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Analysis of gene expression profiles for metal tolerance protein in rice seedlings exposed to both the toxic hexavalent chromium and trivalent chromium

Xiao-Zhang Yu*, Wei-Jia Fan[1], Yu-Juan Lin[1]

The Guangxi Key Laboratory of Theory & Technology for Environmental Pollution Control, College of Environmental Science & Engineering, Guilin University of Technology, Guilin 541004, People's Republic of China

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ABSTRACT

The mechanisms of heavy metal tolerance in plants have been characterized extensively and metal tolerance proteins (MTPs) are one group of specific transporters involved in sequestration of heavy metal ions. This study focused on transcriptome analysis of *osMTP* genes in rice seedlings exposed to hexavalent chromium Cr(VI), or trivalent Cr(III) using real-time quantitative RT-PCR. Distribution of Cr and other mineral elements (Fe, Cu and Mn) in rice seedlings tissues was also determined. Results showed that exposures to both valents of Cr caused significantly more accumulation of Cr in roots rather than shoots, while accumulation and distribution of Fe, Zn and Mn in rice tissues was variable between two Cr treatments. PCR analysis displayed that heterologous expression patterns of individual isogenes from the *osMTP* gene family were observed in plant tissues as well as in Cr variants. Homologues, LOC_Os02g53490.1 (*osMTP8*)/LOC_Os03g12530.1 (*osMTP8.1*) and LOC_Os01g62070.1 (*osMTP11*)/LOC_Os05g38670.1 (*osMT11.1*), were also expressed differently under treatments with both forms of Cr. Data analysis indicated that distribution of mineral elements in rice tissues did not agree with changes of expression patterns of these corresponding *osMTP* genes. These results suggest that inconsistent expression of *osMTP* genes in rice tissues is largely due to Cr accumulation; at nutrient levels, the role of OsMTPs in storage and transport of mineral elements in plant materials is still open to discussion.

1. Introduction

Industrialization has resulted in an ever-increasing release of heavy metals into the environment from anthropogenic sources. In some cases, high-volume input of these harmful chemicals can alleviate biomolecules, impede proteins or substitute other essential ions, eventually cause cell damage of living organisms (Damodaran et al., 2013; Han et al., 2016; Zhang and Liu, 2017). It has been reported that plants have evolved two different strategies to cope with metal ions stresses either through an avoidance mechanism or tolerance pathway. The avoidance channel is a resistance-based process, in which heavy metals are mainly compartmentalized in cell walls in plants (Cobbett, 2003; Lai, 2015; Aryal et al., 2016; Yu et al., 2016). Tolerance pathway is largely dependent on sequestration of heavy metals in vacuoles, on binding them with appropriate ligands, and on the presence of different endogenous enzymes (Clemens, 2001; Sharma and Dietz, 2006; Verbruggen et al., 2009; Park et al., 2012; Yu et al., 2017a). In fact, these mechanisms in plants function never independently; their contributions to detoxification and biofortification of heavy metals are also most likely in a metal-specific manner (Sharma et al., 2016).

In order to avoid cellular damage, metal tolerance proteins (MTPs) play a vital role in transporting metal ions from the cytosol either by sequestration into intracellular compartments (vacuoles) or by export to excellular spaces in plants (Gustin et al., 2011; Sharma et al., 2016). Such a role in regulating cellular cation homeostasis has been experimental proven to influence cation content and tolerance, signal transduction flux, oxidative stress avoidance and protein metabolism in plants (Bruinsma et al., 2002; Ellis et al., 2004; Gustin et al., 2011). Indeed, the importance of MTPs cross-talk in the responses and resistance of plants to heavy metal stresses, such as Cu^{2+}, Zn^{2+}, Cd^{2+} and Mn^{2+}, has been demonstrated (Delhaize et al., 2007; Chen et al., 2013; Menguer et al., 2013; Migocka et al., 2014; Ueno et al., 2015; Fu et al., 2017; Zhang and Liu, 2017). Chromium (Cr) is one of most frequently detected heavy metals in the environment either from natural input or from anthropogenic activities. As Cr is non-essential elements for plants and lack biological function, entry inside it can exert toxicity

* Corresponding author.
E-mail address: xzyu@glut.edu.cn (X.-Z. Yu).
[1] Wei-Jia Fan and Yu-Juan Lin have contributed equally to this study.

