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Differences of Cd uptake and expression of Cd-tolerance related genes in two varieties of ryegrasses

T. Li[1], W. H. Xu[1]*, Y. R. Chai[2]*, Z. Y. Wang[1], D. T. Xie[1]

[1]*College of Resources and Environmental Sciences, Southwest University, Chongqing 400715, P. R. China*
[2]*College of Agronomy and Biotechnology, Southwest University, Chongqing 400715, P. R. China*

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Effects of different Cd levels (0, 75, 150, 300 and 600 mg·kg^{-1}) on biomass, Cd content and accumulation and cadmium tolerance related gene expression levels of two cultivars of ryegrasses (Bond and Aberd) were studied in soil culture experiment. The results showed that dry weights of shoot and the plant of Bond and Aberd increased by 10.06% and 4.04%, 25.84% and 16.89%, respectively compared with the control when exposed to 75 mg·kg^{-1} and 150 mg·kg^{-1} Cd, respectively. Cadmium concentration and Cd accumulation of shoot and root were significantly increased with the increase of soil Cd level (P <0.05). When exposed to 150 mg·kg^{-1} Cd, Cd concentrations of shoot and root in Bond and Aberd were 171.83 mg·kg^{-1}, 374.49 mg·kg^{-1}and 169.12 mg·kg^{-1}, 229.68 mg·kg^{-1}, respectively. Cadmium accumulation in Abed was greater than that in Bond at the same Cd level. The trend of the expression of OAS and IRT gene was consistent with the bimodal curve by increasing of cadmium levels. 75-150 mg·kg^{-1} Cd stress promoted the expression of OAS and IRT genes in ryegrasses. Considering two cultivars of ryegrasses, Abed has more suitable as a phytoremediation materials to repair soil Cd pollution due to higher biomass and cadmium accumulation.

Keywords: cadmium; ryegrasses; cadmium uptake; OAS gene; IRT gene

AIMS AND BACKGROUND

Cadmium (Cd) is the first heavy metal contaminant due to strong mobility in the soil and high toxic to the crop [1] of soil. According to statistics, the world releases about 30,000 tons of Cd to the environment each year, of which, about 82% -94% Cd penetrate into the soil [2]. China's annual discharge of cadmium to the environment by industrial waste totals 680 t, Cd pollution area of farmland reaches 280,000 hm^2, and annual production of agricultural products with exceeded cadmium approaches1.5 million t [3-4]. Cadmium content of approximately 24.1% vegetable garden soil exceeds the national soil environmental quality secondary standard [5]. Studies have shown that Cd will be enriched in the human body through the food chain and damage human health when crops exposed to Cd-contaminated soils [6]. Therefore, Soil Cd pollution control and remediation have been widely concerned by scholars at home and abroad in the field of environmental science.

Phytoremediation technology, as a green biotechnology, has the advantages of simple operation, economy and technical possibility of large area implementation [7]. Ryegrasses (*Lolium multiflorum* L.) is an ideal heavy metal restoration plant due to high growth rate, strong tillering ability and high yield, and strong enrichment effect on soil heavy metals [8]. There are significant differences in uptake and accumulation of Cd between different species and different cultivars, which is mainly related to genotype [9]. So far, there have been many metal ion transporter genes isolated and cloned from plants. Their transporters are closely related to absorption, transport, accumulation and fixation of metal ions, and play an important role in plant tolerance to Cd or Cd accumulation. ZIP gene, i.e. zinc-iron regulating protein gene, includes two types of genes of ZRTP (Zinc Regulated Transporter) and IRT (Iron Regulated Transporter), which are respectively responsible for the transport of Zn and Fe. Iron deficiency-induced increased expression of IRT1 in the root is beneficial to the uptake of Fe by root, and also causes more Zn^{2+} and Cd^{2+} accumulation in the root, indicating that IRT1 is related to absorption of Cd by root [10]. The report by He [11] has shown that RsIRT1 gene in radish is induced by exogenous Cd stress. RsIRT1 is involved in absorption and transport of cadmium under Cd stress. Phytochelatins (PCs) are a class of sulfhydryl-containing polypeptides of varying lengths consisting of cysteine (Cys), glutamic acid (Glu), glycine (Gly), etc.[12]. PCs can decrease Cd activity by chelating with the heavy metal ion Cd^{2+} in plant cells via sulfhydryl group, and alleviate Cd toxicity to plant [13]. Dominguez-Solis et al. [14] found that Cd stress strongly induced the expression of OASTL in *Arabidopsis thaliana*, whereas overexpression of OASTL also increased the tolerance of *Arabidopsis thaliana* to Cd. Unfortunately, there are few studies

* To whom all correspondence should be sent:
E-mail: xuwei_hong@163.com; chaiyourong1@163.com

on the differences in the expression levels of Cd tolerance related genes among different cultivars of ryegrasses Therefore, pot experiment was conducted to study the difference in biomass, Cd content and accumulation, the expression level of Cd tolerance related gene among different Cd levels and between two cultivars of ryegrasses (Bond and Aberd).

