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Original Research

Salix matsudana Koidz Tolerance Mechanisms to Cadmium: Uptake and Accumulation, Subcellular Distribution, and Chemical Forms

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Abstract

Salix matsudana roots exposed to 10, 50, and 100 μM Cd solutions for 24 h were carried out in order to understand the mechanisms involved in Cd tolerance and detoxification. 50 and 100 μM Cd inhibited root length significantly ($P < 0.05$). Cd levels in roots increased significantly with increasing Cd concentrations, and the contents of Fe, Mn, Zn, and Ca decreased significantly. A Cd-specific Leadmium Green AM dye probe showed that the meristem zone was the absorption and accumulation site of Cd in the roots. Subcellular fractionation of Cd-containing tissues indicated that about 53% of the Cd was accumulated in the cell wall of *S. matsudana* roots at 10 μM Cd and 65% of the Cd at 100 μM Cd, indicating that Cd binding and/or precipitation in the cell wall in roots may serve as the first barrier to reduce the cytosolic-free Cd ions. The proportion of CdE and Cdw in roots is low when compared with the other Cd chemical forms. CdHCl, Cdr, and CdHAc represent 46% (10 μM Cd), 49% (50 μM Cd), and 59% (100 μM Cd) of total Cd, and CdNaCl represents 42% (10 μM Cd), 44% (50 μM Cd), and 32% (100 μM Cd).

Keywords: cadmium (Cd), chemical forms, *Salix matsudana* Koidz, subcellular distribution, uptake and accumulation

Introduction

Cadmium (Cd) is inorganic minerals in the earth's crust [1]. Cd contamination is considered a major environmental problem due to its great toxicity to all organisms and its high mobility and risk to the food chain [2, 3]. Cd is a non-essential element, and excessive Cd can target and damage many cellular activities and processes such as photosynthesis, carbohydrate and nitrate metabolism, water balance, and DNA and lipid matrix, resulting in growth inhibition, morphological alterations, and plant senescence or even death [4]. Cd interferes with the uptake, transport, and use of different macro- and micronutrients [5]. Microelements (zinc (Zn), iron (Fe), manganese (Mn), and selenium (Se)) interfering with Cd uptake may decrease Cd concentrations in plants [1].

Recent research has focused on the accumulation and tolerance mechanisms of heavy metals in plants. Subcellular partitioning of metals within living cells has

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attracted great interest because of their importance in eco-toxicological and trophic transfer studies [6]. Non-essential metals accumulated in the cell wall and vacuole is another detoxification process that prevents them from entering more sensitive cell metabolic sites [7]. The vacuole can be considered the most important component in the soluble fraction of a cell [8]. In order to avoid Cd toxicity, plants have developed intra and extra cellular mechanisms for metal detoxification, such as binding and precipitation in the cell wall and/or compartmentalization in vacuoles [9-10].

Regionalization of cell wall deposition and vacuolar compartmentation play a major role in heavy metal detoxification, tolerance, and hyperaccumulation in plants [11]. Cell walls and vacuoles in plant roots are considered to have great potential for Cd accumulation [6]. There is some evidence that subcellular distribution and chemical forms of heavy metals may be associated with metal tolerance and detoxification in plants [12]. Cd phytotoxicity was related to the Cd chemical forms in plant tissues and their mobility in the plants [13]. Ernst et al. [14] and Rauser [15] indicated that Cd bound by pectate and chelated by peptides, polypeptides, or proteins is a defense mechanism to reduce the Cd biological activity in plants. Evidence on Cd integration in pectates and proteins and Cd ligands in vacuoles was reported [8, 11]. However, the stored forms of Cd in different plant tissues are concerned with the capacity of plant Cd tolerance and accumulation [13, 16, 17]. Larger proportions of NaCl-extractable Cd in plants can be thought to play an important role in the alleviation of Cd toxicity. The complexation of metals with organic ligands can result in decreased free ion activity and thus reduce their toxicity [18].