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at multiple levels (Sharma et al., 2016; Yu et al., 2017a). Indeed, physiological and biochemical changes occurring during Cr exposure has been extensively reported (Dixit et al., 2002; Damodaran et al., 2013; Yu et al., 2017a). Until now, rice is the most staple protein source for vegetable-based diet population. Foci of previous works were mainly on investigation of the role of MTPs in heavy metal homeostasis in plants exposed to divalent heavy metals, such as Fe^{2+}, Zn^{2+}, Mn^{2+}, and Cd^{2+}. However, very few works have been conducted on genetic responses of OsMTPs (metal tolerance proteins in *Oryza sativa*) to Cr exposure. The aims of this study were to: 1) measure the changes of mRNA levels of individual isoforms from the rice MTP genomes under Cr exposure by qRT-PCR; 2) investigate the possible role of OsMTPs in detoxification of Cr in rice seedlings.

2. Materials and methods

2.1. Preparation of rice seedlings

Seedlings preparation of rice (*Oryza sativa* L. cv. XZX 45) was similar to our previous study with some modification (Zhang et al., 2014). Briefly, seedlings irrigated with a modified ISO 8692 nutrient medium were grown in a plant growth chamber with constant temperature of 25 ± 0.5 °C, illumination intensity of 20,000 lux, and a relative humidity of 60 ± 2% under continuous artificial light. After 16 d of growth, young seedlings collected were incubated in a modified ISO 8692 nutrient solution for 24 h, after that, seedlings were kept in a pre-treated solution containing 1 mM $CaCl_2$ + 2 mM MES-Tris buffer (pH 6.0) for 4 h to remove the excess ions from the cell wall space (Ebbs et al., 2008). All pre-treated young seedlings were collected for the subsequent experiments.

2.2. Exposure regime of rice seedlings

Exposure regime was identical to our previous study (Feng et al., 2017). Ten pre-treated young rice seedlings with similar size were chosen and kept into a 50 mL Erlenmeyer flask spiked with exactly 50 mL of Cr solution containing either Cr(VI) or Cr(III). Potassium chromate (K_2CrO_4) and chromium nitrate ($Cr(NO_3)_3{\cdot}9H_2O$) of analytical grade with ≥95% purity was used. Three different doses of Cr were selected. The nominal Cr concentrations of Cr(III) treatments were 0, 12.0, 24.0, and 40.0 mg Cr/L, while initial Cr concentrations in Cr (VI) treatments were 0, 2.0, 8.0, and 16.0 mg Cr/L. Each Cr treatment was conducted in 4 independent replicates. In order to prevent minimum evaporation of water and to inhibit algal growth, all flasks were wrapped with aluminum foil, and placed in a plant growth chamber.

2.3. Determination of Cr and nutrient elements in rice tissues

After 3 d of exposure, seedlings from different Cr treatments collected were rinsed with deionized water and separated into roots and shoots. The remaining procedure was identical to our previous work (Yu et al., 2017a). The content of total Cr, Fe, Mn, and Zn in different plant materials was determined by ICP-AES.

2.4. Identification of OsMTP genomes

Identification of OsMTP genomes was conducted through BLAST-P search in the rice database RGAP (http://rice.plantbiology.msu.edu/analyses_search_blast.shtml), based on their respective sequences from AtMTP (metal tolerance protein in *Arabidopsis thaliana*) in *Arabidopsis*. After removing redundant and query hits using an *e*-value ≤ e^{-10} as the threshold, ten isoforms were identified in rice MTP family from the rice database RGAP.

