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Responses to cadmium stress in two tomato genotypes differing in heavy metal accumulation

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Abstract: Our previous research defined two tomato (*Solanum lycopersicum*) genotypes: high cadmium (Cd) accumulator YSL189 and low Cd accumulator HZ903. Further hydroponics experiments investigated the different tolerance mechanisms to Cd stress between YSL189 and HZ903 at the seedling stage. When Cd concentration was >20 μM in the growing medium, the uptake rate of Cd was significantly higher in roots of YSL189 than it was in roots of HZ903. When plants were supplied with 50 and 100 μM Cd in the growing medium, there were higher Cd concentration, higher biomass and plant height, shorter roots, and higher expression levels of transporter genes natural resistance associated macrophage proteins (*Nramp*)*2*, *Nramp3*, and zinc and iron regulated transporter (*ZIP*) in roots of YSL 189 compared to HZ903. We infer that the high Cd accumulation in YSL189 was partly due to the higher Cd uptake rate and higher expression levels of *Nramp2*, *Nramp3*, and *ZIP* in its roots. At the same time, the degree of cell injury indicated by thiobarbituric acid reactive substance showed no significant differences in roots and stems between the two genotypes. We attribute this to the higher activities of superoxide dismutase, peroxidase, and catalase in roots and stems of YSL189 compared to HZ903.

Key words: Cadmium, tomato, transporter, accumulation, antioxidant enzymes

1. Introduction

Cadmium (Cd) is recognized as a significant pollutant due to its high toxicity (Pan and Wang, 2011). Higher soil Cd concentrations can occur either naturally or through anthropogenic activities (Kirkby and Johnson, 2008; Smeets et al., 2008). Cd is a nonessential heavy metal in plants and human beings and can have toxic effects on crop production. Cd can be transferred to the food chain by plant uptake and is particularly troublesome as it has often been implicated in deterioration of human health (Anjum et al., 2008; Cherif et al., 2012). Leaf concentrations greater than 5–10 μg Cd g^{-1} DM are toxic to most plants (White and Brown, 2010), although some ecotypes of a few plant species have adapted to grow on soils with high Cd concentrations and can tolerate leaf concentrations in excess of 100 μg Cd g^{-1} DM (Verbruggen et al., 2009).

Studies in different plant species have revealed that Cd can interfere with a number of metabolic processes. It negatively affects water and nutrient uptake, photosynthesis, and growth, resulting in visible symptoms of injury in plants such as chlorosis and necrosis of leaves, and reduced length and browning of roots (Khan et al., 2006; Mobin and Khan, 2007; Li et al., 2008). Cd toxicity also involves its inclusion in place of essential metals in plant metabolism, modifying the active conformation of macromolecules and disrupting the structural integrity of biomolecules (Schutzendubel and Polle, 2002). High Cd concentration leads to the formation of reactive oxygen species (ROS), causing oxidative stress that leads to damage of membranes indicated by an increase in thiobarbituric acid reactive substance (TBARS) as evidenced by enhanced lipid peroxidation, hydrogen peroxide generation, and ion leakage (Rodríguez-Serrano et al., 2006; Anjum et al., 2008). As a result, plants have evolved various mechanisms to tolerate excessive metal concentrations, such as binding Cd ions in cell walls, effluxing the metal ions from symplasm, reducing metal uptake by root immobilization or mycorrhizal action, exuding organic ligands that can inactivate toxic metal ions, sequestrating by specifically produced organic compounds, forming metal–peptide ligand complexes and compartments in vacuoles, and forming metal-resistant enzymes or metabolites to minimize metal-induced severe metabolic injuries (Lux et al., 2011; Chaffai and Koyama, 2011). The roles of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)

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have been shown to be important in plant Cd tolerance (Romero-Puertas et al., 2007; Chaffai and Koyama, 2011). Other heavy metals can enter plants via uptake systems for essential cations including different metal transporters such as *ZIP* (ZRT, IRT like proteins) and *Nramp* (natural resistance associated macrophage proteins) (Plaza et al., 2007; DalCorso et al., 2008; Pedas et al., 2008; Verbruggen et al., 2009). Studies have identified that transporters including the *ZIP* and *Nramp* transporters participate in Cd accumulation in *Arabidopsis*, and the balance between transport processes is one important factor determining heavy metal tolerance (Klatte et al., 2009; Lux et al., 2011; Chaffai and Koyama, 2011).

All these complex mechanisms of both toxicity and tolerance to heavy metals indicate that various bioprocesses, including absorption, transport, and defense mechanisms, determine the tolerant phenotypes in plants. Our previous results identified two tomato (*Solanum lycopersicum*) genotypes that differed significantly in Cd accumulation: the higher accumulator YSL189 and the lower accumulator HZ903 (Zhao et al., 2015). To investigate the different tolerance mechanisms between these two genotypes, we determined the Cd uptake rate and related gene expression in roots, activities of defense enzymes, and other parameters that indicate tolerance or toxicity of heavy metals to a plant in YSL189 and HZ903 supplied with Cd in growing medium at the seedling stage. Our results will provide useful information for plant molecular breeding to reduce Cd accumulation or improve tolerance to Cd toxicity in plants.