Willows (Salix spp.) is known to have several characteristics that make them ideal plant species for phytoremediation application, including easy propagation and cultivation, large biomass, fast growing, deep root system, high transpiration rate, tolerance to hypoxic conditions, and high metal accumulation capability [19]. *Salix matsudana* Koidz is one of the most widely distributed and commonly cultivated willow species in China [20]. It also has a deep root system compared to grasses, which can act as a biological filter. These traits make it a potential ideal candidate for phytoremediation of Cd-contaminated waters and soils [21, 22].

Root is the plant organ for the uptake of various nutrients as well as metals, including Cd. Resistance to Cd stress is associated with two aspects: avoidance and tolerance. Avoidance is a basic mechanism to mitigate Cd toxicity, which involves decreasing the amount of Cd entering the cell by extracellular precipitation, biosorption to cell walls, reduced uptake, or increased efflux. Tolerance means plants survive in the presence of high internal metal concentration [1, 23]. Excessive Cd has often caused poisoning and environmental contamination. Therefore, the evaluation of action mechanisms of Cd toxic to *S. matsudana* roots and their consequences on absorption site, uptake, accumulation, and its effects on elements, subcellular distribution, and chemical form, as performed in the present investigation, is important. Therefore, the aims of this study were to investigate the characteristics of Cd uptake and accumulation, localization, and subcellular distribution, and chemical forms in *S. matsudana* roots and their implication in Cd tolerance.

Materials and Methods

Plant Material and Growth Conditions

Healthy and equally sized cuttings (25 cm long) from 1-year-old shoots of *S. matsudana* were collected and fully rinsed with distilled water before starting the experiments. After dipping in distilled water at room temperature, 10-day-old healthy seedlings were transferred to a half-strength Hoagland nutrient solution and grown for a week. Then Cd was added to the corresponding containers to form four treatments: basal nutrient solution (control, without Cd) and 10, 50, and 100 μM Cd. for 24 h. Cadmium was provided as cadmium chloride ($CdCl_2$).

The nutrient solution consisted of 5 mM Ca $(NO_3)_2$, 5 mM KNO_3, 1 mM KH_2PO_4, 1 mM $MgSO_4$, 50 μM H_3BO_3, 10 μM FeEDTA, 4.5 μM $MnCl_2$, 3.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$, and 0.1 μM $(NH_4)6Mo_7O_{24}$, adjusted to pH 5.5. Control seedlings were grown in the nutrient solution alone. The solutions were continuously aerated with an aquarium air pump every day.

Determination of Cd and Other Minerals

Seedlings exposed to 10, 50, and 100 μM Cd solutions for 24 h and control were harvested respectively based on uniformity of size and colour (removing the greatest and the smallest seedlings and then selected randomly). The seedlings were washed with 20 mM EDTA, thoroughly with running tap water, and then with deionized water, and roots were separated manually and dried in an oven at 80ºC for one week for metal analysis. Accumulations of Cd, Mn, Zn, Ca, and Fe were analyzed using inductively coupled plasma atomic emission spectrometry (ICP-AES, LEEMAN LABS Inc., USA) [24].

Tissue Fractionation

Cells were separated into three fractions (cell wall, organelle-containing, and soluble fractions) according to the improved method reported by [8, 25]. *S. matsudana* roots exposed to 10, 50, and 100 μM Cd solutions for 24 h were separated, then rinsed cleanly with 20 mM EDTA and deionized water. Each sample was homogenized in 20 mL cooled extraction buffer (50 mM Tris-HCl, 250 mM sucrose and 1.0 mM $C_4H_{10}O_2S_2$, pH 7.5) with a chilled mortar and a pestle. The homogenate was centrifuged at 4,000 rpm for 15 min; centrifugation was repeated twice and the precipitate was designated as “cell wall-containing fraction” consisting mainly of cell walls and cell wall debris. The supernatant

solution was further centrifuged at 12,000 rpm for 45 min. The resultant deposition and supernatant solution were referred to as the “organelle-containing fraction” and the “soluble fraction,” respectively. All steps were performed at 4ºC. The different fractions were wet-digested with HNO_3:$HClO_4$ (4:1, v/v), and the content of Cd in the solutions was measured using ICP (ICP-AES, LEEMAN LABS Inc., USA).