Table 1
Sequence of forward and reverse primers used in gene expression analysis of this study.

Gene	Locus identifier	Primer sequences (5′-3′)	Amplicon size (bp)
osMTP1	LOC_Os05g03780.1	TGGCTGTCTTGCTTGGTC CAGTGCCTGGATGGTGAT	416
osMTP5	LOC_Os02g58580.1	GTTTCTTTCATTGGGGGT GGTTTGGATGGTCAGGTC	346
osMTP6	LOC_Os03g22550.1	TATCTGTCAAAGAAGGGC TGATTGGTTGTAGAAGCG	282
osMTP7	LOC_Os04g23180.1	AGCAGCAGAAGGAATGAG TGAACAGCCACCAAAGAT	132
osMTP8	LOC_Os02g53490.1	CATCCACGCTTGATTCCT TCTCTCCCGCCTTGTTCT	216
osMTP8.1	LOC_Os03g12530.1	GAGCAAAAGCAGAGTGAG CGAGAGATGTGTGAACCA	174
osMTP9	LOC_Os01g03914.1	GCCAGTGGGCATAATAGT TGAGTCCCGAAGGTGTAG	416
osMTP11	LOC_Os01g62070.1	CAAACACCAAATCCCTAC AACTCATCCCCATCAGAC	365
osMTP11.1	LOC_Os05g38670.1	CTTGTGGTTTACTGCCTT CTTCTCCTGCTCTTTTGT	360
osMTP12	LOC_Os08g32650.1	TCCTGAACGAACGGAAGT GAGTGGGAATGTGAATGG	218
osGAPDH	LOC_Os08g03290.1	GACAGCAGGTCGAGCATCTTC CAGGCGACAAGCTTGACAAAG	74

2.5. Quantitative real-time PCR

After Cr exposure, collection procedure of rice tissues was similar to our previous study (Yu et al., 2017b). Total RNA in different parts of rice seedlings was extracted using Trizol (Invitrogen, Carlsbad, CA, USA). The remaining procedure and qRT-PCR analysis were identical to those described previously (Yu et al., 2017b). The cycling condition was an initial denaturation at 95 °C for 10 s, annealing 58 °C for 30 s and extension at 72 °C for 32 s, with 40 cycles. qRT-PCR was determined by the 7500 Fast Real-Time PCR system (Applied Biosystems), using SYBR green chemistry. Rice *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase, LOC_Os08g03290.1) was selected as a house keeping gene (Hussain et al., 2016). The relative expression of each targeted genes was determined by the standard $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). All values referred to mean of 4 independent biological replicates ± SD. Primer sequences for all *osMTP* genes are listed in Table 1.

2.6. Statistical analysis

Analysis of variance (ANOVA) and Tukey's multiple range tests were used to determine the statistical significance at 0.01 or 0.05 between the treatments (Zar, 1999).

3. Results and discussion

3.1. Identification of OsMTP genomes

According to 12 known AtMTP protein sequences (AtMTP1: At2g46800.2, AtMTP2: At3g58810.1, AtMTP3: At3g58810.2, AtMTP4: At2g29410.1, AtMTP5: At3g12100.1, AtMTP6: At2g47830.1, AtMTP7: At1g51610.1, AtMTP8: At3g58060.1, AtMTP9: At1g79520.1, AtMTP10: At1g16310.1, AtMTP11: At2g39450.1, AtMTP12: At2g04620.1) (Fu et al., 2017) from the *Arabidopsis* database TAIR (http://www.arabidopsis.org/, v10.0), a BLAST-P search was conducted to identify all MTP isoforms in the rice genome through the rice database RGAP (http://rice.plantbiology.msu.edu/analyses_search_blast.shtml). After removing redundant and query hits using an *e*-value ≤ e^{-10} as the threshold, 10 potential MTP isoforms were identified in rice. Their molecular details are given in Table 2. The amino acid length of the 10 OsMTP isoforms ranged from 277 (OsMTP5) to