Physiological Responses and Transcriptome Analyses of Upland Rice following Exposure to Arsenite and Arsenate

Xuerong Di[a#], Feng Zheng[b#], Gareth J. Norton[c], Luke Beesley[d], Zulin Zhang[d], Hui Lin[e], Suli Zhi[a], Xuncheng Liu[b], Yongzhen Ding[a*]

[a] Agro-Environmental Protection Institute, Ministry of Agriculture and Rural Affairs, Tianjin 300191, China

[b] South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

[c] School of Biological Sciences, University of Aberdeen, Aberdeen AB24 3UU, UK

[d] The James Hutton Institute, Aberdeen AB15 8QH, UK

[e] The Institute of Environment, Resource, Soil and Fertilizers, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, P.R. China

[#] Both authors have equal contributions

* Corresponding author: dingyongzhen@caas.cn (YZ Ding)

Abstract

This study examines the molecular response of upland rice when exposed to different inorganic arsenic (iAs) species. One-week-old upland rice (*Oryza sativa* indica DOURADOAGULHA) seedlings were separately exposed to 100 μM arsenite [As(III)] and arsenate [As(V)] in hydroponic culture for 12 h. Genome-wide transcriptomic analysis revealed that 2983 and 1844 genes were responsive under As(III) and As(V) stresses, respectively. There were 915 shared genes between As(III) and As(V) treatments. Arsenic stress induced changes in complicated gene regulatory pathway of upland rice seedlings, including transcriptional regulation, hormone signaling, redox, transporters and detoxification process. In upland rice separately exposed to As(III) and As(V) for 12, 24, 48 and 72 h, qRT-PCR analysis showed that *WRKY4*, *bHLH* and *AP2-EREBP* associated with transcriptional regulation were significantly down-regulated, while NAC [NAM (no apical meristem), ATAF (*Arabidopsis thaliana* activating factor) and CUC (cup-shaped cotyledon)] transcription factor was up-regulated. The hormone signaling related gene *OZG2*, the redox related genes encoding Prx, GST, oxidoreductase and cytochrome P450, the transporter process related genes *OsABCC9* and *ZIP3* were affected under As stress. Under As(III) and As(V) treatments for 1 and 3 d, the malondialdehyde (MDA) contents in upland rice were increased, while catalase (CAT), peroxidase (POD),

superoxide dismutase (SOD) and glutathione reductase (GR) contents were significantly declined. In addition, transcriptomic analysis showed that the genes encoding SOD, Prx, Grx and TrxR were mostly down-regulated. In conclusion, the combination of transcriptome and physiological response may provide a deeper understanding about the molecular mechanism in the response to As in upland rice.

Keywords: Antioxidant enzyme; Arsenate; Arsenite; Gene expression; Transcriptome; Upland rice

1. Introduction

Arsenic (As) poses a threat to human health in excessive and prolonged exposure. As the staple food for over half of the world's population, rice (*Oryza sativa* L.) is a major dietary source of inorganic arsenic (Carey et al., 2012). Sun et al. (2008) observed that inorganic arsenic was the dominant form of As in rice grains, whereas Zheng et al. (2013) revealed that the accumulation of dimethylarsinic acid (DMA) in rice grains was more than twice that of inorganic arsenic species (iAs).

The main forms of As in soils are inorganic (iAs) including arsenite [As(III)] and arsenate [As(V)], which affect solubility, mobility and uptake by plants. As(III) has similar physiochemical properties to silicic acid, so it can be taken up by rice roots through the aquaporins transporter and silicon (Si) transporter system such as Lsi2 (Ma et al., 2008). The rice nodulin 26-like intrinsic membrane proteins (NIP), such as OsNIP1;1, OsNIP2;1 (Lsi1), OsNIP3;1, OsNIP3;2 and OsNIP3;3, were reported to have an impact on arsenite accumulation (Ma et al., 2008; Sun et al., 2018). Specifically, Ma et al. (2008) found that, although Lsi6 showed permeable ability to As(III), it had no effect on arsenite uptake by rice roots. In addition, Lsi2 was involved not in the influx of arsenite into roots, but rather in the efflux of arsenite towards the xylem (Ma et al., 2008). Sun et al. (2018) observed that overexpression of *OsNIP1;1* and *OsNIP3;3* in rice restricted As(III) loading into the xylem. Duan et al. (2016) revealed that AtINT2 or AtINT4 mediated As(III) loading into the phloem and the disruption of these two genes reduced the accumulation of arsenic in the phloem.

In the cytosol, As(III) can react with sulfhydryl groups (-SH) of enzymes and proteins, influencing biochemical functions (Meharg and Hartley, 2002). However, as a phosphate analog, As(V) was taken up by the phosphate uptake system, such as OsPT1, OsPT4, OsPT8 in rice (Kamiya et al., 2013; Wang et al., 2016; Cao et al., 2017).

Arsenic exposure can also inhibit plant growth (Chakrabarty et al., 2009). Tripathi et al. (2013) showed that arsenic affected physiological and metabolic activities in plants, with both iAs species generating reactive oxygen species (ROS), resulting in oxygen stress (Requejo et al., 2005). Plants enhance their antioxidant system under As(V) stress whilst they increase thiol metabolism under As(III) stress (Srivastava et al., 2007; Mishra et al., 2008; Dave et al., 2013a). After being taken up by rice roots, As(V) can readily be reduced to As(III) by OsHAC1;1, OsHAC1;2 and OsHAC4 (Shi et al., 2016; Xu et al., 2017). As(III) can bind phytochelatin synthase (PCs), then be transported into the vacuoles by OsABCC1 for sequestration (Song et al., 2014). In particular, a part of As(III) can be extruded into the external medium by ACR3 in plants, thus reducing the As content in plants (Indriolo et al., 2010; Chen et al., 2017).

At present, many genes have been reported to be responsive to arsenic in plants. Norton et al. (2008) observed that the genes involved in metabolism, transporter and glutathione synthesis were responsive to As(V) in rice by using a DNA microarray chip. Chakrabarty et al. (2009) identified that a large number of genes which related

to plant hormone signaling were differentially regulated in rice seedlings after As(III) and As(V) exposure. Liu et al. (2012) identified a set of arsenite-responsive miRNAs including transcription factors and protein kinases. Yu et al. (2012) found that JA signaling and lipid metabolism may play an important role in response to As(III) in rice by using Illumina sequencing. In *Arabidopsis*, the phytochelatin synthase AtPCS1 functions in increasing the tolerance of plants to As stress (Li et al, 2004), whereas transcription factor AtWRKY6 acts to restrict arsenate uptake of the seedlings (Castrillo et al., 2013). Apart from the above studies, semiquantitative RT-PCR analysis has also found that a set of genes related to sulphur assimilation pathway and antioxidant system were responsive to As(III) and As(V) stresses (Rai et al., 2011).

To date, the processes of arsenic uptake, transport and detoxification have been extensively studied in lowland rice varieties, however, the mechanism of how upland rice response to arsenic stress was rarely reported. On the other hand, in aerobic condition, As(V) was the main form, and can be absorbed by clay-sized mineral and oxides such as ferromanganese in soil solutions, thus limiting the movement of arsenic and reducing the bioavailability of arsenic (Kim et al., 2014). Aerobic condition, in which upland rice is usually grown, has been found to contribute less to arsenic accumulation in grains than in flooded condition (Hu et al., 2015). Xu et al. (2008) also observed that the arsenic uptake and accumulation in rice roots and grain were much enhanced in flooding conditions compared with non-flooding ones. In addition, Tan et al. (2016) found that arsenic contents in the grain of all upland rice

cultivars were less than the standard for food security (0.2 $mg \cdot kg^{-1}$) under non-flooding condition (Ding et al., 2017). Therefore, upland rice grown in aerobic condition seems an appropriate measure to reduce arsenic accumulation in grains, and ensure the reduced risk from As accumulation and consequent food chain transfer.

The aims of this study were to investigate the effects of arsenite and arsenate on the molecular mechanisms in upland rice, with particular attention to transcription regulation, hormone signalling, redox, transport and detoxification mechanisms regulated under As(III) and As(V) stresses. The expression patterns of genes which affected by both As(III) and As(V) were further investigated by qRT-PCR assay. In addition, we examined the ROS accumulation and the activities of antioxidant system in upland rice seedlings under As(III) and As(V) stresses.

2. Material and methods

2.1. Seedling cultivation and experimental treatments

The Indica rice variety (DOURADOAGULHA) was selected as the experimental material and was provided by Shanghai Agricultural Biogene Center (China). After sterilization with 3% NaClO for 20 minutes and three-fold washing with deionized water, the seeds were germinated on moist filter paper at 25 ℃ for 3 days. The germinated seeds were then transferred to culturing pots (24 rice seeds per pot) containing 20 mL modified Hoagland's nutrient solution for 4 days with germinated seeds floated on netting. The solution was not aerated but was renewed every 2 days. Each pot was replicated three times.

