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Full Length Article

Effect of Iron on Forms and Concentration of Cadmium and Expression of Cd-tolerance-related Genes in Tomato

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Abstract

Pot experiment was conducted to determine the effect of different iron (Fe) levels viz. 0, 50, 100, 200 and 400 μmol·L^{-1} on biomass, the chemical forms and concentration of Cd in two varieties of tomato when exposed to Cd-contaminated soil (10 mg·kg^{-1}). The expressions of Cd-tolerance related genes in both tomato (*Solanum lycopersicon* L.) varieties, including Yu Powder 109 (YP109) and variant 4641 (V4641), were also determined. The results showed that dry weights of the fruits, roots, stems and leaves significantly increased by spraying with Fe-containing solution. Among seven Cd-tolerance related genes, the highest expression level was found for *OAS* and *CaM2*, followed by *MT2* and *NRAMP*; however, the expression of *HMA* and *PCS* were the lowest. The expression of *MT2* in V4641 leaves was higher than in YP109 leaves. In contrast, the expression levels of *NRAMP* and *PCS* in YP109 leaves were higher than in V4641 leaves. The concentrations of Cd in the tomato fruits were in the order of residual Cd (F_R)>HCl extractable Cd (F_{HCl})>ethanol extractable Cd (F_E)>NaCl extractable Cd (F_{NaCl})>acetic acid extractable Cd (F_{HAC})>water extractable Cd (F_W). Cadmium concentration of leaves, stems, roots and fruits reduced by 7.1–25.3%, 30.8–50.4%, 13.0–45.1% and 2.8–11.7%, respectively in the presence of Fe. However, the total extraction of Cd, and concentration of F_E, F_{NACl}, F_R in fruits increased at high Fe level (400 μmol·L^{-1}). The Cd concentration of fruits in YP109 was higher than in the V4641. The interaction between Fe and Cd showed antagonistic and synergistic co-existence maybe due to Fe levels.

Keywords: Iron; Forms and concentration of cadmium; Expression of Cd-tolerance-related genes; Tomatoes

Introduction

Cadmium (Cd) is the most common heavy metal trace element (Hu *et al*., 2016) and also a major pollutant in the soil (Hirzel *et al*., 2017). Cadmium can be easily absorbed and accumulate in vegetables, thereby entering the food chain and affecting human health either directly or indirectly. Previous work has showed that Cd released into the environment reaches up to 30,000 tons annually worldwide, of which 82–94% enters the soil. In China, as much as 680 tons of Cd is discharged from industrial wastes into the environment every year (Liu *et al*., 2013). Cd contamination in vegetables is found in large- and medium-sized cities and becoming a serious problem for human health (Song *et al.*, 2006; Qin *et al*., 2017). Approximately 24.1% of vegetable samples come from soil containing Cd exceeded the limits allowed in the national soil environment standards (Zeng *et al*., 2007; Ullah *et al.*, 2016; Qin *et al.*, 2017). Liu *et al.* (2013) reported that the absorption and accumulation of Cd in tomato fruits severely affects food safety. The absorption and accumulation capacity of Cd in vegetables differs among varieties of the same species; similarly, Cd accumulation reveals the differences among different species of vegetables (Li *et al.*, 2004; Sun and Shen, 2007; Chen *et al*., 2010; Antoniadis *et al*., 2017). Moreover, some studies have compared Cd absorption and accumulation between different tomato varieties (Ammar *et al.*, 2008; Hasan *et al.*, 2011; Luo *et al*., 2012).

The absorption and accumulation of Cd differ greatly among the different vegetable species and different varieties from the same species due to differences of the genotypes (Antoniadis *et al*., 2017). The metal ion transporter genes that have been isolated and cloned from plants are closely correlated with the absorption, transport, accumulation, and fixation of metal ions, and play important roles in Cd tolerance and accumulation in plants. The zinc and iron-regulated transporter protein (*ZIP*), heavy metal ATPase (*HMA*), ATP-binding cassette transporter (*ABC*), cation diffusion facilitator (*CDF*), cation/H^+ antiporters (*CAX*), and natural resistance-associated macrophage protein (*Nramp*) families have been isolated and identified (Wu *et al*., 2005).

In order to control Cd pollution in the soil, the antagonistic effect between basic ions and Cd^{2+} that inhibits the absorption or transference of Cd in the edible parts of plants has gained extensive attention (Zhang *et al*., 2011). Iron (Fe), as one of the nutritional elements required for

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plant growth, is correlated with the formation of chlorophyll and is also a basic component of the oxidation-reduction system involved in plant growth processes such as photosynthesis and respiration (Chien *et al*., 2001). The interaction between Fe and Cd in plants is currently a hot topic in the fields of soil science, ecology, and environmental science. Early in 1997, Chlopecka and Adriano (1997) reported that the addition of Fe to soil at a concentration of 50 $g \cdot kg^{-1}$ can significantly reduce Cd absorption in barley and corn. Furthermore, Shao *et al.* (2007) indicated that the addition of fertilizers containing Fe can significantly reduce Cd concentrations in the roots, stems, and fruits of paddy rice. However, the opposite results have also been reported (Zhang, 2006).