2. Materials and methods

2.1. Plant materials

Tomato genotypes YSL189 and HZ903 were used. Our previous field and hydroponics experiments showed high Cd accumulation of YSL189 and low Cd accumulation of HZ903 at the adult and seedling stages, regardless of the Cd concentration in the growing medium (Zhao et al., 2015).

2.2. Growth conditions

Hydroponically grown tomato seedlings were used for all experiments. Tomato seeds were surface-sterilized in 1% NaOCl for 30 min, rinsed with deionized water several times, and then allowed to imbibe in aerated water at 28 °C for 24 h in a water bath in darkness. The germinating seeds were placed onto quartz sand in a pot, and the pot was placed into a container filled with 1/2 Hoagland solution. The full-strength Hoagland solution contained 945 mg L^{-1} $Ca(NO_3)_2{\cdot}4H_2O$, 506 mg L^{-1} KNO_3, 493 mg L^{-1} $MgSO_4$, 80 mg L^{-1} NH_4NO_3, 136 mg L^{-1} KH_2PO_4, 1.12 mg L^{-1} Fe, and microelements (6.2 mg L^{-1} H_3BO_3, 0.83 mg L^{-1} KI, 22.3 mg L^{-1} $MnSO_4$, 8.6 mg L^{-1} $ZnSO_4$, 0.25 mg L^{-1} Na_2MoO_4, 0.025 mg L^{-1} $CuSO_4$, and 0.025 mg L^{-1} $CoCl_2$). The container was placed in a controlled-environment growth room with a temperature of 28 ± 2 °C, relative humidity of 70%, and irradiance of 300 μE m^{-2} s^{-2} under fluorescent lighting on a light/dark cycle of 14/10 h. After 15 days the seedlings were used for Cd treatment and absorption rate determination.

2.3. Treatment of plants for determination of Cd absorption rate

After 15 days of growth, seedlings were cultivated in full Hoagland solution for another 10 days, and then seedlings of uniform size were divided into six groups and provided with 50 mL of absorption solution: 5, 10, 20, 50, 100, and 200 μM Cd (added as $CdCl_2{\cdot}2.5H_2O$) with 0.2 mM $CaSO_4$ as solvent. The total weights of plants and solutions were quickly measured, and then placed in the controlled-environment growth room described in 'Growth conditions' above. The total weights of plants and solutions were again obtained quickly 5 h later. The roots were then cut from the plants and weighed, and the solution sampled to determine the concentration of Cd remaining. The Cd concentration in the absorption solution and Cd concentration in the absorption solution after 5 h were determined by graphite furnace atomic absorption spectrometry (contrAA 700 Analytik Jena, Germany). We calculated the rate of Cd absorption as follows:

$$V\ (\mu mol\ g^{-1}\ fresh\ weight\ h^{-1}) = \{C1 \times 50 - C2 \times [50 - (M1 - M2)]\} \times 10^{-3}/(5 \times M3),$$

where V is the Cd uptake rate, 50 is the volume of the absorption solution (mL), $C1$ is the Cd concentration in the absorption solution (μM), $C2$ is the Cd concentration in the absorption solution after 5 h (μM), $M1$ is the total weight of absorption and plant (g), $M2$ is the total weight of absorption and plant after 5 h (g), and $M3$ is the weight of the plant roots (g).

2.4. Plants for determination of morphological character, Cd concentration, gene expression, antioxidant enzyme activities, and TBARS

After 15 days of growth, seedlings were divided into three groups cultivated in Hoagland with Cd (added as $CdCl_2{\cdot}2.5H_2O$) 0, 50, and 100 μM separately, with three replicates per group. The growing solution was refreshed every 24 h to maintain the Cd concentration in the growing medium. The plants were harvested 10 days later. The roots, stems, and leaves were frozen quickly in liquid nitrogen. Part of all three samples was stored at −80 °C for determination of gene expression, part was stored at −40 °C for determination of antioxidant enzyme activity and TBARS, and part was stored at −4 °C for determination of Cd concentration. Root length, plant height, and biomass were measured at the same time.

Cd concentrations were determined by graphite furnace atomic absorption spectrometry (contrAA 700 Analytik Jena, Germany). The activity of antioxidant enzymes (POD, SOD, and CAT) was determined according to Shah

et al. (2001) and concentration of TBARS was determined according to Zhang (1998). Total RNA samples were isolated using the guanidine isothioyanate method. We used 5 μg of total RNA to synthesize cDNA by Reverse Transcriptase PowerScript™ following the manufacturer's protocol. The cDNA samples were used as a template to quantify the target gene expression levels, and we designed the TaqMan probes for each gene according to information in the GenBank database (Table 1). All specific RT-PCR products were cloned, sequenced, and compared with the sequence of the respective genes to confirm the specificity of the RT-PCR products. Taking GAPDH as a house-keeping gene, the TaqMan probes were synthesized by Bioasia Co., Shanghai (China), and purity was >99%. Taq, $MgCl_2$, and dNTP were bought from TaKaRa Biotechnology (Dalian) Co. Ltd., China. Gene expression was calculated according to Zhao et al. (2006).

