RESEARCH

Gene regulation and ionome homeostasis in rice plants in response to arsenite stress: potential connection between transcriptomics and ionomics

**Guobing Lin · Li Ma · Xiaoman He · Jie Tang ·
Lin Wang**

Abstract Ionomics and transcriptomics were applied to demonstrate response of rice to arsenite [As(III)] stress in the current study. Rice plants were cultured in nutrient solutions treated with 0, 100 and 500 µg/L As(III) coded as CK, As1 and As5, respectively. The rice ionomes exhibited discriminatory response to environmental disturbances. Solid evidence of the effects of As(III) stress on binding, transport or metabolism of P, K, Ca, Zn and Cu was obtained in this work. Differentially expressed genes (DEGs) in the shoots were identified in three datasets: As1 vs CK, As5 vs CK and As5 vs As1. DEGs identified simultaneously in two or three datasets were selected for subsequent interaction and enrichment analyses. Upregulation of genes involved in protein kinase activity, phosphorus metabolic process and phosphorylation were detected in the rice treated with As(III), resulting in the maintenance of P homeostasis in the shoots. Zn and Ca binding genes were up-regulated since excess As inhibited the translocation of Zn and Ca from roots to shoots. Increased expression of responsive genes including *HMA*, *WRKY*, *NAC* and *PUB* genes conferred As tolerance in the rice plants to cope with external As(III) stress. The results suggested that As(III) stress could disturb the uptake and translocation of macro and essential elements by rice. Plants could regulate the expression of corresponding genes to maintain mineral nutrient homeostasis for essential metabolic processes.

G. Lin · X. He · L. Wang (✉)
College of Chemistry and Chemical Engineering, Central South University, Changsha 410083, China
e-mail: linwang@csu.edu.cn

L. Ma
College of Forestry, Henan Agriculture University, Zhengzhou 450002, China

J. Tang (✉)
Department of Clinical Pharmacology, Xiangya Hospital, Central South University, Changsha 410008, China
e-mail: jietang@csu.edu.cn

Introduction

Rice and its byproducts are the staple food for half of the world population (Stone 2008). Compared to other terrestrial crops, paddy rice has a tendency to accumulate more arsenic (As) from flooded paddy fields (Hussain et al. 2021). Rice consumption was considered as one of the important pathways for As exposure to human beings (Cubadda et al. 2017). Biotoxicity and mobility of As are mainly dependent on its chemical species. Inorganic As forms including arsenite [As(III)] and arsenate [As(V)] are the most

toxic species, of which As(III) is much more toxic and mobile than As(V). In contrast, organic As forms such as monomethylarsenic acid (MMA), dimethylarsenic acid (DMA), arsenobetaine (AsB) and arsenocholine (AsC) are less or non-toxic species. In rice-soil system, inorganic As is the dominant species while As(III) is predominant under anaerobic condition and As(V) is predominant under aerobic condition (Hussain et al. 2021).

Uptake of As(III) by rice root was confirmed through the aquaporin OsNIP2;1 which is the same transport pathway as silicate (Ma et al. 2008). Application of Si was able to significantly alleviate As(III) toxicity in rice plants through reducing As influx in root and inhibiting As transfer from root to shoot (Chen et al. 2021; Wu et al. 2016). Effects on the accumulation of other elements such as P, Zn, Cu, Mn and Fe were also observed in the rice plants supplied with Si (Banerjee et al. 2021). Due to the similar structure to phosphate, bioaccumulation and translocation of As(V) in rice plants were found to be mediated by phosphate transporters (Cao et al. 2017). However, As stress could not only restrict P translocation, the concentrations of Ca, Mg, Mn, Zn, Fe and Cu in rice plants were also altered (Shaibur et al. 2016). Meanwhile, treatment with additional P would simultaneously alter the contents of As and some other elements in plants (Chutia et al. 2019). Due to the fact that plant is an organic integrity, elements in plant tissues are not independent to each other. Plants regulate their element balance to adapt external stress. Accumulation of one element such as As would inevitably disturb the homeostasis of elements and subsequently lead to variation of other elements in the plant (Lin et al. 2023). Therefore, it is necessary to take into consideration all the elements as a whole rather than focusing solely on individual element.