During preliminary testing we had observed that the growth of upland rice seedlings was inhibited under 100 μM $NaAsO_2$ [As(III)] or $Na_2HAsO_4·7H_2O$ [As(V)] stress when the seedlings exposed to a series of As(III) or As(V) concentrations from 10 to 100 μM. Uniformly-sized rice seedlings (12 seedlings per pot) were exposed to 100 μM $NaAsO_2$ [As(III)] and $Na_2HAsO_4·7H_2O$ [As(V)] (Dubey et al., 2014) for 0, 12, 24, 48, 72, and 96 h, respectively. The modified Hoagland's nutrient solution contained the following composition (mM): $CaCl_2$ (4.00), KNO_3 (6.00), $NH_4·H_2PO_4$ (1.00), $MgSO_4·7H_2O$ (2.00), H_3BO_3 (0.46), $MnCl_2·4H_2O$ (0.09), $ZnSO_4·7H_2O$ (0.007), $CuSO_4·5H_2O$ (0.003), $(NH_4)_6Mo_7O_{24}·4H_2O$ (0.0002), $FeSO_4·7H_2O$ (0.10), Na_2EDTA (0.11). The pH of the solution was adjusted to 5.5 using 1 mM HCl or NaOH. Controls without As(III) or As(V) were also included(CK). The seedlings were grown in a greenhouse at 25 ℃, with 16 h light/ 8 h dark photoperiod, light intensity of 115 $\mu mol·m^{-2}·s^{-1}$ and relative humidity of 80%.

2.2. RNA extraction and RNA sequencing (RNA-seq) assay

The gene expression patterns are sometimes subject to change in an earlier stage after upland rice seedlings are exposed to As(III) or As(V). One-week old seedlings were treated with 0, 100 μM As(III) and As(V) respectively for 12 hours. The shoots of seedlings were harvested and frozen in liquid nitrogen, then preserved at -80^{o}C. Total RNA was extracted for the three biological replicates from the shoot tissues collected from four seedlings for each biological replicate (treatments without As(III) and As(V) were used as blank controls) using Plant Total RNA Isolation Kit (Magent

company, China). The concentration and purity of the total RNA were determined using a Nanodrop 2000, and the integrity was verified by agarose gel electrophoresis. RNA-seq was performed by the Macrogen company in Shenzhen, China. In brief, 2 μg total RNA was used for generating an mRNA-seq library by using an mRNA Seq Kit (Illumina). IlluminaHiseq 4000 platform was utilized for high-throughput sequencing with a read length of Pair-end 150 bp. Genes with more than 2.0-fold changes with statistically significance (p-value < 0.05) were selected. The Rice Information GateWay (http://rice.hzau.edu.cn/rice_rs1/) was used for the exchange of rice ID. Heatmaps were made by the HemI software. The Database for Annotation, Visualization and Integrated Discovery (DAVID) enrichment was analyzed in website (https://david.ncifcrf.gov/tools.jsp). Gene ontology (GO) biological process enrichment analysis was made by using AGRIGO2 at the following webpage: http://systemsbiology.cau.edu.cn/agriGOv2/. For GO biological process enrichment results (p-value < 0.05) see Supplementary materials_2.

2.3. qRT-PCR analysis

In order to prove the accuracy of RNA-seq analysis, qRT-PCR was performed by using the same sample RNA as that of RNA-seq. Using 1 μg RNA as templates, the Monad's reverse transcriptase kit (MonScript ^{TM} RTIII All-in-One Mix with dsDNase) was utilized for reverse transcription. The reverse transcription was conducted at 37℃ for 2 min and then 55℃ for 15 min and finally 85℃ for 10 s. The 200 ng of cDNA was used as the templates for real-time PCR amplification using gene specific primers

(Supplementary materials Table 1) and MonAmp ™ Fast SYBR ® Green qPCR Mix (None Rox). Each sample was analyzed three times. The qRT-PCR conditions were as follows: 95ºC for 30 s followed by 40 cycles at 95ºC for 15 s and 60ºC for 35 s. The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method and normalization with the rice gene *OsActin1* (*Os03g0718100*) (Supplementary materials Table 1).

To further examine the expression pattern of As(III) and As(V)-responsive genes, the seedlings after 0, 12, 24, 48, 72 h As(III) and As(V) treatments were collected for gene expression patterns analysis using qRT-PCR. The seedlings after 0 h (without) As(III) and As(V) treatments were used as blank control. Information on the gene specific primers is in Supplementary materials Table 1.

2.4. Physiological assay

Seven-day-old seedlings were treated with 0, 100 μM As(III) and As(V) for 24 and 72 h. A total of 0.25 g of roots and shoots were harvested individually, then frozen in liquid nitrogen, and conserved at -80ºC for physiological index analyses. The plant samples were homogenized in 150 mM PBS buffer (pH 7.4) at 4ºC. Homogenate was centrifuged at 10,000×g for 10 min 4ºC. Supernatant obtained was used to measure the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and glutathione reductase (GR).

SOD activity was measured according to the method of Dave et al. (2013b) with modification. The test tubes containing 5.9 mL of reaction mixture (3.5 mL phosphate buffer saline (PBS) (pH 7.4), 0.6 mL 130 mM methionine, 0.6 mL 750 μM

nitroblue-tetrazolium (NBT), 0.6 mL 0.1 mM Na_2EDTA, 0.6 mL 20 μM riboflavin) and 0.1 mL enzyme extract were placed below a light source and after 10 min absorbance was recorded at 560 nm. POD activity was assayed by taking 1.5 mL reaction mixture (780 μL 200 mM PBS buffer (pH 6.0), 290 μL a-methoxyphenolsalicyl methyl ether, 430 μL 30% H_2O_2) and 15 μL enzyme extract. The absorbance was recorded at 470 nm. The activity of CAT was measured using 1.5 mL reaction mixture (150 mM PBS buffer (pH 7.4) and 30% H_2O_2) and 30 μL enzyme extract. The absorbance was recorded at 240 nm. GR activity was measured by the combination of 1.97 mL reaction mixture (150 mM PBS buffer (pH 7.4), 2 mM Na_2EDTA, 10 mM GSSG, 0.15 mM NADPH) and 30 μL enzyme extract, and the absorbance was recorded at 340 nm.

Malondialdehyde (MDA) was measured according to the method of Dave et al. (2013b) with modification. A total of 0.25 g plant shoot samples was homogenized in 5 mL of 5% trichloroacetic acid (TCA). Homogenate was centrifuged at 10,000×g for 10 min 4ºC. 2 mL 0.67% 4,6-dihydroxy-2-mercaptopyrimidine (TBA) was added to the 2 mL supernatant. The mixture was heated at 100ºC for 30 min. After cooling, the mixture was centrifuged at 10,000×g for 10 min 4ºC again. The absorbance was recorded at 450 nm, 532 nm and 600 nm.

2.5. Data analysis

The data were subject to analysis of variance (ANOVA) ($p \leq 0.05$) using SPSS 22.0 Statistics. Plots were prepared using SigmaPlot 12.5 software.

3. Results

3.1. Differentially expressed genes (DEGs) analysis

The number of clean reads under the CK treatment was 59845106, 69340856 and 60489846. Under As(III) treatment, the number of clean reads was 69845352, 62204260 and 67740720. The number of clean reads under As(V) treatment was 62267932, 51706214 and 64833728 (see Supplementary materials_2). In total, 2983 and 1844 DEGs were obtained from As(III)_vs_CK and As(V)_vs_CK groups, respectively (Fig. 1a). A total of 1072 DEGs were up-regulated whereas 1911 DEGs were down-regulated in As(III) treatment, whilst 802 DEGs were up-regulated but 1042 DEGs were down-regulated in As(V) treatment (Fig. 1b). A greater number of DEGs were observed in the As(III) treatment compared to the As(V) treatment (Fig. 1b). There were 915 shared DEGs between As(III)_vs_CK and As(V)_vs_CK groups. Among shared DEGs, 36 DEGs were differentially regulated, and the others were co-regulated with 294 genes up-regulated and 585 genes down-regulated (Fig. 1c).

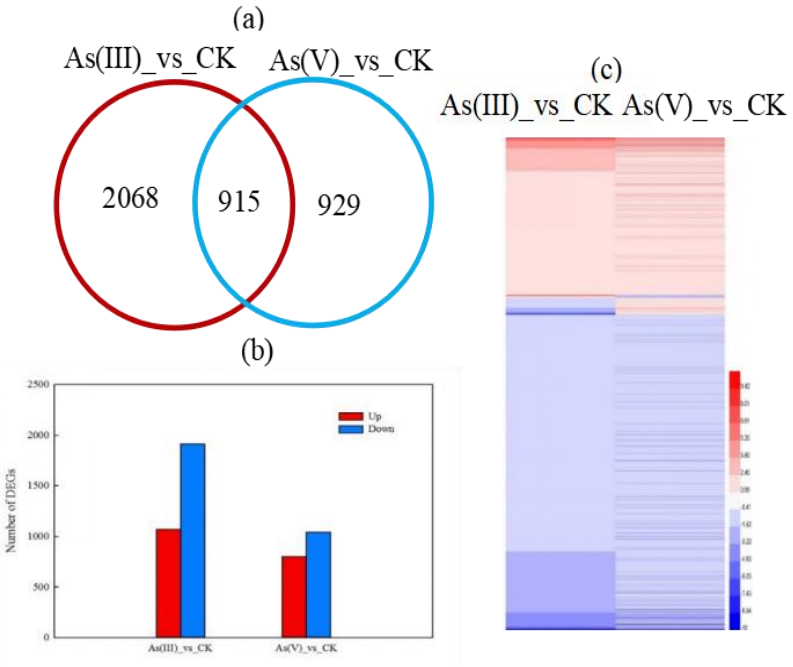


Fig. 1 Differently expressed genes (DEGs) in one-week old upland rice seedlings exposed to As(III) and As(V) treatments for 12 hours. (a), Venn diagram of DEGs that were unique to, and shared by, plants exposed to As(III) or As(V); (b), Numbers of up- and down-regulated genes in one-week old upland rice seedlings exposed to As(III) and As(V); (c), Heatmap of the relative expression levels of DEGs in rice exposed to As(III) or As(V).

