were oven-dried at 100℃ for 1 h and at 60℃ for 48 h, weighed and then ground. Cd content was measured using an inductively coupled argon-plasma emission spectrometry (ICAP 61E Trace Analyzer, Themo-Jarrell Ashe, Franklin, MA, USA). Malondialdehyde (MDA) content, superoxide dismutase (SOD) and peroxidase (POD) activities in leaf and root were measured according to Gao [17].

Statistical analysis

All data presented were the mean values. Statistical analysis was carried out by two-way ANOVA and the Duncan's multiple-range test (SSR) was used to test the significance of the difference between means. Means were considered significantly different at $P \leqslant 0.05$.

RESULTS AND ANALYSIS

Plant growth parameters

The plants grown under low Cd level (0.1 μmol/L) did not show reduction in the three growth parameters (plant height, SPAD value and dry weight) in comparison with the control, and even showed significant increase for some cases, e.g. SPAD value for *bc1* and shoot weight for Shuangkezao (Table 1). High Cd level (≥ 1.0 μmol/L) caused significant reduction in all examined growth parameters, irrespective of the genotype. There were dramatic differences in these growth parameters and the responses to Cd stress between *bc1* and the wild type. The mutant *bc1* was consistently lower than the wild type in plant height, irrespective of Cd stress or not. For SPAD value, the mutant was significantly higher than the wild type in the treatments without Cd addition

Table 1. Plant growth parameters under different Cd treatments.

Cd (μmol/L)	Plant height (cm)		Chlorophyll content (SPAD value)		Dry weight (g/10 plants)			
					Shoot		Root	
	bc1	Shuangkezao	*bc1*	Shuangkezao	*bc1*	Shuangkezao	*bc1*	Shuangkezao
0.0	36.00 a	38.40 a	32.15 b	31.04 a	3.080 a	3.024 b	0.432 a	0.483 a
0.1	35.41 a	37.95 a	34.15 a	31.30 a	2.929 a	3.224 a	0.421 a	0.485 a
1.0	26.91 b	27.80 b	28.02 c	28.84 b	2.174 b	3.022 b	0.368 b	0.472 b
5.0	22.85 c	23.35 c	26.36 d	26.64 c	1.686 c	2.286 c	0.273 c	0.413 c
Mean	30.29 b	31.88 a	30.17 a	29.46 a	2.467 b	2.889 a	0.374 b	0.463 a

The values followed by the same letters within a column for the four Cd treatments and the mean values in a row for a parameter for the two genotypes mean no significant difference at 95% probability.

Table 2. Cd concentration and accumulation in rice plants.

Cd (μmol/L)	Cd concentration (mg/kg)				Cd accumulation (mg /10 plants)			
	Root		Shoot		Root		Shoot	
	bc1	Shuangkezao	*bc1*	Shuangkezao	*bc1*	Shuangkezao	*bc1*	Shuangkezao
In hydroponics								
0.0	0.308 d	0.189 d	0.070 d	0.034 d	0.949 d	0.572 c	0.030 c	0.016 d
0.1	16.230 c	12.393 c	2.981 c	1.976 c	47.538 c	39.955 b	1.255 b	0.958 c
1.0	91.089 b	63.498 b	16.388 b	10.823 b	198.027 a	191.891 a	6.031 a	5.108 b
5.0	102.294 a	81.059 a	21.008 a	14.755 a	172.468 b	185.301 a	5.735 a	6.094 a
Mean	52.480 a	39.285 b	10.112 a	6.897 b	129.468 a	113.494 b	3.782 a	3.193 a
On Cd-contaminated soil								
0.329[a]	18.195 a	13.172 b	0.609 a	0.285 b				

The values followed by the same letters within a column for the four Cd treatments and the mean values in a row for a parameter for the two genotypes mean no significant difference at 95% probability.

[a] DTPA-extractable Cd (mg/kg).

and 0.1 μmol/L Cd, while there were no significant differences between them in the treatments with higher Cd levels. In contrast, no significant differences in both shoot and root weights could be detected between the two genotypes in the control (without Cd addition), but the mutant had significantly lower values than the wild type in all Cd stress treatments.

Cd accumulation

Cadmium content significantly increased with increasing Cd level in growth medium, and the two genotypes showed the same trend (Table 2). There were significant differences in Cd concentrations in both shoot and root between the two genotypes, with the mutant being significantly higher than the wild type. Moreover, it may be seen that the difference between the two genotypes became more predominant with increasing Cd level in the growth medium. Cd accumulations were also higher in *bc1* plants than in Shuangkezao at all Cd levels except for at 5.0 μmol/L Cd due to lower dry weight of *bc1*. In addition, when the plants of the two genotypes were grown on Cd-contaminated soil (Table 2), *bc1* accumulated much more Cd than its wild type in both shoot and root, which was consistent with the case of hydroponics.