Ionome is defined as the element composition of plant tissue including all inorganic elements (Baxter and Dilkes 2012). Compared to single element, ionome of rice plant was proved to be more sensitive to external As stress (Du et al. 2020). Distinct separation was observed between ionomic profiles of different rice tissues treated with different As species, indicating that plant ionomics has good application prospects in detecting environmental disturbances (Baxter et al. 2008; Du et al. 2020). Accumulation and translocation of elements in plants are mediated

by various transporters. One ion can be transferred by a series of transporters and one transporter can mediate translocation of one or more ions (Cao et al. 2017). Transporter expressions are regulated by correlated genes which response to external stress directly. That means ionomic network of plant is essentially controlled by the genetic network (Kumari et al. 2018). Moreover, the response of genes to environmental stresses can be characterized by transcriptome through RNA sequencing (Di et al. 2021). Therefore, revealing the relationship between ionomics and transcriptomics in rice plant will obtain a deeper understanding of the molecular mechanism of rice plant coping with As stress (Xiao et al. 2021).

In previous studies, transcriptomic analyses were performed between control samples and treated samples under different external stresses, for instance, cold stress, salt stress and arsenic stress (Di et al. 2021; Guan et al. 2019; Li et al. 2018). Thousands of differentially expressed genes (DEGs) between two groups were identified followed by bioinformatic analyses such as Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment. Biological functions and metabolic pathways enriched in the bioinformatic analyses were considered as the genetic responses to stress or stimulus (Li et al. 2018; Zhao et al. 2020). It should be noted that the number of correlated genes involved in stress response was still too large to recognize representative biomarkers. In order to establish the connection between ionomics and transcriptomics, the reservoir for stress response genes should be simplified to match ionomic variation more precisely. In the current study, rice plants were categorized into three groups treated with different As(III) concentrations. DEGs and elements with significant variation obtained from transcriptomic and ionomic analyses, respectively, were devoted to further comparative analysis.

Materials and methods

Plant growth

The *japonica* cultivar Nipponbare (*Oryza sativa* L.) was employed for hydroponic cultivation. The mature rice seeds were obtained from Assistant Professor Zhoufei Luo at the College of Agriculture, Hunan

Agricultural University, China. After dehusked, sterilized in 1% active NaClO solution and washed by ultrapure water, the seeds were germinated on moist filter paper at 28 °C for 7 days. The germinated seedlings with similar shoot lengths were selected and transferred into 500-mL cultivating boxes filled with Yoshida nutrient solution (Du et al. 2019). Exposure experiment was categorized into three groups: Group CK—nutrient solution without any addition; Group As1—nutrient solution amended with 100 μg/L As(III) and Group As5—nutrient solution amended with 500 μg/L As(III). The rice seedlings in three groups were cultivated in a growth chamber at 28:20 °C (day:night) with a 16-h light photoperiod. The nutrient solutions were renewed every 3 days during the growth period. After 30-days growth, the rice plants were harvested and rapidly cleaned with ultrapure water for further analysis.