Chemical Forms Extraction

The Cd associated with different chemical forms was successively extracted by designated solutions in the following order [8, 26]:

1. 80% ethanol, extracting inorganic Cd giving priority to nitrate, chloride, and aminophenol cadmium (CdE).
2. Deionized water, extracting water-soluble Cd with organic acids and $Cd(H_2PO_4)_2$ (Cdw).
3. 1 M NaCl, extracting pectate- and protein-integrated Cd (CdNaCl).
4. 2% HAc, extracting insoluble $CdHPO_4$, $Cd_3(PO_4)_2$, and other Cd-phosphate complexes (CdHAc).
5. 0.6 M HCl, extracting oxalic acid bound Cd (CdHCl).
6. Cadmium in residues (Cdr).

The Roots of *S. matsudana* Seedlings exposed to 10, 50, and 100 μM Cd solutions for 24 h were separated, then rinsed cleanly with 20 mM EDTA and deionized water. Each sample was extracted with 20 mL of buffer solution that contained designated solutions (as shown above). Firstly, the homogenate was shaken for 22 h at 25ºC and centrifuged at 5,000 rpm for 10 min. Then the supernatant was collected. After that, 10 mL of buffer solution was added to the centrifuge tube and the tube was shaken for 2 h at 25ºC. At last, the homogenate was centrifuged under the same conditions for another 10 min and the supernatant was collected. This procedure was repeated twice. The Cd content of the plant material remaining after all of the extractions and all of extracting solution had been conducted was determined by wet-digesting it with HNO_3:$HClO_4$ (4:1, v/v). The content of Cd in different chemical forms was measured by ICP (ICP-AES, LEEMAN LABS Inc., USA).

Fluorescence Labeling of Cd

The Cd Probe Leadmium Green AM dye (Molecular Probes, Invitrogen, Calsbad, CA, USA) was used to investigate the distribution of Cd in roots of plants exposed to 10, 50, and 100 μM Cd solutions for 24 h. Fresh and intact root tips of *S. matsudana* were incubated for 10 min in excess of 20 mM disodium ethylenediamine tetra-acetic acid (Na_2-EDTA) at room temperature, and then thoroughly washed with deionized water. A Cd-specific probe was made by adding 50 μL of dimethyl sulfoxide to one vial of the dye. This stock solution was then diluted 1:10 with 0.85% NaCl [27]. The roots were immersed in diluted stock solution at 40ºC for 90 min in the dark. The sections were examined with a laser confocal scanning microscope (ECLIPSE 90i, Nikon, Japan) with excitation and emission wavelengths at 488 and 515 nm, respectively. The fluorescence density was measured using the analyze and measure function of the Image J software (NIH, Bethesda, MD, USA).

Statistical Analysis

Data from this investigation were analyzed with Sigma Plot 13.0 using means ±standard error (SE). For equality of averages the t-test was applied. Results were considered statistically significant at $P < 0.05$.

Results

Effects of Cd on Root Growth

The effects of Cd on the root growth of *S. matsudana* varied with the different treatment concentrations used (Fig. 1). Compared with control, Cd had no toxic effect on root growth at 10 μM Cd during 24 h treatment. However, in concentrations of 50 and 100 μM Cd an obvious toxic effect appeared and Cd inhibited root growth significantly ($P < 0.05$).

Cd Accumulation and its Effects on Other Minerals

Statistical analysis showed the presence of significant correlations between the concentrations of Cd and microelements (Fe, Mn, Zn, and Ca). The accumulation of Cd in *S. matsudana* roots varied with the different treatment concentrations. The level of Cd in roots increased significantly ($P < 0.05$) with increasing Cd concentrations when compared with control (Table 1). Cd had obvious inhibitory effects on uptake and accumulation of Fe, Mn, Zn, and Ca in roots. The contents of Fe, Mn, Zn, and

