Short Communication

Uptake and Accumulation of Cadmium and Relative Gene Expression in Roots of Cd-resistant *Salix matsudana* Koidz

**Hangfeng Wu, Jiayue Wang, Binbin Li, Yangjie Ou,
Wusheng Jiang, Donghua Liu, Jinhua Zou***

Tianjin Key Laboratory of Animal and Plant Resistance, College of Life Sciences, Tianjin Normal University,
Tianjin 300387, People's Republic of China

Abstract

Salix matsudana is thought to be an ideal woody plant for use in phytoremediation programs in China. This study deals with the characterization of early responses to Cd in accumulation and its effects on other metals, and relative gene expression in roots exposed to 50 μM of Cd for 1 to 24 hours. The Cd content in roots exposed to Cd for 1, 3, 6, 12, and 24 hours of treatment was approximately 280, 587, 605, 622, and 795 μg/g DW, respectively. After 24 hours, Cd stress caused a decrease of iron (Fe) (34.1%), manganese (Mn) (60.1%), zinc (Zn) (40.7%) and calcium (Ca) (26.5%). After 24 hours of exposure, the relative expression of IRT1 was 6.7 times that of control treatment ($P<0.05$). A 160.8% increase was detected for the relative expression of NRAMP1 after exposure to Cd treatment for one hour. After three hours of stress, the expression of ZIP1 was 10 times that of control ($P<0.05$). The tolerance of plants to Cd involves gene expression, protein modification, and alterations in the coordination of major and secondary metabolites, which is a complex physiological and biochemical process.

Keywords: cadmium (Cd), elements, gene expression, *Salix matsudana* Koidz, uptake and accumulation

Introduction

With the rapid development of modern agriculture, industry, and anthropogenic activities, trace concentrations of cadmium (Cd) in the environment tend to accumulate to toxic concentrations. Cd is used in mining, electroplating, iron and steel manufacturing, and the battery production process, and is often discharged into the environment [1]. Cd is considered the most harmful and common pollutants in agricultural soils and other plant environments [2-3]. In China, nearly 5 million acres of arable land have been contaminated by heavy metals [4]. According to reports, Cd concentrations in soil near smelters in China is extremely high – up to 11.2-197.3 mg/kg [5-6]. Although Cd is a non-essential element of crops, it is easily absorbed by plants growing in Cd-polluted soils.

Several genes have been demonstrated to be involved in metal accumulation and tolerance, detoxification, vacuolisation, translocation (to shoots), and balance of nutrients in plant roots [7-10]. Recent studies have indicated that Zn-regulated transporter-like proteins and Fe-regulated transporter-like proteins (ZIPs) are involved

*e-mail: zjhmon@163.com

in the hyperaccumulator Thlaspi caerulescens [11], the natural resistance-associated macrophage protein (NRAMP), the cation diffusion facilitator protein (CDF) family [12], and the heavy metal-transporting P1B-type ATPase (HMA) [13]. Cd is most likely crossing the plasma membrane via members of the ZIP transporter family (ZRT-IRT like protein; zinc-regulated transporter, iron-regulated transporter protein) (Guerinot, 2000). IRT1 (iron-regulated transporter protein) is not only the main root transporter of Fe taken from the soil, but is also the main entry route of Cd [14]. Besides IRT1, ZIP1 (zinc-regulated protein) has been shown to transport Cd in plants [15-16].

The NRAMP family metal transporters function as Fe transporters. NRAMP5 is involved in the transportation of Cd from the soil to root cells and is also a transporter of Mn [12]. NRAMP1 is localized in the plasma membrane of root cells, functioning as a high-affinity transporter for Mn uptake [17]. AtNRAMP1 expression induced by Fe deficiency in roots is thought to control Fe homeostasis in plants [18-19] and may be involved in an enhanced influx of Cd into the cells, causing Cd toxicity [20-21]. HMA3 may be involved in the detoxification of metals by chelating Cd into the vacuole [22]. Plant roots are well known as an organ sensitive to environmental stress [23]. Gene expression differences can reveal metal absorption, accumulation, and tolerance traits. Therefore, monitoring differentially relative expression levels of genes under Cd stress is very useful for understanding Cd hyperaccumulation/tolerance mechanisms.