Transcriptomic analysis

Once harvested, three rice plants were randomly selected from each treatment group and immersed immediately into liquid nitrogen for 30 min. The samples were then stored at -80 °C until transcriptomic analysis. RNA extraction and sequencing assay was conducted by Majorbio Biopharm Technology Co., Ltd. (Shanghai, China). The detailed procedures are provided in Text 1 in Supplementary Materials. In brief, total RNA was extracted from pre-homogenized shoot tissue by TRIzol® Reagent (Plant RNA Purification Reagent for plant tissue). The RNA sample with high quality examined by NanoDrop ND-2000 was qualified for following RNA sequencing. RNA-seq sequencing library was prepared and then sequenced on an Illumina HiSeq Xten/NovaSeq 6000 platform. The raw reads were trimmed to obtain clean reads followed by alignment and assembling. DEGs between two groups were identified according to adjusted p value ≤ 0.05 and |log$_2$(fold change)|> 1 using DESeq2 (Love et al. 2014). To verify the transcriptomic results, qRT-PCR was carried out using a LightCycler® 480 II Real-time PCR Instrument (Roche, Switzerland). The primer sequences used for qPR-PCR are summarized in Table S1. For each sample, qRT-PCR was performed in triplicate. Relative expression levels of mRNAs normalized to the internal control gene OsActin1 were determined using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). The

relative expression levels of the selected significant DEGs are shown in Fig. S1.

Ionomic analysis

The rest rice plants were pretreated for digestion and determination of total elements. The rice roots were firstly immersed into ice-cold desorption solution containing 1 mM K$_2$HPO$_4$, 0.5 mM Ca(NO$_3$)$_2$ and 5 mM MES at pH 6.0 for 10 min to removing surface binding As. The rice plants were washed by tap water thoroughly and rinsed with ultrapure water several times. The blotted dry samples were separated into shoots and roots. All the tissue samples were freezing-dried. In order to obtain sufficient amount, 4–6 shoot or root samples were combined as one composite sample and then homogenized in an agate mortar. At least three composite samples were obtained for each tissue in each treatment group. Approximately 0.2 g (for shoot) or 0.1 g (for root) homogenized sample (precise to 0.001 g) was weighted into a polytetrafluoroethylene (PTFE) digestion vessel containing 9 mL HNO$_3$ and 2 mL H$_2$O$_2$ as digestant. The sealed PTFE vessels were digested at 190 °C for 30 min according to the microwave assisted method described in our previous studies (Du et al. 2018). The digested solutions were cooled down to room temperature, filtered through 0.45 μm membranes and diluted up to 50 mL using ultrapure water. Total concentrations of elements including B, Mg, Al, P, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Mo, Cd, Sb, Ba and Pb were determined using ICP-MS (Agilent 7700x). The operating parameters for ICP-MS are listed in Table S2.

Data analysis

One-way analysis of variance (ANOVA) was used to evaluate significant differences in element concentrations of rice tissues from different treatment groups. Principal component analysis (PCA) was employed to characterize ionomic variations between two tissues in three groups. ANOVA and PCA were performed using R software (4.2.0).

Three datasets of DEGs were constructed as follow: D1 (As1/CK)—Group As1 in comparison with Group CK; D2 (As5/CK)—Group As5 in comparison with Group CK; and D3 (As5/As1)—Group As5 in comparison with As1. Venn diagram was generated

based on DEGs using ggplot2 and ggvenn packages in R (Wickham 2011). Biological functions of shared DEGs were identified through GO enrichment analysis. ClueGO app in Cytoscape was employed to perform GO analysis according to default parameters. Cluster min #Gene and %Gene was set to 4 and 1.000, respectively. Clusters with pV ≤ 0.01 were visualized in Cytoscape. Protein–protein interaction (PPI) network was established using STRING (https://string-db.org/) to evaluate the correlations between DEGs which were significantly up-regulated in all three datasets. Required score and FDR stringency were set to 0.150 and 5%, respectively.