3.2. DAVID and GO enrichment analysis of DEGs

DAVID enrichment analysis revealed that “Oxidoreductase”, “Flavonoid biosynthetic process” and “Lignin degradation” were the top three rankingup-regulated genes with exposure to As(III) (Supplementary materials Fig. 1a), and “ABC transporter-like”, “Oxidoreductase” and “Terpenoid synthase” were the top three ranking in the As(V) treatment (Supplementary materials Fig. 1b). With

exposure to As(III) or As(V), DEGs enrichment score of “Ribonucleoprotein” was the highest in the down-regulated genes (Supplementary materials Fig. 1c, d). GO enrichment showed that there were similar biological processes enriched in the rice shoots exposed to As(III) and As(V), including metabolic processes, biosynthetic processes, stimulus response, translation, stress response and redox processes (Supplementary materials_3). In addition, some different biological processes were enriched in the As(III) or As(V) treatment, respectively. For example, regulation of transcription (GO:0045449), regulation of biosynthetic process (GO:0009889) and hormone stimulus response (GO:0009725) were only enriched in the plants exposed to As(III); However, lipid metabolic process (GO:0006629), oxidative stress response (GO:0006979) and lipid transport (GO:0006869) were only enriched in the As(V) treatment (Supplementary materials_3).

3.3. Specific genes responsive to As(III) and As(V) in upland rice seedlings identified by RNA-seq analysis

Transcriptomic analysis showed that arsenic stress induced changes incomplicated gene regulatory pathways in upland rice seedlings, including transcriptional regulation, hormone signaling, redox, transporters and detoxification processes. In the process of transcriptional regulation, the genes, encoding bHLH (basic helix-loop-helix protein), MYB, NAC, WRKY, bZIP, AP2-EREBP, HSF, GRAS and Dof transcription factors, were affected under As(III) and As(V) stresses (Table 1). In 915 common DEGs, 36 genes were related to gene expression regulation,

among which, 17 genes were up-regulated, 13 genes were down-regulated, and 6 genes were expressed in opposite directions in response to As(III) and As(V), respectively (Supplementary materials Fig. 2a). In addition, among the 96 regulation related genes specific to As(III) stress, DEGs with upregulation (26 genes) were fewer than were that of downregulation (70 genes) (Table 1). Most of the genes related to transcriptional factors were down-regulated under As(III) stress. In contrast, among the 24 DEGs specific to As(V) stress, 15 were up-regulated and 9 were down-regulated. Furthermore, all of NAC transcription factors were up-regulated under As(V) stress, indicating that NAC may play a positive regulation role in response to As(V) exposure. Compared to As(V) stress, more genes related to transcriptional factors were expressed under As(III) stress, and most of them were down-regulated, indicating that As(III) stress could mainly inhibit the expression of down-stream genes by inhibiting the expression level of the transcription factors. These results revealed that As(III) and As(V) treatments may cause differentially transcriptional regulatory modes in upland rice seedlings.

Table 1 The number of transcriptional factor related genes in rice shoots exposed to either As(III) or As(V) for 12 hours compared to unexposed plants (CK)

Gene pathway	Gene name	As(III)_vs_CK		As(V)_vs_CK	
		Up	Down	Up	Down
Transcription factors	bHLH	4	18	2	2

MYB	10	14	6	8
NAC	10	7	10	0
WRKY	0	24	5	2
bZIP	6	5	4	3
AP2-EREBP	6	19	7	6
HSF	4	2	2	0
GRAS	1	0	0	1
Dof	2	0	2	0

Under arsenic stress, redox related genes (SOD, Prx, Grx, oxidoreductase, NADPH, TrxR, cytochrome P450 and GST) were affected (Table 2). Among the identified redox related genes, 46 were co-regulated by As(III) and As(V) stresses, while 61 and 50 genes were specifically regulated by As(III) and As(V) stresses, respectively. Among the 46 genes associated with redox that were both regulated by As(III) and As(V), 28 and 16 were up-regulated and down-regulated, respectively (Supplementary materials Fig. 2b). The other two genes were expressed conversely in response to As(III) and As(V). Among the 61 DEGs specific to As(III) stress, 34 and 27 were up-regulated and down-regulated, respectively, while 23 and 27 genes differentially expressed specific to As(V) stress were up-regulated and down-regulated, respectively. Compared to As(V) stress, more genes related to redox were expressed under As(III) stress. Furthermore, the genes, encoding SOD, Prx, Grx and TrxR were down-regulated under As(III) and As(V) stresses in an early stage.

However, the genes encoding cytochrome P450 and GST were mostly up-regulated, indicating GST and cytochrome P450 may play a positive regulation role in responding to As stress.

Table 2 The number of redox related genes in rice shoots exposed to either As(III) or As(V) for 12 hours compared to unexposed plants (CK)

Gene name	As(III)_vs_CK		As(V)_vs_CK	
	Up	Down	Up	Down
SOD	0	3	0	1
Prx	6	10	5	12
Grx	1	7	1	5
oxidoreductase	5	7	4	6
NADPH	0	1	0	0
TrxR	0	4	0	2
cytochrome P450	41	11	30	17
GST	10	1	12	1

In the process of the hormone signaling, the genes involved with auxin (AUX) signaling pathway (SAUR, ARP, AUX/IAA and ARF), gibberellin (GA) signaling pathway (GID1L2, GA20ox, GA2ox and CIGR), abscisic acid (ABA) signaling pathway (PP2C, LEA, ASR and PYL6), cytokinin (CTK) signaling pathway (OZG2, CKX and RR), ethylene signaling pathway (EIN3 and ACO7) and jasmonic acid (JA) signaling pathway ((LOX, OPR and JAZ) were affected in upland rice shoots under

As(III) and As(V) stresses (Table 3). Among the identified genes involved in hormone signaling, 22 common genes were expressed both under As(III) and As(V) stress, while 35 and 11 DEGs were specific to As(III) and As(V) stress, respectively. The hormone signaling related genes in rice shoots that responded to both As(III) and As(V), 14 genes, included genes encoding protein phosphatase 2C (PP2C), late embryogenesis abundant protein (LEA), were up-regulated, and 6 DEGs were down-regulated under As(III) and As(V) stress (Supplementary materials Fig. 2c). The other two genes were expressed in opposite directions in response to As(III) and As(V). Among the 35 DEGs specific to As(III) stress, 14 and 21 were up-regulated and down-regulated, respectively, while 6 and 5 genes differentially expressed specific to As(V) stress were up-regulated and down-regulated, respectively. Compared to As(V) treatment, many more genes related to hormone signaling were expressed under As(III) treatment. Furthermore, AUX hormone signaling related genes *ARP*, *AUX*/*IAA* and *ARF,* as well as GA related gene *CIGR* were only affected under As(III) stress for 12 h in upland rice shoots, but these genes were not identified under As(V) stress. However, ABA signaling pathway related gene *PYL6* was only identified under As(V) stress. These results showed As(III) and As(V) treatments may cause differential hormone signaling regulatory modes in rice seedlings.

Table 3 The number of hormone signaling related genes in rice shoots exposed to either As(III) or As(V) for 12 hours compared to unexposed plants (CK)

Gene pathway	Gene name	As(III)_vs_CK		As(V)_vs_CK	
		Up	Down	Up	Down
AUX signaling pathway	SAUR	1	6	0	2
	ARP	0	1	0	0
	ARF	1	0	0	0
	AUX/IAA	0	2	0	0
GA signaling pathway	GID1L2	3	0	1	0
	GA20ox	1	0	1	1
	GA2ox	1	1	2	1
	CIGR	0	2	0	0
	GASR10	0	1	0	0
ABA signaling pathway	PP2C	4	5	6	0
	LEA	6	2	6	0
	ASR	1	1	0	0
	PYL6	0	0	0	1
CTK signaling pathway	OZG2	5	2	2	2

	CKX	1	1	0	2
	RR	0	1	0	0
ethylene	EIN3	1	0	0	0
	ACO7	0	1	0	0
	LOX	1	2	1	1
JA signaling pathway	OPR	2	0	3	1
	JAZ	0	1	0	0

Under arsenic stress, transporter related genes, including aquaporin protein, ATP-binding cassette (ABC) family (ABCB, ABCC and ABCG), metallothionein family (MT1a, MT1g and MT2a) and MATE efflux family protein were responsive (Table 4). Among all identified genes involved in transporter processes in the rice shoots, 13 were responsive to both As(III) and As(V) stress, while 37 were specifically responsive to As(III), and 26 specific to As(V). Among the 13 genes related to detoxification process that responded to both As(III) and As(V) stress, 11 were up-regulated, while two were down-regulated (Supplementary materials Fig. 2d). Moreover, among the 37 DEGs specific to As(III) stress, 26 and 11 were up-regulated and down-regulated, respectively. Among the 26 genes specific to As(V) stress, 22 were up-regulated and 4 were down-regulated. Above results showed that most of the genes related to transporter process were up-regulated under As(III) or As(V) stress.