The objectives of the present study were to investigate uptake and accumulation of Cd and relative gene expression (IRT1, NRAMP1, and ZIP1 genes) in roots of Cd-resistant *S. matsudana* exposed to 50 μM Cd over a short period of treatment (1, 3, 6, 12, and 24 hours). The identified physiological and molecular data provide novel insight into Cd tolerance in woody plants, which can in turn play an important role in phytoremediation investigations.

Materials and Methods

Plant Material and Growth Conditions

Woody cuttings (25 cm long) from one 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 plants were transferred to half-strength Hoagland nutrient solution and grown for a week. In the previous investigation, we treated *S. matsudana* with a different concentration of Cd and found that 50 μM Cd is only just a little influence on phenotype and growth. Thus, we chose this concentration in the present investigation. They were spiked with 50 μM Cd for 1, 3, 6, 12, and 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

Both control plants and plants exposed to 50 μM of Cd for 1, 3, 6, 12, and 24 hours were harvested based on uniformity of size and colour (removing the greatest and the smallest plants, followed by random selection). The roots for each treatment were washed thoroughly with running tap water for 30 minutes, incubated for 10 minutes in 20 mM of disodium ethylenediamine tetra-acetic acid (Na_2-EDTA) at room temperature, and then washed with deionized water to remove traces of nutrients and Cd ions from their surfaces. The roots were dried at 45ºC for 72 hours, 80ºC for 24 hours, and 105ºC for 12 hours. After weighing, dried-root material (0.2 g) was digested with a mixture of HNO_3 and $HClO_4$ (4:1, v/v) at a controlled temperature of 160ºC; the ratio of the volume (mL) of the mixture acid/mass to the tissue (g) was 20:1. After dry-ashing, concentrations of Cd, Mn, Zn, Ca, and Fe were analyzed using inductively coupled plasma atomic emission spectrometry (ICP-AES, Leeman Labs Inc., USA).

Total RNA Extraction and First-Strand cDNA Synthesis

Roots of control and treatment groups exposed to 50 μM of Cd for 1, 3, 6, 12, and 24 hours were used for gene expression analysis. They were separated and washed with deionized water. Total RNA was extracted using the easy-spin Plant RNA Extraction Kit (Aidlab, China), and First-Strand Synthesis SuperMix (TransScript, China) was used for cDNA synthesis – both according to the manufacturers' instructions. RNA integrity was verified by running agarose gel electrophoresis and detecting the three bands corresponding to ribosomal RNA 25S, 18S, and 5S. Total extracted RNA was quantified with a NanoDrop 200ºC Spectrophotometer (Biochrom, England) and stored at -80ºC. Each treatment was replicated twice.

Quantitative Real-Time Reverse Transcription-PCR (qRT-PCR)

Roots of control and treatment groups exposed to 50 μM of Cd for 1, 3, 6, 12, and 24 hours were used. Every cDNA template for the qRT-PCR reaction with 7500 Real-Time PCR (Applied Biosystems, USA) was measured using a SYBR Select Master Mix (Applied Biosystems, USA). The programme used for qRT-PCR was as follows: UDG activation at 50ºC for 2 minutes, Amplitaq fast DNA polymerase, up activation at 95ºC for 2 minutes, followed by 40 cycles of denaturation at 95ºC for 15 seconds and annealing/extension at 50ºC for one minute. The gene-specific forward and reverse primers and cDNA

Table 1. Sequences of primers for qRT-PCR.