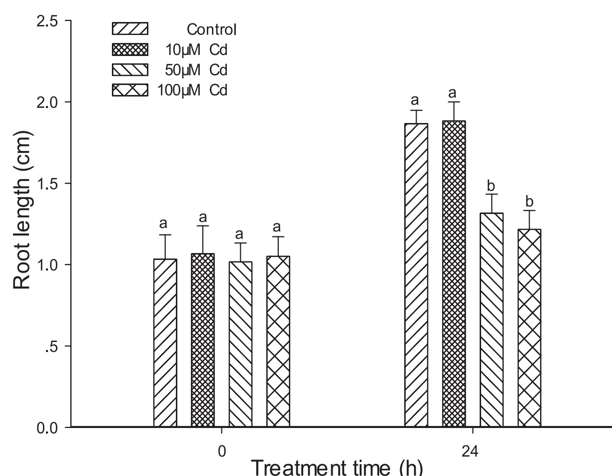

Fig. 1. Effects of Cd on root length of S. matsudana exposed to 10, 50, and 100 μM Cd for 24 h. Vertical bars denote SE. Values with different letters differ significantly from each other (n = 10, $P < 0.05$).

Table 1. Cd, Mn, Fe, Zn, and Ca accumulation by *S. matsudana* roots exposed to different concentrations of Cd for 24 h.

Element	μg g^{-1} dry weight			
	Treatment μM			
	Control	10	50	100
Cd	0.00±0.00a	274.93±0.90b	866.38±3.48c	1477.338±2.91d
Fe	723.55±10.64a	474.83±3.27b	449.37±2.58c	359.04±3.97d
Mn	547.56±0.96a	421.96±2.28b	389.37±6.47c	120.38±1.04d
Zn	590.85±2.28a	397.0±4.57b	365.77±11.52c	249.17±6.83d
Ca	5614.95±24.49a	4777.89±44.18b	4457.88±15.41c	3109.67±10.20d

Values followed by different letters are significantly different ($P < 0.05$). Vertical bars denote SE (n = 4).

Ca in roots decreased significantly with increasing Cd concentrations ($P < 0.05$). In concentrations of 100 μM Cd group, Cd stress caused a 50.4% decrease of Fe content, 78.0% of Mn, 57.8% of Zn, and 44.6% Ca in roots when compared with the control group.

Cd Distribution in Different Zones

The distribution of Cd in the root tips of *S. matsudana* exposed to 10, 50, and 100 μM Cd for 24 h was investigated using the Cd-specific Leadmium Green AM dye probe (Invitrogen, Carlsbad, CA, USA; Fig. 2). The fluorescent dye showed a bright and clear green fluorescence in the root tip cells of Cd-treated roots, whereas there was no green fluorescence signal in control root tips (Figs 2A1-A2). The fluorescent dye loaded into the roots showed different fluorescence intensity in the root tip cells exposed to different concentrations of Cd for 24 h. A weak green fluorescence labeling of Cd was distributed in the meristematic cells exposed to 10 μM (Figs 2 B1-B2). At 50 μM Cd, the labeling of the meristematic cells increased (Figs 2C1-C2). The strongest fluorescence in meristematic cells was observed at 100 μM Cd of incubation (Figs 2D1-D2).

These results indicated that Cd ions were localized in meristematic cells and increased with increasing Cd concentrations. The fluorescence density analysis of Cd was carried out by Image J software, confirming the observations mentioned above (Fig. 3). The data showed that the meristem zone was the absorption and accumulation site of Cd in the roots of *S. matsudana* under Cd stress.

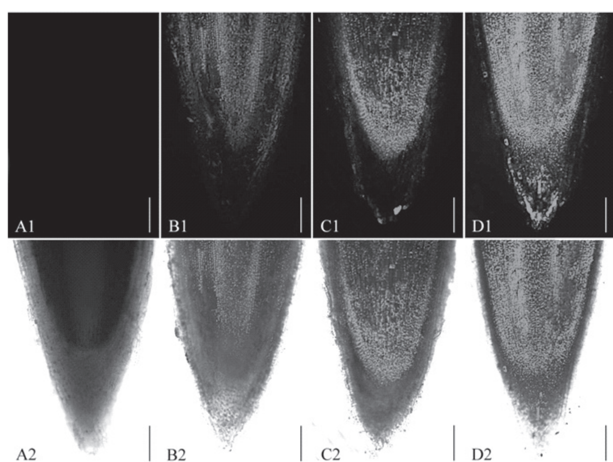

Fig. 2. Micrographs of roots from *S. matsudana* exposed to Cd using Leadmium Green AM dye at longitudinal section (A1-D1). Showing Cd detection images of the roots exposed to 0 (A), 10 (B), 50 (C), and 100 (D) μM Cd for 24 h, respectively (A2-D2). Showing fluorescence labeling merged with a bright field. All images were taken with decuple magnification, Scale bars = 200 μm, and green fluorescence represents the binding of the dye to Cd.