All of these significantly affect the internal transportation, accumulation, and toxicity of heavy metals in plants. On the other hand, the effects of Fe on the growth of tomato and the accumulation and chemical formation of Cd in tomato plants have rarely been reported. In order to further explore the correlation between Fe and Cd, the impact of Fe on Cd absorption and transference in tomato; two varieties (V4641 and YP109) were planted in pots containing Cd pollution and then analyzed.

Materials and Methods

Two tomato (*Lycopersicon esculentum* Mill.) varieties were used in the present study: YP109 with a high capacity for Cd accumulation, and V4641 have low capacity. The tested soil samples were collected from Baishiyi Vegetable Base in Jiulongpo District, Chongqing. The contents of organic matter, total nitrogen, alkali-degradable nitrogen, available K, available P, Cd and Fe in soil, the cation exchange capacity (CEC) of soil, and the soil pH were 33.3 $g \cdot kg^{-1}$, 1.21 $g \cdot kg^{-1}$, 110.8 $mg \cdot kg^{-1}$, 104.6 $mg \cdot kg^{-1}$, 10.6 $mg \cdot kg^{-1}$, 0.005 $mg \cdot kg^{-1}$, 57.08 $mg \cdot kg^{-1}$, 0.209 $mol \cdot kg^{-1}$ and 6.90, respectively.

The pot experiment was conducted with Fe levels of 0, 50, 100, 200, and 400 $\mu mol \cdot L^{-1}$ ($FeSO_4 \cdot 7H_2O$) from March 10, 2015 to July 9, 2015 in the greenhouse of College of Resources and Environmental Sciences at Southwest University, China. 5 kg of air-dried soil was passed through a 40-mesh screen and then treated with $CdCl_2 \cdot 2.5H_2O$ (10 $mg \cdot kg^{-1}$). After uniform mixing, the Cd-treated soil was placed into plastic pots with a diameter of 25 cm and a height of 17 cm for an equilibration period of 2–3 weeks. One seedling was planted in each pot. Fe-containing nutritional solution was sprayed on the seedling leaves during florescence, and a sample sprayed with de-ionized water was used as the control. Spraying was performed every 5 days, at 100 $mL \cdot pot^{-1}$ per day, for 7 days.

The base fertilizers included were P as $NH_4H_2PO_4$, K as KCl, and N as $NH_4H_2PO_4$ and urea at concentrations of 100 $mg \cdot kg^{-1}$, 150 $mg \cdot kg^{-1}$ and 180 $mg \cdot kg^{-1}$, respectively. The soil moisture was measured using a soil moisture tachometer before watering. The mean moisture content in the soil was calculated over three watering sessions to evaluate the amount of water required to maintain the maximum water-holding capacity of the soil at up to 60%. The experiment was performed in triplicate, and arranged randomly. During the middle and late stages of tomato growth, fresh leaves from the control plants were subjected to snap freezing with liquid nitrogen, and kept in the freezer at -80°C for RNA extraction. The plants were harvested on July 9, 2015, and kept at 105°C for 15 min for denaturing the enzymes, and then oven-dried at 60°C until there was no further change in the weight of the sample.

Analysis of Cd Concentrations in Soil and Plants

Soil containing Cd was first boiled with HNO_3-$HClO_4$ (*v*:*v*=4:1) (Sinha *et al*., 1997), and its concentration was determined using an atomic absorption spectrophotometer (SIMMA 6000; PerkinElmer, Norwalk, CT, USA). The plant samples were first air-dried, grounded and the Cd concentration in the plants was determined using a similar method used to quantify the levels of Cd in the soil. The results were monitored for quality control in following with plant standard reference material (GBW08513) and soil (GBW08303) obtained from the National Institute of Standards and Technology, China. The recovery rates of all the plants and soils were higher than 95%, and the relative standard deviation (RSD) for the precision of the tests was less than 10%.

Determination of Cd Forms in Fruits

The concentration of Cd in its various forms in tomato fruits was determined through continuous leaching method (Alarcón *et al*., 1998). The measurement limit of Cd was 0.005 $mg \cdot kg^{-1}$. The sequence of extraction agents used was as follows: 80% ethyl alcohol (F_E for extracting nitrate, inorganic salt, chloride, and cysteine salt), de-ionized water (F_W for extracting water-soluble organic salt and heavy metallic phosphate), 1 $mol \cdot L^{-1}$ NaCl solution (F_{NaCl} for extracting pectin salt bound to protein or absorbed with heavy metals), 2% acetic acid (F_{HAC} for extracting indissoluble heavy metallic phosphates including secondary phosphates), and 0.6 $mol \cdot L^{-1}$ HCl (F_{HCl} for extracting oxalate). A flame atomic absorption spectrometer (SIMMA 6000; PerkinElmer) was used to determine Cd concentration. The plant standard reference material (GBW08513) obtained from the National Institute of Standards and Technology was adopted to monitor and control the quality of the measurement results. The recovery rates of all the plant samples were higher than 95% and the RSD was less than 10%.