Target	Primer sequences (5'-3')	GenBank accession No.
PtActin	F: CCCCTCAACGCTAAGGCTAACAG R: CAGAATCTTCATCAAAGCATCGGTG	XM_002298674.2
PeIRT1	F: TTTACACGTTCGATCCCTGCTT R: CTGATAACATAGCTCCAAACCC	XM_011042073.1
PtNRAMP1	F: GCAGAACACTGTAGGAATGA R: GCAGAACACTGTAGGAATGA	XM_006368452.1
PtZIP1	F: TGAAATACAAAGCCGCAGCAAT R: TCGGGAAGCACATGAATAAAGG	XM_002307824.2

Note: 'F' was defined as forward primer and 'R' as reverse primer.

template were added to the SYBR Select Master Mix. Primers for the genes were designed by Primer 5.0 with gene sequences from GenBank in NCBI. The *Populus trichocarpa* actin gene was used as a reference for all genes. Primers designed for the target genes and reference gene are detailed in Table 1. Real-time PCR experiments were conducted on the two biological replicates, with three technical replicates for each sample. Melting curves were used to assess amplification specificity. The relative quantities of the transcripts were analyzed using the comparative Ct method [24].

Statistical Analysis

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

Results

Cd Accumulation and its Effects on Other Minerals

Statistical analysis from inductively coupled plasma atomic emission spectrometry (ICP-AES) showed significant correlations between concentrations of Cd and microelements (Fe, Mn, Ca, and Zn). The level of Cd in roots increased significantly ($P<0.05$) with prolonged treatment times when compared with control (Table 2). The roots of *S. matsudana* exposed to 50 µM of Cd for 1, 3, 6, 12, and 24 hours rapidly absorbed and accumulated large amounts of Cd: approximately 280, 587, 605, 622, and 795 µg/g (DW), respectively. Concentrations of Mn, Fe, Ca, and Zn in roots decreased ($P<0.05$) in the presence of Cd (Table 2) when compared to control, except for Zn at six hours. After 24 hours of treatment, Cd stress caused a decrease of Fe (34.1%), Mn (60.1%), Zn (40.7%), and Ca (26.5%).

Expression of IRT1, NRAMP1, and ZIP1 Genes

In order to elucidate the different durations of Cd uptake at the genetic level, changes in relative expressions of IRT1, NRAMP1, and ZIP1 genes in the roots of *S. matsudana* exposed to 50 µM of Cd from 1 hour to 24 hours were examined. The expression of IRT1 in roots was higher than in control ($P<0.05$) during the whole Cd treatment period (Fig. 1). After 24 hours of exposure, the relative expression of IRT1 peaked: an increase 6.7 times higher than that of control treatment ($P<0.05$). With regard to NRAMP1, relative expression in the roots varied according to the duration of treatment. A sharp increase of 160.8% for the relative expression of

Table 2. Concentrations (µg/g DW) of Cd, Fe, Mn, Zn, and Ca in roots of S. matsudana exposed to 50 µM Cd for different treatment times.

Element	µg g⁻¹ dry weight ± standard error					
	0 h	1 h	3 h	6 h	12 h	24 h
Cd	0.00±0.00a	280.53±0.84b	587.38±4.25c	605.51±2.11d	622.95±5.05e	795.58±1.00f
Fe	730.18±4.02a	701.03±3.57b	612.73±2.97c	614.94±1.71c	531.51±2.30d	481.14±0.84e
Mn	547.56±0.96a	498.70±6.13b	507.85±1.38b	410.95±2.33c	405.07±1.90c	218.70±1.54d
Zn	590.85±2.28a	490.10±1.68b	469.59±7.03c	485.84±5.02d	434.21±1.21e	350.34±8.96f
Ca	5,614.95±24.49a	5,145.33±30.12b	4,989.86±22.34c	5,046.76±5.40c	4,738.24±11.93d	4,127.69±15.59e