2.5. Statistical analysis

Statistical analysis of the experimental data was performed with Microsoft Excel 2000 and SPSS 11.5, with significant differences determined by one-way ANOVA and Duncan's multiple range test.

3. Results

3.1. Morphological characteristics and Cd accumulation

Despite the different responses to Cd stress, compared to Cd low-accumulator HZ903, the Cd high-accumulator YSL189 possessed higher biomass, plant height, and shorter roots than HZ903 did, without the influence of different Cd supplied (Table 2). The addition of Cd significantly reduced plant height, root length, and biomass in YSL189 and HZ903, and these three characteristics showed different adaption patterns to Cd stress (Table 2). Addition of Cd significantly reduced root length in YSL189 and HZ903, but there were no significant differences in root length between 50 and 100 μM Cd treatments. We found that only 100 μM Cd treatment significantly reduced plant height, and there were no significant differences between 0 and 50 μM or between 50 and 100 μM Cd treatments. YSL189 and HZ903 had the same response to Cd stress in plant height and root length; however, the response in biomass varied with genotype. In YSL189, the biomass decreased significantly with 50 μM Cd treatment compared to 0 μM Cd, and there was no significant difference in biomass between 50 and 100 μM Cd treatments, while in HZ903, a significant decrease only occurred for 100 μM Cd treatment. All our data suggested that root length was more sensitive to Cd stress than plant height or biomass was, and that YSL189 biomass seems more sensitive to Cd than that of HZ903.

The Cd concentration was significantly higher in YSL189 than in HZ903 in roots, stems, and leaves, regardless of the amount of Cd addition in growing medium (Table 3). Compared with the controls (0 μM Cd), the Cd accumulation increased significantly with addition of Cd to the growing medium in all parts of plants of both YSL189 and HZ903 (Table 3). Although the Cd concentrations in plant roots, stems, and leaves increased

Table 1. Gene-specific primers used for RT-PCR analysis.

Gene	Access number	Sequence for primers	Product size (bp)
Nramp1	AY196091	5′ ggagccaccgctgatgctatc 3′ 5′ gtacaagggcagagtgaaggaagaga 3′	79
Nramp2	AY562196	5′ gggattttatggcagtgagcaagc 3′ 5′ ggcacaatggcacaacttcgagta 3′	246
Nramp3	AY196092	5′ cgagggttcttgcctctatggtct 3′ 5′ ccctacaattcccactgcctgct 3′	246
IRT1	AF136579	5′ gggctatcactaggtgcgtcaag 3′ 5′ gatgcaaccaccaaggcccattc 3′	105
IRT2	AF136580	5′ gtggttgcattctccaggctgag 3′ 5′ gcccgtgggctagtttcctcat v	136
ZIP	EF026083	5′ cagtcgcacaggggcagtca 3′ 5′ gctctccaagcatcggcttgtga 3′ 5′ ccacaagtgctcgtccaacaactga v	181
GAPDH	U97257	5′ ctgctgatgtctccgttgtcg 3′ 5′ ccctctgattcctccttgattgc 3′	94

Table 2. Comparison of plant height, root length, and total biomass.

Genotypes Treatments	Plant height (cm)		Root length (cm)		Total biomass (g)	
	YSL189	HZ903	YSL189	HZ903	YSL189	HZ903
0 μM Cd	14.8 ± 2.11 a (100%)	10.5 ± 1.11 a (100%)	9.00 ± 1.01 a (100%)	10.8 ± 1.20 a (100%)	1.32 ± 0.12 a (100%)	0.53 ± 0.08 a (100%)
50 μM Cd	11.8 ± 1.32 ab (80%)	8.75 ± 1.02 ab (83%)	7.00 ± 0.85 b (78%)	8.50 ± 0.99 b (79%)	0.76 ± 0.08 b (57%)	0.44 ± 0.03 ab (83%)
100 μM Cd	10.5 ± 1.05 b (71%)	8.25 ± 0.97 b (79%)	6.50 ± 0.47 b (72%)	7.25 ± 0.73 b (67%)	0.64 ± 0.07 b (48%)	0.39 ± 0.03 b (74%)

Two tomato genotypes (YSL189 and HZ903) seedlings of 15 days growth were divided into three groups and cultivated in Hoagland solution containing 0, 50, and 100 μM Cd separately with three replicates per group. The plant height, root length, and total biomass were measured after 10 days.
Values are mean ± SE (n = 3). The data in brackets are the percent changes along with the actual data. Means with the same lower-case letters in the same column are not significantly different among treatments at $P < 0.05$ according to Duncan's multiple range test.

Table 3. Cd accumulation in plants treated with different amounts of Cd.