Detection of Gene Expression

Total RNA extraction and RNA detection: Total RNA

was extracted from the plant material using an RNA extraction kit purchased from Huashun Biotechnology Company (Qingdao, China). RNase-free DNase I (Code No.D2215) (TARALA) was used to remove trace amounts of DNA in the RNA samples. The purified RNA (1 μL) was used to determine the optical density (OD) values at 260 nm and 280 nm using a Nanodrop 2000 UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Synthesis of cDNA for reverse transcription: The reaction system (20 μL) included 2 μg of total RNA, 1 μL of Olig (dT) 18, and 11 μL of diethylpyro carbonate-H_2O in ice-bathed microcentrifuge tubes. The materials were gently mixed until uniform, and then centrifuged at 2000×*g* for 5 s. The reaction mixture was placed in a 70°C water bath for 5 min, and then rapidly cooled for 5 min and again centrifuged at 2000×*g* for 5 s. Under ice-bath conditions, 4 μL of 5× buffer, 1 μL of RNase inhibitor (40 $U \cdot \mu L^{-1}$), 1 μL of MMLV (200 $U \cdot \mu L^{-1}$), and 2 μL of dNTP (10 mM) were sequentially added. The mixture was subjected to gentle shaking until uniform, centrifuged at 2000×*g* for 5 s, incubated in a 42°C water bath for 1 h, and then incubated at 70°C for 15 min. Finally, the reaction was terminated at 4°C for 1 min, and the reaction mixture was stored at -20°C until future analysis.

Design and synthesis of primers: In order to examine the genes associated with Cd metabolism, the reverse transcription polymerase chain reaction (RT-PCR) primers for the seven above-mentioned genes were designed with Oligo6.0 software using the cDNA sequence of tomato (*Solanum lycopersicum* L.) from the NCBI Genbank and other cDNA sequences of *Solanaceae* and synthesized by Invitrogen (Carlsbad, CA, USA; Table 1).

PCR Amplification of cDNA

cDNA was specifically amplified with an ABI-9700 PCR (Applied Biosystems, Foster City, CA, USA) for the reaction system containing 2.5 μL of 10× buffer (including Mg^{2+}), 0.5 μL of dNTP (10 m*M*), 0.5 μL of forward primer (10 μ*M*), 0.5 μL of reserve primer (10 μ*M*), 0.3 μL of Taq DNA polymerase (5 $U \cdot \mu L^{-1}$), and 0.25 μL of cDNA template, with double distilled H_2O added to make up the total volume to 25 μL (Table 1).

The PCR reaction was conducted using a program that included 35 cycles of amplification with pre-denaturation at 94°C for 2 min, denaturation at 94°C for 40 s, annealing at 52–60°C for 1 min (may be adjusted), extension at 72°C for 45 s, and a final extension at 72°C for 10 min. The PCR products were evaluated through agarose gel electrophoresis (1.2% gel, containing Gold View), and then photographed with an ultraviolet transmitter. The PCR annealing temperatures were 52°C for MT2, 56°C for OAS, 56°C for HMA, 56°C for NRAMP, 58°C for PCs, 58°C for CAM2, and 60°C for IRT. The obtained cDNA was specifically amplified using the ABI-9700 PRC instrument. The reaction system included 2.5 μL of 10× buffer (containing Mg^{2+}), 0.5 μL of dNTP (10 m*M*), 0.5 μL of forward primer (10 μ*M*), 0.5 μL of reverse primer (10 μ*M*), 0.3 μL of Taq DNA polymerase (5 $U \cdot \mu L^{-1}$), and 0.25 μL of cDNA template, with ddH_2O added to make up the total volume to 25 μL.

Real-time Quantitative PCR

The cDNA obtained by reverse transcription was diluted with double distilled H_2O by 2.5 times, and then its specificity was detected using SYBR Premix Ex TaqTM II (Perfect Real Time) reagent from TaKaRa Company (Kyoto, Japan), with 28S as the interior labeled gene. The PCR amplification reaction system included 10 μL of SYBR®Premix Ex Taq™ II (2×), 0.8 μL of forward primer (10 $\mu mol \cdot L^{-1}$), 0.8 μL of reverse primer (10 $\mu mol \cdot L^{-1}$), 0.4 μL of ROX Reference Dye II (50×), and 100 ng of cDNA template, with ddH_2O added to make up the total volume to 20 μL.