Values followed by different letters are significantly different ($P<0.05$). n = 4.
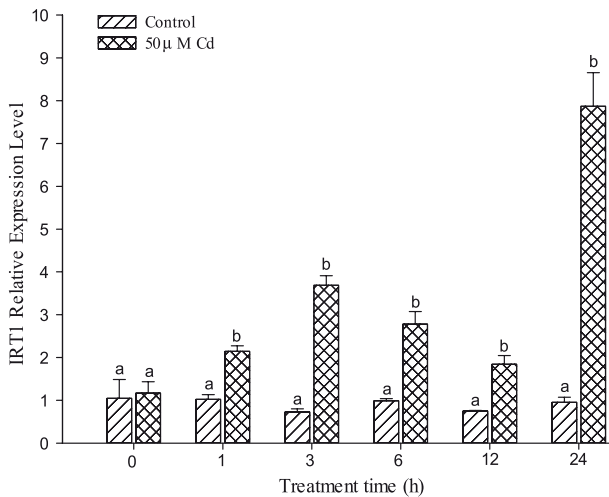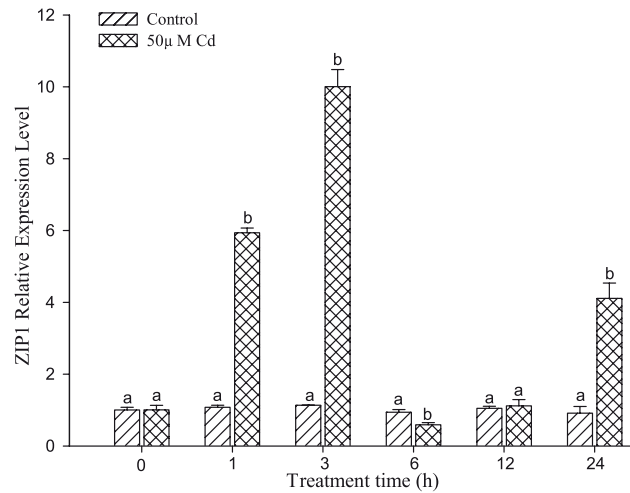
Fig. 1. qRT-PCR analysis of IRT1 expression level in roots of *S. matsudana* exposed to 50 μM Cd with different treatment times. Values with different letters differ significantly from each other (n = 3, *P*<0.05). Data are means ± SE.

Fig. 3. qRT-PCR analysis of ZIP1 expression level in roots of *S. matsudana* exposed to 50 μM Cd with different treatment times. Values with different letters differ significantly from each other (n = 3, *P*<0.05). Data are means ± SE.
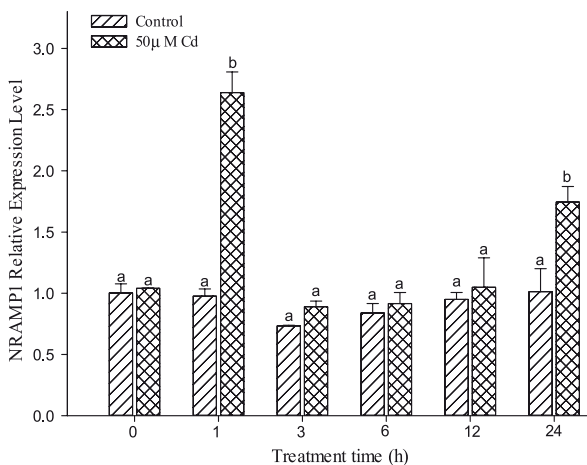

Fig. 2. qRT-PCR analysis of NRAMP1 expression level in roots of *S. matsudana* exposed to 50 μM Cd with different treatment times. Values with different letters differ significantly from each other (n = 3, *P*<0.05). Data are means ± SE.

NRAMP1 was detected after exposure to Cd treatment for 1 hour (*P*<0.05) when compared to control, after which the expression declined (Fig. 2). However, the relative expression of NRAMP1 was again higher (*P*<0.05) after exposure for 24 hours, increasing by 78.8%. In addition, it is certain that the expression of ZIP1 increased sharply after exposure to 50 μM Cd for one hour, which is 5.9 times that of control treatment (*P*<0.05) (Fig. 3). After three hours of the stress, the expression of ZIP1 reached a maximum, which is 10 times that of control (*P*<0.05).

Discussion

Some heavy metals such as cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn) are metal micronutrients that play an essential role in plant cell growth and development [25]. Dynamic equilibrium of these heavy metals in plant cells is necessary to avoid deficiency and toxicity [1]. To achieve this, plants utilize some stringent regulatory mechanisms. It is well known that the absorption and maintenance of heavy metal ions in cells are controlled by specific transporters and metal pumps [26]. Cd ion uptake occurs via transmembrane carriers engaged in the uptake of Ca, Fe, magnesium (Mg), Cu, and Zn [27-28]. At the root level, IRT1, ZIP1, and NRAMP1 are the best-studied non-specific transporters responsible for Cd uptake and other divalent metal nutrient transporters [29-32].

