DE GRUYTER

Biologia **72**/4: 388—397, 2017
Section Botany
DOI: 10.1515/biolog-2017-0048

Transcriptome analysis in leaves of rice (*Oryza sativa*) under high manganese stress*

Ping Li[1,2], Alin Song[2], Zhaojun Li[2], Fenliang Fan[2] & Yongchao Liang[2,3]**

[1] *College of Agriculture, Shanxi Agricultural University, Taigu,Shanxi, 030801, P.R. China; e-mail: lipinghxy@126.com*
[2] *Ministry of Agriculture Key Laboratory of Crop Nutrition and Fertilization, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, P.R. China*
[3] *Ministry of Education Key Laboratory of Environment Remediation and Ecological Health, College of Environmental & Resource Sciences, Zhejiang University, Hangzhou, 310058, P.R. China*

Abstract: Elucidating molecular mechanisms of rice responses to high manganese (Mn) stress is crucially important to manganese-resistant gene cloning, functional analysis and other research work. In the present study, we analyzed the gene expression in leaves of Mn-sensitive rice (*Oryza sativa* cv. Xinxiangyou 640) exposed to high Mn stress by high-throughput sequencing. There were about 2831 differently expressed genes [the false discovery rate (FDR) ≤ 0.001 and $\log_2$ratio(Mn/CK) ≥ 1] among 21258 genes, in which 1336 appeared to be up-regulated and 1495 appeared to be down-regulated in rice treated with high level of Mn compared with the normal level of Mn. Under high Mn stress, the differentially expressed genes were relating to various transcription factors (TFs), large number of transporters, numerous transferase proteins, catalytic proteins, etc, involving in the major primary and secondary metabolisms. Among the rest, the genes of WRKY family were all significantly up-regulated whereas all the Aux/IAA genes were strongly down-regulated. Potassium transporters were also significantly up-regulated whereas sodium transporters were strongly down-regulated. The expression patterns of representative related genes were further confirmed by fluorescent quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR). Manganese resistance mechanism in rice is very complex and is a consequence of coordinated expression of a large number of genes. This data resource contributed substantially to biological and physiological research on rice genes, and to comparative analysis of Mn-specific responses in plants.

Key words: gene expression; high manganese stress; rice.

Introduction

Manganese (Mn) is an essential trace element for plant growth and development. Mn plays roles in catalyzing the water-splitting reaction as a component of the oxygen-evolving complex in photosystem II (PSII). Mn acts as a cofactor that activates ~ 35 different enzymes (Williams et al. 2010), such as Mn-superoxide dismutase (MnSOD), a principal antioxidant enzyme involved in protection against oxidative stress in plants. However, Mn toxicity caused by excess Mn supply is one of the most limiting factors for crop production on acid and insufficiently drained soils with low redox potential (Schlichting & Sparrow 1988; Conyers et al. 1997). High Mn exposure affects some physiological and biochemical processes associated with plant growth and development. High (1.5 mM) Mn treatment significantly decreased the plant growth in Chinese cabbage (*Brassica rapa* L. ssp. *campestris*) (Lee et al. 2011). High Mn concentrations lead to a reduction in dry weight and a decline in biomass of *Arabidopsis* plants (Millaleo et al. 2013). Manganese toxicity can induce oxidative stress in rice (Li et al. 2012), in barley (Demirevska-Kepova et al. 2004), in *Cucumis sativus* (Shi et al. 2005), and in perennial ryegrass (Ribera et al. 2013). Excess Mn impairs photosynthesis in plants. Rate of photosynthesis was decreased in both cultivars of *Triticum aestivum* (Macfie et al. 1992). High Mn can inhibit the redox transients of PSI reaction centre (P700) and the abundance of PSI reaction centre polypeptides (PsaA and PsaB) in *Arabidopsis thaliana* (Millaleo et al. 2013). Under excess Mn, quantum yield of PSII (Φ PSII), electron transport rate, CO_2 assimilation, was decreased in leaves of perennial ryegrass, particularly in the sensitive cultivar (Ribera et al. 2013). In addition, Mn toxicity can also inhibit the uptake and translocation of other essential elements such as Ca, Mg, Fe (Lee et al. 2011; Marschner 2012).

Rice is the primary staple food for over two billion people in Asia, Africa, and Latin America (Salekdeh et

* Electronic supplementary material. The online version of this article (DOI: 10.1515/biolog-2017-0048) contains supplementary material, which is available to authorized users.
** Corresponding author

al. 2002). Manganese stress (> 0.55 mg L^{-1}) reduced biomass and grain yield in rice (Dube et al. 2002). Mn toxicity symptoms in rice usually appeared as dark-brown spots, necrotic lesions, and chlorosis of leaf edges and tips. Our previous study also showed that high Mn significantly reduced non-protein thiols and glutathione concentrations, leading to oxidative damage in cv. XXY rice cultivar (Li et al. 2012).

In the past decade, gene microarray technology and particularly second-generation DNA sequencing technology developed more recently are high-throughput technology aimed at studying gene structure and function, promoting the development of functional genomics and systems biology. More recently, novel high-throughput deep sequencing technology has dramatically changed the way that the functional complexity of transcriptomes can be studied. The rice species is considered to be a model plant because of its small genome size, extensive genetic map, relative ease of transformation and synteny with other cereal crops (Sasaki et al. 2002). The rice transcript map has been a valuable resource for genetic study, gene isolation, and genome sequencing at the Rice Genome Research Program and should become an important tool for comparative analysis of chromosome structure and evolution among the cereals (Wu et al. 2002). High-throughput sequencing technology, will promote the development of functional genomics research on rice and other crops.

High-throughput sequencing technology are used in plant responses to heavy metals stresses, such as copper stress in rice (Sudo et al. 2008; Lin et al. 2013), cadmium stress in rice (Lin et al. 2013) and soybean (Zhang et al. 2012), lead (Pb) stress in radish (Wang et al. 2013) and maize (Shen et al. 2013), arsenic (As) stress in *Arabidopsis thaliana* (Abercrombie et al. 2008) and rice (Chakrabarty et al. 2009). However, large scale microarray-based expression profiling of rice genes in responses to Mn toxicity has not yet been reported in refereed publications. By high-throughput deep sequencing technology, in the present study we investigated effects of excess manganese on gene expression in leaves of Mn-sensitive rice (*Oryza sativa* L. cv. Xinxiangyou 640) to better understand the molecular mechanisms of rice response to Mn-induced stress and provide a basis on unearthing and utilizing gene-resistant Mn.

Material and methods

Plant material and growth conditions

Based on these preliminary screening studies (Li et al. 2012), i.e. cv. Xinxiangyou 640, a manganese-sensitive cultivar was used in this investigation. Rice seeds were surface-sterilized with 10% $NaClO_3$ for 15 min, followed by rinsing thoroughly with distilled water, then germinated on moist filter paper in an incubator in the dark at 28 °C for 3 days. The germinated seeds were sown in the plastic containers filled with quartz sand and watered with 1/2-strength Kimura B nutrient solution. The composition of the basic nutrient solution was in accord with that in previous study (Li et al. 2012). Then they were grown in a growth chamber under controlled environmental conditions at 25–30 °C/18–22 °C day/night temperatures. The light period was about 12 h. After 7 days, the seedlings were moved to 4 L plastic pots containing full-strength nutrient solution (18 plants per pot). The experiment was started at the onset of the fifth leaf stage when the plants were transferred to solutions with either normal (6.7 μM) or high (2.0 mM) Mn (added as $MnSO_4$). The nutrient solutions were renewed every 3 days. There were two treatments that were arranged in a randomized, complete block design with 3 replicates.