The RT-PCR conditions included a 40-cycle amplification program with pre-denaturation at 95°C for 30 s, denaturation at 95°C for 5 s, annealing at a suitable temperature (dependent on the specific reaction: MT2 at 52°C, OAS at 56°C, HMA at 56°C, NRAMP at 56°C, PCS at 58°C, CAM2 at 58°C and IRT at 60°C) for 1 min, extension at 72°C for 45 s and a final extension program at 72°C for 10 min. Each sample was subjected to repeated amplification three times, with 26S as the interior labeled gene.

Statistical Analysis

Data were subjected to three-way univariate ANOVA using SPSS version 21.0 (IBM Corp., Armonk, NY, USA). The parameters for the roots, stems, leaves and fruit biomass, and Cd concentration tomatoes were analyzed. Prior to ANOVA, normal probability and residual plots were constructed for each dataset and examined for unequal variance and deviations from normality among the residuals. All data fulfilled the conditions for equal variance and normality. The means were considered statistically significant ($P \leq 0.05$) using Fisher's least significant difference test.

Results

Plant Biomass

Significance differences of dry weights of the fruits, roots, stems, leaves, and dry weight of plant were found between two tomato varieties and among Fe levels were found (Fig. 1). Under heavy metal Cd pollution (10 $mg \cdot kg^{-1}$) in soil, spraying Fe solution significantly increased the total dry weights of the fruits, roots, and leaves of the two tomato

Table 1: Primers for RT-PCR of cadmium tolerance associated genes in tomato

Gene	Primers	Sequence (5' - 3')
MT2	FSMT2RT(Forward primer)	ATGTCTTGCTGTGGAGGAAGCTG
	RSMT2RT(Reverse primer)	CACACTTGCAGTCAGATCCACATTTGCA
OAS	FSOASRT	CAGCAATTTGAAAACCCTGCTAACCC
	RSOASRT	ACCAATCCCCTGAATCTTATGTGGAC
HMA	FSHMART	TGGGCTGGGACTACGAATCTAAATG
	RSHMART	GTTATTGTTCCAGTTTTGTCAAAAGCCA
NRAMP	FSNRAMPRT	ATGAGTATAGCTTTTTTGGATCCGGG
	RSNRAMPRT	GCTTCCAGCTTCCTTACACCATAGTTCTC
PCS	FSPCSRT	GTCTTGAATGCCCTTGCTATTGATCC
	RSPCSRT	TAACCACCAATAGGTGAAAAGTGGCC
CAM2	FSCAM2RT	GATCAGCTCACCGACGATCAGATCTC
	RSCAM2RT	TCCTCCTCAGAGTCAGTGTCCTTCAT
IRT	FSIRTRT	TGGCACAAATTCCCCTTACATGGATTTGT
	RSIRTRT	GCTATACCAAATGGAGTTGTTACTGCGAAG

Table 2: Effects of different Fe levels on chemical forms and concentration of Cd in tomato$^{\Psi}$

Fe concentration/μmol·L^{-1}	F_E/mg·kg^{-1}		F_W/mg·kg^{-1}		F_{NaCl}/mg·kg^{-1}		F_{HAC}/mg·kg^{-1}		F_{HCl}/mg·kg^{-1}		F_R/mg·kg^{-1}		Total extracted Cd/mg·kg^{-1}	
	V4641	YP109	V4641	YP109	V4641	YP109	V4641	YP109	V4641	YP109	V4641	YP109	V4641	YP109
0	0.345	0.170	0.115	0.229	0.334	0.222	0.220	0.167	0.232	0.310	0.839	1.239	2.085	2.337
50	0.274	0.105	0.057	0.047	0.123	<0.001	0.124	0.138	0.213	0.544	0.827	1.172	1.618	2.006
100	0.200	0.112	0.024	<0.001	0.022	0.034	0.110	0.082	0.201	0.336	0.799	1.309	1.356	1.873
200	0.070	0.140	0.013	<0.001	0.088	0.134	0.095	0.138	0.193	0.367	0.819	1.380	1.278	2.159
400	<0.001	0.174	<0.001	0.172	0.225	0.233	0.082	0.157	0.312	0.405	1.104	1.346	1.723	2.487
$LSD_{0.05}$														
V	0.068			0.009		0.016		0.011		0.004		0.037		0.221
C	0.021			0.003		0.005		0.003		0.015		0.006		0.079
V×C	0.013			0.005		0.008		0.012		0.021		0.019		0.113

$^{\Psi}F_W$, F_E, F_{HAc}, F_{NaCl}, F_{HCl} and F_R represent water extractable Cd, ethanol extractable Cd, acetic acid extractable Cd, NaCl extractable Cd, HCl extractable Cd and residual Cd, respectively

varieties by 5.0–48.7%, 13.3–71.1%, 16.0–63.1%, 9.8–54.6% and 18.5–40.3%, respectively except for the dry weight of the roots (50 μmol·L^{-1} for V4641) and the dry weight of the leaves (400 μmol·L^{-1} for YP109). Highest dry weights of the fruits, roots, stems, leaves, and dry weight of plant were found at the 100 or 200 μmol·L^{-1} Fe treatments, and then gradually reduced. Compared with the two tomato varieties, the dry weight of the fruits was higher in V4641 than in YP109 with the Fe levels in the range of 0–100 μmol·L^{-1}; however, the dry weight of the fruits was higher in YP109 than in V4641 at Fe levels of 200 or 400 μmol·L^{-1}.