Treatments of Cd (μM)	Plant organs	Cd concentration (mg kg^{-1})	
		YSL189	HZ903
0	Root	26.8 ± 3.79 e	16.0 ± 2.11 g
	Stem	4.80 ± 0.52 h	2.25 ± 0.36 i
	Leaf	1.59 ± 0.21 j	0.796 ± 0.09 k
50	Root	657 ± 35.8 a	328 ± 32.0 b
	Stem	81.5 ± 5.88 c	47.4 ± 4.55 d
	Leaf	47.9 ± 3.91 d	21.6 ± 3.41 ef
100	Root	673 ± 49.1 a	412 ± 44.8 b
	Stem	95.4 ± 8.22 c	53.6 ± 6.29 d
	Leaf	46.9 ± 4.67 d	19.9 ± 1.54 f

Seedlings of 15 days growth of two tomato genotypes (YSL189 and HZ903) were divided into three groups and cultivated in Hoagland solution contain 0, 50, and 100 μM Cd separately with three replicates per group. The roots, stems, and leaves were harvested after 10 days and the Cd concentrations measured by graphite furnace atomic absorption spectrometry.
Values are means ± SE (n = 3). Values denoted by different lower-case letters significantly differ among Cd concentrations at $P < 0.05$ according to Duncan's multiple range test.

with the addition of Cd from 50 to 100 μM in growing medium in YSL189 and HZ903, there were no significant differences between 50 and 100 μM Cd treatments in roots, stems, and leaves (Table 3).

Accumulation of Cd decreased significantly according to the order: roots > stems > leaves (Table 3), which means it was easier for Cd to accumulate in roots than in stems or leaves.

3.2. Cd absorption and related transporter genes

The different concentrations of Cd in YSL189 and HZ903 may be related to the different rates of Cd uptake in their roots (Figure 1). The Cd uptake rate in YSL189 was significantly higher than that in HZ903 only when the Cd concentration in growing medium was >20 μM. There was no significant difference between YSL189 and HZ903 in Cd uptake rate when the Cd concentration in growing medium was <20 μM.

Real-time PCR results revealed that Cd transporters may play an important role in the different Cd accumulation of YSL189 and HZ903 (Figure 2). *Nramp2* (natural resistance-associated macrophage), *Nramp3*, and *ZIP* (zinc-regulated transporters) were significantly more expressed in roots of YSL189 than in HZ903 for 50 or 100 μM Cd treatments (Figure 2). This agreed with the trends for Cd accumulation and uptake rates in the genotypes (Table 3; Figure 1). This suggested that the three transporters *Nramp2*, *Nramp3*, and *ZIP* may play important roles in the higher Cd accumulation and uptake rate in YSL189.

Although the expression levels of *Nramp1* and *IRT1* in HZ903 were higher than those in YSL189 in our experiment,

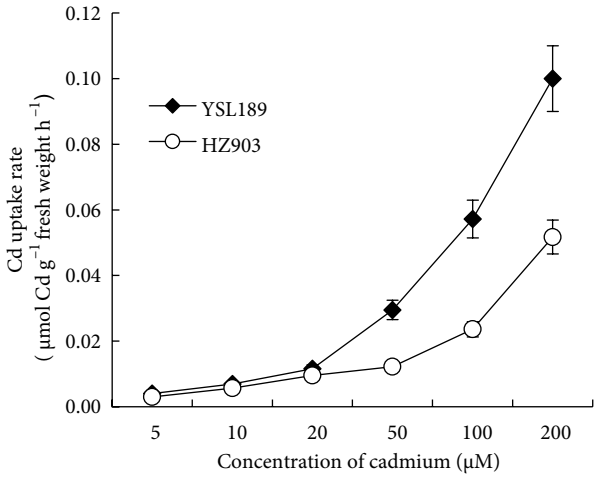

Figure 1. The uptake rate of Cd in roots of tomato genotypes YSL189 and HZ903. Seedlings with 25 days growth and uniform size were divided into six groups and provided with 50 mL of absorption solution: 5, 10, 20, 50, 100, and 200 μM Cd. The roots were weighed 5 h later and the concentrations of Cd in solution were also determined; uptake rate was then calculated. Values are mean ± SE (n = 3).

there were differences in the significance levels. There was no significant difference between genotypes in the expression of *Nramp1* with 50 μM Cd treatment and also of *IRT1* with 100 μM Cd, and the corresponding expression levels of *Nramp1* and *IRT1* in HZ903 were higher than those in YSL189 by 28% and 10%, respectively. There were significantly higher expression levels of *Nramp1* in HZ903 than in YSL189 for 0 and 100 μM Cd treatments and *IRT1* for 0 and 50 μM Cd treatments. The expression of *IRT2* did not follow a regular pattern with the addition of Cd to the growing medium. The expressions of *Nramp1*, *IRT1*, and *IRT2* were significantly higher in HZ903 than in YSL189 with 0 μM Cd treatment.