The plants were harvested 3 days after the onset of Mn treatments for analysis of gene expression by high-throughput sequencing.

Pipeline of experiment

The high-throughput sequencing experimental process includes sample preparation and sequencing in Huada Genomics Company, Shenzhen, China. One treatment was used. The main reagents and supplies are Illumina Gene Expression Sample Prep Kit and Solexa Sequencing Chip (flowcell), and the main instruments are Illumina Cluster Station and Illumina Genome Analyzer System.

Huada Genomics Company extracted 6 μg of the total RNA and used Oligo (dT) magnetic beads adsorption to purify mRNA, and then used Oligo (dT) to guide reverse transcription to synthesize double-stranded cDNA. mRNA in the total RNA was enriched by beads of OligodT, and then transferred into double-stranded cDNA through reverse transcription. This cDNA was digested by four base recognition enzyme NlaIII, and Illumina adapter 1 was linked. Mmel was used to digest at 17bp downstream of CATG site; Illumina adapter 2 was linked at 3' end. Primer GX1 and Primer GX2 were added for PCR. Then, 85bp strips were regained through 6% TBE PAGE. The DNA was purified and followed by Solexa sequencing. 2 repeat treatments were used.

Libraries were sequenced using the standard Solexa pipeline (version RTA1.6).

Pipeline of bioinformatics analysis:

We identified differentially expressed genes between two samples referring to "The significance of digital gene expression profiles" (Monni et al. 2001). To identify statistically significant differentially expressed genes, a combined criterion of FDR < 0.001 and the absolute value of $\log_2$Ratio (Mn/CK) ≥ 1 in the Poisson distribution were adopted. Mn/CK means multiples of differentially expression.

In gene functional-enrichment analysis, GO and KEGG were performed to identify differentially expressed genes (DEGs). Compared with the whole-transcriptome background, DEGs were significantly enriched in GO terms and metabolic pathways at corrected P-value ≤ 0.05 using the formula described in the previous studies (Hao et al. 2011; Gao et al. 2012).

Confirmation of gene expression patterns by RT-PCR

RT-PCR analysis was employed to confirm expression patterns of selected genes based on their known functions. Gene Specific Primers (supplementary Table 1) were designed and synthesized by Baosheng Corporation, China. Total RNA isolated from rice tissues was first converted to cDNA using MMLV reverse transcriptase (Tiangen Corporation, China), according to the manufacturer's specifications, with random hexamer primers from Baosheng. The cDNA products generated were from three biological replicates for each sample. Reactions (20 μL) were performed in triplicate in 96-well plates (TempPlate Scientific, Bio-Rad, China). The program consisted of a Hot-Start activation step at 95 °C for 10 s,

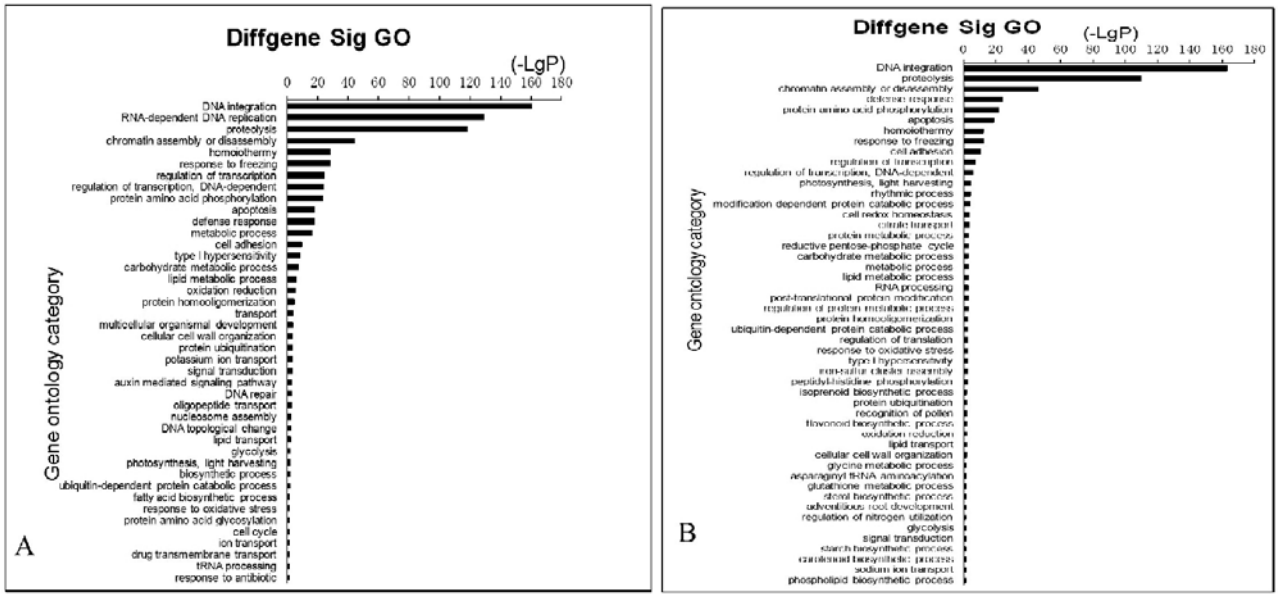

Fig. 1. The go-enrichment classification of molecular function of differentially expressed genes in rice. A means up-regulated genes. B means down-regulated genes.

followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. Amplification of the actin internal control was performed in the same 96-well plate as the other genes. Reactions were performed with the iCycler Real-Time PCR Detection System (Bio-Rad Laboratories INC., USA) employing the two-step amplification plus melting curve protocol. The values for threshold cycle (Ct) determination were generated automatically by iCycler software (Bio-Rad Laboratories). Relative quantitative method delta-delta CT ($2^{-\Delta\Delta CT}$) (Livak et al. 2001) was used to describe expression patterns of selected genes by comparing the gene expression levels between the Mn-stressed samples and healthy samples.

Results

Identification of differentially expressed genes under high Mn stress

There were about 2831 differently expressed genes among 21258 genes, in which 1336 appeared to be up-regulated and 1495 appeared to be down-regulated under high Mn stress by high-throughput sequencing. Supplementary table 2 lists the some differentially expressed genes. A set of up- and down-regulated genes had different molecular functions related to binding, catalytic activity, transporters, transcription factors, signal transducer activity and otherwise (Fig. 1).