MDA content

MDA contents in shoot and root of the two rice genotypes varied with Cd level (Fig. 1). At lower Cd level (0.1 μmol/L), MDA content was little affected in the leaves of both rice genotypes and the root of *bc1*, while for the root of Shuangkezao MDA content was markedly increased. At the high Cd treatments (1.0 and 5.0 μmol/L), MDA contents in both genotypes were greatly increased, irrespective of plant tissue.

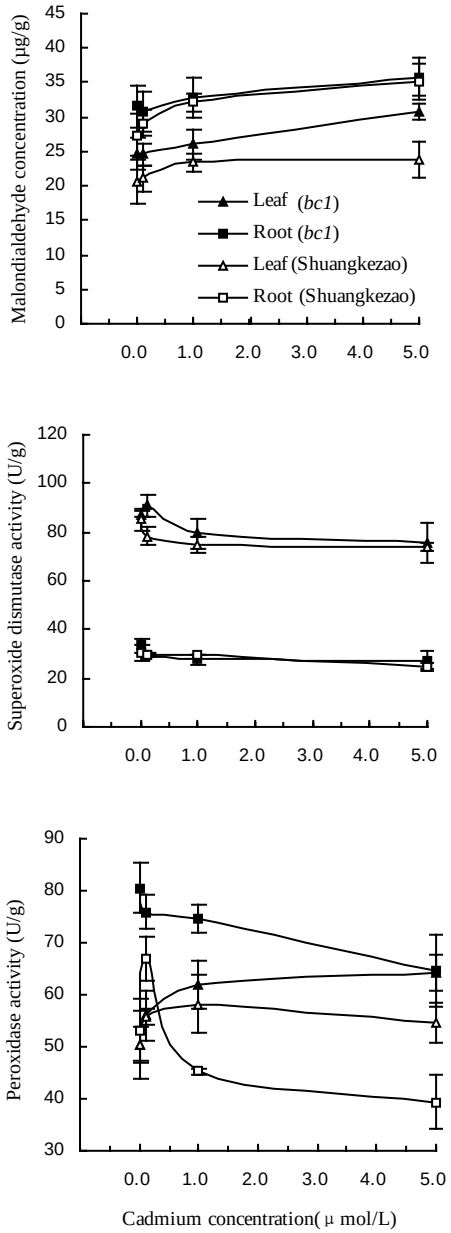

Fig. 1. MDA, SOD and POD concentrations in rice plants as affected by Cd stress.

Compared with the wild type, *bc1* had consistently higher MDA contents in all Cd treatments.

Superoxide dismutase (SOD) activity

SOD activity in rice plants was affected markedly by Cd stress and also showed significant difference between the two rice genotypes (Fig. 1). SOD activity in rice plants was suppressed under Cd stress with exception of that in the leaf of *bc1* at low Cd level. Moreover, *bc1* had higher SOD activities in both shoot and root than Shuangkezao generally, and the change trends of SOD activities caused by Cd stress were similar in shoot and root.

Peroxidase (POD) activity

POD activity in rice plants was also affected by Cd stress. However, Cd-induced POD activity changes were much different in shoot and root (Fig. 1). POD activity in the leaves of both genotypes increased markedly with the increase of Cd level, but those in the root were decreased at all Cd levels with exception of that in the root of Shuangkezao at low Cd level. Meanwhile, POD activities in the leaf and root of *bc1* were significantly higher than those of Shuangkezao, especially in root at high Cd level.

DISCUSSION

The experiment was conducted to characterize and identify the effects of cell wall in rice Cd accumulation and toxicity at the whole plant level. We used a classical rice mutant brittle culm 1 (*bc1*) as material, which was significantly different from its wild type (Shuangkezao, indica rice) in cell wall structure and components [14]. According to our results, *bc1* was lower than its wild type in plant height when grown on normal soil (data not showed) or in nutrient solution, and which indicated *BC1* gene may also influence plant height at whole plant level besides cell wall structure and components at cell level.