Some investigations have proven that transporters (including the ZIP family transporters) participate in Cd absorption and accumulation in plants, and the balance between transport processes [31-33]. IRT1, the earliest discovered member of the ZIP family, is not only the primary root iron uptake system in plants but can also transport significant amounts of Cd [34-35]. In the present investigation, the relative expression of IRT1 in the roots of *S. matsudana* exposed to 50 μM of Cd during the whole treatment period increased significantly (*P*<0.05) and large amounts of Cd were absorbed, suggesting that IRT1 plays an important role in Cd uptake. ZIP1 belong to the same transporter family with IRT1. ZIP1, ZIP3, ZIP4, ZIP5, ZIP9, and ZIP10 genes in *Arabis thaliana* have been demonstrated to be an important factor to control Zn uptake, as they encode a plasma membrane-localized Zn-specific transporter. [36-38]. The current studies have shown Cd transport in rice, tomato, tobacco, and radish by ZIP1 [16, 32, 39-40]. In the present investigation, expression of ZIP1 increased significantly and quickly in the roots of *S. matsudana* for the early stage of 50 μM Cd treatments (Fig. 3), suggesting that ZIP1 may play crucial roles in the early absorption of Cd. NRAMP1 can be considered a kind of

Fe transporter in plants, and its expression can be induced by Fe deficiency in roots [19]. Cailliatte et al. [18] demonstrated that the metal transporter NRAMP1 was high-affinity Mn uptake that is essential for growth in conditions of low Mn availability. The results from Xiao et al. [30] and Thomine et al. [21] also showed that NRAMP1 encodes a functional metal transporter responsible for mediating the distribution of ions as well as the transportation of Cd in plants. In these studies, the increased relative expression of IRT1, ZIP1, and NRAMP1 in Cd-treated roots led to increased Cd uptake. IRT1 and ZIP1 plays a dominant role in the absorption of Cd compared with NRAMP1 in *S. matsudana's* early exposure to Cd. Meanwhile, the relative expression of IRT1 and NRAMP1 is likely stimulated by Cd-induced Fe, Zn, and/or Mn deficiency.

Different concentrations of Cd may lead to different expressions of these genes. It was reported that IRT1, Nramp1, and ZIP1 expression increased in two tomato genotypes with increases in the Cd concentration [32]. The results from other studies showed that high Cd accumulators had high expression of IRT1 and ZIP1 when in comparison with low Cd accumulators [32, 35, 41]. More studies, however, are required in this direction.

Evidence has confirmed that adding excessive Fe and Mn to a solution containing Cd can reduce Cd uptake, ameliorate phytotoxicity, and alleviate (to some extent) Cd-induced inhibitory effects on growth parameters of kidney bean seedlings, maize plants, and rice by preventing impairment in pigment synthesis [42]. Choppala et al. [1] found that Fe and Mn were conducive to internal defensive mechanisms in plants. They compete with Cd in the active transporters and can minimize Cd transportation into plants. Here, the contents of Fe, Mn, and Cd levels in the roots showed a negative correlation during the whole experiment, which may explain why concentrations of Fe and Mn gradually declined with increased Cd concentrations in the roots (Table 2). Chemical similarities and interactions between Zn and Cd are thought to be the main causes of Cd toxicity in higher plants. Cd and Zn belong to Group IIB in the periodic table and form tetrahedral complexes, competing for the same binding sites and/or ligands in biological systems [43]. Zn is known as a cofactor in many enzymes and regulatory protein. Interference by Cd in Zn homeostasis may result in serious effects on cell growth, development, and functioning [42]. Zn and Cd likely pass into the cell membrane through transporter protein family members by ZIP (zinc-regulated transporter-like proteins or iron-regulated transporter-like proteins) [44]. Other studies have reported that Cd ions could enter root cells via orthologues of AtIRT1, TcZNT1/TcZIP4, and TaLCT1, or via cation channels [7, 45-46]. The results of the present investigation have shown that the concentration of Zn in the roots of *S. matsudana* decreased ($P<0.05$) during the whole experiment, except for the treatment group exposed to 50 μM of Cd for six hours, which is consistent with previous investigations where Cd induced a marked reduction of Zn levels in *Allium cepa* [47] and maize

[48]. Cd and Ca have similar ionic radii (0.099 nm and 0.097 nm, respectively) and compete for the same Ca channels in plants [49]. Shortly after Cd enters the cytoplasm, it binds to certain sites in the root tip apoplast, affecting the function of the plasmalemma pumps transporting Ca ions and resulting in the interference of Ca uptake.