A large number of genes encoding transcription factors (TFs) were differentially expressed under high Mn stress. Among these TFs, eight genes encoding AUX/IAA proteins (LOC_Os08g01780, LOC_Os01g08320, LOC_Os01g53880, LOC_Os05g14180, LOC_Os07g08460, LOC_Os01g13030, LOC_Os03g53150 and LOC_Os05g48590) were all significantly down-regulated. In contrast to AUX/IAA proteins, six genes encoding WRKY proteins (LOC_Os01g14440, LOC_Os09g25070, LOC_Os01g61080, LOC_Os01g40260, LOC_Os11g29870, and LOC_Os08g29660) were all significantly up-regulated. Of genes encoding C_2H_2 zinc finger proteins, two (LOC_Os03g05690 and LOC_Os06g48530) were significantly up-regulated, whereas other two (LOC_Os01g62460 and LOC_Os08g40560) were down-regulated. Four genes encoding CCT/B-box zinc finger proteins (LOC_Os09g06464, LOC_Os02g39710, LOC_Os06g15330 and LOC_Os02br g49880) were significantly up-regulated, whereas two genes encoding B-box zinc finger family proteins (LOC_Os05g11510 and LOC_Os04g41560) were strongly down-regulated. One gene encoding GATA zinc finger domain containing protein (LOC_Os06g37450) was down-regulagted, whereas one (LOC_Os02g05510) was up-regulated. One gene encoding zinc-finger C3HC4-type protein (LOC_Os11g18947) was significantly up-regulated. Three genes encoding TUB proteins (LOC_Os07g47110, LOC_Os01g48370 and LOC_Os05g43850) were all strongly down-regulated. One gene encoding helix-loop-helix (HLH) DNA-binding-domain containing proteins (LOC_Os01g01840) was significantly up-regulated, while the other one (LOC_Os11g06010) was down-regulated. Two genes encoding GRAS proteins (LOC_Os05g49930 and LOC_Os07g39470) were significantly up-regulated. Among genes encoding MYB family proteins, three genes (LOC_Os05g48010, LOC_Os02g46030 and LOC_Os10g41200) were significant up-regulated, whereas other 17 genes (LOC_Os09g31454, LOC_Os11g35390, LOC_Os06g43090, LOC_Os04g30890, LOC_Os12g37970, LOC_Os04g40930, LOC_Os01g63460, LOC_Os02g47190, LOC_Os01g59660, LOC_Os05g10690, LOC_Os05g37730, LOC_Os07g02800, LOC_Os06g51260, LOC_Os05g40960, LOC_Os03g31230, LOC_Os08g25799 and LOC_Os09g01960) were strongly down-regulated. Two genes encoding HMG (high mobility group) boxes (LOC_Os02g27060 and LOC_Os04g47690) were significantly up-regulated, whereas the other one (LOC_Os06g51220) was down-regulated. One gene encoding DDT

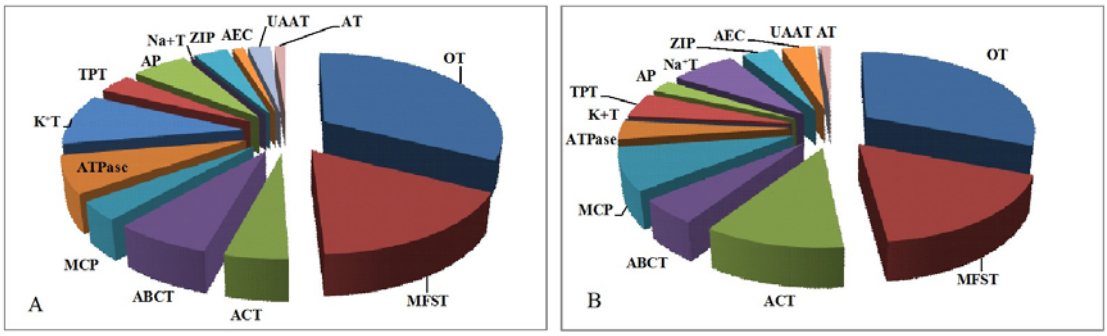

Fig. 2. The classification of differentially expressed transporters in rice. Different genes were divided in to amino acid transporter (ACT), mitochondrial carrier protein (MCP), MFS general substrate transporter (MFST), potassium ion transporte (K^+T), UAA transporter family (UAAT), ABC transporter (ABCT), aquaporin protein (AP), ZIP Zinc transporter (ZIP), triose-phosphate transporter (TPT), ammonium Transporter Family (AT), xenobiotic-transporting ATPase activity (ATPase), sodium transporter (Na^+T), auxin efflux carrier component (AEC) and others (OT). A means up-regulated genes. B means down-regulated genes.

protein (LOC_Os01g48180) was strongly down-regulated. One gene encoding TCP family transcription factor (LOC_Os05g43760) was strongly down-regulated. One gene encoding CCAAT-binding transcription factor (LOC Os12g41880) was significantly up-regulated, and the other one (LOC_Os03g48970) was strongly down-regulated.

A total of 164 genes encoding transporters were differently expressed under high Mn stress compared with the normal Mn level (Fig. 2). Of these 28 genes encoding major facilitator superfamily (MFS) general substrate transporters, half were significantly up-regulated whereas the others were strongly down-regulated. Among genes encoding ATP-binding cassette (ABC) transporters, six (LOC_Os08g45010, LOC_Os01g50100, LOC_Os11g05700, LOC_Os01g07870, LOC_Os11g07600 and LOC_Os06g03770) were up-regulated, whereas other four (LOC_Os09g39910, LOC_Os03g06139, LOC_Os05g04610 and LOC_Os01g73530) were down-regulated. Of genes encoding six ZIP zinc transporters, two ZIP zinc transporters (LOC_Os06g41770 and LOC_Os10g01470) were significantly up-regulated, whereas others (LOC_Os02g49700, LOC_Os09g29460, LOC_Os09g35910 and LOC_Os05g41540) were strongly down-regulated. All six genes encoding sodium transporters were strongly down-regulated (LOC_Os04g51820, LOC_Os06g48810, LOC_Os05g02240, LOC_Os12g07270, LOC_Os12g42300 and LOC_Os01g45750), whereas eight genes encoding potassium ion transporters were significantly up-regulated (LOC_Os10g31330, LOC_Os01g70490, LOC_Os04g55080, LOC_Os01g45990, LOC_Os04g40950, LOC_Os05g13940, LOC_Os09g27580, LOC_Os02g57240).

Among these transferase proteins, ten genes encoding glutathione S-transferase proteins (GSTs) were differently expressed, of which five (LOC_Os10g38140, LOC_Os10g38150, LOC_Os10g38580, LOC_Os10g38710 and LOC_Os10g38610) were down-regulated, and other five GSTs genes (LOC_Os12g02960, LOC_Os10g38780, LOC_Os09g29200, LOC_Os07g28480 and LOC_Os12g10730) were significantly up-regulated. Nine genes encoding UDP-glucoronosyl and UDP-glucosyl transferase proteins (LOC_Os02g36830, LOC_Os04g46970, LOC_Os03g55030, LOC_Os04g12960, LOC_Os04g25490, LOC_Os07g13634, LOC_Os01g53390, LOC_Os07g30610 and LOC_Os04g25440), three genes encoding nucleotidyl transferase proteins (LOC_Os03g52460, LOC_Os09g12660 and LOC_Os08g25734), two genes encoding glycosyl transferases groups (LOC_Os06g06560 and LOC_Os01g52250), two genes encoding galactosyltransferase proteins (LOC_Os02g06840 and LOC_Os09g27950), one gene encoding glucanotransferase protein (LOC_Os07g46790) and one gene encoding gamma-glutamyltranspeptidase protein (LOC_Os01g05810) were significantly affected under high Mn stress.

Cytochrome P450-related genes were also differently expressed under high Mn stress compared with the normal Mn level. Among these, twenty genes were significantly up-regulated, and other ten genes were strongly down-regulated.

Pathway enrichment analysis for DEGs

Different genes usually co-operate with each other to exercise their biological functions. Pathway-based analysis helps us to further understand genes' biological functions. There were 26 significantly differentially expressed genes enriched metabolic pathways (FDR $\leq$ 0.05) in 118 pathways in the leaves of rice under high Mn stress (Table 1). A set of common up- and down-regulated genes belonged to various gene families related to regulatory proteins, growth and developments and secondary metabolisms. The pathway analysis showed that Mn-stress response significantly influenced the pathways relating to the primary metabolisms such as glycolysis/gluconeogenesis, citrate cycle (TCA cycle), oxidative phosphorylation, pentose phosphate pathway, pyruvate metabolism and so on. High Mn stress affected not only the primary metabolisms in rice, but also the secondary metabolisms, which significantly affected the biosynthesis of alkaloids derived from shikimate, ornithine, lysine, nicotinic acid, histidine, purine, terpenoid, polyketide and so on. High Mn stress also affected

Table 1. List of significantly enriched pathway analysis in the leaves of rice under excess Mn stress (FDR $\leq$ 0.05).