Table 4 The number of transporter related genes in rice shoots exposed to either As(III) or As(V) for 12 hours compared to unexposed plants (CK)

Gene pathway	Gene name	As(III)_vs_CK		As(V)_vs_CK	
		Up	Down	Up	Down
ATP-binding cassette (ABC)	ABCB	5	0	3	0
	ABCC	3	0	4	0
	ABCG	4	0	7	0
Aquaporin	TIP4;2	1	0	0	0
	TIP3;1	1	0	1	0
	TIP4;3	0	0	0	1
	TIP2;1	0	1	0	1
	PIP1;3	1	0	0	0
	PIP2;4	1	0	1	0
	PIP2;3	0	1	0	0

Table 4 (Forward)

Gene pathway	Gene name	As(III)_vs_CK		As(V)_vs_CK	
		Up	Down	Up	Down
Phosphate transporter	PHO1;2	1	0	0	0
	Pht1;4	1	0	0	0
	PT19	0	0	1	0
Metallothionein	MT1a	0	0	2	0

Metallothionein	MT1g	1	0	1	0
	MT2a	0	1	0	0
	heavy metal transport/ detoxification protein	1	3	1	0
metal transport/detoxification protein	IRT2(Fe)	1	0	0	0
	ZIP1	0	0	1	0
	ZIP3(Zn)	1	0	1	0
	ZIP10(Zn)	1	0	0	0
	NCX15(Na/Ca)	1	0	0	0
	HAK1	0	0	1	0
	HAK12(K)	1	0	0	0
	HAK27	1	0	0	0
	HMA5	1	0	0	0
	ATPase, P-type	0	1	0	0
	Nramp1	1	1	1	1
	ACA8(Ca)	0	1	0	0
	MTP11.1	0	0	0	1
	BOR2(B)	0	1	0	0
MATE efflux family protein		6	2	5	1
Plasma membrane H+ ATPase	AHA1	1	0	1	0

Table 4 (Forward)

Gene pathway	Gene name	As(III)_vs_CK		As(V)_vs_CK	
		Up	Down	Up	Down
citrate transporter		1	1	0	0
Major facilitator superfamily protein	ZIFL2	0	0	0	1
	ZIFL8	0	0	1	0
	ZIFL12	0	0	1	0
	ENA1	1	0	0	0

To confirm the reliability of RNA-seq results, we randomly chose some genes related to transcription factors, hormone signaling, redox or detoxification process. We found the expression modes of genes in RNA-seq analysis were consistent with those in qRT-PCR results under As(III) and As(V) stress (see Supplementary materials Fig. 3), indicating that RNA-seq results were reliable and could be used for further analysis.

3.4. qRT-PCR analysis of the dynamic changes of As(III) and As(V)-inducible genes

To further study the regulatory modes of genes related to transcription factors, hormone signaling, redox and transporter, the genes screened from transcriptome data were analyzed under As(III) and As(V) treatments for 0, 12, 24, 48 and 72 h. Under arsenic stress, transcription factors related genes *Os03g0758900* (*WRKY4*), *Os08g0471401* (*bHLH*) and *Os03g0182800* (*AP2-EREBP*) were continuously

inhibited under As(III) and As(V) stresses (Fig. 2). However, the gene *Os07g0684800* encoding NAC transcription factor was induced to express. These results indicated that both As(III) and As(V) could affect stress response of upland rice seedlings by changing the expression modes of different kinds of transcription factors.

In the redox process, the genes, *Os03g0235000* encoding Prx and *Os01g0692100* encoding GST, were induced in an early and middle stage (12 and 48 h) under As(III) treatment, while both of them were induced only in an early stage (12 h) under As(V) treatment (Fig. 2). The gene *Os10g0525200*, encoding cytochrome P450, was induced in an early stage (12 h) under both As(III) and As(V) treatments. However, the gene *Os04g0339400*, encoding oxidoreductase was continuously induced under As(III) and As(V) treatments. These results indicated that both As(III) and As(V) treatments differentially induced the reaction of the antioxidant system in response to oxidative stress.

Hormone signaling related gene *OZG2* (*Os02g0755900*), encoding cytokinin-O-glucosyltransferase 2, was significantly induced under As(III) and As(V) stress at 12 h, but the gene expression was significantly inhibited at 24 h and 48 h, indicating that arsenic induced the defense reaction mediated by *OZG2* of upland rice seedlings in an early stage. After 72 h under As(III) and As(V) treatments, the expression of *OZG2* was up-regulated and down-regulated, respectively. The results showed that As(III) and As(V) caused different a defense reaction mediated by *OZG2* in a later stage (Fig. 2).

The gene *OsABCC9* (*Os04g0209200*) was induced in an early and middle stage (12 and 48 h) under As(III) treatment and in an early stage under As(V) treatment (Fig. 2). The gene *ZIP3* (*Os04g0613000*) was induced under As(III) and As(V) stresses for 12 h, but the gene was inhibited continuously for 24, 48 and 72 h under both treatments. The gene *Nramp1* (*Os07g0258400*) was induced under As(III) stress for 12, 24 and 48 h, but the gene expression showed no significant change under As(V) stress. The above results showed that the expression patterns of detoxification related genes were quite different under As(III) and As(V) stresses.

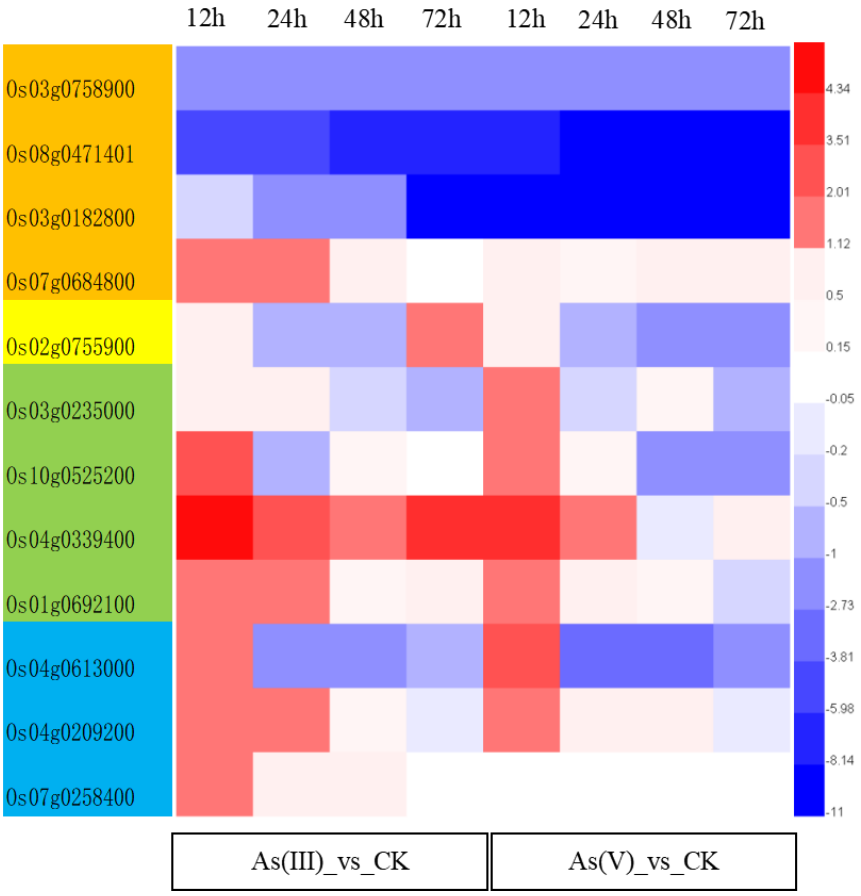


Fig. 2 Expression profiles of genes in rice shoots exposed to either As(III) or As(V) for 12，24，48 and 72 hours compared to unexposed plants (CK). Orange indicates

regulation related genes; yellow indicates hormone signaling related genes; light green indicates redox related genes; lightblue indicates transporter and detoxification related genes.

3.5. Analysis of the antioxidant activity and MDA content of upland rice seedlings after As(III) and As(V) treatment

To determine the physiological processes that were likely affected by As(III) and As(V), antioxidants and ROS in the roots and shoots were analyzed. The root CAT contents with As(III) exposure led to a significant reduction of 23.6% on day 1 and 74.9% on day 3, respectively (Fig. 3). The CAT content in roots was significantly decreased by 34.4% compared to the CK treatment at 3 d exposure to As(V). In shoots CAT activities were unaffected with As(III) and As(V) exposure at 1 d, compared with CK exposure. However, in shoots CAT content decreased significantly to 35.8% and 22.3% after 3 d exposure to As(III) and As(V), respectively. After As(V) exposure for 3 d, CAT activity in roots increased significantly more than three folds compared to As(III) treatment.

