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Accumulation and detoxification of cadmium in *Brassica pekinensis* and *B. chinensis*

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Abstract

The effect of excessive Cd on the growth and metal uptake by leafy vegetables *Brassica chinensis* L. (cv. Wuyueman) and *Brassica pekinensis* (Lour.) Rupr. (cv. Qingyan 87-114) were studied in hydroponic solution culture. The Cd concentration higher than 10 μM significantly decreased the root elongation and leaf chlorophyll contents of both plant species. The shoots of *B. pekinensis* had significantly higher concentrations of total and water-soluble Cd than *B. chinensis*. The roots of both species accumulated more Cd than the shoots in all the Cd treatments. Most of the Cd in the roots was found in the cell walls. The shoot/root ratio of Cd concentrations in *B. pekinensis* was always greater than that in *B. chinensis*. But, the concentration and proportion of Cd in the cell walls in *B. chinensis* were higher than that in *B. pekinensis*. Cadmium treatments also increased the concentrations of total non-protein thiols (NPT) in the shoots of the both species. A significant correlation was found between the concentrations of soluble Cd and NPT in plant shoots.

Additional key words: Cd tolerance, cell wall, non-protein thiols, toxicity.

Introduction

Cadmium (Cd) is a highly toxic nonessential element, and is of particular concern to human health as it can be readily absorbed by plant roots, and be concentrated by many cereals, potatoes, vegetables and fruits (Wagner 1993). The most common consequences of Cd phytotoxicity in plants are leaf chlorosis, cell and plant growth inhibition, respiratory and nitrogen metabolism changes, and nutrient uptake reduction (Sanità di Toppi and Gabbrielli 1999, Zhang *et al*. 2000, 2003, Vassilev *et al*. 2004). Cadmium also produces oxidative stress by generating free radicals and reactive oxygen species (Sanità di Toppi and Gabbrielli 1999, Hall 2002, Skórzyńska-Polit *et al*. 2003/4, Zhang *et al*. 2003). These species react with lipids, proteins, and nucleic acids and cause lipid peroxidation, membrane damage, inactivation of enzymes, thus affecting cell viability (Scandalios 1993).

Depending on plant species, metal tolerance may result from two basic strategies: metal exclusion and metal accumulation (Baker 1987). Plants with exclusion strategy can avoid excessive metal uptake and restrict metal transport from roots to shoots. An important way of heavy metal exclusion is the metal-binding capacity of cell walls (Ramos *et al.* 2002, Zornoza *et al*. 2002, Lou *et al.* 2004). In most plant species, Cd and other metals are mainly accumulated in roots, although the translocation to shoots may vary considerably among different species (Sanità di Toppi and Gabbrielli 1999, Stolt *et al.* 2003, Lou *et al.* 2004). Some plants can translocate heavy metals from roots and accumulate them in shoots (Reeves and Baker 2000).

In metal accumulating plants, it is essential that intracellular level of free Cd be kept at a minimum level due to irreversible changes to protein conformation by forming metal thiolate bonds (Van Assche and Clijsters 1990), and alteration of cell walls and membrane permeability (Ramos *et al.* 2002). An active detoxification mechanism developed by plants to avoid

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Abbreviations: GSH - glutathion; NPT - non-protein thiols; PC - phytochelatins.
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heavy metal poisoning involves intracellular sequestration of metal ions. It has been reported that Cd induces the synthesis of phytochelatins (PCs), which bind cadmium in the cytosol and form a complex, which is then transported into the vacuole where it is accumulated with organic acids or as a high molecular mass PC-Cd complex (Rauser 1995, Hall 2002). The PC-Cd complex is up to 1000 times less toxic to many plant enzymes than free Cd ion (Kneer and Zenk 1992). Phytochelatins are low molecular mass polypeptides and are synthesized using glutathione (GSH) as a substrate by PC synthase (Rauser 1995, Hall 2002).

In this study, the effects of Cd on the seedling growth, non-protein thiols concentration, and content of Cd and its distribution in *B. chinensis* and *B. pekinensis* were investigated. The possible mechanisms of Cd tolerance in these two species were discussed based on the hydroponic culture experiments.