Chemical Forms and Concentrations of Cd in Tomato Fruits

A significant differences of Cd concentrations in its various chemical forms and the total amount of extracted Cd between the two tomato varieties, and among Fe levels (Table 2). The average Cd concentrations of chemical forms in fruits of the two tomato varieties were in order of residual Cd (F_R)>HCl extractable Cd (F_{HCl})>alcohol extractable Cd (F_E)>NaCl extractable Cd (F_{NaCl})>acetic acid extractable Cd (F_{HAC})>water extractable Cd (F_W). The average concentrations of F_R in the two tomato varieties were 0.23 and 0.39 mg·kg^{-1}, respectively accounting for 14.5 and 18.1% of the total amounts of extracted Cd, respectively, while the average concentrations of F_W and F_E were 0.16 and 0.07 mg·kg^{-1}, accounting for only 8.4 and 3.5% of the total amounts of extracted Cd, respectively.

In the present study, spraying with a suitable amount of Fe (<100 or 200 μmol·L^{-1}) reduced Cd concentrations of various chemical forms in fruits both tomato varieties. However, as the Fe level increased, different trends were found in Cd concentration of various chemical forms. For example, increasing Fe levels, F_E and F_W in V4641 fruits decreased from 0.345 mg·kg^{-1} and 0.115 mg·kg^{-1} to less than 0.001 mg·kg^{-1}. Furthermore, the concentration of F_{HAc} gradually reduced by 0.096–0.138 mg·kg^{-1} as the Fe level increased (Table 2). However, the amounts of total extracted Cd and concentrations of various chemical forms of Cd (excepted of F_{HCl}) in the YP109 fruits, and the concentrations of F_{NaCl}, F_{HCl}, and F_R in V4641 fruits decreased at first with increasing Fe levels (≤ 50, 100 or 200 μmol·L^{-1}, respectively) and then increased (Table 2). High levels of Fe (400 μmol·L^{-1}) resulted in increases of F_{HCl} and F_R in V4641 fruits and F_E, F_{NACl}, and F_R in YP109 fruits, with enhancements of 0.08 mg·kg^{-1}, 0.27 mg·kg^{-1}, 0.004 mg·kg^{-1}, 0.011 mg·kg^{-1} and 0.15 mg·kg^{-1}, respectively. In addition, spraying Fe solution (50-400 μmol·L^{-1}) resulted in an increase of F_{HCl} in YP109 fruits, with enhancements of 0.06–0.23 mg·kg^{-1}.

Cd Concentration in Tomato

Cadmium concentrations in the two tomato varieties were in order of leaves>roots>stems>fruits (Fig. 2). In the control group not sprayed with Fe-containing solution, Cd

Table 3: Measurement of contents of total RNA in tomato leaves

Sample	OD_{230}	OD_{260}	OD_{280}	RNA concentration/ng·μL^{-1}	OD_{260}/OD_{280}	OD_{260}/OD_{230}
V4641	15.630	22.664	10.691	906.5	2.12	1.45
YP109	28.769	25.317	11.862	1012.7	2.13	0.88

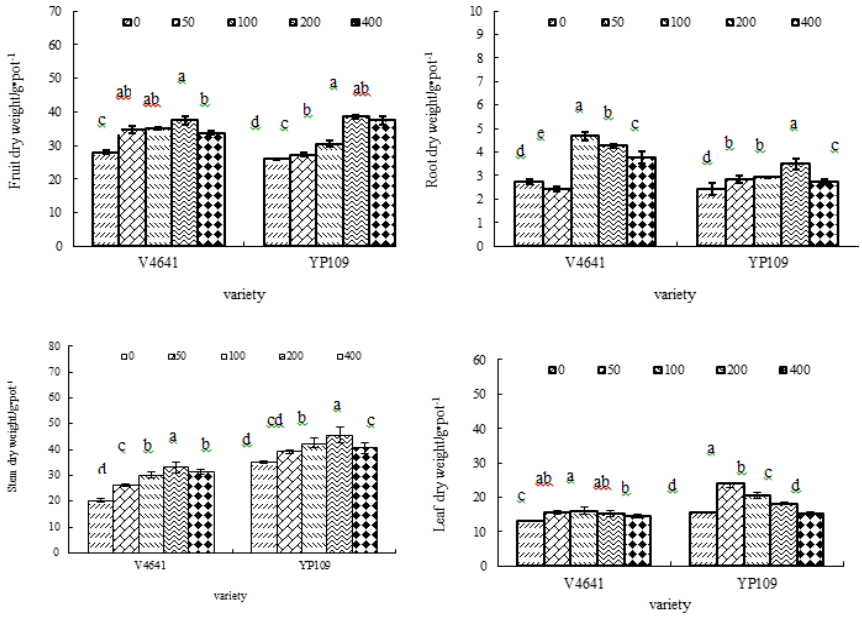

Fig. 1: Effects of different Fe levels (μmol·L^{-1}) on dry weight of tomatoes (g·pot^{-1})
[ψ]Different letters (a, b, c) indicate significant difference at P≤0.05 among different Fe levels in the same variety