EXPERIMENTAL

Plant material, soil and Cd treatments

The soil pH was 5.23, organic matter content was 8.87 $g \cdot kg^{-1}$, total nitrogen content was 1.54 $g \cdot kg^{-1}$, available nitrogen content was 74.60 $mg \cdot kg^{-1}$, available potassium content was 65.07 $mg \cdot kg^{-1}$, available phosphorous content 38.59 $mg \cdot kg^{-1}$, cation exchange capacity (CEC) was 0.178 $mol \cdot kg^{-1}$. The total cadmium 0.11 $mg \cdot kg^{-1}$, and available cadmium was not detected. Seeds of two cultivars of ryegrasses (Bond and Aberd) were purchased from Jiangxi Scarecrow Agricultural Garden.

Five levels s of Se (0, 75, 150, 300 and 600 $mg \cdot kg^{-1}$) prepared from $CdCl_2 \cdot 2.5H_2O$ were set up for the pot experiment. Moreover, air-dried soil (5 kg) was sieved using a 40-mesh sieve, and treated with the $CdCl_2 \cdot 2.5H_2O$ solution and mixed to be homogeneously put in a plastic pot (diameter, 25 cm; height, 17 cm) and kept balance for two weeks. The seedlings of ryegrasses (*Lolium multiflorum* L.) with 10 cm high were then transplanted 30 plant for each pot. The moisture content in soil was kept 60% of the maximum moisture in the fields with deionized water. Fast- measurement of Soil Moisture (TZS-IW, Zhejiang Tuopu Instrument Co., Ltd., China) was used to determine the moisture content in soil. In the basic fertilizer the used amount of P ($NH_4H_2PO_4$) and K (KCl) were 100 and 150 $mg \cdot L^{-1}$ respectively and Nitrogen content ($NH_4\ H_2\ PO_4$ and urea) was 180 $mg \cdot L^{-1}$. N fertilizer was applied in three installments: 40% for basal and 60% each for seedling stages which applied with 15 days' interval after transplanting, each time 30%. All experiments were performed in triplicate and arranged at random. The harvested plants were oven-dried at 105 ˚C for 15min, and oven drying to constant weight in 60˚C.

ANALYSIS OF SOIL CD CONCENTRATIONS

Soil total Cd was digested with HNO_3-$HClO_4$ (5: 1 by volume) and determined by atomic absorption spectrophotometry (Perkin Elmer SIMMA 6000, Norwalk, USA). Soil available Cd content was determined by DTPA extraction (GBT 23739-2009) and atomic absorption spectrophotometry (Perkin Elmer SIMMA 6000, Norwalk, USA). Soil reference materials (GBW # 08303) provided by National Institute of Standards and Technology were used for quality monitoring of the determined results. The Cd recovery of all soil samples was higher than 95% and accuracy of relative standard deviation (RSD) was within 10%.

ANALYSIS OF CD CONCENTRATIONS IN THE PLANTS

Plant Cd concentration was digested with HNO_3:$HClO_4$ (4: 1), and Cd^{2+} solution after digestion was determined by atomic absorption spectrophotometry (Perkin Elmer SIMMA 6000, Norwalk, USA). The detection limit was 0.005 $mg \cdot kg^{-1}$. Plant reference materials (GBW # 08513) provided by National Institute of Standards and Technology were used for quality monitoring of the determined results. The Cd recovery of all plant samples was higher than 95% and accuracy of relative standard deviation (RSD) was within 10%.

DETECTION OF GENE EXPRESSION

Total RNA extraction

RNA was extracted from each leaf tissue sample. The specific procedure was performed with reference to the Biomed RNA Extraction Kit operation manual.

Purification of total rna and detection

Reverse transcription of RNA used TaKaRa's PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time). First, use DNase I to treat the remaining DNA in RNA, and the treatment time was extended from 2 min in manual to 20 min, for sufficient removal of heavily contaminated total genome DNA from RNA and reverse transcription into cDNA. The specific operation is: (1) RNA purification: take 200μl RNase-free centrifuge tube configuration reaction system mixture, 5×gDNA Eraser Buffer 2.0 μl, gDNA Eraser 1.0 μl, Total RNA 1.0 μg, RNase Free dH_2O Up to 10 μl. After configuration of mixture, PCR instrument was heated at 42°C for 20 min, and then cooled to 4°C for1~2 min. (2) RNA reverse transcription into cDNA. 20 μl reaction system: 5× PrimeScrip Buffer 4 μl, PrimeScrip RT EnzymeMix I 1 μl, RT Primer MIX 1 μl, purified RNA 10 μl, RNase Free dH2O supplemented to 20 μl. Reverse transcription was performed on a PCR instrument at 37°C for 15 min → 85°C for 5 sec → 4°C for1 min. The resulting cDNA was stored at -20°C refrigerator for standby use.