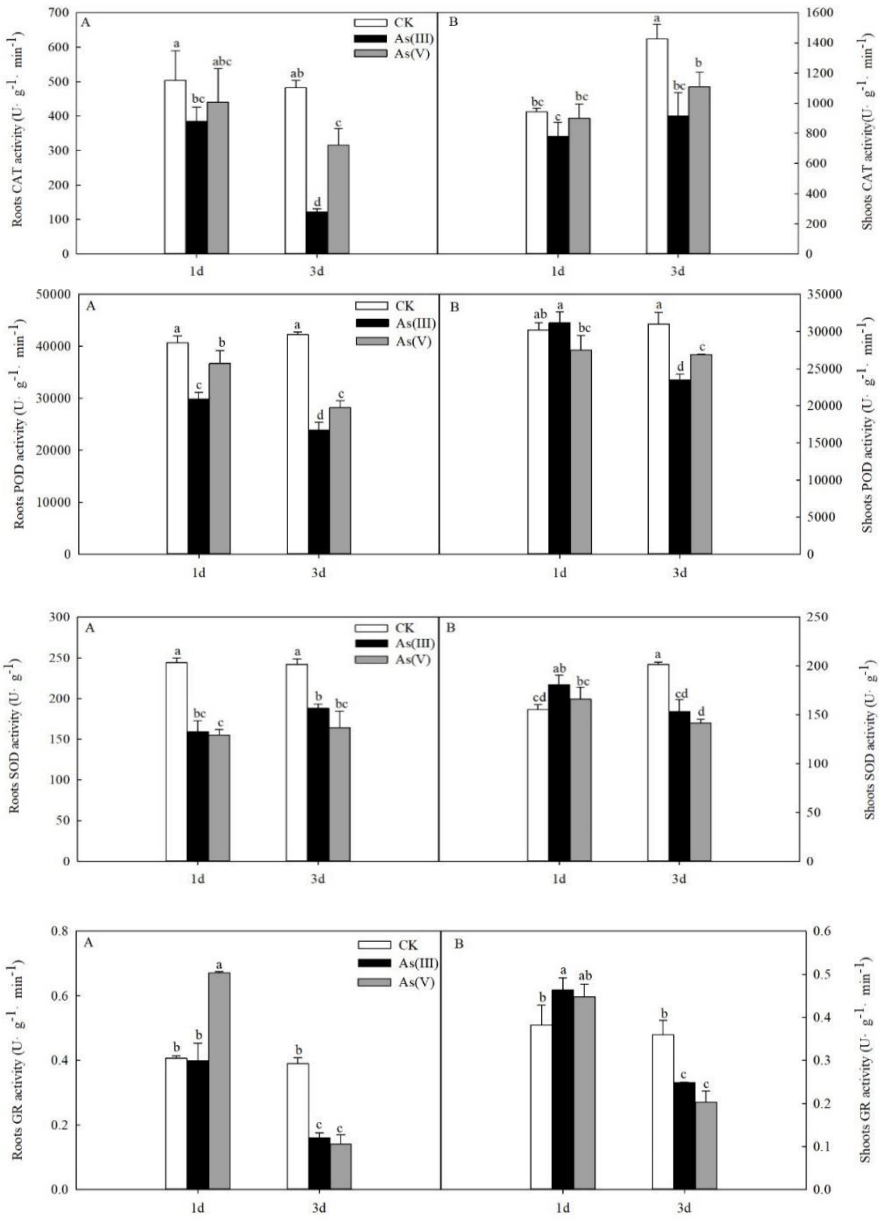
A duration dependent reduction in POD activity in roots and shoots (Fig. 3) was seen in this study. In roots, As(III) and As(V) exposure led to a significant reduction of 26.7% and 9.7% respectively on day 1 and 43.4% and 33.27% respectively on day 3. For shoot POD activity, As(III) and As(V) exposure for 1 d was not significantly different compared to CK. However, in shoots POD content decreased significantly by 24.3% and 13.3% on 3 d exposure to As(III) and As(V), respectively. Compared

with As(III) treatment, POD activity in roots with As(V) exposure had a significant increase.

A duration dependent reduction in SOD activity in shoots was also seen (Fig. 3). In roots, compared with CK, As(III) and As(V) exposure led to a significant reduction of 34.8% and 36.5% respectively on day 1 and 22.4% and 32.2% respectively on day 3 (Fig. 3). Compared with CK treatment, SOD content in shoots reduced significantly by 23.8% and 29.6% on 3 d exposure to As(III) and As(V), respectively.

Likswise, a duration dependent reduction in GR activity roots and shoots was also measured (Fig. 3). In roots, As(V) exposure led to a significant increase of 65.19% on day 1. In roots GR content decreased significantly by 59.01% and 63.87% on 3 d exposure to As(III) and As(V) compared to CK, respectively. In shoots, As(III) exposure led to a significant increase of 21.16% on day 1. In shoots GR content decreased significantly by 30.90% and 43.40% on 3 d exposure to As(III) and As(V) compared to CK, respectively.

A time-dependent increase in MDA contents under As stress was detected (Fig. 3). Compared with 1 day exposure, MDA contents in As(III) and As(V) treatments with exposure to 3 days increased by 37.8% and 43.7%, respectively. In As(III) and As(V) treatments, MDA contents on day 1 and day 3 were 2.7-3.9 times more than those in CK treatment. MDA content in As(III) treatment was higher than that in As(V) treatment on day 1 and day 3.


A
B
CK
As(III)
As(V)
Roots CAT activity (U· g-1· min-1)
Shoots CAT activity(U· g-1· min-1)
Roots POD activity (U· g-1· min-1)
Shoots POD activity (U· g-1· min-1)
Roots SOD activity (U· g-1)
Shoots SOD activity (U· g-1)
Roots GR activity (U· g-1· min-1)
Shoots GR activity (U· g-1· min-1)
1d
3d

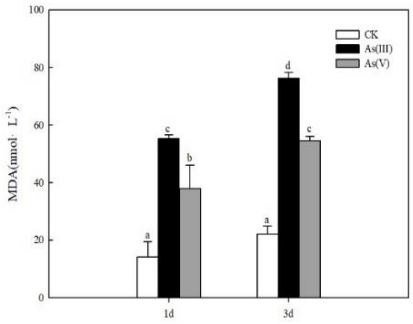


Fig. 3 The antioxidant system (CAT, POD, SOD, GR) in roots (A) and shoots (B) and MDA contents in shoots of 7-day-old upland rice seedlings treated with As(III) and As(V) for 1 and 3 days. Different letters mean significant differences between treatments at $p \leq 0.05$.

4. Discussion

4.1. Transcriptome analysis

Under various abiotic stresses, plants have developed complicated mechanism to adapt to their local environment (Wang et al., 2019). In the present work, we analyzed the transcriptome of upland rice in response to As(III) and As(V) exposure separately. We identified more As-regulated genes in upland rice in comparison with other

studies on rice (for example: Chakrabarty et al., 2009; Huang et al., 2012; Huang et al., 2019). Chakrabarty et al. (2009) observed that the DEGs (428) under 250 μM As(V) treatment outnumbered that (171) of 25 μM As(III) for 10 days by using a DNA microarray chip. Furthermore, only 66 (about 29.6%) genes of the 223 up-regulated DEGs under As(V) treatment that Chakrabarty et al. (2009) found were represented in our set of 802 As(V)-up-regulated genes, and 22 (about 25.9%) of the 85 DEGs with upregulation under As(III) treatment was the same as our 1,072 As(III)-up-regulated genes (Supplementary Table 4a). The main reason for this difference may be that the bias of the DNA chip in low-intensity genes was higher than that of the RNA-seq (Robinson et al., 2015) or the difference of rice varieties. Lowland and upland rice (*Oryza sativa* L.) represent two of the most important rice ecotypes adapted to agro-ecosystem. Lowland rice is cultivated in deep-water and paddy systems and usually possesses shallow and thin roots, while upland rice is cultivated in dry fields, and usually has deeper and thicker roots (Courtois et al., 2013).

Of the 1,690 genes that Huang et al. (2012) found with ≥ two-fold-increased change in expression exposed to 25 μM As(V) treatment for 1 and 3 h, only 149 (about 8.8%) were represented in our set of 802 As(V)-up-regulated genes, and there were 37 (about 2.2%) up-regulated genes were the same as our set of 1,042 As(V)-down-regulated genes (Supplementary Table 4b). Because of the difference of time treatment (1-3 h vs 12 h), genes with upregulation were likely to be down-regulated. Arsenic-induced genes associated were with transcriptional

regulation, hormone signaling, redox, glutathione metabolism and transporters, which is consistent with previous studies which reported genes involved in heat-shock proteins, metallothioneins, regulation, transporters in rice under As stress (Chakrabarty et al., 2009; Dubey et al., 2014; Huang et al., 2019). Gene microarray chip analysis revealed that the genes involved in glutathione synthesis, metabolism, and transport were responsive to As(V) stress in rice roots (Norton et al., 2008). In rice roots subjected to As(III) stress, RNA-seq analysis revealed that the expression of genes associated with transcription factors, protein kinases, stress responses, and metabolic process was affected (Liu et al., 2012).