Subcellular Distribution of Cd

The subcellular distribution of Cd in roots was expressed as Cd concentration in the different fractions. In the present investigation, the subcellular distribution of Cd in *S. matsudana* roots exposed to 10, 50, and 100 μM Cd for 24 h was in the following order: cell wall > soluble fraction > cell organelle (Table 2). The levels

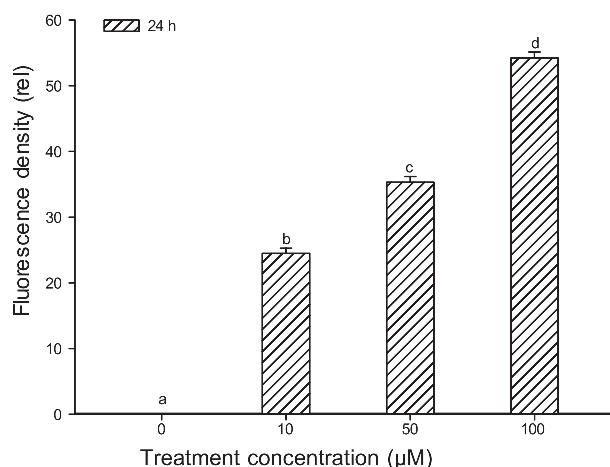

Fig. 3. Leadamium Green AM dye fluorescence density from the longitudinal section in *S. matsudana* on roots exposed to 10, 50, and 100 μM Cd for 24 h. Values with different letters differ significantly from each other (n = 5, $P < 0.05$). Data are means ±SE.

Table 2. Subcellular distribution of Cd content in different organs in roots of *S matsudana* exposed to 10, 50, and 100 μM Cd for 24 h.

Treatment (μM)	Cd in subcellular distribution (μg/g FW)		
	Cell wall	Cell organelle	Soluble fraction
10	142.05±1.99a	33.54±1.74a	90.36±0.37a
50	507.64±2.59b	95.844±0.71b	240.46±0.50b
100	947.64±7.06c	129.19±3.71c	381.37±1.02c

Values followed by different letters differ significantly from each other (P <0.05, t-test). Means ±SE, n = 4.

of Cd increased significantly (P < 0.05) with increasing Cd concentrations. Data also revealed that the contents of Cd in the cell wall, soluble fractions, and cell organelle of *S. matsudana* roots were 142.0, 90.4, and 33.5 μg/g, respectively, after 24 h treatment with 10 μM Cd. However, the accumulation of Cd increased by 5.7-, 3.2-, and 2.9-fold in the roots exposed to 100 μM Cd for 24 h in comparison with the 10 μM Cd treatment (Table 2).

During the whole experiment the maximum proportion of Cd in roots was related to the cell wall, followed by the soluble cell organelle fraction. With the increase of Cd concentration, the proportion of Cd in cell wall increased, while the proportion of Cd in soluble and cell organelle fraction decreased (Fig. 4). For instance, with exposure to 100 μM Cd, on average 65.0, 26.0, and 9.0% Cd was distributed in the cell wall and soluble and cell organelle fractions, respectively. The evidence indicated that the cell wall was the main accumulation site for Cd.

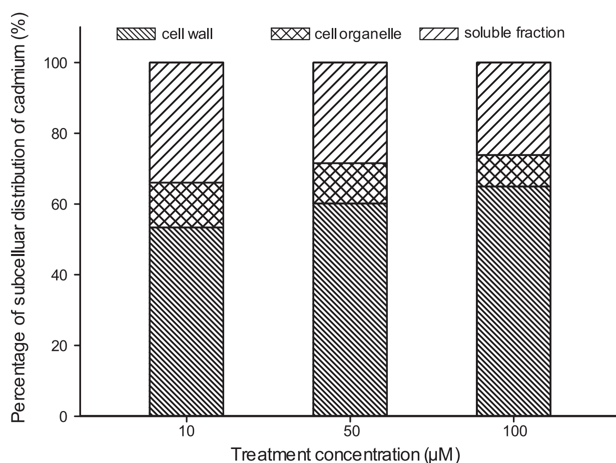

Fig. 4. Proportional changes of Cd ion contents in cell wall, soluble, and cell organelle fraction in roots of *S. matsudana* exposed to 10, 50, and 100 μM Cd for 24 h.