Number	Pathway	DEGs (1732)	All genes (21258)	Pvalue	FDR	Pathway ID
1	Ribosome	80 (4.62%)	457 (2.15%)	4.84E-11	5.71E-09	ko03010
2	Biosynthesis of alkaloids derived from shikimate pathway	55 (3.18%)	336 (1.58%)	4.92E-07	2.90E-05	ko01063
3	Pyruvate metabolism	27 (1.56%)	128 (0.6%)	3.73E-06	1.47E-04	ko00620
4	Glycerolipid metabolism	24 (1.39%)	109 (0.51%)	5.84E-06	1.72E-04	ko00561
5	Glycolysis / Gluconeogenesis	38 (2.19%)	224 (1.05%)	1.20E-05	2.82E-04	ko00010
6	Glutathione metabolism	27 (1.56%)	140 (0.66%)	2.11E-05	4.16E-04	ko00480
7	Biosynthesis of alkaloids derived from ornithine, lysine and nicotinic acid	43 (2.48%)	283 (1.33%)	5.30E-05	8.77E-04	ko01064
8	Biosynthesis of plant hormones	91 (5.25%)	744 (3.5%)	5.95E-05	8.77E-04	ko01070
9	Ubiquinone and other terpenoid-quinone biosynthesis	13 (0.75%)	47 (0.22%)	6.74E-05	8.83E-04	ko00130
10	Biosynthesis of alkaloids derived from histidine and purine	39 (2.25%)	258 (1.21%)	0.000129	1.52E-03	ko01065
11	Biosynthesis of phenylpropanoids	86 (4.97%)	722 (3.4%)	0.0002309122	2.477058e-03	ko01061
12	Citrate cycle (TCA cycle)	16 (0.92%)	79 (0.37%)	0.0005491799	5.400269e-03	ko00020
13	Butanoate metabolism	21 (1.21%)	121 (0.57%)	0.0007397392	6.714556e-03	ko00650
14	Amino sugar and nucleotide sugar metabolism	27 (1.56%)	173 (0.81%)	0.0008037268	6.774269e-03	ko00520
15	Metabolic pathways	402 (23.21%)	4316 (20.3%)	0.001086438	8.546646e-03	ko01100
16	Phenylalanine, tyrosine and tryptophan biosynthesis	15 (0.87%)	79 (0.37%)	0.001612468	1.158335e-02	ko00400
17	Oxidative phosphorylation	31 (1.79%)	218 (1.03%)	0.001668788	1.158335e-02	ko00190
18	Pentose phosphate pathway	14 (0.81%)	73 (0.34%)	0.002062038	1.351780e-02	ko00030
19	Biosynthesis of alkaloids derived from terpenoid and polyketide	33 (1.91%)	242 (1.14%)	0.002425511	1.506370e-02	ko01066
20	Metabolism of xenobiotics by cytochrome P450	20 (1.15%)	125 (0.59%)	0.002684974	1.584135e-02	ko00980
21	Fructose and mannose metabolism	28 (1.62%)	207 (0.97%)	0.005520845	3.102189e-02	ko00051
22	Galactose metabolism	17 (0.98%)	107 (0.5%)	0.005789762	3.105418e-02	ko00052
23	Endocytosis	23 (1.33%)	162 (0.76%)	0.006302631	3.233524e-02	ko04144
24	Glycerophospholipid metabolism	25 (1.44%)	182 (0.86%)	0.0069338	3.331725e-02	ko00564
25	Arachidonic acid metabolism	8 (0.46%)	36 (0.17%)	0.007341088	3.331725e-02	ko00590
26	Folate biosynthesis	8 (0.46%)	36 (0.17%)	0.007341088	3.331725e-02	ko0079

ubiquinone and other terpenoid-quinone biosynthesis.

Characterization and expression confirmation of selected genes

In order to verify the accuracy of high-throughput sequencing data, we selected gene sequencing results relating to the significantly differentially expressed genes enriched metabolic pathways to perform real-time fluorescence quantitative PCR. The results obtained from all the genes tested by RT-PCR agreed with the trend of regulation identified by high-throughput sequencing testing (Table 2), indicating that the gene expression profile of information by high-throughput sequencing had high reliability.

Discussion

Although many previous studies have been conducted on the effects of excess Mn stress, the precise molecular mechanisms relating to both the effects of Mn phytotoxicity and the defense reactions of plants against excess Mn remain still poorly understood. Excess Mn stress decreased the chlorophyll content in wheat seedlings (Moroni et al. 1991) and in pea plants (Rezai et al. 2008). Our studies also showed the contents of Chl a, Chl b were significantly decreased by high Mn stress, then we found that LOC_Os03g08730 endoding HemD was down-regulated that inhibited Chl synthesis metabolism (Li et al. 2015). This can explain at the molecular level why the chlorophyll content was decreased significantly under excess Mn. The expressions of the genes (LOC_Os04g58200, LOC_Os08g39840 and LOC_Os12g24650) relating to chloroplast precursor were significantly up-regulated under high Mn stress. This may be affected Chl synthesis catabolism.

It was demonstrated that apoplastic water-soluble peroxidase in the apoplastic washing fluid was enhanced under excess Mn (Fecht-Christoffers et al. 2003). It has also been shown that the H_2O_2-producing NADH peroxidase in the leaf apoplast plays a major role in Mn toxicity and Mn tolerance in cowpea (Fecht-Christoffers et al. 2006). Our results showed that high Mn stress induced the significantly up-regulated expression of the precursor of peroxisome (LOC_Os07g48050) ($\log_2$Ratio(Mn/CK) = 12.36) (Supplementary Table 1), suggesting the molecular effects of manganese stress on antioxidant defense system. Führs et al. (2008) reported that proteins involved in pathogenesis-response reactions were of lower abundance upon Mn stress in *Vigna unguiculata* L. We also found the gene expression of pathogenesis-related protein 10 (LOC_Os12g36860) was significantly up-regulated under high Mn stress ($\log_2$Ratio (Mn/CK) = 10.94).

Table 2. Comparison and validation of selected genes related to the significantly differentially expressed genes enriched metabolic pathways with RT-PCR data in leaves of rice.