Primer design and synthesis

Seven Cd-resistant genes (OAS, IRT, HAM, NRAMP, MT, PCS and CAM) were studied in this study (Table 1). Based on BLAST and multiplex alignment (Vector NTI Advance 11.51) of sequence of Cd-resistant gene family members in ryegrasses, RT-PCR specific primers of seven genes and 25S rRNA primers of reference genes were designed (Table 2), which were synthesized by Nanjing Kingsley Biotech Co., Ltd.

PCR amplification of CDNA

The obtained cDNA was specifically amplified with an ABI-9700 PCR instrument. The total volume of the reaction system was 25 μl, including 2.5 μl 10 × PCR Buffer (containing Mg^{2+}), 0.5 μl dNTPs (10 mM), 0.5 μl forward and reverse primers (10 μM), 0.25 μl Easy-Taq DNA polymerase (5 $U \cdot \mu l^{-1}$), 0.5μl cDNA template, and the rest was ddH_2O. PCR reaction procedure: pre-degeneration at 94˚C for 2 min, degeneration at 94˚C for 30 s, annealing (at 61.5˚C) for 45 s, extension at72˚C for30 s, to be repeated 35 cycles, with last extension at 72˚C for 3 min, at 16˚C for 5 min. PCR products were subject to 1.0% agarose electrophoresis detection. If clear band of predicted target size (about 250 bp) is obtained, then the reverse transcription is successful.

Real-time quantitative PCR

The cDNA after reverse transcription was diluted 30 times with ddH_2O, and the transcriptional expression level of the target gene was detected by qRT-PCR using FastStart Essential DNA Green Master kit of F.Hoffmann-La Roche AG. The data was analyzed on Bio-Rad CFX Manager 3.0 software via CFX96TM Real-Time System of real-time quantitative PCR. The operation was carried out with reference to the instruction manual. The reaction system is as follows: FastStart Essential DNA Green Master (2X) 5μl, F-primer (10 μM) 0.5 μl, R-primer (10 μM) 0.5 μl, cDNA template 3 μl, ddH_2O 1μl. Reaction conditions: pre-degeneration at 95˚C for 10 min, degeneration at 95˚C for 10s, annealing at 61.5˚C for 30 s, to be repeated 40 cycles. The product melting curve was tested from 65˚C to 95˚C, and repeat 3 times with 25 s as internal reference gene.

THE STATISTICAL ANALYSIS

Three-way analysis of univariate ANOVA and correlation analysis were performed using SPSS version 21.0 package (SPSS, 2009). The variables analyzed separately were Cd concentration and Cd uptake in ryegrass. The level of significant was 0.05.

Table 1. Selection of Cd-tolerance related genes

Gene name	Gene symbol	Encoded protein	Literature resources
Calmoduline gene	*TcCaM2*	Calmoduline	Han et al. [15]
Plant synthase gene	*AtPCS1* *BjPCS*1	Plant synthase	Clemens et al. [16]
Metallothionein gene	*BjMT2*	Metallothionein	Zhang et al. [17] An et al. [18] (2006)
OASTL gene	*OAS*	O-acetyl-ser(thi0l)-lyase	Dominguez-Solis et al. [14]
HMA family	*HMA2* *HMA3* *HMA4*	Related ATP enzyme	Mills et al. [19] Hussain et al. [20] Gravot et al. [21] Bernard et al. [22]
Nramp family	*AtNramp3* *AtNramp4*	Encoded metal ion transport protein	Thomine et al. [23]
ZIP family	*IRT1*	Encoded plasma membrane transporter	Eide et al. [24](1996) Clemens et al. [25] (2006)