Cd is a toxic heavy metal which has been widely reported to inhibit plant growth through triggering the burst of active oxygen free radicals, disturbing the nutrient ion homeostasis such as iron and zinc, reducing chlorophyll content and plant dry weight and so on [9-11]. In our experiment, low Cd level didn't much affect the plant height, chlorophyll content and both shoot and root dry weight and even stimulated in some cases, e.g. chlorophyll content of *bc1* and shoot dry weight of Shuangkezao. When Cd concentration was up to 1.0 and 5.0 μmol/L, the plant height, chlorophyll content and both shoot and root dry weights of both rice genotypes were substantially reduced. In comparison to Shuangkezao, *bc1* appeared to suffer more seriously from Cd stress according to the reduced chlorophyll content, and both shoot and root weights, though no significant difference was detected between the two genotypes based on the

reduced plant height by Cd stress. The results indicated *bc1*-induced cell wall changes in components and structure produced some adverse effects on rice plant to resist the disadvantageous environment.

Cadmium is a high mobile metal in the soil-root-shoot system. Cd uptake and accumulation in plant is dependent on phytoavailable Cd concentration in soil solution. In present study, Cd accumulation in both shoot and root of two rice genotypes was increased markedly with the increase of Cd levels. Meanwhile, there was a significant difference in Cd accumulation in plants between the two genotypes grown in nutrient solution. The results showed *bc1* had accumulated much more Cd than its wild type, Shuangkezao, irrespective of shoot or root. In order to compare the Cd accumulation between two genotypes grown on Cd-contaminated soil, *bc1* and Shuangkezao were planted in Cd-contaminated paddy field in Fuyang, Zhejiang Province, China. The results showed *bc1* also accumulated much more Cd than Shuangkezao in both shoot and root. These results suggested that *BC1*-induced changes in cell wall structure and components might play an important role in Cd uptake and accumulation in root and subsequent transportation to shoot. Previous studies suggested cell wall could accumulate most of Cd in plant, and therefore, might play certain role in Cd uptake and accumulation by root [1, 13]. However, cell wall has a finite capacity for binding Cd [13] and continued or increased exposure to Cd may result in the saturation of available binding sites of cell wall [17]. Based on our results, it could be hypothesized that the role of cell wall might not only accumulate high concentration of Cd for Cd accumulation in plant, but the most important was to impede Cd in soil solution or in apoplast to arrive at root cell membrane and to be taken up into symplast.

MDA content, SOD activity and POD activity are three important parameters for estimating what extent plant suffered from adverse stress [19-22]. The production of MDA is usually the results of cell membrane lipid peroxidation. In present study, MDA content in both shoot and root of two rice genotypes increased dramatically with the increase of Cd level, indicating that Cd triggered the burst of active oxygen free radical. Meanwhile, the MDA content in plant, in particular in shoot, was much higher in *bc1* than in Shuangkezao at all Cd levels, which might be contributed to *bc1*-induced injury in cell wall and Cd-induced burst of active oxygen free radical. SOD and POD are two key antioxidative enzymes for scavenging active oxygen free radical and hydrogen peroxide in plant [19-22]. Cd-induced changes of SOD and POD activities were reported widely but without a decisive result [17, 22-24]. In present study, Cd stress lowered SOD activity in both rice plants and *bc1* was higher in SOD activity than Shuangkezao as a whole. However, Cd stress promoted POD activities in shoot of both rice genotypes, but inhibited those in root, which might imply that POD played an important role in detoxifying active oxygen free radical caused by Cd stress. Moreover, both SOD and POD activities were higher in *bc1* than in Shuangkezao at all Cd levels, and the same case was showed when the two rice genotypes grown on Cd-contaminated soil (data not shown). The above results also suggested that *bc1*-induced injury in cell wall resulted in the generation of active oxygen free radical and subsequent triggering of enhanced SOD activity and POD activity, which might be an important mechanism for that Cd-induced active oxygen free radical was cleaned up based on lower degree of increased MDA content in root of *bc1* than that of Shuangkezao under Cd stress.

ACKNOWLEDGMENTS

We are very grateful to the National Natural Science Foundation of China (Grant No. 30600379), Zhejiang Natural Science Foundation (Grant No. Z304104) and the Research Funds for National Non-profit Research Institution (Grant No. CNRRI10023) for their financial support.

REFERENCES

1 Vázquez S, Goldsbrough P, Carpena R O. Assessing the relative contributions of phytochelatins and the cell wall to cadmium resistance in white lupin. *Physiol Plant*, 2006, **128**: 487-495.

2 Cohen C K, Fox T C, Garvin D F, Kochian L V. The role of

iron-deficiency stress responses in stimulating heavy-metal transport in plants. *Plant Physiol*, 1998, **116**: 1063-1072.

3 Vert G A, Grotz N, Dedaldechamps F, Gaymard F, Guerinot M L, Briat J F, Curie C. IRT1, an *Arabidopsis* transporter essential for iron uptake from the soil and for plant growth. *Plant Cell*, 2002, **14**: 1223-1233.