Results and discussion

Element concentrations in the rice plants

Total concentrations of 22 elements in the rice tissues from different treatment groups are demonstrated in Fig. 1. Significant differences in the rice tissues between two groups were identified by ANOVA. The concentrations of As in the shoots and roots were significantly elevated along with exposed As concentration. However, other elements exhibited different distribution patterns from As. In consideration of rice tissues, the shoots accumulated high contents of B, Mg, P, K, Ca and Mn while more Al, Ti, V, Cr, Fe, Co, Ni, Cu, As, Sr, Mo, Cd, Sb, Ba and Pb were found in the roots. This is because plant roots play an important role on sequestering toxic elements and preventing them from translocated to arial parts (Du et al. 2018; Nocito et al. 2011). For Mg, P, K and Ca and B, no significant difference was observed in the shoots from different treatment groups. The contents of these elements except Ca in the roots had a tendency to decrease when exposure to As(III). Plants are readily to translocate essential minerals into growing tissues for biochemical functions and biophysical balance (Karley and White 2009). Moreover, translocation ability of macro elements such as P, Ca and Mg were found to be enhanced in the rice seedlings exposure to As (Shaibur et al. 2016). It is suggested that uptake of B, Mg, P and K in the rice roots was hindered by exogenous As in this work, leading to decreasing concentrations of B, Mg, P and K in the rice roots treated with As(III). The concentrations of Mn in the rice shoots and roots were simultaneously reduced when increasing exogenous As. Uptake and translocation of Mn as well as Fe and Cu were proved to be mediated by Zn transporters based on their identical ionic charge and similar chemical properties (Gindri et al. 2020). Exposure to As disturbed the homeostasis of nutrient elements such as Zn, Mn, Fe and Cu in rice plants (Khan and Gupta 2018). Translocation of Zn was hampered by As stress in this work, resulting in lower Zn contents in the rice shoots from Group As1 and As5.