Table 2. Primers for qRT-PCR of Cd-tolerance related genes in ryegrass

Gene	Primer	Sequence (5'→ 3')	Tm˚C
OAS1	FLmOAS1q（Forward primer）	5'-GCTGGTTGGAATATCTTCTGGC-3'	61.5
	RLmOAS1q（Reverse primer）	5'-CCATGCTCTCAGCCTCCTTCT-3'	
OAS2	FLmOAS2q	5'-GCTGGTTGGAATATCTTCCGGT-3'	61.5
	RLmOAS2q	5'-CATGTTCTCGGCCTTCCTCC-3'	
OAS3	FLmOAS3q	5'-GCAAAGCAGTTGGCTCTTCAG-3'	61.5
	RLmOAS3q	5'-CTGCTCGCACTCTTCTCTGATG-3'	
OAS4	FLmOAS4q	5'-GTTACCACGGGAGAGGCAGT-3'	61.5
	RLmOAS4q	5'-CGGAACAGGATGCTAGAGATGT-3'	
OAS5	FLmOAS5q	5'-AGGTGAAAGGTGAGGATGCTG-3'	61.5
	RLmOAS5q	5'-CAGCTTCCTTCCTCAAACCCT-3'	
OAS6	FLmOAS6q	5'-CACTGAGGATGCAATGACGAAC-3	61.5
	RLmOAS6q	5'-CAGTGGCAAAGAGGTCCGAGTT-3'	
OAS7	FLmOAS7q	5'-AGTCATCGACGAAGTGGTCACT-3'	61.5
	RLmOAS7q	5'-TGCTGCAAAGAGGTGTGAGTC-3'	
OAS8	FLmOAS8q	5'-GGTGATTGACGAGATCCTTGCA-3'	61.5
	RLmOAS8q	5'-TTCCACGAAGAGGTCAGAGGAA-3'	
OAS9	FLmOAS9q	5'-GGTCACACAAGATTCAGGGTACA-3'	61.5
	RLmOAS9q	5'-GTCACATTCCTCCCTAACAAGTG-3'	
IRT4	FLmIRT4q	5'-CCGAAACGATCCGTCACAGA-3'	61.5
	RLmIRT4q	5'-AAGAAGGTCGCCATGAGCAC-3'	
ITR6	FLmIRT6q	5'-GAAGCAGAAGATGGTCTCCAAG-3'	61.5
	RLmIRT6q	5'-CACATGTAACCCACTGTTGCCA-3'	
ITR7	FLmIRT7q	5'-GCTCCGTCGTGGTGTCACAG-3'	61.5
	RLmIRT7q	5'-GCTCCGTCGTGGTGTCACAG-3'	
ITR8	FLmIRT8q	5'-TCCGAGGACGAAAAGGACAC-3'	61.5
	RLmIRT8q	5'-CAGAAGAAGAGGATCATGGTCAC-3'	
ITR10	FLmIRT10q	5'-CCATGGGAGCGAGGAGAGAC-3'	61.5
	RLmIRT10q	5'-AGCCATGAGGAGTGCAGAGA-3'	
HMA2	FLmHMA2q	5'-CTGCCGCCCATCATCCTCA-3'	61.5
	RLmHMA2q	5'-CTTCACATCCTGGCAAGCAAC-3'	
HMA3	FLmHMA3q	5'-TCGAGACCCTGGCTTGCAC-3'	61.5
	RLmHMA3q	5'-CTGCTTGGGCACCGGATAA-3'	
NRAMP2	FLmNRAMP2q	5'-GTGGTTACGAGCAATGATCACAC-3'	61.5
	RLmNRAMP2q	5'-CGGACTTCGTCGGTATAGAAGGA-3'	
NRAMP6	FLmNRAMP3q	5'-CTGAGGGCGCTGATAACCAGA-3'	61.5
	RLmNRAMP3q	5'-CAGCCACTGTCCAGGTTACAG-3'	
NRAMP6L	FLmNRAMP6Lq	5'-AGCTGTCGCTCTGTACTTCAAC-3'	61.5
	RLmNRAMP6Lq	5'-TTGATCACGATTGGCAGAGACG-3'	
MT1	FLmMT1q	5'-GGATGTCTTGCAGCTGTGGAT-3'	61.5
	RLmMT1q	5'-CCGGAGGCCATCTCAAACT-3	
MT2A	FLmMT2Aq	5'-CATCATGTCGTGCTGCGGT-3'	61.5
	RLmMT2Aq	5'-CACTTGCAGCCTCCGTTCT-3'	
MT2B	FLmMT2Bq	5'-GGAAGGAGAATGTCTTGCTGCA-3'	61.5
	RLmMT2Bq	5'-ACTTGCAGGTGGTGCAGTC-3'	
MT2C	FLmMT2Cq	5'-GAAGATGTCTTGCTGCTCAGGA-3'	61.5
	RLmMT2Cq	5'-TGGTGCCGCAGTTGCACTT-3'	
PCS	FLmPCSq	5'-CGCTCTCCGTCGTCCTCAAC-3'	61.5
	RLmPCSq	5'-TGGATGGTGGTCTGGTCTGC-3'	
CAM	FLmCAMq	5'-GAGCAGATCGCCGAGTTCAAGGA-3'	61.5

RESULTS

Biomass

As shown in Fig. 1, significantly different of dry weight in shoots, roots and the plant in Bond and Aberd were found between cultivars and among different Cd levels (P <0.05). The dry weight of shoots in Bond and Aberd increased first and then decreased with the increase of soil Cd level. The highest dry weight of shoots and the plant were observed at 75mg·kg^{-1} Cd, increased by10.06% and 4.04% compared with the control respectively. The dry weight of shoots and the plant of Aberd reached highest value at 150mg·kg^{-1} Cd, increased by

25.84% and 16.89% respectively compared with the control.