concentrations in the leaves, stems, and fruits of YP109 were higher than those in V4641, with enhancements of 1.13, 1.23, and 1.11 times, respectively. Spraying Fe-containing solution reduced Cd concentrations in the tomato leaves, stems, and fruits, with reductions of 15.7–21.9% and 7.1–25.3%, 30.8–47.2% and 35.6–50.4%, 29.1–45.1% and 13.0–17.1%, 2.8–11.7% and 4.3–9.9%, when compared with the control, respectively, except for the YP109 plant at 400 μmol·L^{-1} Fe treatment. However, as the Fe level increased, Cd concentrations in the leaves, stems, roots, and fruits initially decreased, and then increased when Fe level more than 100 μmol·L^{-1} or 200 μmol·L^{-1}.

Genes Associated with Cd Accumulation and Tolerance in Tomato

The total RNA concentration in the leaves of both tomato varieties was approximately 1000 ng·μL^{-1} (Table 3). The OD_{260}/OD_{280} ratios for V4641 and YP109 RNA were 2.13 and 2.12, respectively. The rRNA accounted for more than 90% of the RNA in plant cells. After being dyed with l coeruleum bromophenolis, two clear stripes with different sizes on colloid matrix (28S and 18S) were observed.

Based on the semi-quantitative analysis, seven genes associated with Cd accumulation were expressed in the leaves of the tomato plants. As shown in Fig. 3, except for the *IRT* (including *IRT1* and *IRT2*) genes, the other six genes associated with Cd tolerance were expressed in the tomato leaves. Among these genes, we found high expression levels for *OAS* and *CaM2*, medium expression levels for *MT2* and *NRAMP*, and low expression levels for *HMA* and *PCS*. In addition, as shown in Fig. 3, *MT2*, *OAS*, *HMA*, *NRAMP*, *PCS* and *CaM2* were approximately 220, 250, 440, 400, 320, and 280 bps in length, respectively, which were similar to the cDNA lengths of the related genes found in Genbank.

When comparing two tomato varieties, the expression of the genes associated with *MT2* in the V4641 leaves was obviously higher than in the YP109 leaves, and the expression levels of *NRAMP* and *PCS* in YP109 were a little higher than in V4641. The expression of *MT2* in the leaves was higher than of *NRAMP* in V4641, while the expression of *NRAMP* was higher than of *MT2* in YP109.

Discussion

Iron, as one of the necessary nutritional elements for plant growth, is not only involved in the formation of chlorophyll, but also is an important part of oxidation-reduction enzymes such as ferroprotein and molybdo-Fe protein. Meanwhile, it

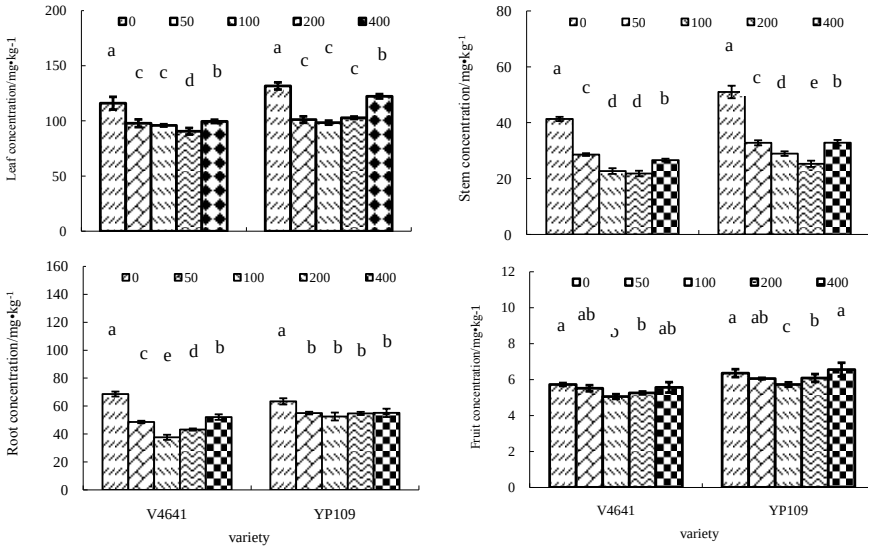

Fig. 2: Effect of different Fe levels (μmol·L^{-1}) on Cd concentration in tomatoes
$^{\Psi}$Different letters (a, b, c) indicate significant difference at P≤0.05 among different Fe levels in the same variety