Materials and methods

Plants: Seeds of *Brassica pekinensis* (Lour.) Rupr. (cv. Qingyan 87-114) and *Brassica chinensis* L. (cv. Wuyueman) were surface-sterilized in 0.5 % NaClO and rinsed thoroughly with deionized water, then germinated for 3 d. Afterwards, the seedlings were transferred to 2 dm^3 pots containing Hoagland nutrient solutions about 20 plants per pot. Plants were allowed to grow for 30 d before treatments were initiated. The $CdCl_2$ was added into solution at the concentration of 0, 10, 20, 50, and 100 μM. Each treatment was replicated three times. The experiments were carried out in a greenhouse with day/night temperature of 23/15 ºC and natural light. The pH of nutrient solutions was adjusted to 6.0 with 1 mM HCl or 1 mM NaOH. Nutrient solutions were renewed every 3 d. The root lengths were determined on day 0, 5, 7 after the Cd treatment. The plants were harvested 7 d after the Cd treatment for analysis of the concentrations of non-protein thiols, contents of chlorophyll and Cd. The shoots and roots were washed with tap water for 10 min, then rinsed with deionized water. The plant materials for Cd analysis were dried at 80 °C for 72 h, and then were grounded in a Carnelian mortar.

Analytical methods: Chlorophylls were extracted with 80 % acetone, and estimated according to the method of Arnon (1949) by spectrophotometer (*UV-2450/2550*, *Shimadzu*, Kyoto, Japan). Non-protein thiols (NPT) were extracted by homogenising 0.5 g fresh leaves in 2 cm^3 of extraction buffer solution containing 5 % sulphosalicylic acid. The acid extract of the plant material was centrifuged at 10 000 *g* for 30 min at 4 ºC, and the NPT content was measured in the supernatant according to method of Ellman (1959).

The concentrations of Cd in plants were determined by the flame atomic absorption spectrometer (*TAS-986*, *Purkinje General Co.*, Beijing, China). The plant materials were acid-digested with a mixture of HNO_3:$HClO_4$ (87:13 v/v). Water-soluble Cd was extracted with 1 mM 2-morpholinoethanesulphonic acid buffer solution at pH 6.0 for 5 h (Cakmak and Marschner 1988). Cadmium bound on the root cell walls was measured with the method of Hart *et al.* (1992). Washed roots were dipped in a mixture of methanol:chloroform (1:1, v/v) solution for 3 d, and then washed with deionized water. The materials were dried at 80 °C, and then acid-digested with a mixture of HNO_3 and $HClO_4$ (87:13 v/v) for Cd.

Data reported in this paper were the mean values based on the three replicate results. Analysis of variance was performed on all data sets. Least significant difference (LSD) was used for multiple comparisons between different treatment means.

Results

Plant growth and chlorophyll content: 30-d-old seedlings were grown in the solutions with different Cd concentrations. The root length was determined at the 5th and 7th day after the Cd treatment. All Cd treatments significantly decreased the root elongation of *B. chinensis* and *B. pekinensis*, and the decrease was more pronounced with the increasing Cd concentrations in solution and the exposure time (Table 1). The Cd treatments with 10 to 50 μM Cd for 7 d decreased the net root elongation from 42 to 70 % for *B. chinensis,* and from 35 to 61 % for *B. pekinensis*, respectively. Compared with the control, 10 to 50 μM Cd in solutions had no significant effect on the shoot dry mass of *B. pekinensis*, whereas the negative effect on *B. chinensis* was observed at 20 μM Cd and above (Table 2). The root dry mass of *B. pekinensis* and

Table 1. The root elongation of *B. pekinensis* and *B. chinensis* during the Cd treatments. Different letters in the same column indicate a significant difference at $P \leq 0.05$ according to the LSD tests.