Concentration of CD in shoot and root

The concentrations of Cd in shoot and root of cultivars increased significantly with the increase of soil Cd (P <0.05) (Fig. 2). At different Cd levels, Cd concentrations of roots were higher than that of shoots. When soil Cd level was at 150 mg·kg^{-1}, Cd concentrations of shoots and roots of Bond and Aberd were 171.83 mg·kg^{-1}, 374.49 mg·kg^{-1} and 169.12 mg·kg^{-1}, 229.68 mg·kg^{-1} respectively, with Cd concentrations exceeding the critical value of cadmium hyper-accumulator (100 mg·kg^{-1}).

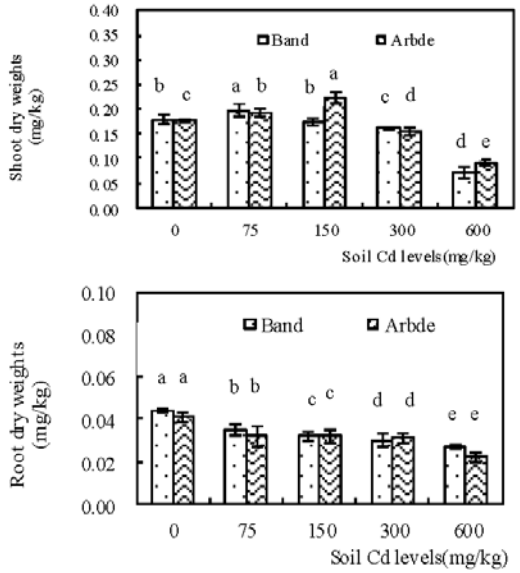

Fig.1. Effects of different Cd levels on dry weights of shoot and root in ryegrass. Different letters (a, b, c) indicate significant difference at P≤0.05 among different Cd levels in the same variety.

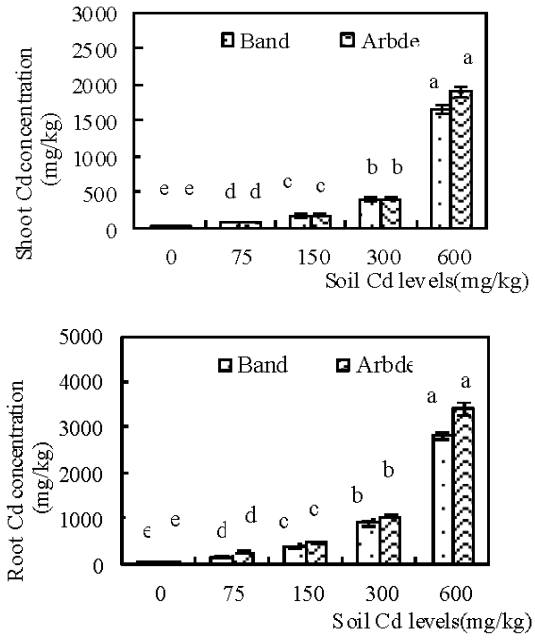

Fig. 2. Cadmium concentration in shoot and root of ryegrass. Different letters (a, b, c) indicate significant difference at P≤0.05 among different Cd levels in the same variety

CD accumulation in shoot and root

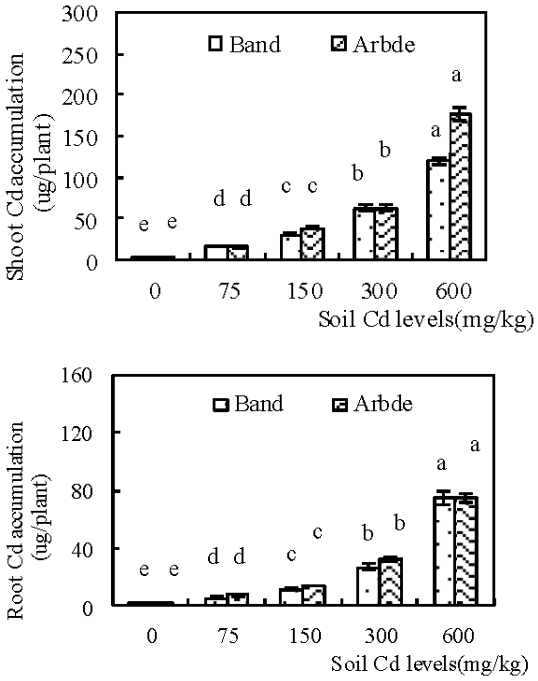

Fig. 3. Effect of different Cd levels on Cd accumulation of shoot and root in ryegrass. Different letters (a, b, c) indicate significant difference at P≤0.05 among different Cd levels in the same variety

As shown in Fig. 3, Cd accumulation in each part of plant increased with the increase of soil Cd level, and significant difference was found between cultivars and among soil Cd levels(P <0.05). The accumulations of cadmium in the shoots and roots of Bond were 53.84-422.93 times and 8.10- 116.56 times higher than that of the control respectively at 75, 150, 300 and 600 mg·kg^{-1} Cd.; while the accumulations of cadmium in shoots and roots of Aberd were 25.72- 304.23 and 6.94-70.04 times higher than that of the control respectively. At different Cd levels, the total of Cd accumulation in Aberd was greater than that in Bond.