References

1. CHOPPALA G., SAIFULLAH, BOLAN N., BIBI S., IQBAL M., RENGEL Z., KUNHIKRISHNAN A., ASHWATH N., SIK OK Y. Cellular mechanisms in higher plants governing Tolerance to Cadmium Toxicity. Crit. Rev. Plant Sci. **33**, 374, **2014**.
2. MONTEIRO C.C., CARVALHO R.F., GRATAO P.L., CARVALHO G., TEZOTTO T., MEDICI L.O., PERES L.E.P., AZEVEDO R.A. Biochemical responses of the ethylene-insensitive Never ripe tomato mutant subjected to cadmium and sodium stresses. Environ. Experi. Bot. **71**, 306, **2011**.
3. TOMAR P.C., LAKRA N., MISHRA S.N. Effect of cadaverine on *Brassica juncea* (L.) under multiple stress. Indian J. Experi. Biol. **51**, 758, **2013**.
4. GAO F. The mystery of heavy metal pollution in farmland. China Land. **2**, 14, **2014**.
5. CUI S., ZHOU Q., CHAO L. Potential hyperaccumulation of Pb, Zn, Cu and Cd in endurant plants distributed in an old smeltery, northeast China. Environ. Geol. **51**, 1043, **2007**.
6. BI X.Y., FENG X.B., YANG Y.G., LI X.D., SHIN G.P.Y., LI F., QIU G.L., LI G.H., LIU T.Z., FU ZH.Y. Allocation and source attribution of lead and cadmium in maize (*Zea mays* L.) impacted by smelting emissions. Environ. Pollut. **157**, 834, **2009**.
7. VERBRUGGEN N., HERMANS C., SCHAT H. Molecular mechanisms of metal hyeraccumulation in plants. New Phytol. **181**, 759, **2009**.
8. HASSAN Z., AARTS M.G.M. Opportunities and feasibilies for biotechnological improvement of Zn, Cd or Ni tolerance and accumulation in plants. Environ. Experi. Bot. **72**, 53, **2011**.
9. NA G., SALT D.E. The role of sulfur assimilation and sulfur-containing compounds in trace element homeostasis in plants. Environ. Experi. Bot. **72**, 18, **2011**.
10. RASCIO N., NAVARI-IZZO F. Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? Plant Sci. **180**, 169, **2011**.
11. PENCE N.S., LARSEN P.B., EBBS S.D., LETHAM D.L.D., 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. **97**, 4956, **2000**.
12. SASAKI A., YAMAJI N., YOKOSHO K., MA J.F. Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. Plant Cell. **24**, 2155, **2012**.

13. PARK W., AHN S.J. How do heavy metal ATPases contribute to hyperaccumulation? J. Plant Nutr. Soil Sci. **177**, 121, **2014**.

14. BARBERON M., DUBEAUX G., KOLB C., ISONO E., ZELAZNY E., VERT G. Polarization of iron-regulated transporter 1 (IRT1) to the plant-soil interface plays crucial role in metal homeostasis. P. Natl. Acad. Sci. USA. **111**, 8293, **2014**.

15. RAMESH S.A., SHIN R., EIDE D.J., SCHACHTMAN D.P. Differential metal selectivity and gene expression of two zinc transporters from rice. Plant Physiol. **133**, 126, **2003**.

16. XU X.Y., DING Z.J., CHEN L., YAN J.Y., LI G.X., ZHENG S.J. An eukaryotic translation initiation factor, AteIF_5A-2, affects cadmium accumulation and sensitivity in Arabidopsis. J. Integr. Plant Boil. **57**, 848, **2015**.