Cd conc. [μM]	Net root elongation [cm d^{-1}]			
	B. pekinensis		*B. chinensis*	
	day 0 - 5	day 5 - 7	day 0 - 5	day 5 - 7
0	0.29a	0.43a	0.45a	0.65a
10	0.23b	0.18b	0.31b	0.27b
20	0.17c	0.12c	0.22c	0.12c
50	0.13d	0.13c	0.16d	0.10cd
100	0.11d	0.09c	0.15d	0.08d

Table 2. The effects of various Cd concentrations on the dry mass and chlorophyll content of *B. chinensis* and *B. pekinensis*. Different letters in the same column indicate a significant difference at $P \leq 0.05$ according to the LSD tests.

Cd concentration [μM]	*B. pekinensis* Shoot [g plant^{-1}]	Root [g plant^{-1}]	Chl (*a*+*b*) [mg kg^{-1}(f.m.)]	*B. chinensis* Shoot [g plant^{-1}]	Root [g plant^{-1}]	Chl (*a*+*b*) [mg kg^{-1}(f.m.)]
0	0.354a	0.133a	1.06a	0.296a	0.103a	1.29a
10	0.346a	0.118ab	0.85b	0.276ab	0.069b	1.06b
20	0.330ab	0.102b	0.74bc	0.250bc	0.057bc	0.96c
50	0.318ab	0.084bc	0.71c	0.234c	0.049c	0.91c
100	0.297b	0.071c	0.68c	0.223c	0.046c	0.80c

B. chinensis decreased significantly with Cd concentrations ≥ 20 and ≥ 10 μM, respectively. These results indicated that the species *B. pekinensis* was more tolerant to Cd than *B. chinensis*. The root growth of both species was clearly more sensitive to high concentrations of Cd in solution than the shoot growth. Thus, the shoot/root ratio of dry matter increased with the increasing Cd supply in solutions.

The content of chlorophyll in the leaves of the both species decreased significantly with increasing Cd concentrations in the nutrient solution. The contents of chlorophyll were always lower in the leaves of *B. pekinensis* than in *B. chinensis* (Table 2).

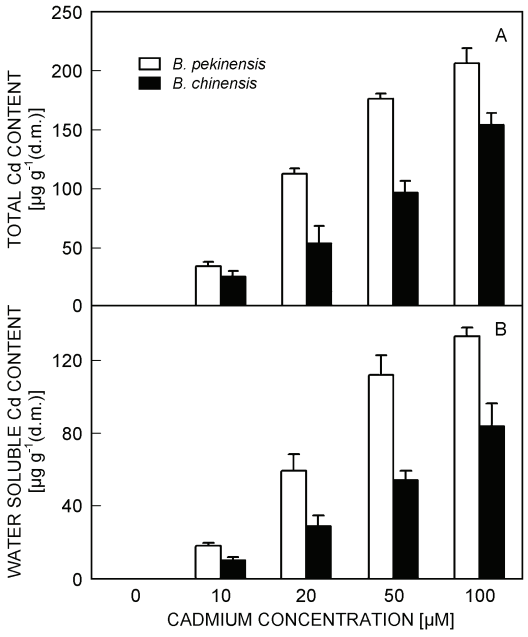

Fig. 1. The content of total Cd (*A*) and water-soluble Cd (*B*) in the shoots of *B. chinensis* and *B. pekinensis* 7 d after Cd treatment. Means of three replicates ± SE.