4.1.1. Transcriptional regulation related genes

Transcriptional regulation is an essential molecular mechanism of plant stress response. Huang et al. (2012) observed that the number of upregulated genes related to transcription factor was more than twice that of downregulated genes under As(V) stress, and the 168 upregulated transcription factor genes mainly belong to AP2/ERF (19.6%) and WRKY (10.1%) and MYB (10.1%) families in rice. However, our data indicated that significant upregulation of 38 transcription factors genes predominantly belonged to NAC (26.3%), AP2-EREBP (18.4%) and MYB (15.8%) families in upland rice. In addition, we observed that all of the NAC transcription factors were up-regulated under As(V) stress, which indicated that NAC may play a positive regulation role in response to As(V). This was consistent with other observations that NAC transcription factor genes with upregulation were more than twice that of

downregulation under As(V) stress (Huang et al., 2012). Huang et al. (2016) found that *ONAC095* played opposite roles in cold and drought stresses. Therefore, NAC in plants showed a differential expression model under abiotic stresses (Nuruzzaman et al., 2015). The WRKY transcription factor family is one of the largest gene families in plants' response to various biotic and abiotic stresses (Pandey et al., 2009). It has been reported that the WRKY6 transcription factor has the ability to restrict As(V) uptake in *Arabidopsis* (Castrillo et al., 2013). Interestingly, lots of WRKY transcription factors, such as WRKY1, WRKY2, WRKY4, WRKY7 were observed except WRKY6. Therefore, the expression of WRKY6 and the other WRKY transcription factors in upland rice under As stress should be further studied. For bHLH transcription factors, it has been reported that bHLH is not expressed in any tissues of rice (Li et al., 2006). When exposed to As(III) and As(V) separately, 22 and 4 DEGs encoding bHLH transcription factors were responsive in rice shoots, respectively (Table 1). Chakrabarty et al. (2009) also observed that bHLH transcription factors were responsive under As stress in rice roots. This suggested that bHLH transcription factors could be regulated by As(III) and As(V) in rice roots and shoots.

4.1.2. Redox related genes

In this study, DEGs associated with antioxidant system, such as SOD, Prx, Grx, oxidoreductase, NADPH, TrxR and cytochrome P450, were responsive under As(III) and As(V) stress. With exposure to As(III), most genes encoding cytochrome P450 were up-regulated. Different from previous reports that cytochrome P450 responds

specifically to As(V) in rice roots (Chakrabarty et al., 2009), cytochrome P450 family were expressed under both As(III) and As(V) stress. In addition, most of DEGs encoding SOD, Prx, Grx and TrxR under As(III) and As(V) stress were down-regulated. However, Huang et al. (2019) observed that the DEGs associated with redox-related enzymes were mostly up-regulated in rice roots under As stress. This may be due to the different application dose of As. In addition, glutathione functions as cofactors of antioxidant enzyme, and can directly quench ROS, thus glutathione plays a very important role under abiotic stress (Fujita., 2012; López-Climent et al., 2014). In the present study, DEGs encoding glutathione-S-transferase (GST) were mostly up-regulated under both As(III) and As(V) stress in rice shoots (Table 3). These suggest that the up-regulation of cytochrome P450 and GSTs in rice shoots may play a crucial role in protecting plants from ROS damage.

4.1.3. Hormone signaling related genes

Phytohormones have proven to be involved in reconfiguration of developmental patterns under various stresses (Zwack et al., 2015). Srivastava et al. (2013) observed ABA and JA contents were significantly increased under As stress, indicating that ABA and JA may be involved in the induction of protective mechanisms against As toxicity (Bücker-Neto et al., 2017). Jasmonates played an important role in the perception and response mechanisms to As stress (Srivastava et al., 2009). Sko´rzyn´ska-Polit et al. (2006) reported that jasmonic acid (JA) and its biosynthesis-related genes were responsive under metal stress. In the present work,

the JA related gene OPR was mostly upregulated under As stress, which was consistent with the previous work of Paulose et al. (2010) in *Crambe abyssinica*, suggesting the significance of OPRs in protection against As toxicity. In addition, cytokinin (CTK) is also an important hormone component in plants adaption to As stress (Mohan et al., 2016). Under As(III) stress, Yu et al. (2012) observed that JA related genes *LOX*, *JAZ*, *AOS* and *AOC*, ABA related genes *NCED* and GA related genes *GA2ox3* were all responsive. Huang et al. (2012) observed that JA, ABA, GA and CTK-related genes in rice were regulated by As(V). In *Arabidopsis*, there have been similar results reported by Fu et al. (2014) where ABA, CTK and ethylene related genes were regulated by As(III). In the present study, we found that the genes associated with AUX, GA, ABA, CTK, ethylene and JA signaling were responsive under As(III) and As(V) stress (Table 2). In addition, the genes related to hormone signaling under As(III) stress were much more than that of As(V) stress. These data suggested that As stress may change the development processes of upland rice seedlings via multiple hormonal pathways.

4.1.4. Transporter and detoxification process related genes

With exposure to external stress, the activities of transporters related with detoxification processes are altered (Pontigo et al., 2015). In the present study, DEGs associated with ATP-binding cassette (ABC), metallothionein, MATE efflux family protein and citrate transporter were responsive under As(III) and As(V) stresses in upland rice seedlings. In total, the up-regulated genes associated with detoxification

process were much more than the down-regulated genes (Table 4), especially ABC-family transporter and MATE efflux family protein, indicating an involvement of ABC-family and MATE transporters in the induction of protective mechanisms against As toxicity. Huang et al. (2012) also observed ABC-family transporters induced by As. It has been reported that *AtABCC1* and *AtABCC2* exhibited As tolerance in *Arabidopsis* (Song et al., 2010) and *OsABCC1* reduces As accumulation in rice grains (Song et al., 2014), because ABC transporters can transport As-phytochelatin complexes from the cytosol into vacuoles. Some transporters involved in As uptake and transport have been verified, such as phosphate uptake system OsPT1, OsPT4, OsPT8 (Kamiya et al., 2013; Wang et al., 2016; Cao et al., 2017), NIP transporters OsNIP1;1, OsNIP2;1 (Lsi1), OsNIP3;1, OsNIP3;2 and OsNIP3;3, (Ma et al., 2008; Sun et al., 2018), and silicon transporter OsLsi2 (Ma et al., 2008). Interestingly, NIP transporters were not identified, but tonoplast intrinsic protein (TIPs) TIP4;2, TIP3;1, and plasma membrane intrinsic proteins (PIPs) PIP1;3, PIP2;4 were induced in upland rice exposed to As(III) and As(V) (Table 4). The main reason may be the difference of upland and lowland rice varieties. Mosa et al. (2012) observed that *OsPIP2;4*, *OsPIP2;6* and *OsPIP2;7* were involved in As(III) permeability in plants. However, there has been a paucity of research on TIPs involved in As permeability. In the present work, in addition to known transporters, we observed that OsPHO1;2 and OsPT19 were specifically induced under As(III) and As(V) stress. Previous studies have reported that OsPT19 was induced in rice roots exposed to As(III) (Yu et al., 2012). For metallothionein, in the study, two genes

(*Os11g0704500* and *Os12g0570700*), encoding OsMT1a, were up-regulated specifically to As(V) exposure, while one gene (*Os01g0149800*), encoding OsMT2a, was down-regulated specifically to As(III), indicating that upland rice with exposure to As(III) and As(V) had a different transporter response mechanism.

4.2. Arsenic may inhibit antioxidant activities from both transcriptional and translational processes

It has been reported that As exposed plants have generated measurable lipid peroxidation and reactive oxygen species (Ahmad et al., 2012). MDA contents are used to measure the level of lipid peroxidation, which is indicative of oxidative stress. Gupta et al. (2014) observed that MDA contents in rice were increased under As stresses. In the present study, As stress also significantly increased the MDA contents (Fig. 3), but reduced the SOD, POD, CAT and GR activities in upland rice (Fig. 3), which suggested that As treatment may induce severe oxidative stress in upland rice seedlings. Consistent with the physiological responses, the antioxidant enzyme related genes, such as *SOD*, *Prx*, *Grx* and *TrxR* were detected to be down-regulated after exposure to As(III) and As(V) (Table 2). Previous studies reported that transcription factors such as NAC may also affect the accumulation of ROS. Xu et al. (2015) reported that wheat NAC transcription factor TaNAC29 modulated the antioxidant enzyme activities under salt stress. In the present study, a number of NAC transcription factors were responsive under As stress (Table 1), which suggested that As stress may decrease the expression levels of genes related to antioxidant enzymes

by NAC transcription factors.

Moreover, DAVID enrichment analysis revealed the process related to ribonucleoprotein was repressed under both As(III) stress and As(V) stress (see Supplementary materials Fig.1c, d), indicating that As may also decrease the antioxidant activities by inhibiting the translation process. Similarly, Rai et al. (2011) reported that As stress decreased the ascorbate peroxidase (APX) activity by reducing its protein level and transcription level in rice cultivar IET-4786. Together, these findings suggested that As may decrease the antioxidant activities from both transcriptional and translational processes in upland rice seedlings.