Detection of cd-resistance related gene expression

The results of testing of OAS gene family of two cultivars of ryegrass were seen from Fig. 4. The trends of the expression of OAS5, OAS6 and OAS7 at different Cd levels were basically consistent with bimodal curve. The expression levels of OAS5, OAS6 and OAS7 of Bond cultivar were significantly increased by 13.63, 15.42 and 41.31 times higher than that of the control at 75 mg·kg^{-1} Cd, respectively. Expression levels of OAS5, OAS6 and OAS7 in Bond cultivar decreased at the level of

150-300 mg·kg^{-1}Cd, but increased at 600 mg·kg^{-1} Cd; Expression levels of OAS5, OAS6 and OAS7 in Aberd increased at the level of 75-150 mg·kg^{-1}Cd, decreased at 300 mg·kg^{-1}Cd, but increased at 600 mg·kg^{-1} Cd.

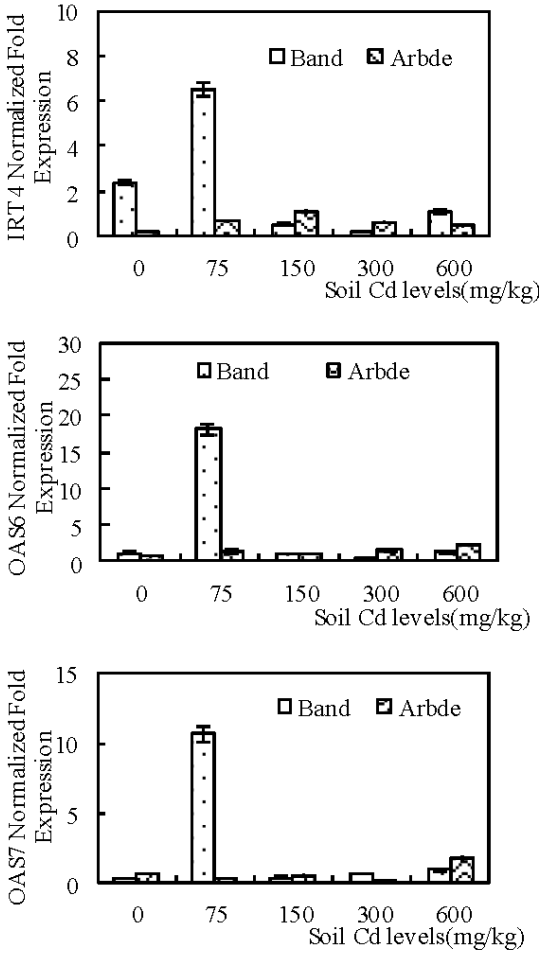

Fig. 4. Real-time PCR analysis of OAS in leaf of Ryegrass

As shown in Fig. 5, the expression trends of IRT gene family of two ryegrass cultivars at different cadmium levels were different. The expression trend of IRT gene family of Bond was basically consistent with bimodal curve. Expression level of IRT gene family in Bond significantly increased by 2.77, 3.60, 9.84, 4.40 and 1.80 times higher than that of the control at the level of 75 mg·kg^{-1} Cd respectively; while the expression level of IRT gene family in Bond decreased at the level of 150-300 mg·kg^{-1} Cd, then increased at the level of 600 mg·kg^{-1} Cd. The expression level of IRT gene family in Aberd increased first and then decreased, showing unimodal curve at 75-300 mg·kg^{-1} cadmium. The highest of expression level of IRT4 and IRT6 was observed at the level of 150 mg·kg^{-1} Cd, and then decreased when Cd level ≥ 300 mg·kg^{-1}. The expression level of IRT4 was significantly increased at 300 mg·kg^{-1} Cd. The highest of expression level of IRT8 and IRT10 was found at the level of 75 mg·kg^{-1} Cd, but significantly decreased when Cd level ≥ 150 mg·kg^{-1}. Significantly differences of expression level of IRT4, IRT6, IRT7, IRT8 and IRT10 genes were observed between cultivars of ryegrass and among soil Cd levels (P <0.05).