Ionomic variations of the rice tissues

PCA was carried out based on the ionomic matrix without As to discriminate the tissue samples in different groups. As shown in Fig. 2a, the first principal component (PC1) could explain 70.4% of the total variation while the explanation of second principal component (PC2) was 9.1%. All the shoot samples were located in positive PC1 with high loadings of B, Mg, P, K, Ca and Mn. The root samples with high loadings of other elements were located in negative PC1, indicating that discrimination of plant tissues was capable by their ionomes (Du et al. 2020). Differentiation of the shoot ionomes from different groups was also exanimated by PCA. The shoots in Group As5 located in positive PC1 and negative PC2 were separated from the shoots in other groups as shown in Fig. 2b. Element loadings represented that the rice plants exposure to 500 µg/L As(III) preferred to accumulate more contents of B, Fe, Cu and Mo mineral nutrients. The shoots in Group CK inclined to accumulate more Zn could be separated from Group As1 to some extent. With regard to roots, distinct separation was observed among three groups as shown in Fig. 2c. The roots in Group CK were mainly located in positive PC1 with high loading of most nutrient elements. That means uptake of most nutrients in the rice roots were inhibited by As stress. The results of PCA exhibited good resolution on discriminating plant tissues under external As stress through ionome. Multi-elements have more sensitivity on genomic and environmental factors than single element in plants (Baxter 2015). Compared to environmental disturbance, genomic composition was proved to be the dominant factor on determination of plant ionomes (Du et al. 2020; Xiao et al. 2021). This can explain the samples were preferentially
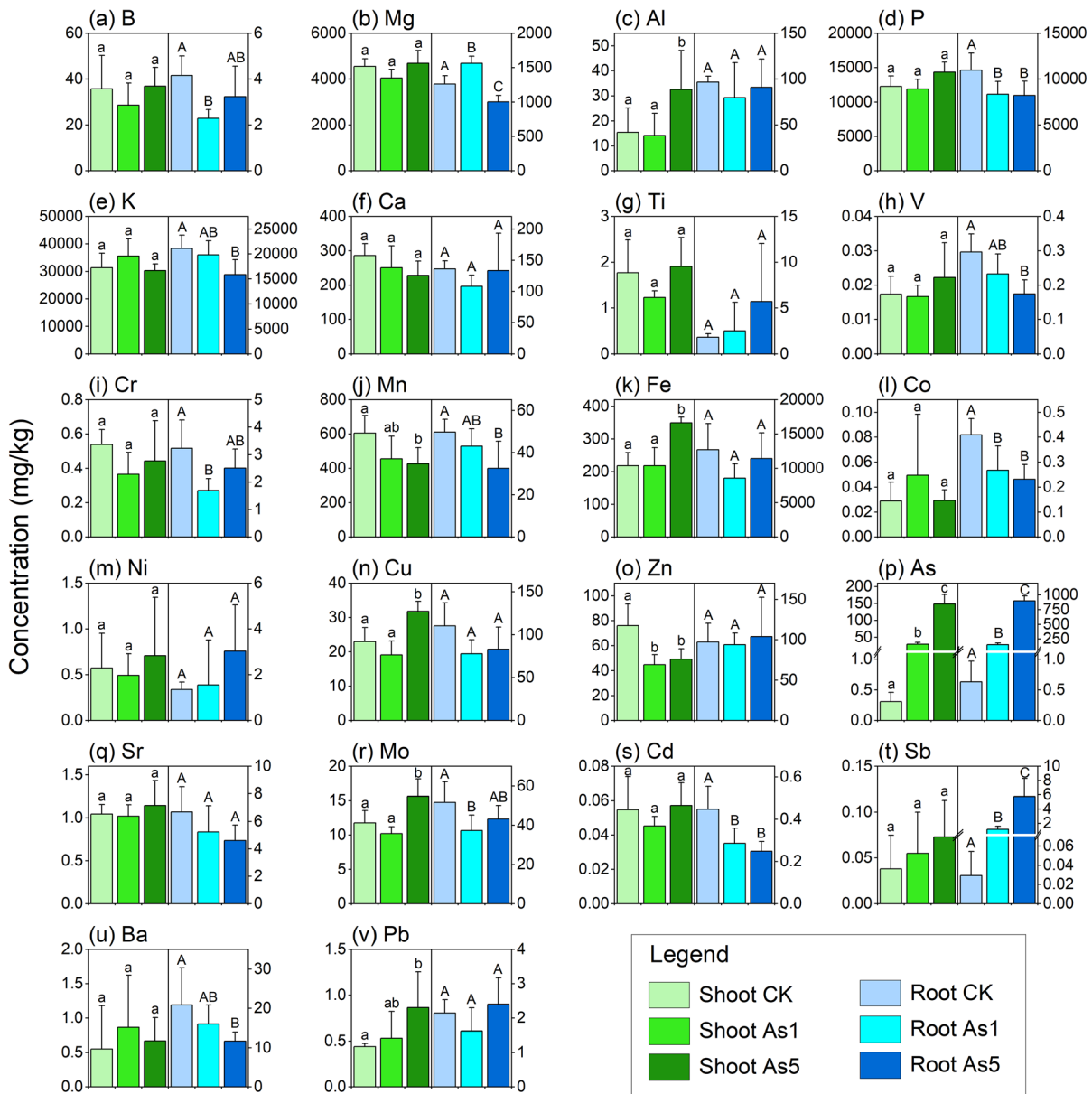
Fig. 1 Element concentrations in the rice shoots and roots from three groups **a** B, **b** Mg, **c** Al, **d** P, **e** K, **f** Ca, **g** Ti, **h** V, **i** Cr, **j** Mn, **k** Fe, **l** Co, **m** Ni, **n** Cu, **o** Zn, **p** As, **q** Sr, **r** Mo, **s** Cd, **t** Sb, **u** Ba, **v** Pb. The different letters denote values significantly different at $p < 0.05$ by ANOVA

categorized according to tissue type rather than exposed As(III) concentration in this work. Plants regulate genomic expressions in response to external stress, which simultaneously mediate ion transfer and translocation (Jeyasingh et al. 2017). Different plants or tissues presented different response characteristics, resulting in their specific ionomic networks. Due to more responsive genes to external stress, plant shoot was suggested to be more sensitive in transcriptomics than root (Li et al. 2018; Yu et al. 2012). Therefore, transcriptomic analyses were conducted on the rice shoots and DEGs were identified and classified to evaluate the relationships between responsive genes and ionome.