5. Conclusion

This study reports the transcriptome analysis of upland rice under arsenic stress. In upland rice shoots exposed to As(III) or As(V) treatments, most DEGs performed gene downregulation. Furthermore, the DEGs of upland rice shoots response to As(III) stress were much greater than those of As(V) stress. A novel set of DEGs associated with transcription factors, hormone signaling, redox processes and detoxification processes were responsive under As(III) and As(V) stress.

Under As(III) and As(V) stress, MDA contents were increased while antioxidant enzyme activities were reduced, and most of the genes related to SOD, Prx, Grx and TrxR were down-regulated, which indicated that arsenic stress may reduce the activities of antioxidant enzymes by repressing these genes expressions. These results provide new insights into the molecular mechanisms at play within upland rice in

response to As exposure, and may therefore aid the safer cultivation of rice within As rich environments.

Acknowledgements

This research was financially supported by the National Natural Science Foundation of China (No. 41471274) and the Scottish Government's Rural and Environment Science and Analytical Service Division (RESAS).

References

Ahmad, M.A., Gaur, R., Gupta, M., 2012. Comparative biochemical and RAPD analysis in two varieties of rice (*Oryza sativa*) under arsenic stress by using various biomarkers. Journal of Hazardous Materials 217, 141-148.

Bücker-Neto, L., Paiva, A.L.S., Machado, R.D., Arenhart, R.A., Margis-Pinheiro, M., 2017. Interactions between plant hormones and heavy metals responses. Genetics and Molecular Biology 40, 373-386.

Cao, Y., Sun, D., Ai, H., Mei, H., Liu, X., Sun, S., Xu, G., Liu, Y., Chen, Y., Ma, L.Q., 2017. Knocking out OsPT4 gene decreases arsenate uptake by rice plants and inorganic arsenic accumulation in rice grains. Environmental Science & Technology 51, 12131-12138.

Carey, A. M., Lombi, E., Donner, E., Jonge, M., Punshon, T., Jackson, B., Guerinot, M., Price, A., Meharg, A., 2012. A review of recent developments in the speciation and location of arsenic and selenium in rice grain. Analytical and Bioanalytical Chemistry 402, 3275-3286.

Castrillo, G., Sanchez-Bermejo, E., De Lorenzo, L., Crevillen, P., Fraile-Escanciano, A., Tc, M.,

Mouriz, A., Catarecha, P., Sobrino-Plata, J., Olsson, S., Leo Del Puerto, Y., Mateos, I., Rojo, E., Hernandez, L.E., Jarillo, J.A., Pineiro, M., Paz-Ares, J., 2013. WRKY6 transcription factor restricts arsenate uptake and transposon activation in *arabidopsis*. The Plant Cell 25, 2944-2957.

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., Hua, C. Y., Jia, M.R., Fu, J.W., Liu, X., Han, Y.H., Liu, Y., Rathinasabapathi, B., Cao, Y., Ma, L.Q., 2017. Heterologous expression of Pteris vittata arsenite antiporter *PvACR3;1* reduces arsenic accumulation in plant shoots. Environmental Science & Technology 51, 10387-10395.

Courtois, B., Audebert, A., Dardou, A., Roques, S., Herrera, T., Droc, G., Frouin, J., Rouan, L., Goze, E., Kilian, A., 2013. Genome-wide association mapping of root traits in a japonica rice panel PLoS One 8, 78037.

Dave, R., Singh, P., Tripathi, P., Shri, M., Dixit, G., Dwivedi, S., Chakrabarty, D., Trivedi, P., Sharma, Y., Dhankher, O., Corpas, F., Barroso, J., Tripathi, R., 2013a. Arsenite Tolerance is Related to Proportional Thiolic Metabolite Synthesis in Rice (*Oryza sativa* L.). Archives of Environmental Contamination and Toxicology 64, 235-242.

Dave, R., Tripathi, R. D., Dwivedi, S., Tripathi, P., Dixit, G., Sharma, Y. K., Prabodh, K.T., Corpas, F.J., Barroso, J.B., Chakrabarty, D., 2013b. Arsenate and arsenite exposure modulate

antioxidants and amino acids in contrasting arsenic accumulating rice (*Oryza sativa* L.) genotypes. Journal of Hazardous Materials 262, 1123-1131.

Ding, Y., Wang, Y., Zheng, X., Cheng, W., Shi, R., Feng, R., 2017. Effects of foliar dressing of selenite and silicate alone or combined with different soil ameliorants on the accumulation of As and Cd and antioxidant system in Brassica campestris. Ecotoxicology and Environmental Safety 142, 207-215.

Duan, G. L., Hu, Y., Schneider, S., McDermott, J., Chen, J., Sauer, N., Rosen, B.P., Daus, B., Liu, Z., Zhu, Y.G., 2016. Inositol transporters AtINT2 and AtINT4 regulate arsenic accumulation in *Arabidopsis* seeds. Nature Plants 2, 15202.

Dubey, S., Shri, M., Misra, P., Lakhwani, D., Bag, S., Asif, M., Trivedi, P., Tripathi, R., Chakrabarty, D., 2014. Heavy metals induce oxidative stress and genome-wide modulation in transcriptome of rice root. Functional & Integrative Genomics 14, 401-417.

Fu, S.F., Chen, P.Y., Nguyen, Q.T.T., Huang, L.Y., Zeng, G.R., Huang, T.L., Lin, C.Y., Huang, H.J., 2014. Transcriptome profiling of genes and pathways associated with arsenic toxicity and tolerance in *Arabidopsis*. BMC Plant Biology 14, 94.

Fujita, M., 2012. Molecular Mechanism of Heavy Metal Toxicity and Tolerance in Plants: Central Role of Glutathione in Detoxification of Reactive Oxygen Species and Methylglyoxal and in Heavy Metal Chelation. Journal of Botany 2012, 31-37.

Gupta, M., Ahmad, M.A., 2014. Arsenate induced differential response in rice genotypes. Ecotoxicology and Environmental Safety 107, 46-54.

Hu, P., Ouyang, Y., Wu, L., Shen, L., Luo, Y., Christie, P., 2015. Effects of water management on arsenic and cadmium speciation and accumulation in an upland rice cultivar. Journal of Environmental Sciences 27, 225-231.

Huang, L., Hong, Y., Zhang, H., Li, D., Song, F., 2016. Rice NAC transcription factor ONAC095 plays opposite roles in drought and cold stress tolerance. BMC Plant Biology 16, 203.

Huang, T.L., Nguyen, Q., Fu, S.F., Lin, C.Y., Chen, Y.C., Huang, H.J., 2012. Transcriptomic changes and signaling pathways induced by arsenic stress in rice roots. Plant Molecular Biology 80, 587-608.

Huang, Y.M., Chen, H.Q., Reinfelder, J.R., Liang, X.Y., Sun, C.J., Liu, C.P., Li, F.B., Yi, J.C., 2019. A transcriptomic (RNA-seq) analysis of genes responsive to both cadmium and arsenic stress in rice root. Science of the Total Environment 666, 445-460.

Indriolo, E., Na, G., Ellis, D., Salt, D.E., Banks, J.A., 2010. A vacuolar arsenite transporter necessary for arsenic tolerance in the arsenic hyperaccumulating fern Pteris vittata is missing in flowering plants. Plant Cell 22, 2045-2057.

Kamiya, T., Islam, R., Duan, G., Uraguchi, S., Fujiwara, T., 2013. Phosphate deficiency signaling pathway is a target of arsenate and phosphate transporter OsPT1 is involved in As accumulation in shoots of rice. Soil Science & Plant Nutrition 59, 580-590.

Kim, E.J., Yoo, J.C., Baek, K., 2014. Arsenic speciation and bioaccessibility in arsenic-contaminated soils: sequential extraction and mineralogical investigation. Environmental Pollution 186, 29.

Li, X., Duan, X., Jiang, H., Sun, Y., Tang, Y., Yuan, Z., Guo, J., Liang, W., Chen, L., Yin, J., Ma, J., Zhang, D., 2006. Genome wide analysis of basic/helix-loop-helix transcription factor family in rice and Arabidopsis. Plant Physiology 141, 1167-1184.

Li, Y., Dhankher, O.P., Carreira, L., Lee, D., Chen, A., Schroeder, J.I., Balish, R.S., Meagher, R.B., 2004. Overexpression of phytochelatin synthase in Arabidopsis leads to enhanced arsenic tolerance and cadmium hypersensitivity. Plant Cell Physiology 45, 1787-1797.

Liu, Q.P., Zhang, H.M., 2012. Molecular Identification and Analysis of Arsenite Stress-Responsive miRNAs in Rice. Journal of Agricultural and Food Chemistry 60, 6524-6536.

López-Climent, M.F., Arbona, V., Pérez-Clemente, R.M., Zandalinas, S.I., Gómez-Cadenas, A., 2014. Effect of cadmium and calcium treatments on phytochelatin and glutathione levels in citrus plants. Plant Biology 16, 79-87

Ma, J.F., Yamaji, N., Mitani, N., Xu, X.Y., Su, Y.H., McGrath, S.P., Zhao, F.J., 2008. Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. Proceedings of the National Academy of Sciences of the United States of America 105, 9931-9935.