17. CAILLIATTE R., SCHIKORA A., BRIAT J.F., MARI S., CURIE C. High-affinity manganese uptake by the metal transporter NRAMP1 is essential for Arabidopsis growth in low manganese conditions. Plant Cell. **22**, 904, **2010**.

18. CURIE C., ALONSO J.M., JEAN M.L., ECKER J.R., BRIAT J.F. Involvement of NRAMP1 from Arabidopsis thaliana in iron transport. J. Biochem. **347**, 749, **2000**.

19. TAKAHASHI R., ISHIMARU Y., SENOURA T., SHIMO H., ISHIKAWA S., ARAO T., Nakanishi H, Nishizawa N.K. The OsNRAMP1 iron transporter is involved in Cd accumulation in rice. J. Exp. Bot. **62**, 4843, **2011**.

20. CAILLIATTE R., LAPEYRE B., BRIAT J.F., MARI S., CURIE, C. The NRAMP6 metal transporter contributes to cadmium toxicity. J. Biochem. **422**, 217, **2009**.

21. THOMINE S., WANG R., WARD J.M., CRAWFORD N.M., SCHROEDER J.I. Cadmium and iron transport by members of a plant metal transporters family in Arabidopsis with homology to NRAMP genes. Proc. Natl. Acad. Sci. USA. **97**, 4991, **2000**.

22. UENO D., MILNER M., YAMAJI N., YOKOSHO K., KOYAMA E., ZAMBRANO M.C., KASKIE M., EBBS S., KOCHIAN L.V., MA J.F. Elevated expression of TcHMA3 plays a key role in the extreme Cd tolerance in a Cd-hyperaccumulating ecotype of Thlaspi caerulescens. Plant J. **66**, 852, **2011**.

23. JIANG Z., QIN R., ZHANG H.H., ZOU J.H., SHI Q.Y., WANG J.R., JIANG W.S., LIU D.H. Determination of Pb genotoxic effects in *Allium cepa* root cells by fluorescent probe, microtubular immunofluorescence and comet assay. Plant Soil **383**, 357, **2014**.

24. LIVAK K.J., SCHMITTGEN T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2 (-Delta Delta C(T)) method. Methods **25**, 402, **2001**.

25. HANSCH R., MENDEL R.R. Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). Curr. Opin. Plant Biol. **12**, 259, **2009**.

26. PILON M., COHU C.M., RAVET K., ABDEL-GHANY S.E., GAYMARD F. Essential transition metal homeostasis in plants. Curr. Opin. Plant Biol. **12**, 347 **2009**.

27. ROTH U., VON ROEPENACK-LAHAYE E., CLEMENS S. Proteome changes in Arabidopsis thaliana roots upon exposure to Cd^{2+}. J. Experi. Bot. **57**, 4003, **2006**.

28. CLEMENS S. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie. **88**, 1707, **2006**.

29. LIN Y.F., ARTS M.G.M. The molecular mechanism of zinc and cadmium stress response in plants. Cell Mol. Life Sci. **69**, 3187, **2012**.

30. XIAO H.H., YIN L.P., XU X.F., LI T.Z., HAN Z.H. The iron-regulated transporter, MbNRAMP1, isolated from Malus baccata is involved in Fe, Mn and Cd trafficking. Ann.

Bot. **102**, 881, **2008**.

31. CHAFFAI R., KOYAMA H. Chapter 1-Heavy Metal Tolerance in Arabidopsis thaliana. Adv. Bot. Res. **60**, 1, **2011**.

32. ZHAO S.P., ZHANG Y.Z., YE X.Z., ZHANG Q., XIAO W.D. Responses to cadmium stress in two tomato genotypes differing in heavy metal accumulation. Turk. J. Bot. **39**, 615, **2015**.

33. LUX A., MARTINKA M., VACULIK M., WHITE P.J. Root responses to cadmium in the rhizosphere. J. Experi. Bot. **62**, 21, **2011**.