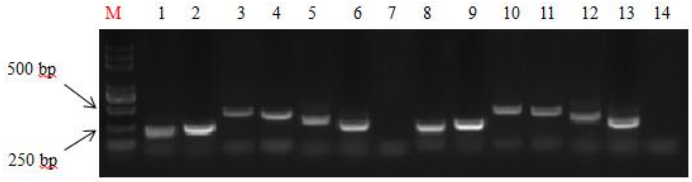

Fig. 3: Electrophoresis figure of RT-PCR of cadmium tolerance associated genes in tomato leaves$^{\Psi}$
$^{\Psi}$Number1-7 indicated *MT2*, *OAS*, *HMA*, *NRAMP*, *PCS*, *CAM2* and *IRT* of YP109, respectively; number 8-14 indicated *MT2*, *OAS*, *HMA*, *NRAMP*, *PCS*, *CAM2* and *IRT* of V4641, respectively

can combine with protein in various chemical forms in plants as an important electronic transporter or catalyst for improving processes such as photosynthesis and respiration, promoting plant growth and enhancing anti-stress capacity in plants (Lu *et al*., 2006), thereby mitigating the growth inhibition of plants caused by stress from heavy metals. Under experimental conditions with polluted soil (10 mg·kg^{-1} Cd), the spraying of Fe-containing solution resulted in a significant increase in the dry weights of the tomato fruits, leaves, and roots compared with plants not treated with Fe. It may be due to antagonism of Fe and Cd could effectively reduce the toxicity of Cd on tomato plants (Shao *et al.*, 2007). On the other hand, the excessive levels of Fe (>100 or 200 μmol·L^{-1}) resulted in the reduction of biomass, which may be due to excess iron and its compounds in plants induced various active free radicals, resulting in lipid peroxidation and membrane damage (Sinha *et al*., 1997). Moreover, the synergistic effect between Fe and Cd could increase the toxicity levels in tomato plants (Siedlecka and Krupa, 1999). It showed that the interaction between Fe and Cd was closely correlated with the level of exogenous Fe. In the present study, the suitable level for the addition of exogenous Fe was 100 or 200 μmol·L^{-1}, which could get higher biomass for both tomato varieties.

Usually, dominant chemical forms of heavy metals in plant were inactive, such as sodium chloride, acetate, or hydrochloride (Qin *et al*., 2017). In the present study, we found that highest concentration of various Cd chemical forms was F_R in tomato fruits, with an average concentration of 1.08 mg·kg^{-1}, accounting for 57.2% of the total amount of extracted Cd (Table 2). Followed by F_{HCl}, its average concentration was 0.31 mg·kg^{-1}, accounting for 16.5% of the total extracted Cd. The sum of both forms, as the major forms of Cd in tomato, was 1.39 mg·kg^{-1}, accounting for 73.7% of the total amount of extracted Cd. F_R and F_{HCl} were dominant chemical forms of Cd in tomato fruits and the low active chemical forms of Cd; conversely, F_W and F_E, were the high active forms of Cd. The sum of average concentrations of F_W and F_E were 0.23 mg·kg^{-1}, accounting for 11.9% of the total extracted Cd. It greatly reduced Cd toxicity on tomato plants. In the controls plants not sprayed with Fe-containing solution, the total amount of extracted Cd in tomato fruits, Cd concentrations of leaves, stems, and fruits in YP109 were more than in V4641, showed obvious genotype differences in absorption of Cd.

IRT is mainly responsible for the absorption of Fe and the transmission of metallic ions, including Cd and Zn ions. Some studies have also shown that the *IRT1* gene can be

induced and expressed in large amounts in plant roots to promote the absorption of Fe in stressful environments with insufficient Fe (Vert *et al*., 2002; Li *et al*., 2006). According to the study by Yoshihara *et al.* (2006), Cd stress can prevent Fe transference from the roots to the parts above the ground, thereby resulting in shortage of Fe in the plant parts above ground level. Similarly, Cd stress can also induce the expression of *NtIRT1* and *NtFRO* as the genes responding to the shortage of Fe in tobacco roots. In the present study, we did not observe the expression of *IRT* in tomato leaves, which may be because it is not necessary in plants and other transferring proteins involving the transference of Fe. Maybe*IRT1* can be expressed in tomato roots to improve the absorption of Fe (Yang and Poovaiah, 2003). Type II *MT* gene is mainly expressed in plant parts such as the leaves above the ground, with less expression in the roots (Whitelaw *et al.*, 1997). In the present study, high levels of *MT* expression were found in the tomato leaves, similar to reported previous researchers. It indicated that Cd stress can induce *MT2* expression. In addition, we found higher expression of *MT2* in V4641 leaves than in YP109 leaves, which is possibly due to more Cd^{2+} chelated by *MT2* under Cd stress and hence so Cd toxicity to plant is reduced. The detoxification mechanism of *MT2* for Cd is more important for V4641. Cd^{2+} has a stronger reaction with CaM and is capable of combining with CaM to activate downstream target enzymes, thereby leading to toxicity of plant cells. Furthermore, Cd can also combine with CaM, resulting in the accumulation of Cd in plant cells. In the present study, the difference of CaM expression in the leaves of both tomato varieties may be correlated with the different ability of inducing CaM synthesis between the two varieties under Cd stress.