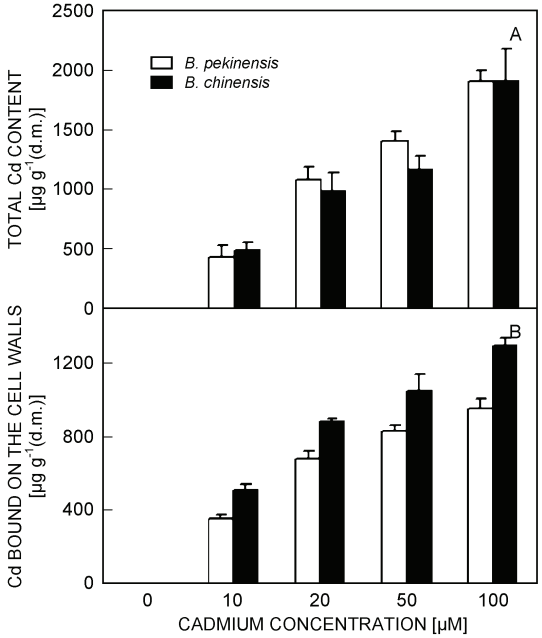

Fig. 2. The content of total Cd (*A*) and Cd bound on the cell walls (*B*) in the roots of *B. chinensis* and *B. pekinensis* 7 d after Cd treatment. Means of three replicates ± SE.

Accumulation and distribution of Cd in plants: The Cd concentrations in shoots and roots increased with the increasing Cd concentrations in the nutrient solution. In the 100 μM Cd treatment, the concentration of Cd in the shoots reached 206 and 154 μg g^{-1} dry mass in *B. pekinensis* and *B. chinensis* (Fig. 1*A*), respectively. In the shoots, a larger proportion of Cd was water-soluble, accounting for 40 - 64 % of the total Cd (Fig. 1*B*). The percentages of water-soluble Cd in the shoots increased with the increasing Cd supply in solution. Compared with *B. chinensis*, the shoots of *B. pekinensis* had significantly higher concentrations of total and water-soluble Cd. The percentages of water-soluble Cd in the shoots were also significantly higher in *B. pekinensis* than in *B. chinensis* in the treatments with ≥ 50 μM Cd in solution ($P < 0.05$). The roots of both species accumulated more Cd than the shoots in all Cd treatments (Fig. 2*A*). The shoot/root ratio of Cd concentrations in *B. pekinensis* was always greater than that in *B. chinensis*, indicating that the former was able to translocate more Cd from roots to shoots than the latter. In addition, most of the Cd in the roots of the two species was found on the cell walls (Fig. 2*B*). For the 10 μM Cd treatment in solution, Cd bound on the cell

walls accounted for 84.5 and 67.1 % of the total Cd in the roots of *B. chinensis* and *B. pekinensis*, respectively. The proportion of Cd bound on the cell walls decreased significantly with the increasing Cd concentration in solution (Fig. 2). No significant difference of the total Cd content in the roots was found between the two species (Fig. 2). However, the content and proportion of Cd bound on the cell walls were significantly higher in *B. chinensis* than in *B. pekinensis* (Fig. 2).

Non-protein thiols in shoots: The 10 μM Cd treatment had no significant effect on the NPT concentrations of the species. At 20 μM Cd, only in *B. pekinensis*, NPT significantly increased. At greater Cd concentrations (>20 μM), the NPT concentrations in the shoots increased significantly with the increasing Cd concentration. A significant correlation was found between the concentrations of soluble Cd and NPT in the shoots (r = 0.967 for *B. pekinensis*; r = 0.928 for *B. chinensis*; $P < 0.01$). The differences of the NPT concentration between the both species were highly significant ($P < 0.01$).

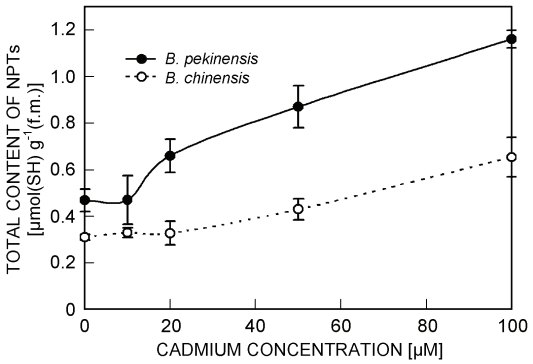

Fig. 3. The content of total non-protein thiols (NPTs) in the shoots of *B. pekinensis* and *B. chinensis* 7 d after Cd treatment. Means of three replicates ± SE.