Chemical Forms of Cd

Cd bound to the different chemical forms in the roots of *S. matsudana* can be measured with different extracting agents. The Cd content and the percentage of the variety of chemical forms were shown in Table 3 and Fig. 5. The Cd content of the different chemical forms (CdE, Cdw, CdNaCl, CdHAc, CdHCl, and Cdr) in roots all increased significantly (P < 0.05) with increasing concentrations of Cd (Table 3). The proportion of the chemical forms varied with Cd concentration used during the whole experimental treatment (Fig. 5). The proportion of CdNaCl in roots exposed to 10 and 5o μM Cd was the highest, follow by CdHCl, CdHAc, Cdw, CdE, and Cdr. However, at 100 μM Cd, the proportion of Cd chemical form from high to low is in the order: CdNaCl > CdHAc > CdHCl > Cdw > CdE > Cdr, respectively. During the whole experiment, the proportion of Cd extracted by 0.6 M HCl and 2% HAc increased with increasing Cd concentrations, while the proportion of Cd extracted by 1 M NaCl decreased (Fig. 5).

Discussion

Recently, fluorescent Cd reagent has been applied only rarely in plant cytological studies. Cd ions have a high affinity for sulphur-, nitrogen-, or oxygen-containing ligands. They can bind strongly to SH-containing enzymes/proteins inside the cell. Obviously, Leadmium Green AM dye has higher affinity for Cd than internal proteins [28] and is not sensitive to other divalent ions

Table 3. Different chemical forms of Cd in roots of *S. matsudana* exposed to 10, 50, and 100 μM Cd for 24 h.

Treatment (μM)	Extractable form (μg/g FW)					
	CdE	CdW	CdNaCl	CdHAc	CdHCl	CdR
10	7.85±0.15a	24.36±0.80a	115.12±0.70a	55.18±2.29a	60.52±0.21a	7.64±0.06a
50	17.36±0.19b	45.20±0.55b	374.92±1.19b	196.92±1.124b	208.24±3.59b	10.06±0.43b
100	40.50±0.97c	85.75±2.11c	493.53±2.29c	446.52±2.62e	427.19±2.67c	32.49±0.31c

Values followed by different letters differ significantly from each other (P < 0.05, *t*-test). Means ±SE, n = 4.

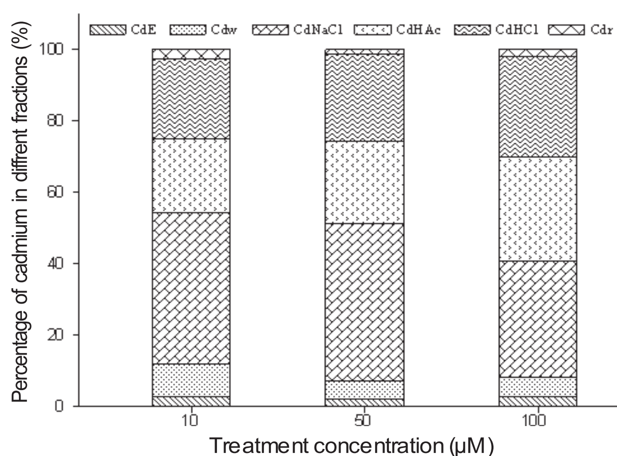

Fig. 5. Different chemical forms of Cd proportion in roots of *S. matsudana* exposed to 10, 50, and 100 μM Cd for 24 h.

except for lead. In this work, the uptake of Cd into root cells of *S. matsudana* using Cd-sensitive Leadmium Green was studied, suggesting that the meristematic zone is the primary site of Cd uptake and accumulation, which supported our previous observations, where Cd was found to be absorbed within hours in the root cells of *Allium sativum* exposed to Cd [29]. As for excessive Cd ions accumulating in the meristem (50 and 100 μM Cd), they disturb cell division and result in the inhibition of the root growth of *S. matsudana*.