34. RAMESH S.A., SHIN R., EIDE D.J., SCHACHTMAN D.P. Differential metal selectivity and gene expression of two zinc transporters from rice. Plant Physiol. **133**, 126, **2003**.

35. WU Z.C., ZHAO X.H., SUN X.C., TAN Q.L., TANG Y.F., NIE Z.J., HU C.X. Xylem transport and gene expression play decisive roles in cadmium accumulation in shoots of two oilseed rape cultivars (Brassica napus). Chemosphere **119**, 17, **2015**.

36. VAN DE MORTEL J.E., VILLANUEVA L.A., SCHAT H., KWEKKEBOOM J., COUGHLAN S., MOERLAND P.D., VAN THEMAAT E.V.L, KOORNNEEF M., AARTS M.G.M. Large expression differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots of Arabidopsis thaliana and the related metal hyperaccumulator Thlaspi caerulescens. Plant Physiol. **142**, 1127, **2006**.

37. TALKE I.N., HANIKENNE M., KRAMER U. Zinc-dependent global transcriptional control, transcriptional deregulation, and higher gene copy number for genes in metal homeostasis of the hyperaccumulator Arabidopsis halleri. Plant Physiol. **16**, 1, **2006**.

38. ASSUNÇÃO A.G.L., HERRERO E., LIN Y.F., HUETTEL B., TALUKDAR S., SMACZNIAK C., IMMINK R.G.H., VAN ELDIK M., FIERS M., SCHAT H. Arabidopsis thaliana transcription factors bZIP19 and bZIP23 regulate the adaptation to zinc deficiency. Proc. Nat. Acad. Sci. **107**, 10296, **2010**.

39. SHIMO H., ISHIMARU Y., AN G., YAMAKAWA T., NAKANISHI H., NISHIZAWA N.K. Low cadmium (LCD), a novel gene related to cadmium tolerance and accumulation in rice, J. Experi. Bot. **62**, 5727, **2011**.

40. GONG Y.Q., LIU L.W. De novo sequencing of root transcriptome reveals complex cadmium-responsive regulatory networks in radish (Raphanus sativus L.). Plant Sci. **236**, 313, **2015**.

41. ROGERS E.E., EIDE D.J., GUERINOT M.L. Altered selectivity in an Arabidopsis metal transporter. P. Natl. Acad. Sci., 12356, **2000**.

42. SHAO G.S., CHEN M.X., WANG D.Y., XU CH.M., MOU R.X., CAO ZH.Y., ZHANG X.F. Using iron fertilizer to control Cd accumulation in rice plants: A new promising technology. Sci. China Life Sci. **51**, 245, **2008**.

43. MATOVIĆ V., BUHA A., BULAT Z., ĐUKIĆ-ĆOSIĆ D. Cadmium toxicity revisited: Focus on oxidative stress induction and Interactions with zinc and magnesium. Arh. Hig. Rada Toksikol. 62, 65, **2011**.

44. GUERINOT M.L. The ZIP family of metal transporters. Biochim. Biophys. Acta. 1465, 190, **2000**.

45. DAL CORSO G., FARINATI S., MAISTRI S., FURINI A. How plants cope with cadmium: Staking all on metabolism and gene expression. J. Int. Plant Biol. 50, 1268, **2008**.

46. PEDAS P., YTTING C.K., FUGLSANG A.T., JAHN T.P., SCHJOERRING J.K., HUSTED S. Manganese efficiency in barley: identification and characterization of the metal ion transporter HvIRT1. Plant Physiol. 148, 455, **2008**.

47. ZOU J.H., YUE J.Y., ZHANG Z.G., JIANG W.S, LIU D.H. Effects of cadmium stress on root tip cells and some physiological indexes in *Allium cepa* var. agrogarum L. Acta. Biol. Cracov. Bot. 54, 129, **2012**.

48. WANG M., ZOU J.H., DUAN X.C., JIANG W.S., LIU D.H. Cadmium accumulation and its effects on metal uptake in maize (Zea mays L.). Bioresource Tech. 98, 82, **2007**.

49. NELSON M.T. Interactions of divalent cations with single calcium channels from rat brain synaptosomes. J. Gen. Physiol. 87, 986, **2011**.