3.3. TBARS: a product of membrane lipid peroxidation

When the Cd concentration in plants reaches toxic levels, it can cause direct disturbance to biomolecules, resulting in ROS production and oxidative stress. Lipid peroxidation as the measure of cell oxidative injury was determined by TBARS measurement. In our experiment, the increased TBARS concentration in plants indicated that membrane lipid peroxidation injury, especially to roots, resulted from addition of Cd to the growing medium (Figure 3). There was no significant difference between genotypes in the TBARS concentration with 0 μM Cd. When the Cd concentration in the growing medium was increased, the Cd concentration in plants also increased (Table 3), accompanied by increased TBARS concentration (Figure 3), indicating increasingly severe lipid peroxidation damage. The roots accumulated Cd most readily and suffered a corresponding degree of lipid peroxidation damage. Stems were less damaged and leaves were least damaged. The TBARS concentration was slightly higher in roots and stems of YSL189 than in those of HZ903 when 50 and 100 μM of Cd were supplied, but a significant difference in TBARS concentration between genotypes only occurred in the leaves.

3.4. Differences in defense systems

In roots and stems, the activities of antioxidant enzymes including POD, SOD, and CAT were all higher in YSL189 than in HZ903 for 50 and 100 μM Cd treatments (Table 4). Although there was no significant difference between genotypes in the activities of SOD in stems and CAT in roots with 50 μM Cd treatment, the corresponding activities in HZ903 were higher than those in YSL189 by 2.6% and 13%, respectively (Table 4). This corresponded to the higher Cd concentrations in the roots and stems in YSL189 compared to HZ903 (Table 3). In leaves with 50 and 100 μM Cd treatments, the activities of POD and SOD were also significantly higher in YSL189 than in HZ903, while significant higher activity of CAT occurred in HZ903 than in YSL189 (Table 4).

4. Discussion

The different levels of Cd accumulation between plant species or genotypes of the same species are recognized by the current research, while the mechanism leading to these differences has not been clarified. Genetic studies have identified the role of genes encoding metal transporters (e.g., *AhZIP9*) involved in hyperaccumulation of heavy metals in *Arabidopsis halleri* (Becher et al., 2004; Hanikenne et al., 2005). Overexpressing *AtIRT1* results in higher concentrations of Cd and Zn in transgenic plants than in the wild type under Fe-deficient conditions (Connolly et al., 2002). Genes of *AhIRT3*, *AhZIP3*, *AhZIP6*, and *AhZIP12* in shoots and roots and *AhZIP9* in roots were predominantly expressed in *A. halleri*, which could be responsible for the Zn/Cd uptake ability in this hyperaccumulator plant (Becher et al., 2004; Weber et al., 2004). In the present study, the higher Cd uptake rate and higher expression levels of *Nramp2*, *Nramp3*, and *ZIP* in roots may have greatly contributed to the higher Cd accumulation in YSL189 than in HZ903 (Table 3; Figures 1 and 2). In our results, only one of three *ZIPs* (*ZIP*) showed a high expression level in the high accumulator YSL189 (Figure 2), and the other two *ZIPs* (*IRT1* and *IRT2*) seemed to contribute little to the different levels of Cd between the genotypes. This still needs further research.

Researchers agreed that Nramp mediates the sequestration of heavy metals in vacuoles, thereby leading to enhanced metal tolerance (Hanikenne et al., 2005). Our results reveal higher expression levels of *Nramp2* and *Nramp3* in YSL189 than in HZ903, which may partly