The subcellular distribution of Cd in plants is thought to be important in influencing the accumulation, migration, and phytotoxicity of Cd in different species or genotypes [16, 30]. Plant cell walls contain polyoses such as cellulose, hemicellulose, lignin, and mucilage glue as well as proteins, and can potentially bind to Cd ions and restrict Cd from entering the cell protoplast and moving to the upper parts of the plant [16]. In the present investigation about 53% of the Cd was accumulated in the cell wall of *S. matsudana* roots at 10 μM Cd and 65% of the Cd at 100 μM Cd, indicating that Cd binding and/or precipitation in the cell wall in roots may serve as the first barrier to reduce the cytosolic free Cd ions and lessen the damage to the root cells (Table 2).

The results obtained here are consistent with earlier findings [7, 11, 31, 32]. However, Carrier et al. [33] reported that approximately 80% of Cd was found predominantly in the soluble fraction, and relatively little Cd was found in the cell wall (11%). Lozano-Rodríguez et al. [34] indicated that maize was more tolerant to Cd than pea by incorporating more Cd into the cell wall, while pea showed more severe damage caused by higher concentration of Cd in the soluble fraction. Thus plant responses to Cd may be affected by the species, cultivar, and stress level [35, 36]. The retention of Cd in root cell walls and compartmentalization of Cd into vacuoles are the most important mechanisms involved in the detoxification of Cd in *S. matsudana*.

The next large proportion of Cd in *S. matsudana* roots was observed in soluble fractions in this work (Fig. 4). Once Cd ions are inside the cytosol, plants can avoid Cd stress by minimizing the concentration of free Cd in the cytosol by forming metal chelates/complexes [37-38]. The high proportion of Cd in the soluble fraction may limit root-to-shoot translocation of Cd, resulting in low Cd levels in the shoots [39].

It has been reported that Cd chemical forms in plants are considered important factors that affect the characteristics of Cd migration, accumulation, and phytotoxicity degree [13, 30]. Water-soluble Cd in inorganic form (extracted by 80% ethanol) and organic form (extracted by H_2O) are thought to have more deleterious and induced stunted growth and chlorosis in the plants due to its highest capacity to migrate, followed by pectate-and protein-integrated Cd (NaCl fraction), undissolved Cd phosphates (HAC fraction), oxalic-acid bound form (HCl fraction), and residues [13, 30, 32, 40].

The high Cd concentrations of the toxic forms of Cd in the cell organelles could seriously damage the cells and the metabolic processes in plants [7]. In the present investigation we observed that the proportion of CdE and Cdw in roots is low when compared with the other Cd chemical forms. CdHCl, Cdr, and CdHAc represent 46% (10 μM Cd), 49% (50 μM Cd), and 59% (100 μM Cd) of total Cd, indicating that the Cd is transformed into a non-toxic or low toxicity complex to protect the cells. CdNaCl represents 42% (10 μM Cd), 44% (50 μM Cd), and 32% (100 μM Cd). It was demonstrated that NaCl extractant binds to proteins and pectic acids, with Cd being fixed by pectic acids [7]. Vacuolar sequestration and cell wall binding play a major role in hyperaccumulation of heavy metals [8]. The vacuole appears to be the main site for Cd accumulation in the plant. Salt et al. [41] indicated that the organo-ligands responsible for Cd compartmentation in the vacuoles were mainly sulfur-rich peptides and organic acids. Wu et al. [26] reported that a Cd-sensitive barley genotype had a larger amount of Cd in inorganic and water-soluble forms as compared with the Cd-resistant genotypes. The cell wall is the first barrier protecting the protoplast from Cd toxicity because it is composed of polyoses and protein [42]. As Cd has a strong affinity to proteins or sulfhydryl compounds (–SH) and other side chains, it can easily combine with proteins [5] and disturb enzyme activity [7]. It was reported that the cell wall played a role in metal tolerance when the cell wall volume was high compared to the cytosol and vacuole [43].