Meharg, A.A., Hartley, W.J., 2002. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. New Phytologist 154, 29-43.

Mishra, S., Srivastava, S., Tripathi, R.D., Trivedi, P.K., 2008. Thiol metabolism and antioxidant systems complement each other during arsenate detoxification in *Ceratophyllum demersum* L. Aquatic Toxicology 86, 205-215.

Mohan, T.C., Castrillo, G., Navarro, C., Zarco-Fernández, S., Ramireddy, E., Mateo, C., Zamarreño, A.M., Paz-Ares, J., Muñoz, R., García-Mina, J.M., Hernández, L.E., Schmülling, T., Leyva, A., 2016. Cytokinin Determines Thiol-Mediated Arsenic Tolerance and Accumulation. Plant Physiology 171, 1418-1426.

Mosa, K., Kumar, K., Chhikara, S., Mcdermott, J., Liu, Z., Musante, C., White, J., Dhankher, O., 2012. Members of rice plasma membrane intrinsic proteins subfamily are involved in arsenite permeability and tolerance in plants. Transgenic Research 21, 1265-1277.

Norton, G.J., Lou-Hing, D.E., Meharg, A.A., Price, A.H., 2008. Rice–arsenate interactions in hydroponics: whole genome transcriptional analysis. Journal of Experimental Botany 59, 2267-2276.

Nuruzzaman, M., Sharoni, A.M., Satoh, K., Karim, M.R., Harikrishna, J.A., Shimizu, T., Sasaya, T., Omura, T., Haque, M.A., Hasan, S.M.Z., Ahmad, A., Kikuchi, S., 2015. NAC transcription factor family genes are differentially expressed in rice during infections with Rice dwarf virus, Rice black-streaked dwarf virus, Rice grassy stunt virus, Rice ragged stunt virus, and Rice transitory yellowing virus. Frontiers in Plant Science 6, 676.

Pandey, S.P., Somssich, I.E., 2009. The role of WRKY transcription factors in plant immunity. Plant Physiology 150, 1648-1655.

Paulose, B., Kandasamy, S., Dhankher, O., 2010. Expression profiling of *Crambe abyssinica* under arsenate stress identifies genes and gene networks involved in arsenic metabolism and detoxification. BMC plant biology 10, 108.

Pontigo, S., Ribera, A., Gianfreda, L., Mora M., Nikolic, M., Cartes, P., 2015. Silicon in vascular plants: uptake, transport and its influence on mineral stress under acidic conditions. Planta 242, 23-37.

Rai, A., Tripathi, P., Dwivedi, S., Dubey, S., Shri, M., Kumar, S., Tripathi, P.K., Dave, R., Kumar, A., Singh, R., Adhikari, B., Bag, M., Tripathi, R.D., Trivedi, P.K., Chakrabarty, D., Tuli, R., 2011. Arsenic tolerances in rice (*Oryza sativa*) have a predominant role in transcriptional regulation of a set of genes including sulphur assimilation pathway and antioxidant system. Chemosphere 82, 986-995.

Requejo, R., Tena, M., 2005. Proteome analysis of maize roots reveals that oxidative stress is a main contributing factor arsenic to plant toxicity. Phytochemistry 66, 1519-1528.

Robinson, D.G., Wang, J.Y., Storey, J.D., 2015. A nested parallel experiment demonstrates differences in intensity-dependence between RNA-Seq and microarrays. Nucleic Acids Research 43, 131.

Shi, S., Wang, T., Chen, Z., Tang, Z., Wu, Z., Salt, D.E., Chao, D.Y., Zhao, F.J., 2016. OsHAC1;1 and OsHAC1;2 function as arsenate reductases and regulate arsenic accumulation. Plant Physiology 172, 1708-1719.

Sko´rzyn´ska-Polit, E., Pawlikowska-Pawlega, B., Szczuka, E., Drazkie-wicz, M., Krupa, Z., 2006. The Activity and localization of lipoxygenases in Arabidopsis thaliana under cadmium and copper stresses. Plant Growth Regulation 48, 29-39

Song, W.Y., Park, J.Y., Mendoza-Cózatl, D., Suter-Grotemeyer, M., Shim, D., Hörtensteiner, S.,

Geisler, M., Weder, B., Rea, P., Rentsch, D., Schroeder, J., Lee, Y., Martinoia, E., 2010. Arsenic tolerance in Arabidopsis is mediated by two ABCC-type phytochelatin transporters. Proceedings of the National Academy of Sciences of the United States of America 107, 21187-21192.

Song, W.Y., Yamaji, N., Jung, K.H., Ko, D., Fujii-Kashino, M., Lee, Y., An, G., Yamaki, T., Martinoia, E., Ma, J.F., 2014. A rice ABC transporter, OsABCC1, reduces arsenic accumulation in the grain. Proceedings of the National Academy of Sciences of the United States of America 111, 15699-15704.

Srivastava, S., Mishra, S., Tripathi, R.D., Dwivedi, S., Trivedi, P.K., Tandon, P.K., 2007. Phytochelatins and antioxidant systems respond differentially during arsenite and arsenate stress in *Hydrilla verticillata* (L.f.) Royle. Environmental Science & Technology 41, 2930-2936.

Srivastava, S., Srivastava, A.K., Suprasanna, P., D'Souza, S.F., 2009. Comparative biochemical and transcriptional profiling of two contrasting varieties of *Brassica juncea* L. in response to arsenic exposure reveals mechanisms of stress perception and tolerance. Journal of Experimental Botany 60, 3419-3431.

Srivastava, S., Srivastava, A.K., Suprasanna, P., D'Souza, S.F., 2013. Identification and profiling of arsenic stress-induced microRNAs in *Brassica juncea*. Journal of experimental botany 64, 303-315.

Sun, G.X., Williams, P.N., Carey, A.M., Zhu, Y.G., Deacon, C., Raab, A., Feldmann, J., Islam,

M.R., Meharg, A.A., 2008. Inorganic Arsenic in Rice Bran and Its Products Are an Order of Magnitude Higher than in Bulk Grain. Environmental Science & Technology 42, 7542-7546.

Sun, S.K., Chen, Y., Che, J., Konishi, N., Tang, Z., Miller, A.J., Ma, J.F., Zhao, F.J., 2018. Decreasing arsenic accumulation in rice by overexpressing OsNIP1;1 and OsNIP3;3 through disrupting arsenite radial transport in roots. New Phytologist 219, 641-653.

Tan, Y.R., Xu, X.Y., Ding, Y.Z., Zheng, X.Q., Dai, L.H., Feng, R.W., Shi, R.G., Zhou, L., Chen, P.Z., Yang, B., 2016. Genotypic variation of arsenic and cadmium uptake by upland rice. Journal of Agro-Environment Science 35, 1436-1443.

Tripathi, P., Tripathi, R.D., Singh, R.P., Dwivedi, S., Goutam, D., Shri, M., Trivedi, P.K., Chakrabarty, D., 2013. Silicon mediates arsenic tolerance in rice (*Oryza sativa* L.) through lowering of arsenic uptake and improved antioxidant defense system. Ecological Engineering 52, 96-103.

Wang, G.L., Ren, X.Q., Liu, J.X., Yang, F., Wang, Y.P., Xiong, A.S., 2019. Transcript profiling reveals an important role of cell wall remodeling and hormone signaling under salt stress in garlic. Plant Physiology and Biochemistry 135, 87-98.

Wang, P.T., Zhang, W.W., Mao, C.Z., Xu, G.H., Zhao, F.J., 2016. The role of OsPT8 in arsenate uptake and varietal difference in arsenate tolerance in rice. Journal of Experimental Botany 67, 6051-6059.

Xu, J.M., Shi, S.L., Wang, L., Tang, Z., Lv, T., Zhu, X.L., Ding, X.M., Wang, Y.F., Zhao, F.J., Wu, Z.C., 2017. OsHAC4 is critical for arsenate tolerance and regulates arsenic accumulation in

rice. New Phytologist 215, 1090-1101.

Xu, X.Y., Mcgrath, S.P., Meharg, A.A., Zhao, F.J., 2008. Growing rice aerobically markedly decreases arsenic accumulation. Environmental Science and Technology 42, 5574-5579.

Xu, Z., Gongbuzhaxi, Wang, C., Xue, F., Zhang, H., Ji, W., 2015. Wheat NAC transcription factor TaNAC29 is involved in response to salt stress. Plant Physiology & Biochemistry 96, 356-363.

Yu, L.J., Luo, Y.F., Liao, B., Xie, L.J., Chen, L., Xiao, S., Li, J.T., Hu, S.N., Shu, W.S., 2012. Comparative transcriptome analysis of transporters, phytohormone and lipid metabolism pathways in response to arsenic stress in rice (*Oryza sativa*). New Phytologist 195, 97-112.

Zheng, M.Z., Li, G., Sun, G.X., Shim, H., Cai, C., 2013. Differential toxicity and accumulation of inorganic and methylated arsenic in rice. Plant and Soil 365, 227-238.

Zwack, P.J., Rashotte, A.M., 2015. Interactions between cytokinin signaling and abiotic stress responses. Journal of Experimental Botany 66, 4863-4871.