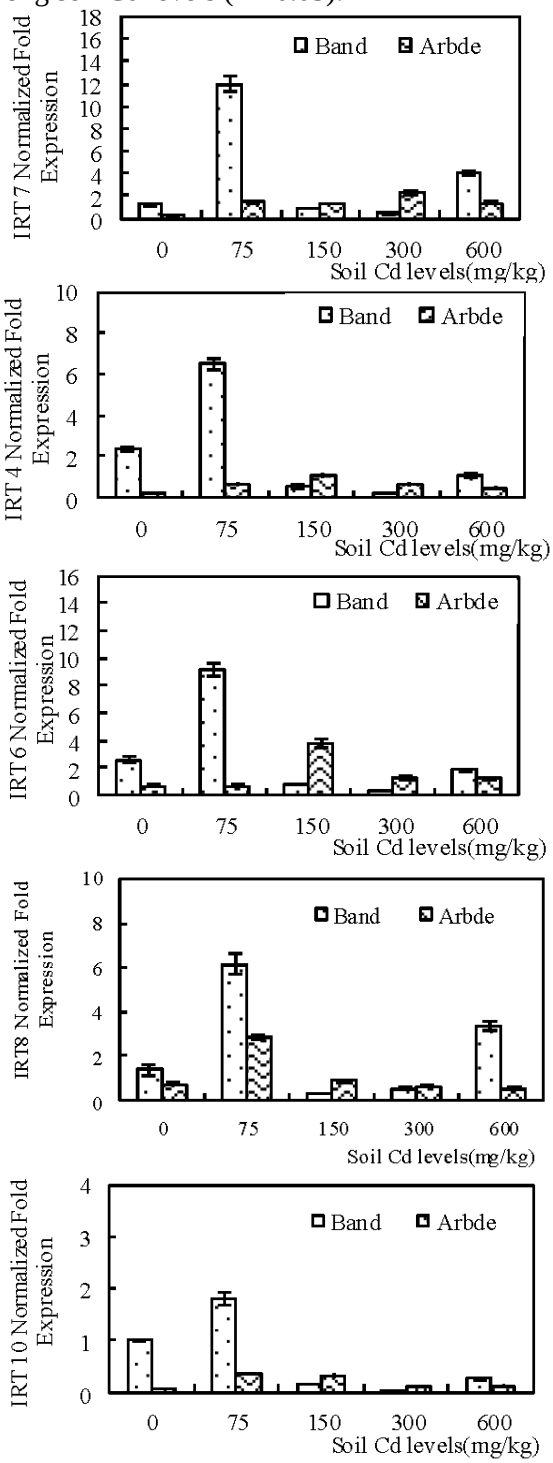

Fig. 5. Real-Time PCR analysis of IRT in leaf of ryegrass

DISCUSSION

Under Cd stress, the growth of plants are often inhibited and the toxic effect increases with the

increase of Cd stress level [26]. However, a few studies also have shown that Cd has no significant effect on plant growth under low Cd concentration [27], and even stimulates plant growth [28]. In our study, with the increase of soil Cd level, the dry weights of shoots of Bond and Aberd increased first and then decreased. Low Cd (≤75mg·kg^{-1} or 150mg·kg^{-1}Cd) stimulates the growth of Bond and Aberd, but high Cd (>75mg·kg^{-1} or 150mg·kg^{-1}Cd) inhibits the growth of shoots and roots of the two cultivars. The result is similar to the report by Shi et al. [28]. Significant differences in tolerance to Cd were found among different plant varieties or cultivars due to genotypic difference [29]. The recent experiment also supported this view, which Cd tolerance of Aberd was greater than that of Bond.

Cadmium hyper-accumulators should have three characteristics: First, under the same growth condition, shoot Cd concentration of plant is 100 times higher than that of ordinary plants, with cadmium critical content at 100 mg·kg^{-1}; second, Cd concentration in root is less than that in shoot; third, plant growth does not show obvious toxic symptoms, and has a strong tolerance to cadmium [30]. Shoot Cd concentrations of the two ryegrass cultivars (Bond and Aberd) exceeded the critical value of hyper-accumulator (100mg·kg^{-1}) when exposed to 150 mg·kg^{-1} Cd. It indicated that ryegrass was one of cadmium hyper-accumulators. The results were similar to those reported by Fang et al. [31]. Cadmium is highly mobile and readily absorbed by plants, and most plants can transfer cadmium from root to shoot [32]. However, the recent experiment showed that Cd concentrations of roots in Bond and Aberd were higher than that of shoot at the same Cd level in soil. It replied that roots of ryegrass had strong ability to enrich soil Cd, while its ability of transferring Cd to shoots from roots was weaker. These results were similar to the report of Sun et al. [33]. Significant differences in uptake and accumulation of cadmium between cultivars due to genotypic difference [34]. At the same level of cadmium, Cd concentrations and Cd accumulations of shoots and roots in Aberd were higher than that in Bond. This result supported to the report of Nesler et al. [34]. Comparing with two cultivars of ryegrass, Aberd has greater repairing ability in Cd pollution soil.