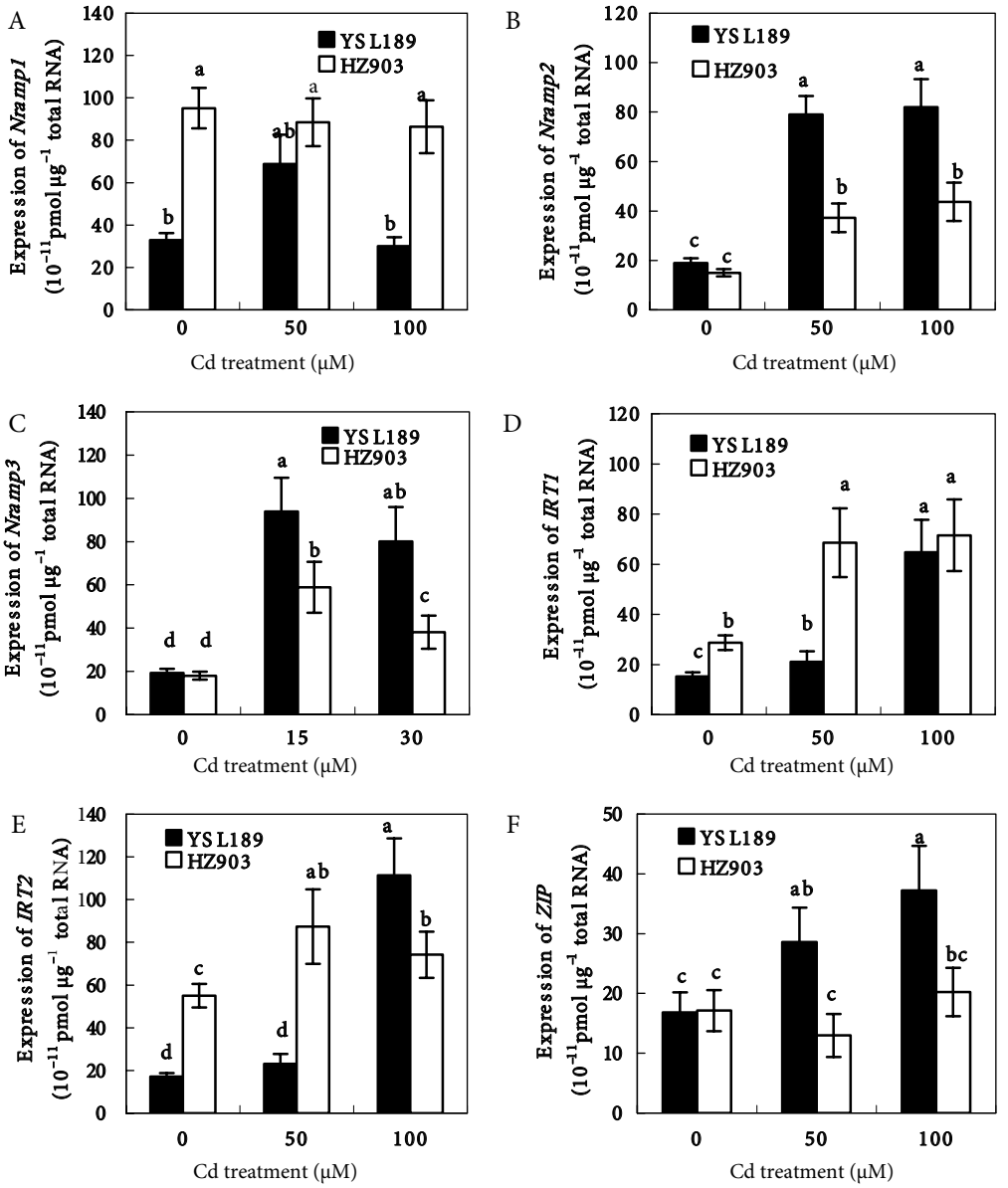

Figure 2. The expression level of Cd transporter genes in roots of YSL189 and HZ903. Seedlings of 15 days growth were divided into three groups and cultivated in Hoagland solution with 0, 50, and 100 μM Cd separately, with three replicates per group. After 10 days the roots, stems, and leaves were harvested and frozen quickly in liquid nitrogen. The expression of Cd transporter genes was determined by real-time PCR. Expression in roots of (A) *Nramp1*, (B) *Nramp2*, (C) *Nramp3*, (D) *IRT1*, (E) *IRT2*, and (F) *ZIP*. Values are mean ± SE (n = 3). Means with the same lower-case letters are not significantly different at $P < 0.05$ according to Duncan's multiple range test.

contribute to the equal level of TBARS between roots of YSL189 and HZ903 with 50 and 100 μM of Cd treatments (Table 2; Figure 3) because of their roles in sequestration of heavy metals in vacuoles. At the same time, we could not exclude possible roles of *Nramp2* and *Nramp3* in higher Cd accumulation in YSL189 than in HZ903. In higher plants, nonessential heavy metals such as Cd are likely to be transported across membranes via nutrient transporters or channels that are not completely selective (Clemens, 2006). Therefore, several plant nutrients have many direct as well as indirect effects on the availability of Cd in the soil and the uptake of Cd into plants (Sarwar et al., 2010). For example, phosphate (Pi) favors the precipitation of Cd^{2+} (Hong et al., 2010) and tomato supplied with NO_3^- can absorb more Cd than those supplied with NH_4^+ (Luo et al., 2012), while ferrous iron (Fe^{2+}) competes with Cd^{2+} for the same membrane transporters in plant cells (Kovacs et al., 2010). Consequently, the higher Cd accumulation

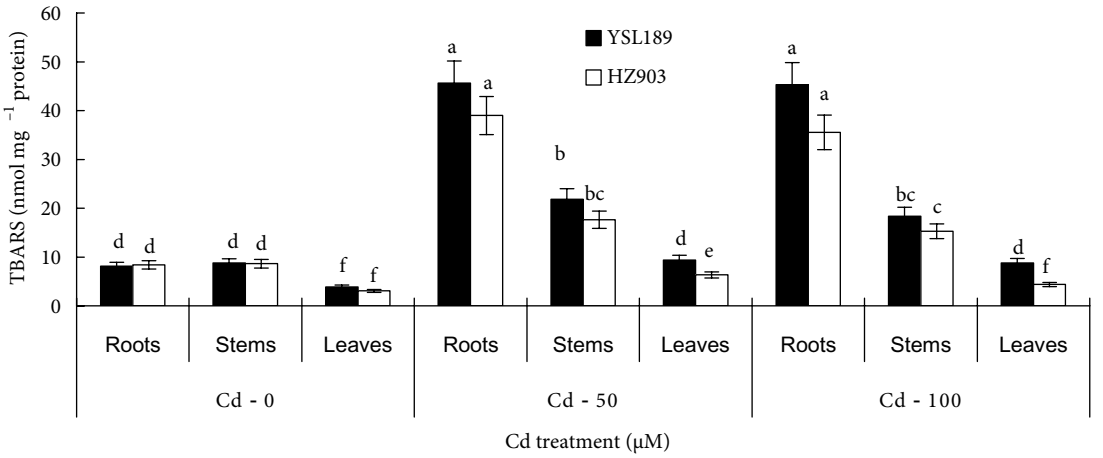

Figure 3. TBARS in YSL189 and HZ903. Seedlings of 15 days growth were divided into three groups and cultivated in Hoagland solution with 0, 50, and 100 μM Cd separately with three replicates per group. After 10 days the roots, stems, and leaves were harvested and frozen quickly in liquid nitrogen. The TBARS concentration was determined (malondialdehyde quantitation). Values are mean ± SE (n = 3). Means with the same lower-case letters are not significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 4. Activities of antioxidant enzymes POD, SOD, and CAT in plants treated with different amounts of cadmium.