Enhanced expression of PCs can promote the biological synthesis of plant-chelating peptides (PCs), which enhanced tolerance of plant on Cd due to combine with Cd to produce PC-Cd complexes, and stored in vacuoles. In the present study, higher expression levels of PCs and NRAMP were found in YP109 leaves than in V4641 leaves, which are consistent with higher Cd concentrations of leaves, stems, and fruits in YP109 than those in V4641at the control without any spray of Fe-containing solution. NRAMP is involved in the transportation of Fe and Cd in plants, but proteins with different gene codes in the same family have different functions. *NRAMP3* can transport Cd from vacuoles to the cytoplasm, thus increasing Cd toxicity (Thomine *et al*., 2003); while *NRAMP* can transport free metal ions to vacuoles, thus reducing the toxicity (Thomine *et al*., 2000). *NRAMP1* is mainly expressed in the roots of plants, while *NRAMP3* and *NRAMP4* are expressed in both the root and shoot (Qi *et al*., 2003). Therefore, we inferred that *NRAMP3* is mainly expressed in tomato leaves. Higher expression of *NRAMP* in YP109 leaves may increase the free Cd concentration in the cytoplasm. It may be the main reason why Cd concentration of leaves, stems, and fruits were higher in YP109 than in V4641.

In the present study, spraying a suitable level of Fe (≤200 μmol·L^{-1}) reduced Cd concentrations of its various chemical forms and the total amount of extracted Cd in tomato fruits. Obvious antagonistic effect between Fe and Cd was observed in this experiment. This result is similar to the report of Krupa *et al*. (1995). The reason may be related to the gene expression of the Fe transporter, or may be the competition between Fe and Cd for the transport site in the roots (An *et al*., 2002). However, high levels of Fe (400 μmol·L^{-1}) increased F_{HCl} and F_R concentrations in V4641 fruits, and increase F_E, F_{NACl} and F_R concentrations, as well as the total amount of extracted Cd in YP109, which further confirms the synergistic effect between Fe and Cd.

Stephan and Grun (1989) reported that the high-Fe-efficient tomato can absorb more heavy metals under high Fe levels. Compared with the two tomato varieties, Cd concentration of fruits in V4641 are less than those in YP109, whether the Fe-containing solution was sprayed on the leaves or not. Therefore, the interaction between Fe and Cd is not only correlated with Fe levels, but is also correlated with plant variety. In addition, competitive absorption of Fe and Cd by the transport site of the root can reduce efficiency of Cd long distance transportation in the xylem of plants. The spraying of suitable amounts of Fe-containing solution (≤100 or 200 μmol·L^{-1}) on tomato leaves reduced Cd concentrations in the leaves, stems, roots, and fruits of tomato is similar to those reported by Chlopecka and Adriano (1997) and Shao *et al.* (2007). The reasons may be the silencing of the Fe transporter genes, enhanced Fe absorption, and reduced passive absorption of Cd under conditions of sufficient Fe in the soil (Wang *et al*., 2005). On the other hand, Cd concentrations in the leaves, stems, roots, and fruits obviously increased when spraying Fe level was more than 100 or 200 μmol·L^{-1}. This result further confirmed that the interaction between Fe and Cd is not a simple antagonistic effect, but showed antagonistic and synergistic coexistence; it may be related to spraying Fe levels.

Conclusion

When exposed to Cd (10 mg·kg^{-1}), Fe (≤200 μmol·L^{-1}) spray improved the dry weights of tomato fruits, roots, stems, leaves, and plant, and reduced Cd concentrations in tomato fruits. Further, the residual Cd (F_R) and F_{HCl} with low activity were dominant chemical forms of Cd in tomato fruits. Spraying suitable Fe (<400 μmol·L^{-1}) reduced Cd concentrations of its various chemical forms in tomato fruits. The Cd concentration of fruits and its accumulation in the YP109 variety were less than in the V4641 variety. Among the seven genes associated with Cd accumulation and tolerance, the highest expression levels were *OAS* and *CaM2*, followed by *MT2* and *NRAMP*, while the lowest expression were for *HMA* and *PCS*. The expression of *MT2* in the V4641 leaves was higher than in the YP109 leaves, while the expression levels of *NRAMP* and *PCS* in the YP109 leaves were higher than in the V4641 leaves.

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