The study of glutathione metabolism is one of the important research contents in resistance / tolerance mechanism of plant under environmental stress [35]. There are two key enzymes, O-acetyl-serine (thiol) ligase (OAS-TL) and glutamylcysteine synthetase (γ-GCS), in the process of biochemical synthesis of glutathione [36]. These two enzymes impacts detoxification of cells and heavy metal enrichment due to limit the amount of glutathione synthesis, and affect synthesis and activity of polypeptide in the plant that can integrate heavy metal ions, i.e. phytochelatins (PCs) [37]. Harada et al. [13] introduced some of the genes into model plant tobacco using transgenic techniques. The results showed that tolerability of stress was much greater than that of the control group, and synthesis ability of cysteine in the plant was significantly improved. In this study, the highest expression level of OAS genes for Bond was found at 75mg·kg^{-1}Cd, while the highest expression level of OAS genes for Aberd was found at 75-150mg·kg^{-1}. It showed that Cd stress level at 75-150 mg·kg^{-1} promoted the expression of OAS genes in ryegrass. However, with the increase of soil Cd level, the expression level of OAS genes in ryegrass decreased gradually. These results were different to Wang‘s report, which the transcription expression of OAS-TL6 gene in collard increased when exposed to Cd-contaminated soil. The expression level of two Cd related genes (family) in ryegrass by Cd induced were significantly different between cultivars and among different cadmium treatments. These results are consistent with the report by Takahashi et al. [38].

IRT was first discovered as an iron transporter in *Arabidopsis thaliana*, which regulates cadmium absorption and transport [39]. The expression product of IRT1 and IRT2 is iron ion transport regulating protein located on the plasma membrane, and it is responsible for absorption of Fe^{2+} in the root and outer cortex. It is also related to Cd^{2+} absorption, and OSIRT1, AtIRT1 and AtIRT2 overexpression can increase accumulation of cadmium [38, 40]. In recent experiment, 75-150 mg·kg^{-1} cadmium stress increased the expression level of IRT genes in ryegrass, while high cadmium stress (≥600 mg·kg^{-1}) decreased the expression level of IRT genes in ryegrass, which was lower than that of the control. It may be the toxicity of Cd on growth and metabolic levels of ryegrass when exposed to high Cd stress. However, these results are inconsistent with the report by Uraguchi and Fujiwara [41].

CONCLUSION

The dry weight of shoot and the plant of the two ryegrass cultivars (Bond and Aberd) increased first and then decreased with the increase of soil cadmium pollution, and reached the peak at 75 mg·kg^{-1} and 150mg·kg^{-1} Cd respectively. Shoot Cd

concentrations of the two cultivars (Bond and Aberd) were higher than the critical value of hyper-accumulator (100mg·kg^{-1}) at 150 mg·kg^{-1} Cd. At the same soil Cd level, Cd concentrations of shoot and root in Aberd were higher than that in Bond. Cadmium stress (75-150 mg·kg^{-1}) promoted the expression levels of OAS and IRT genes in ryegrass. The expression levels of the two cadmium metabolism related genes (family) were significantly different between cultivars and among soil Cd levels (P <0.05).

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РАЗЛИКИ В УСВОЯВАНЕТО НА Cd И ЕКСПРЕСИЯТА НА Cd-ТОЛЕРАНТНИ ГЕНИ В ДВЕ РАЗНОВИДНОСТИ НА РАЙГРАС

Т. Ли[1], У.Х. Су[1]*, И.Р. Чай[2]*, З.И. Уанг[1], Д. Сие[1]

[1] *Колеж по ресурси и екологични науки, Югозападен университет, Чунцин 400715, Н. Р. Китай*
[2] *Колеж по агрономия и биотехнология, Югозападен университет, Чунцин 400715, Н. Р. Китай*

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(Резюме)

Изследван е ефекта на различни нива на кадмий в почвата (0, 75, 150, 300 и 600 $mg \cdot kg^{-1}$) и биомасата, съдържанието на Cd, натрупването му и експресията на Cd-толерантни гени в две култури на райграс (Bond и Aberd) при експерименти с почвени култури. Резултатите показват, че сухото тегло на издънки и растения от Bond и Aberd се повишават с 10.06% и 4.04% и съответно 25.84% и 16.89%, спрямо контролните експерименти с данни от съответно 75 $mg \cdot kg^{-1}$ и 150 $mg \cdot kg^{-1}$ Cd. Концентрациите и натрупването на Cd в издънките и корените значително нарастват с нарастването на нивата на кадмий в почвата (P <0.05). При въздействие от 150 $mg \cdot kg^{-1}$ Cd концентрацията на последния в издънките и корените на културите Bond и Aberd са съответно 171.83 171.83 $mg \cdot kg^{-1}$, 374.49 $mg \cdot kg^{-1}$ и 169.12 $mg \cdot kg^{-1}$, 229.68 $mg \cdot kg^{-1}$. Натрупването наCd в Aberd е по-високо отколкото в Bond при еднакви нива на кадмий. Тенденцията за експресия OAS- и IRT-гените последователна с би-модална крива с повишаването на нивата на кадмия. Стрес от кадмий от 75-150 $mg \cdot kg^{-1}$ промотира експресията на OAS- и IRT-гените в райграса. Културата Aberd е по-подходяща за ремедиация на почвата спрямо кадмия, поради по-голямото количество на биомаса и натрупването на кадмия.