Fig. 2 Score plot and loading plot generated by PCA, **a** all samples, **b** shoot samples and **c** root samples from three treatment groups
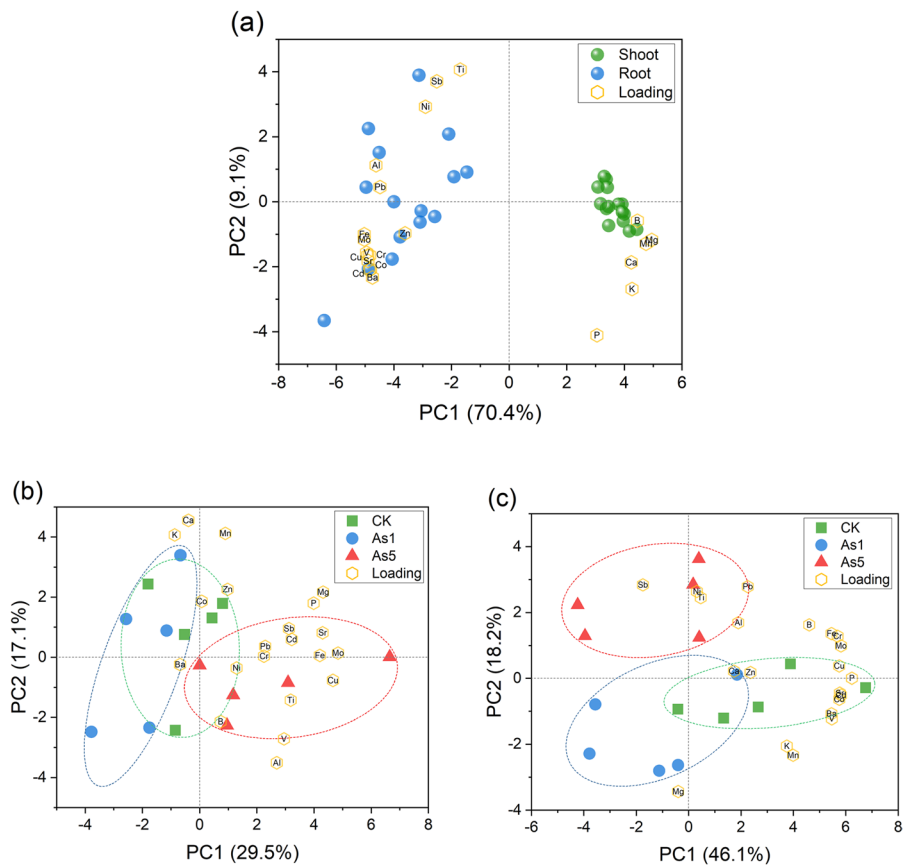

Global expression profiling

In total, 3154, 2584 and 2330 DEGs (adjusted p ≤ 0.05 and |log₂(fold change)| > 1) were identified from As1/CK, As5/CK and As5/As1 datasets, respectively. The volcano plots for expressed genes in three datasets are illustrated in Fig. S2. Distribution of up-regulated and down-regulated DEGs in three datasets is shown in Fig. 3a. Up-regulated genes were less than down-regulated genes from dataset As1/CK, while more up-regulated genes were detected than down-regulated genes in dataset As5/CK and