Gene	Accession Number	Description	Pathway	$\log_2$ Ratio(Mn/CK)	
				High-throughput sequencing	RT-PCR
LOC_Os02g07490	CT832583	glyceraldehyde-3-phosphate dehydrogenase, putative, expressed	Glycolysis / Gluconeogenesis	3.64	3.37
LOC_Os01g62870	AK103729	oxidoreductase, aldo/keto reductase family protein, putative, expressed	Glycolysis / Gluconeogenesis	3.21	2.45
LOC_Os05g39690	AK102864	oxidoreductase, aldo/keto reductase family protein, putative, expressed	Glycolysis / Gluconeogenesis	3.04	0.49
LOC_Os01g40870	AK101427	aldehyde dehydrogenase, putative, expressed	Glycolysis / Gluconeogenesis	1.92	1.28
LOC_Os01g09570	AK121093	6-phosphofructokinase, putative, expressed	Glycolysis / Gluconeogenesis	1.74	2.00
LOC_Os09g33500	CT832669	transketolase, putative, expressed	Glycolysis / Gluconeogenesis	1.90	4.64
LOC_Os03g50130	CT829125	glutathione S-transferase 3, putative, expressed	Glutathione metabolism	1.31	1.64
LOC_Os07g28480	CT828710	glutathione S-transferase, putative, expressed	Glutathione metabolism	2.14	1.49
LOC_Os12g02960	AK240983	glutathione S-transferase, putative, expressed	Glutathione metabolism	9.92	5.45
LOC_Os12g10730	CT832859	glutathione S-transferase, putative, expressed	Glutathione metabolism	1.48	2.71
LOC_Os04g16680	CT833181	fructose-1,6-bisphosphatase, putative, expressed	Glycolysis / Gluconeogenesis	–3.39	–1.56
LOC_Os06g14510	AK068236	glucose-6-phosphate isomerase, putative, expressed	Glycolysis / Gluconeogenesis	–1.04	–1.12
LOC_Os04g33190	AK243122	AMP-binding enzyme, putative, expressed	Glycolysis / Gluconeogenesis	–1.90	–0.42
LOC_Os08g29170	AK069608	dehydrogenase, putative, expressed	Glycolysis / Gluconeogenesis	–1.31	–0.95
LOC_Os10g29470	AK069040	dehydrogenase, putative, expressed	Glycolysis / Gluconeogenesis	–1.77	–1.08
LOC_Os06g04510	AK104904	enolase, putative, expressed	Glycolysis / Gluconeogenesis	–1.50	–1.69
LOC_Os05g44760	AF372831	hexokinase, putative, expressed	Glycolysis / Gluconeogenesis	–3.18	–4.21
LOC_Os06g08670	AK243433	glutathione peroxidase, putative, expressed	Glutathione metabolism	–1.35	–2.70
LOC_Os01g05810	AK120703	gamma-glutamyltranspeptidase 1 precursor, putative, expressed	Glutathione metabolism	–2.33	–4.86
LOC_Os07g22600	AK060916	spermidine synthase, putative, expressed	Glutathione metabolism	–3.13	–3.46
LOC_Os01g27360	CT833497	glutathione S-transferase, putative, expressed	Glutathione metabolism	–1.89	–1.45
LOC_Os10g38140	AF402802	glutathione S-transferase, putative, expressed	Glutathione metabolism	–9.25	–6.90
LOC_Os10g38710	AF402800	glutathione S-transferase, putative, expressed	Glutathione metabolism	–1.25	–4.00
LOC_Os10g38580	CT831574	glutathione S-transferase, putative, expressed	Glutathione metabolism	–2.47	–2.58

The present study showed that the catalase isozyme (LOC_Os02g02400) was significantly up-regulated under high Mn stress ($\log_2$ratio(Mn/CK) = 3.28). This can explain why CAT activity in rice was 5-fold higher at the high Mn stress than at the normal Mn level in our previous study (Li et al. 2012). Our previous study has shown that high Mn stress significantly reduced the GSH concentration (Li et al. 2012), which could be well explained by the strongly down-regulated expression of the genes relating to glutathione peroxidase (LOC_Os06g08670 and LOC_Os02g44500) under high Mn stress.

Transcription factors (TFs) regulate genome expression in response to environmental and physiological signals and are involved in biotic and abiotic stress tolerance, such as heat stress, oxidative stress, osmotic stress, acidic stress, nitrogen depletion, and amino acid starvation (Wu et al. 2007). The *WRKY* gene family represents one of the major groups of plant-specific transcriptional regulators. *WRKY* proteins are involved in the regulation of plant responses to environmental stress such as drought stress (Qiu et al. 2009; Pnueli et al. 2009), osmotic stress (Wei et al. 2008), hormones stress (Xie et al. 2005; Xie et al. 2006) and phosphate stress (Devaiah et al. 2007). In the present study, six *WRKY* pro-

teins *OsWRKY1v2* (LOC_Os01g14440), *OsWRKY62* (LOC_Os09g25070), *OsWRKY24* (LOC_Os01g61080), *OsWRKY77* (LOC_Os01g40260), *OsWRKY72* (LOC_Os11g29870) and *OsWRKY69* (LOC_Os08g29660) were all significantly up-regulated under high Mn stress. This means that the *WRKY* gene family possibly plays an important role in response signaling of Mn stress in rice. Yan et al. (2016) suggested that Mn was affected in *Arabidopsis* wrky46-1 mutant. However, further studies are needed to better understand the endogenous biological function of WRKY gene family in Mn stress signaling.

The Aux/IAA genes are a large gene family, many of which are induced by auxin. In the present study, all the Aux/IAA genes were down-regulated, suggesting that high Mn stress inhibited the formation of auxin and impeded the endogenous developmental processes.

MYB proteins are a superfamily of transcription factors that play regulatory roles in developmental processes and defense responses in plants. The expression patterns of several R2R3-type MYB genes involved in the responses to environmental stress such as drought, salt and cold were characterized in *Arabidopsis* (Zhu et al. 2005; Agarwal et al. 2006; Chen et al. 2006). Overexpression of *OsMYB3R-2* enhanced cold tolerance in rice (Ma et al. 2009). Overexpression of a *R1R2R3* MYB Gene, *OsMYB3R-2*, increased tolerance to freezing, drought, and salt stress in transgenic*Arabidopsis* (Dai et al. 2007). Our study showed that most of MYB transcription factors exposed to Mn stress were strongly down-regulated in rice, indicating that high Mn stress inhibited the activities of MYB transcription factors.

bHLH TF opens the way to the improvement of phosphate or iron deficiency tolerance by manipulating the whole gene regulatory networks in rice (Ogo et al. 2006; Ogo et al. 2007; Yi et al. 2005). Evidence suggests that genes from this family played vital roles in stress response, such as *OsbHLH1* in cold stress (Wang et al. 2003), *RERJ1* in wounding and drought stress (Kiribuchi et al. 2004; Kiribuchi et al. 2005) and *OrbHLH2* in tolerance to salt and osmotic stress. The present study showed that two HLH TF (LOC_Os01g01840 and LOC_Os11g06010) were differently expressed under high Mn stress, indicating HLH may be involved in tolerance to high Mn stress.

It has been reported that overexpression of some C2H2-type zinc finger protein genes enhanced tolerance to salt, cold and drought stresses in rice (Huang et al. 2005, 2007; Xu et al. 2007; Sun et al. 2010). Our study also showed differential expression among the members of C2H2-type zinc finger proteins under high Mn stress, suggesting that C2H2-type zinc finger proteins may be contributed to enhancing rice tolerance to high Mn stress.

Currently studies on Mn-induced gene expression, particularly on transporters, are relatively weak. Two transporter gene family ZIP (ZRT, IRT-like protein) and CDF (Cation diffuse facilitator) have been characterized and identified in plants (Hirschi et al. 2000; Delhaize et al. 2003; Lopez-Millan et al. 2004). Among Mn-CDFs, OsMTP8.1 is an Mn-specific transporter that sequesters Mn into vacuoles in rice and is required for Mn tolerance in shoots (Chen et al. 2013). The high-throughput sequencing data offered a vast reservoir of information of Mn-regulated transporter gene expression on transporters. Twenty-eight MFS general substrate transporter, ten ABC transporters, six ZIP zinc transporters, seven sodium transporters, eight potassium ion transporters and the others were significantly affected under high Mn stress compared with the normal Mn level (Fig. 2). AtZIP1 and AtZIP2 T-DNA transporters play a role in Mn (and possibly Zn) translocation from the root to in *Arabidopsis* (Milner et al. 2013). The present study also showed that ZIP zinc transporters (LOC_Os06g41770, LOC_Os10g01470, LOC_Os02g49700, LOC_Os09g29460, LOC_Os09g35910 and LOC_Os05g41540) were strongly differently expressed under high Mn stress.