Antioxidant enzymes	Treatments of Cd (μM)	Root		Stem		Leaves	
		YSL189	HZ903	YSL189	HZ903	YSL189	HZ903
Activity of POD (U mg^{-1} protein)	0	13.3 ± 1.02 k	58.7 ± 6.21 g	42.8 ± 3.95 h	24.5 ± 2.04 j	22.7 ± 2.30 j	28.6 ± 2.58 i
	50	564 ± 55.9 bc	355 ± 36.2 e	784 ± 77.9 a	494 ± 50.1 cd	184 ± 19.3 f	30.6 ± 3.14 i
	100	645 ± 65.1 b	431 ± 39.7 d	774 ± 78.4 a	637 ± 64.0 b	65.2 ± 6.38 g	26.2 ± 2.57 ij
Activity of SOD (U mg^{-1} protein)	0	43.8 ± 3.96 i	34.9 ± 4.01 i	147 ± 13.5 f	172 ± 19.2 f	263 ± 27.4 de	286 ± 25.3 d
	50	85.0 ± 7.93 g	63.0 ± 6.58 h	425 ± 39.4 b	414 ± 40.5 b	742 ± 65.8 a	375 ± 39.4 bc
	100	97.8 ± 8.37 g	63.1 ± 5.88 h	314 ± 29.1 cd	222 ± 20.9 e	434 ± 39.7 b	330 ± 35.0 cd
Activity of CAT (U mg^{-1} protein)	0	0.306 ± 0.0291hi	0.854 ± 0.0795 f	0.525 ± 0.0531gh	0.464 ± 0.0305 h	0.242 ± 0.0217 i	0.603 ± 0.0594 g
	50	5.39 ± 0.498 a	4.76 ± 0.501 ab	4.31 ± 0.395 b	2.83 ± 0.251 c	1.51 ± 0.136 e	2.54 ± 0.300 cd
	100	2.83 ± 0.213 c	1.330.102 e	2.72 ± 0.220 c	2.06 ± 0.230 d	0.89 ± 0.124 f	2.06 ± 0.218 d

Seedlings of 15 days growth were divided into three groups and cultivated in Hoagland solution with 0, 50, and 100 μM Cd separately with three replicates per group. After 10 days the roots, stems, and leaves were harvested and frozen quickly in liquid nitrogen. The activities of antioxidant enzymes (POD, SOD, and CAT) were determined.
Values are means ± SE (n = 3). Means denoted by different lower-case letters significantly differ among treatments, genotypes, or tissues of the same antioxidant enzyme at $P < 0.05$ according to Duncan's multiple range test.

in YSL189 could be attributed to many factors involved in heavy metal absorption, translocation, and detoxification, and this still need further research. The different expression patterns of *Nramp1*, *IRT1*, and *IRT2* between YSL189 and HZ903 (Figure 2) suggest that these three genes have a role in Cd accumulation under low Cd conditions, especially for HZ903. The higher expression of *Nramp1* and *IRT1* in HZ903 than in YSL189 indicated their different roles in Cd accumulation compared with *Nramp2*, *Nramp3*, and *ZIP*. They may have a role in other Cd transport processes, such as mediating the sequestration of Cd in vacuoles and Cd xylem loading and unloading. This speculation needs considerable further research.

Our results showed higher Cd accumulation in roots than in stems and leaves (Table 2). This agreed with the results reported by Delpérée and Lutts (2008) that Cd

concentrations were higher in roots than in shoots. Cd concentrations are often (but not always) greater in roots than in shoots, suggesting that Cd transport to the xylem is restricted in most plants, and lowest in seeds, fruits, and tubers, suggesting that Cd is not readily translocated in the phloem (Seregin and Kozhevnikova, 2008; Conn and Gilliham, 2010). Moreover, we cannot ignore the role of biomass in heavy metal accumulation, with hyperaccumulator plants having the common characteristic of greater biomass. The high accumulator YSL189 possessed higher aboveground biomass than the low accumulator HZ903 in our experiment. While the biomass decreased 52% and 26% due to 100 μM Cd in YSL189 and HZ903, respectively (Table 2), it seems that HZ903 was more tolerant to Cd than YSL189 was, according to biomass relatively. Although the net biomass decrease was larger in YSL189 than in HZ903, the absolute values of biomass of YSL189 were still higher than those of HZ903 in our experiment (Table 2), and perhaps the higher biomass in YSL189 was also a reason for the high Cd accumulation because of growth force. Although the biomass of YSL189 seems more sensitive to Cd stress, the higher activities of antioxidant enzymes in YSL189 than in HZ903 may mean higher capacity for ROS detoxification. However, more research is required to explain the different tolerance to Cd stress between YSL189 and HZ903.