Fig. 3 Differentially expressed genes **a** detected in datasets As1/CK, As5/CK and As5/As1; **b** shared among three datasets
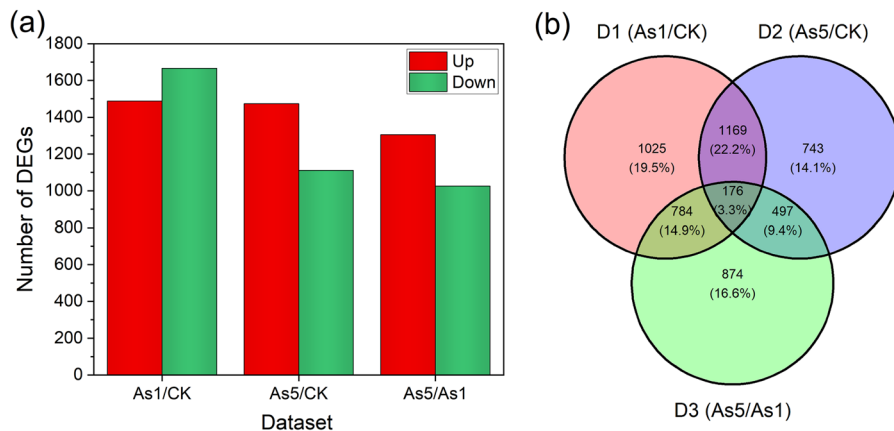
As5/As1. Venn diagram shown in Fig. 3b was generated to demonstrate the DEGs shared among three datasets. There were 176 genes (account for 3.3% of total DEGs) found to be significantly regulated in all three datasets. Except them, dataset As1/CK and As5/CK shared 1169 DEGs, accounting for 22.2% of total DEGs. The numbers of shared DEGs were 784 and 497 between dataset As1/CK and As5/As1, and dataset As5/CK and As5/As1, respectively. In order to establish the connection between transcriptomics and ionomics, DEGs identified in the current work were classified according to their expressions. The classification rules are listed in Table 1. The DEGs were selected and classified into three types: As-sensitive, Low-As-responsive and High-As-responsive type. As-sensitive gene is defined as gene expression increased or decreased with external As(III) increasing. Low-As-responsive gene was defined as up-regulated simultaneously in As1/CK and As5/CK but down-regulated or non-significant in As5/As1; or down-regulated simultaneously in As1/CK and As5/CK but up-regulated or non-significant in As5/As1. Similarly, high-As-responsive gene was defined as up-regulated or down-regulated in As5/CK and As5/As1 as listed in Table 1.

Functional categories of As-sensitive genes

Among all the DEGs, only 43 genes were up-regulated and 4 genes were down-regulated in three datasets. The detailed information on gene id, name and description of As-sensitive genes is listed in Table S3. According to the annotations, 4 down-regulated genes were irrelevant to ion binding or stress response. Therefore, 43 up-regulated genes were chosen for functional analysis. Due to the insufficient number of As-sensitive genes for GO enrichment analysis, protein–protein interaction (PPI) of up-regulated As-sensitive genes was analyzed by STRING with required score 0.15 and FDR stringency 5% to reveal the functions of these genes in response to As(III) stress. The PPI network was constructed by STRING and visualized by Cytoscape as shown in Fig. 4. The coding proteins (n = 38) could be separated into six classes according to their biological functions. Nine proteins were strongly correlated with ion binding and transport. Even though the required score was set to low confidence (0.15), there still were 12 proteins unconnected to other proteins. OS03T0152000-01, OsMYB30 and OsMyb4 were located as the key hub nodes of the PPI network.

OS03T0152000-01 with 10 co-expressed proteins is a heavy metal-associated domain containing protein (HMA). Most of *HMA* genes in rice have been proved to be up-regulated in response to heavy metal stress (Cong et al. 2019). Up-regulation of *OS03T0152000* as well as other 8 *HMA* genes was confirmed in the rice root and shoot under Cd exposure for 24 h (Oono et al. 2017). *OS03T0152000* up-regulated in this work made a contribution on translocation of As in the rice plants due to its sensitivity to external As(III) stress. Three proteins EL5, OsRFPH2-3 and OS06T0192800-01 co-expressed with OS03T0152000-01 are zinc-finger proteins (ZFPs) involved in metal binding with one or more Zn ions (Sun et al. 2019). Increased expression of ZFPs in plants conferred tolerance to biotic and abiotic stresses including heavy metals (Mukhopadhyay et al. 2004). Dramatically reduced Zn concentrations in the rice shoots exposed to As(III) (Fig. 1o) triggered the upregulation of *ZFP* genes *EL5*, *OsRFPH2-3* and *Os06g0192800* due to their crucial roles in plant growth and signal transduction (Sun et al. 2019).