4 Nakanishi H, Ogawa I, Ishimaru Y, Mori S, Nishizawa N K. Iron deficiency enhances cadmium uptake and translocation mediated by the Fe^{2+} transporters OsIRT1 and OsIRT2 in rice. *Soil Sci Plant Nutr*, 2006, **52**: 464-469.

5 Pence N S, Larsen P B, Ebbs S D, Letham D L, Lasat M M, Garvin D F, Eide D, Kochian L V. The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proc Natl Acad Sci*, *USA*, 2000, **97**: 4956-4960.

6 Cosio C, Martinoia E, Keller C. Hyperaccumulation of cadmium and zinc in *Thlaspi caerulescens* and *Arabidopsis halleri* at the leaf cellular level. *Plant Physiol*, 2004, **134**: 716-725.

7 Clemens S, Antosiewicz D M, Ward J M, Schachtman D P, Schroeder J I. The plant cDNA *LCT1* mediates the uptake of calcium and cadmium in yeast. *Proc Natl Acad Sci*, *USA*, 1998, **95**: 12043-12048.

8 Hacisalihoglu G, Hart J J, Kochian L V. High- and low-affinity zinc transport systems and their possible role in zinc efficiency in bread wheat. *Plant Physiol*, 2001, **125**: 456-463.

9 Das P, Samantaray S, Rout G R. Studies on cadmium toxicity in plants: A review. *Environ Pollution*, 1997, **89**: 29-36.

10 Sanità di Toppi L, Lambardi M, Pazzagli L, Cappugi G, Durante M, Gabbrielli R. Response to cadmium in carrot in vitro plants and cell suspension cultures. *Plant Sci*, 1998, **137**: 119-129.

11 Sanità di Toppi L, Gabbrielli R. Response to cadmium in higher plants. *Environ Exp Bot*, 1999, **41**: 105-130.

12 Cobbett C, Goldsbrough P. Phytochelation and metallothioneins: Roles in heavy metal detoxification and homeostasis. *Annu Rev Plant Biol*, 2002, **53**: 159-182.

13 Nishizono H, Ichilawa H, Suzuki S, Ishii F. The role of the root cell wall in the heavy metal tolerance of *Athyrium yokoscense*. *Plant Soil*, 1987, **101**: 15-20.

14 Li Y, Qian Q, Zhou Y, Yan M, Sun L, Zhang M, Fu Z, Wang Y, Han B, Pang X, Chen M, Li J. *BRITTLE CULM1*, which encodes a COBRA-like protein, affects the mechanical properties of rice plants. *Plant Cell*, 2003, **15**: 2020-2031.

15 Schmohl N, Horst W J. Cell-wall composition modulates aluminium toxicity. *In*: Horst W J. Plant Nutrition: Food security and sustainability of agro-ecosystems. Dordrecht, The Netherlands: Kluwer Academic Publishers, 2001: 262-263.

16 Wang K R. Comparative study on Cd phytotoxicity to different genes of rice. *Rural Eco-Environ*, 1996, **12**(3): 18-23. (in Chinese)

17 Gao J F. Experimental Guide of Plant Physiology. Beijing: Higher Education Press, 2006. (in Chinese)

18 Damien B, Bernard B, Michel C. Cadmium uptake and subcellular compartmentation in the ectomycorrhizal fungus *Paxillus involutus*. *Microbiology*, 2000, **146**: 1109-1117.

19 Noctor G, Eoyer C H. Ascorbate and glutathione: Keeping active oxygen under control. *Annu Rev Plant Biol*, 1998, **49**: 249-279.

20 Lin C C, Kao C H. Effect of NaCl stress on H_2O_2 metabolism in rice leaf. *Plant Growth Regul*, 2000, **30**: 151-155.

21 Salin M L. Toxic oxygen species and protective systems of the chloroplast. *Physiol Plant*, 1987, **72**: 681-689.

22 Wu F B, Zhang G P, Dominy P. Four barley genotypes respond differently to cadmium: Lipid peroxidation and activity of antioxidant capacity. *Environ Exp Bot*, 2003, **50**: 67-77.

23 Sandalio L M, Dalurzo H C, Gomez M, Romero-Puertas M C, Rio L A. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *J Exp Bot*, 2001, **52**: 2115-2126.

24 Muhammad J H, Shao G S, Zhang G P. Influence of cadmium toxicity on growth and antioxidant enzyme activity in rice cultivars with different grain cadmium accumulation. *J Plant Nutr*, 2005, **28**: 1259-1270.