There were significantly higher Cd concentrations in roots, stems, and leaves in YSL189 than in HZ903 (Table 3), while there was no significant difference in TBARS concentration between the genotypes in roots and stems (Figure 3), suggesting a stronger ability of YSL189 than HZ903 in detoxifying Cd stress in roots and stems when treated with 50 and 100 μM Cd. Although the direct effects of metal ions may vary because of their chemical and physical properties, ROS-induced stress is exerted by various metal ions. All heavy metals participate in the formation of ROS when they are in excess and then cause oxidative stress (Babula et al., 2008). Therefore, the sensitivity of plants to oxidative stress and their ability to detoxify ROS are important factors determining their tolerance of heavy metals. Some genetic approaches analyzing natural allelic variation provide strong evidence supporting this hypothesis (Chiang et al., 2006). The higher activities of antioxidant enzymes POD, SOD, and CAT in roots and stems in YSL189 than in HZ903 for 50 and 100 μM Cd treatments (Table 4) may indicate a higher capacity to detoxify ROS in YSL189 than in HZ903, and it may be the key reason for the lack of significant differences in TBARS in roots and stems between YSL189 and HZ903 with 50 and 100 μM Cd treatments (Figure 3). In leaves, the TBARS concentration was significantly higher in YSL189 than in HZ903 (Figure 3). This corresponded to the Cd level found in leaves (Table 3), and it seems to indicate that the higher Cd concentrations led to greater damage to cells, despite the significantly higher activities of SOD and POD (Table 4), and this needs further research on the ultrastructure of plant cells to prove it.

Whatever the difference between genotypes, we also found different responses of antioxidant enzymes in plants to the increased addition of Cd in growing medium. The activity of CAT was significantly upregulated by 50 μM Cd treatment and significantly downregulated by 100 μM Cd treatment in roots, stems, and leaves for both YSL189 and HZ903 (Table 4). The same response pattern occurred in stems and leaves for SOD in YSL189 and HZ903 and also for POD in leaves in YSL189 only (Table 4). The activities of SOD in roots of YSL189 and HZ903, of POD in roots and stems of YSL189, and of POD in leaves of HZ903 were significantly increased by 50 μM Cd treatments and with no significant difference between 50 and 100 μM Cd treatments (Table 4). The activity of POD in roots and stems of HZ903 was significantly increased by 50 and 100 μM Cd treatments (Table 4).

These differences in response of antioxidant enzymes to Cd increase in growing medium seem to suggest that these enzymes can tolerate different concentrations of Cd in plants, which rely on the plant tissue and genotypes, and that CAT may be more sensitive to Cd than SOD and POD are. Moreover, in the two tomato genotypes, the antioxidant enzymes seemed able to tolerate higher Cd toxicity in YSL189 than in HZ903, shown by the higher Cd in the plant (Tables 3 and 4). A report on two mustard genotypes differing in Cd accumulation showed different results to the present study, possibly partly because of the different species; the mustard genotypes with higher Cd accumulation exhibited higher values of TBARS and activity of SOD but lower activity of CAT (Noushina et al., 2010). In the present study, the lower activity of CAT in YSL189 than in HZ903 only occurred in leaves with 0, 50, and 100 μM Cd treatments (Table 4), and we attributed this different response in antioxidant enzymes to the different tolerance range to Cd toxicity of defense systems among plant species or genotypes within the same species. Previous research on pea plants in 50 μM $CdCl_2$ showed decreased activities of CAT, CuZn-SOD, and Mn-SOD, and it may be that the structures of CAT, CuZn-SOD, and Mn-SOD were damaged by 50 μM $CdCl_2$ in pea plants (Romero-Puertas et al., 2007), because antioxidant enzymes were also the toxicity target of Cd stress. The different results of these studies showed that various tolerance mechanisms exist in plant species and this requires further research.

Overall, our results proved that the Cd uptake rate in roots of YSL189 was significantly higher than that of HZ903 when the Cd concentration was >20 μM in the growing medium. The expression levels of transporter genes *Nramp2*, *Nramp3*, and *ZIP* were significantly

higher in roots of YSL189 than in HZ903 for 50 and 100 μM Cd treatments. We infer that YSL189 may achieve higher Cd accumulation in the plant partly because of the higher Cd uptake rate and higher expression levels of *Nramp2*, *Nramp3*, and *ZIP* in roots. Despite the higher Cd concentrations in the roots and stems of YSL189 than of HZ903, the concentrations of TBARS were equal between the two genotypes. We attributed this to higher POD, SOD, and CAT activities in roots and stems in YSL189 compared to HZ903 with 50 and 100 μM Cd treatments, which may indicate a higher capacity to detoxify ROS in YSL189 than in HZ903.

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