Discussion

It has been found that some species of the *Brassica* genus, such as *Brassica juncea* (Kumar *et al.* 1995, Salt *et al.* 1995, Ru *et al.* 2004), *Brassica napus* (Carrier *et al.* 2003), *Brassica oleracea* (Pandey and Sharma 2002), are capable of accumulating high levels of Cd as well as other toxic metals in shoots from soil or hydroponic solution. Plants accumulating more than 100 mg kg^{-1} of Cd in their shoots are defined as Cd hyperaccumulator species (Baker and Brooks 1989). In the present study, *B. chinensis* and *B. pekinensis* were found to accumulate more than 100 μg(Cd) g^{-1}(d.m.) in their shoots. The maximum Cd content in the shoots of *B. chinensis* and *B. pekinensis* were two times higher than the criterion set by Baker and Brooks (1989). The results showed that *B. chinensis* and *B. pekinensis* had a high ability to take up Cd and transport it to the shoots as the Cd hyperaccumulator species.

Plant tolerance to heavy metals can be achieved by different strategies (Baker 1987, Sanità di Toppi and Gabbrielli 1999, Hall 2002). After 7 d of exposure to 10 - 100 μM Cd, the roots of *B. chinensis* and *B. pekinensis* contained about 8 - 14 and 15 - 23 times higher Cd than the shoots, respectively. The Cd resistance of the both plant species may be based on an exclusion mechanism, in which the roots accumulate the metal, and then prevent the Cd translocation to the shoots. Similar results were reported for many other plant species (Ramos *et al.* 2002, Zornoza *et al.* 2002, Stolt *et al.* 2003). In the present study, the majority of the Cd accumulated in the roots of the two species appeared to be bound on the cell walls, particularly in the treatments with lower Cd concentrations. The cell walls of plants have the capacity to bind metals by ionic and non-ionic interactions to pectins, glycoproteins and other components of cell walls (Sanità di Toppi and Gabbrielli 1999, Hall 2002). Some tolerant plants can hold heavy metals in cell walls (Ramos *et al.* 2002, Zornoza *et al.* 2002, Lou *et al.* 2004, Wójcik *et al.* 2005), reducing the formation of heavy metal complex with large mass molecules in plant cell plasma. The roots of *B. chinensis* had greater binding capacity of Cd in the cell walls. The amounts of Cd bound to the cell walls of *B. chinensis* and *B. pekinensis* accounted for 56.2 - 84.5 and 41.2 - 67.1 % of the Cd accumulated in roots, respectively. However, the variations of Cd bound to the cell walls did not relate to the total differential tolerance of the two species. Similarly, Macfie and Welbourn (2000) found more Cd accumulated internally by the walled strain than by the wall-less strain of the unicellular green alga (*Chlamydomonas reihardtii*). Thus, the Cd tolerance of the two species appears to involve other mechanisms besides the binding of the metal to the cell walls.

NPTs, which contain a high percentage of cysteine sulfhydryl residues, play an important role in heavy metal detoxification process in plants. Skórzyńska-Polit *et al.* (2003/4) reported that the content of SH-groups increased gradually with increasing Cd concentration in the nutrient solution. GSH and PC are important components of NPTs. In response to Cd stress, PC biosynthesis has been reported in a variety of plants species (Rauser 1995, Sanità di Toppi and Gabbrielli 1999, Hall 2002). GSH protects cells from the oxidative stress induced by heavy metals, and is the direct precursor of PC synthesis. The roles of GSH and PC synthesis in heavy metal tolerance have been well illustrated in Cd-sensitive mutants of *Arabidopsis* (Howden *et al.* 1995). In the present experiments, a marked increase of NPTs was found in the shoots of the both species after the treatment with 10 - 100 μM Cd in solution. The NPT increase was more pronounced in *B. pekinensis* than *B. chinensis*. The different tolerance of *B. chinensis* and *B. pekinensis* to Cd may be explained partially by the accumulation of NPT in

the shoots of the two plants. Lucarini *et al.* (1999) found that *Brassica* vegetables were rich in organic acids. In plant cells, Cd can be complexed by organic acids to reduce its toxicity (Sanità di Toppi and Gabbrielli 1999, Hall 2002).

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