The ABC transporter superfamily is one of the largest transporter protein families in plants (Rea et al. 2007). ABC transporters are involved in the membrane transport of endogenous secondary metabolites in plants and also those mediated the transport of plant products in heterologous systems as well. Huang et al. (2009) reported that two ABC transporters were involved in aluminum tolerance in rice. Moons et al. (2003) reported that PDR-type ABC transporter, was induced by Cd and Zn stress in rice roots. Moons et al. (2008) also showed that PDR-type ABC transporters were involved in plant growth regulators, redox perturbations and weak organic acid stresses. Overexpression of AtPDR8 was more resistant to Cd^{2+} or Pb^{2+} in *Arabidopsis* (Kim et al. 2007). The present study showed that high Mn stress induced differential expression among the eleven members of ABC transporter proteins. These ABC transporter proteins might play roles in Mn tolerance in rice. As is well known, Na^+ and K^+ homeostasis are crucial for plant growth and development. Our study showed that high Mn stress significantly up-regulated potassium transporters but strongly down-regulated sodium transporters, which maybe destroyed ionic balance, especially the Na^+/K^+ ratio, thus impeding rice growth and development.

GSTs catalyze a variety of reactions and accept endogenous and xenobiotic substrates. GSTs have also been reported in numerous stress responses such as pathogenic attack, oxidative stress and heavy-metal toxicity (Marrs et al. 1996; Kilili et al. 2004; Banerjee et al. 2010). GST activity was significantly lower in leaf of *calystegia sepium* under cadmium stress (Lyubenova et al. 2007). Norton et al. (2008) reported that a total of 15 GST genes including LOC_Os10g38140 and LOC_Os10g38610 were up-regulated in rice under arsenate (As) stress. But our study showed that both of them were down-regulated under high Mn stress. This seems to suggest that rice varieties had differential response to As and Mn stress. The results of RT-PCR also showed that seven GSTs (LOC_Os06g08670, LOC_Os01g05810, LOC_Os07g22600, LOC_Os01g27360, LOC_Os10g38140, LOC_Os10g38710 and LOC_Os10g38580) were down-regulated in glutathione metabolism

(Table 2), suggesting that high Mn stress inhibited the activities of "detoxification enzymes", leading to Mn toxicity in rice plants.

The cytochrome P450 superfamily (officially abbreviated as CYP) is a large and diverse group of enzymes. CYPs are involved in many important biological responses such as drug metabolism and bioactivation in a variety of organisms. Chakrabarty et al. (2009) speculated that cytochrome P450 could be used as an important biomarker for arsenic (As) stress in rice. It is interesting that thirty CYPs were differently expressed under high Mn stress compared with the normal Mn level. Of these CYPS, two-thirds were significantly up-regulated, whereas others were strongly down-regulated, making them candidates for regulators of biological progress in rice under high Mn stress.

The results of RT-PCR and high-throughput deep sequencing both showed high Mn stress inhibited the activities of the key enzyme (LOC_Os06g14510, LOC_Os06g14510 and LOC_Os05g44760) and dehydrogenase (LOC_Os08g29170 and LOC_Os10g29470) of glycolysis pathway, thus impeding the metabolic pathway that converts glucose into pyruvate. The free energy released in this process was strongly reduced, inhibiting the growth and development in rice.

Conclusions

In the present study, the molecular basis of the response to Mn stress in rice was comprehensively characterized. The differentially expressed genes involved various transcription factors (TFs), large number of transporters, numerous transferase proteins, catalytic proteins, etc. Six WRKY proteins were all significantly up-regulated under high Mn stress, whereas all the Aux/IAA genes were strongly down-regulated. Potassium transporters were significantly up-regulated whereas sodium transporters were strongly down-regulated. The analysis of differentially-expressed gene profiles provides valuable information for the further functional characterisation of genes associated with Mn stress in rice.

Acknowledgements

This work was partially supported by National Natural Science Foundation of China (31601212), Research on science and technology of Shanxi Province (20150311006-2), Shanxi Agricultural University Doctoral Scientific Research fund (2012YJ05).

References

Abercrombie J.M., Halfhill M.D., Ranjan P., Rao M.R., Saxton A.M., Yuan J.S. & Stewart C. N. 2008. Transcriptional responses of *Arabidopsis thaliana* plants to As (V) stress. BMC Plant Biol. **8:** 87.

Agarwal M., Hao Y., Kapoor A., Dong C.H., Fujii H., Zheng X.W. & Zhu J.K. 2006. A R2R3 type MYB transcription factor is involved in the cold regulation of CBF genes and in acquired freezing tolerance. J. Biol. Chem. **281:** 37636–37645.

Banerjee S. & Goswami R. 2010. GST profile expression study in some selected plants: in silico approach. Mol. Cell. Biochem. **336:** 109–126.

Chakrabarty D., Trivedi P.K., Misra P., Tiwari M., Shri M., Shukla D., Kumar S., Rai A., Pandey A., Nigam D., Tripathi R.D. & Tuli R. 2009. Comparative transcriptome analysis of arsenate and arsenite stresses in rice seedlings. Chemosphere **74:** 688–702.

Chen Y.H., Yang X.Y., He K., Liu M.H., Li J.G., Gao Z.F., Lin Z.Q., Zhang Y.F., Wang X.X., Qiu X.M., Shen Y.P., Zhang L., Deng X.H., Luo J.C., Deng X.W., Chen Z.L., Gu H.Y. & Qu L.J. 2006. The MYB transcription factor superfamily of *Arabidopsis*: expression analysis and phylogenetic comparison with the rice MYB family. Plant Mol. Biol. **60:** 107–124.

Chen Z.H., Fujii Y., Yamaji N., Masuda S., Takemoto Y., Kamiya T., Yusuyin Y., Iwasaki K., Kato S., Maeshima M., Ma J.F. & Ueno D. 2013. Mn tolerance in rice is mediated by MTP8.1, a member of the cation diffusion facilitator family. J. Exp. Bot. **64:** 4375–4387.

Conyers M.K., Uzen N.C., Helyor K.R., Poile G.J. & Culist B.R. 1997. Temporal variation in soil acidity. Aust. J. Soil Res. **35:** 1115–1129.

Dai X.Y., Xu Y.Y., Ma Q.B., Xu W.Y., Wang T., Xue Y.B. & Chong K. 2007. Overexpression of a R1R2R3 MYB Gene, OsMYB3R–2, increases tolerance to freezing, drought, and salt stress in transgenic*Arabidopsis*. Plant Physiol. **143:** 1–13.

Delhaize E., Kataoka T., Hebb D.M., White R.G. & Ryan P.R. 2003.Genes encoding proteins of the cation diffusion facilitator family that confer manganese tolerance. Plant Cell **15:** 1131–1142.

Demirevska–Kepova K., Simova–Stoilova L., Stoyanova Z., Hölzer R. & Feller U. 2004. Biochemical changes in barley plants after excess supply of copper and manganese. Environ. Exp. Bot. **2:** 253–266.