The data from inductively coupled plasma mass spectrometry (ICP-MS) revealed that 56% of total Cd was located in cell walls of *Allium cepa* epidermal cells exposed to Cd [31]. Similarly, the data from energy-dispersive x-ray analyses (EDXA) indicated that Cd ions appeared in walls of root cells of *A. cepa* treated with Cd [42]. Cytochemical evidence also confirmed that cysteine-rich proteins were localized in electron-dense granules in root cell walls of *A. cepa* [44], which seemed to contribute substantially to Cd detoxification.

Cd can alter the uptake of minerals by plants through its effects on the availability of minerals from the soil, or through a reduction in the population of soil microbes

[45]. Microelements such as Zn, Fe, Ca, and Mn play a vital role in mitigating Cd stress to plants by activating certain Cd avoidance and/or tolerance mechanisms in plants [23, 46]. Mn is an essential micronutrient in plants and is associated with some Mn-metalloproteins [47]. The availability of Mn to plants decreases in the presence of Cd in soil [48].

Data from this investigation showed that Mn accumulation was reduced significantly ($P < 0.05$) in *S. matsudana* roots exposed to different Cd concentrations, and progressively decreased with an increase in Cd concentration. Dong et al. [49], through regression analysis, showed that there was a significantly negative correlation between Cd and Mn, implying an antagonistic effect of Cd on Mn absorption and translocation. Peng et al. [50] found that adding Mn to the solution containing Cd significantly improved plant growth and reduced the concentrations of Cd in all organs of the plant. Zn is known to play a crucial role in protein metabolism, gene expression, chromatin structure, and photosynthetic carbon metabolism [51]. In addition, it is associated with the stabilizing and protective effect on biomembranes against oxidative and peroxidative damage, loss of plasma membrane integrity, and alteration of the permeability of the membrane [52, 53]. The results here revealed that Zn accumulation in roots decreased significantly ($P < 0.05$) with increasing Cd concentration. There is a strong competition between Zn and Cd [54], because Cd and Zn are taken up as divalent cations and have a similar chemistry [1, 55]. The Fe level in roots of *S. matsudana* showed significant decrease under Cd stress. Kovács et al. [56] indicated that Fe ions may compete with Cd ions for the same membrane binding (transport) sites in plants, and so an adequate Fe ion supply may decrease Cd uptake and relieve Cd toxicity in plants. Studies have provided clear evidence that Fe can alleviate to some extent the Cd-induced inhibitory effects on plant growth [1]. For instance, adding an Fe supply to plants exposed to Cd can result in increased activity of antioxidative enzymes – an important defensive mechanism against oxidative stress [57]. Data from ICP-AES in the present investigation showed that Ca content in the roots of *S. matsudana* exposed to Cd decreases significantly ($P < 0.05$). It is well known that Cd and Ca have similar ionic radii (0.099 nm and 0.097 nm, respectively). Shortly after Cd enters the cytoplasm, it rapidly binds to certain sites of the root tip apoplast, modifying the concentration of free cytosolic Ca ions, affecting the function of the plasmalemma pumps transporting Ca^{2+}, and resulting in a disturbance of Ca uptake and physiological activities.

Conclusions

Based on the information provided in this article, it is concluded that:

1. Under Cd stress, more Cd ions exist in the meristem zone, suggesting that the meristem zone of root tips is the main site of Cd uptake and accumulation.
2. Contents of Mn, Zn, Fe, and Ca in the root tip cells of *S. matsudana* decrease, while Cd level increases.
3. Cd ions mainly exist in cell walls, suggesting that the cell wall barrier is crucial for the detoxification of Cd.
4. The proportion of CdE and Cdw in roots is very low, although they have more deleterious biological activity. CdHCl, Cdr, and CdHAc have low toxic effects when compared with CdNaCl. CdNaCl represent 42% (10 μM Cd), 44% (50 μM Cd), and 32% (100 μM Cd) of total Cd. It can be suggested that Cd integrated with pectates and proteins in cell walls may be responsible for the adaptation of *S. matsudana* to Cd stress.

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