Devaiah B.N., Karthikeyan A.S. & Raghothama K.G. 2007. WRKY75 transcription factor is a modulator of phosphate acquisition and root development in *Arabidopsis*. Plant Physiol. **43:** 1789–1801.

Dube B.K., Khurana N. & Chatterjee C. 2002. Yield, physiology and productivity of rice under manganese stress. Indian J. Plant Physiol. **7:** 392–395.

Fecht-Christoffers M.M., Führs H., Braun H.P. & Horst W.J. 2006. The role of hydrogen peroxide-producing and hydrogen peroxide-consuming peroxidases in the leaf apoplast of cowpea in manganese tolerance. Plant Physiol. **140:** 1451–1463.

Fecht-Christoffers M.M., Maier P. & Horst W.J. 2003. Apoplastic peroxidases and ascorbate are involved in manganese toxicity and tolerance of *Vigna unguiculata*. Plant Physiol. **117:**237–244.

Führs H., Hartwig M., Molina L.E.B., Heintz D., Dorsselaer A.V., Braun H.P. & Horst W.J. 2008. Early manganese–toxicity response in *Vigna unguiculata* L. – a proteomic and transcriptomic study. Proteomics **8:** 149–159.

Hao Q.N., Zhou X.A., Sha A.H., Wang C., Zhou R. & Chen S.L. 2011. Identification of genes associated with nitrogen–use efficiency by genome–wide transcriptional analysis of two soybean genotypes. BMC Genomics **12:** 525.

Hirschi K.D., Korenkov V.D., Wilaganowski N.L. & Wagner G.J. 2000. Expression of *Arabidopsis* CAX2 in tobacco. Altered metal accumulation and increased manganese tolerance. Plant Physiol. **124:** 125–133.

Huang C.F., Yamaji N., Mitani N., Yano M., Nagamura Y. & Ma J.F. 2009. Bacterial–Type ABC transporter is involved in aluminum tolerance in rice. Plant Cell **21:** 655–667.

Huang J., Wang J.F., Wang Q.H. & Zhang H.S. 2005. Identification of a rice zinc finger protein whose expression is transiently induced by drought, cold but not by salinity and abscisic acid. DNA Sequence **16:** 130–136.

Huang J., Yang X., Wang M.M., Tang H.J., Ding L.Y., Shen Y. & Zhang H.S. 2007. A novel rice C2H2–type zinc finger protein lacking DLN–box/EAR–motif plays a role in salt tolerance. Bioch. Biophys. Acta **1769:** 220–227.

Kilili K.G., Atanassova N., Vardanyan A., Clatot N., Al–Sabarna K., Kanellopoulos P.N., Makris A.M. & Kampranis S.C. 2004.

Differential roles of tau class glutathione S–transferases in oxidative stress. J. Biol. Chem. **279:** 24540–24551.

Kim D.Y., Bovet L., Maeshima M., Martinoia E. & Lee Y. 2007. The ABC transporter AtPDR8 is a cadmium extrusion pump conferring heavy metal resistance. Plant J. **50:** 207–218.

Kiribuchi K., Jikumaru Y., Kaku H., Minami E., Hasegawa M., Kodama O., Seto H., Okada K., Nojiri H. & Yamane H. 2005. Involvement of the basic helix–loop–helix transcription factor RERJ1 in wounding and drought stress responses in rice plants. Biosci. Biotech. Bioch. **69:** 1042–1044.

Kiribuchi K., Sugimori M., Takeda M., Otani T., Okada K., Onodera H., Ugaki M., Tanaka Y., Tomiyama–Akimoto C., Yamaguchi T., Minami E., Shibuya N., Omori T., Nishiyama M., Nojiri H. & Yamane H. 2004. RERJ1, a jasmonic acid–responsive gene from rice, encodes a basic helix–loop–helix protein. Biochem. Biophys. Res. Commun. **325:** 857–863.

Lee T.J., Luitel B.P. & Kang W.H. 2011. Growth and physiological response to manganese toxicity in Chinese cabbage (*Brassica rapa* L. ssp. *campestris*). Hortic. Environ. Biotech. **52:** 252–258.

Li P., Song A.L., Li Z.J., Fan F.L. & Liang Y.C. 2012. Silicon ameliorates manganese toxicity by regulating manganese transport and antioxidant reactions in rice (*Oryza sativa* L.). Plant Soil **354:** 407–419.

Li P., Song A.L., Li Z.J., Fan F.L. & Liang Y.C. 2015. Silicon ameliorates manganese toxicity by regulating physiological processes and expression of genes relating to photosynthesis in rice (*Oryza sativa* L.). Plant Soil **397:** 289–301.

Lin C.Y., Trinh N.N., Fu S.F., Hsiung Y.C., Chia L.C., Lin C.W. & Huang H. J. 2013. Comparison of early transcriptome responses to copper and cadmium in rice roots. Plant Mol. Biol. **81:** 507–522.

Livak K.J. & Schmittgen T.D. 2001. Analysis of relative gene expression data using real–time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods **25:** 402–408.

Lopez–Millan A.F., Ellis D.R. & Grusak M.A. 2004. Identification and characterization of several new members of the zip family of metal ion transporters in *Medicago truncatula*. Plant Mol. Biol. **54:** 583–596.

Lyubenova L., Götz C., Golan–Goldhirsh A. & Schröder P. 2007. Direct effect of Cd on glutathione S–transferase and glutathione reductase from *Calystegia sepium*. Int. J. Phytoremediat. **9:** 465–473.

Ma Q.B., Dai X.Y., Xu Y.Y., Guo J., Liu Y.J., Chen N., Xiao J., Zhang D.J. & Xu Z.H. 2009. Enhanced tolerance to chilling stress in OsMYB3R–2 transgenic rice is mediated by alteration in cell cycle and ectopic expression of stress genes. Plant Physiol. **150:** 244–256.

Macfie S.M. & Taylor G.J. 1992. The effects of excess manganese on photosynthetic rate and concentration of chlorophyll in *Triticum aestivum* grown in solution culture. Physiol. Plant. **85:** 467–475.

Marrs K.A. 1996. The functions and regulation of glutathione S–transferases in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **47:** 127–158.

Marschner P. 2012. Mineral Nutrition of Higher Plants. Academic Press, Boston, MA.

Millaleo R., Reyes–Diaz M., Alberdi M., Ivanov A.G., Krol M. & Huner N.P. 2013. Excess manganese differentially inhibits photosystem I versus II in *Arabidopsis thaliana*. J. Exp. Bot. **64:** 343–354.

Milner M.J., Seamon J., Craft E. & Kochian L. V. 2013. Transport properties of members of the ZIP family in plants and their role in Zn and Mn homeostasis. J. Exp. Bot. **64:** 369–381.

Monni S., Uhlig C., Hansen E. & Magel E. 2001. Ecophysiological responses of *Empetrum nigrumto* heavy metal pollution. Environ. Pollut. **112:** 121–129.

Moons A. 2003. Ospdr9, which encodes a PDR–type ABC transporter, is induced by heavy metals, hypoxic stress and redox perturbations in rice roots. FEBS Lett. **553:** 370–376.

Moons A. 2008. Transcriptional profiling of the PDR gene family in rice roots in response to plant growth regulators, redox perturbations and weak organic acid stresses. Planta **229:** 53–71.

Moroni J.S., Briggs K.G. & Taylor G.J. 1991. Chlorophyll content and leaf elongation rate in wheat seedlings as a measure of manganese tolerance. Plant Soil **136:** 1–9.

Norton G.J., Lou–Hing D.E., Meharg A.A. & Price A.H. 2008. Rice–arsenate interactions in hydroponics**:** whole genome transcriptional analysis. J. Exp. Bot. **59:** 2267–2276.

Ogo Y., Itai R.N., Nakanishi H., Inoue H., Kobayashi T., Suzuki M., Takahashi M., Mori S. & Nishizawa N.K. 2006. Isolation and characterization of IRO2, a novel iron–regulated bHLH transcription factor in graminaceous plants. J. Exp. Bot. **57:** 2867–2878.

Ogo Y., Itai R.N., Nakanishi H., Kobayashi T., Takahashi M., Mori S. & Nishizawa N.K. 2007. The rice bHLH protein OsIRO2 is an essential regulator of the genes involved in Fe uptake under Fe–deficient conditions. Plant J. **51:** 366–377.

Pnueli L., Hallak–Herr E., Rozenberg M., Cohen M., Goloubinoff P., Kaplan A. & Mittler R. 2002. Molecular and biochemical mechanisms associated with dormancy and drought tolerance in the desert legume *Retama raetam*. Plant J. **31:**319–330.

Rea P.A. 2007. Plant ATP–binding cassette transporters. Annu. Rev. Plant Biol. **58:** 347–375.

Rezai K. & Farboodnia T. 2008. Manganese toxicity effects on chlorophyll content and antioxidant enzymes in pea plant (*Pisum sativum* L. cv. *qazvin*). Agricul. J. **3:** 454–458.

Ribera A.E., Reyes–Díaz M.M., Alberdi M.R. & Alvarez–Cortez D.A. 2013. Photosynthetic impairment caused by manganese toxicity and associated antioxidative responses in perennial ryegrass. Crop Pasture Sci. 6**4:** 696–707.

Salekdeh G.H., Siopongco J., Wade L.J., Ghareyazie B. & Bennett J. 2002. A proteomic approach to analyzing drought– and salt–responsiveness in rice. Field Crop. Res. **76:** 199–219.

Sasaki T., Matsumoto T., Yamamoto K., Sakata K., Baba T., Katayose Y., et al. 2002. The genome sequence and structure of rice chromosome I. Nature **420:** 312–316.

Schlichting E. & Sparrow L. 1988. Distribution and amelioration of manganese toxic soils, pp. 277–288. In**:** Graham R.D., et al. (eds), Manganese in Soils and Plants. Kluwer Academic Publishers, Dordrecht, the Nederland.

Shen Y., Zhang Y., Chen J., Lin H., Zhao M., Peng H., Liu L., Yuan G., Zhang S., Zhang Z. & Pan G. 2013. Genome expression profile analysis reveals important transcripts in maize roots responding to the stress of heavy metal Pb. Physiol. Plant. **147:** 270–282.

Shi T., Gao Z., Wang L., Zhang Z., Zhuang W., Sun H. & Zhong W. 2012. Identification of differentially–expressed genes associated with pistil abortion in Japanese apricot by genome–wide transcriptional analysis. PloS One **7:** e47810.

Shi Q.H., Zhu Z.J., Xu M., Qian Q.Q. & Yu J.Q. 2005. Effect of excess manganese on the antioxidant systemin *Cucumis sativus* L. under two light intensities. Environ. Exp. Bot. **58:** 197–205.

Sudo E., Itouga M., Yoshida–Hatanaka K., Ono Y. & Sakakibara H. 2008. Gene expression and sensitivity in response to copper stress in rice leaves. J. Exp. Bot. **59:** 3465–3474.

Sun S.J., Guo S.Q., Yang X., Bao Y.M., Tang H.J., Sun H., Huang J. & Zhang H.S. 2010. Functional analysis of a novel Cys2/His2–type zinc finger protein involved in salt tolerance in rice. J. Exp. Bot. **61:** 2807–2818.

Wang Y.J., Zhang Z.G., He X.J., Zhou H.L., Wen Y.X., Dai J.X., Zhang J.S. & Chen S.Y. 2003. A rice transcription factor OsbHLH1 is involved in cold stress response. Theor. Appl. Genet. **107:** 1402–1409.

Wang Y., Xu L., Chen Y., Shen H., Gong Y., Limera C. & Liu L. 2013. Transcriptome profiling of radish (*Raphanus sativus* L.) root and identification of genes involved in response to Lead (Pb) stress with next generation sequencing. PLoS One **8:** e66539.

Wei W., Zhang Y., Han L., Guan Z.Q. & Chai T.Y. 2008. A novel WRKY transcriptional factor from *Thlaspi caerulescens* negatively regulates the osmotic stress tolerance of transgenic tobacco. Plant Cell Rep. **27:** 795–803.

Williams L.E. & Pittman J.K. 2010. Dissecting pathways involved in manganese homeostasis and stress in higher plant cells, pp. 95–117. In: Hell R. & Mendal R.R., (eds) Cell Biology of

Metals and Nutrients. Plant Cell Monographs 17, Springer, Berlin.

Wu J.Z., Maehara T., Shimokawa T., Yamamoto S., Harada C., Takazaki Y., Ono N., Mukai Y., Koike K., Yazaki J., Fujii F., Shomura A., Ando T., Kono I., Waki K., Yamamoto K., Yano M., Matsumotoand T. & Sasaki T. 2002. A comprehensive rice transcript map containing 6591 expressed sequence tag sites. Plant Cell **14:** 525–535.

Wu W.S. & Chen B.S. 2007. Identifying stress transcription factors using gene expression and TF–Gene association data. Bioinformatics Biol. Insights **1:** 9–17.

Xie Z., Zhang Z.L., Zou X., Huang J., Ruas P., Thompson D. & Shen Q.J. 2005. Annotations and functional analyses of the rice WRKY gene super–family reveal positive and negative regulators of abscisic acid signaling in aleurone cells. Plant Physiol. **137:** 176–189.

Xie Z., Zhang Z.L., Zou X., Yang G., Komatsu S. & Shen Q.J. 2006. Interactions of two abscisic–acid induced WRKY genes in repressing gibberellin signaling in aleurone cells. Plant J. **46:** 231–242.

Xu S., Wang X. & Chen J. 2007. Zinc finger protein 1 (ThZF1) from salt cress (*Thellungiella halophila*) is a Cys–2/His–2–type transcription factor involved in drought and salt stress. Plant Cell Rep. **26:** 497–506.

Yan J.Y., Li C.X., Sun L., Ren J.Y., Li G.X., Ding Z.J. & Zheng S.J. 2016. A WRKY transcription factor regulates Fe translocation under Fe deficiency. Plant Physiol. **171:** 2017–2027.

Yi K., Wu Z., Zhou J., Du L., Guo L., Wu Y. & Wu P. 2005. OsPTF1, a novel transcription factor involved in tolerance to phosphate starvation in rice. Plant Physiol. **138:** 2087–2096.

Zhang M., Liu X.C., Yuan L.Y., Wu K.Q., Duan J., Wang X.L. & Yang L.X. 2012. Transcriptional profiling in cadmium–treated rice seedling roots using suppressive subtractive hybridization. Plant Physiol. Biochem. **50:** 79–86.

Zhu J., Verslues P.E., Zheng X.W., Lee B., Zhan X.Q., Manabe Y., Sokolchik I., Zhu Y., Dong C.H., Zhu J.K., Hasegawa P.M. & Bressan R.A. 2005. HOS10 encodes an R2R3–type MYB transcription factor essential for cold acclimation in plants. Proc. Natl. Acad. Sci. USA **102:** 9966–9971.

Received September 5, 2016
Accepted February 7, 2017