OsMYB30 and OsMyb4 both with 11 co-expressed proteins were identified as myb proteins which were confirmed to be sensitive to cold stress.

Table 1 Classification of As responsive genes

Classification	Regulation sign	Regulation in datasets			Gene number
		As1/CK	As5/CK	As5/As1	
As-sensitive	Up	Up	Up	Up	43
	Down	Down	Down	Down	4
Low-As-responsive	Up	Up	Up	No/down	547
	Down	Down	Down	No/up	717
High-As-responsive	Up	No/down	Up	Up	397
	Down	No/up	Down	Down	133
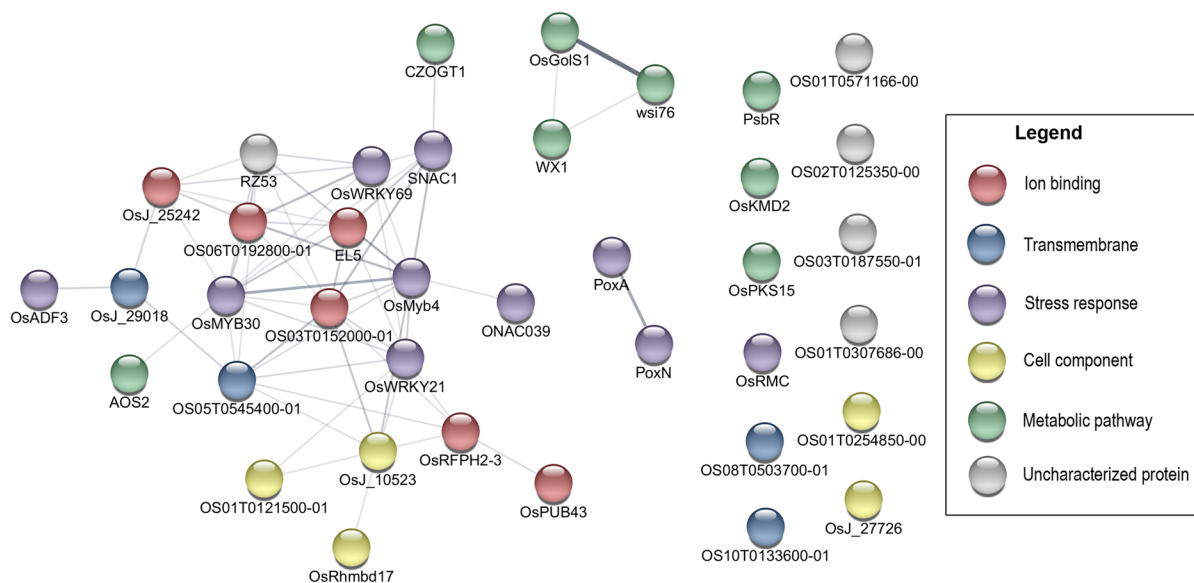
Fig. 4 Protein–protein interaction network generated from As-sensitive gene coding proteins. The width of edge represents the confidence level of co-expression between two proteins

Increased expression of *OsMYB30* would decrease cold tolerance in rice (Lv et al. 2017), while *OsMyb4* was up-regulated under cold stress (Vannini et al. 2004). Moreover, OsMYB30 as an R2R3-type MYB transcription factor was proved to be responsive to P starvation in rice (Gu et al. 2017). Three protein kinase domain containing proteins OS05T0545400-01, OsJ_29018 and OS10T0133600-01 present in the PPI network are also correlated with P translocation. Referring to the ionomic profiles, it suggested that P uptake by the rice roots was inhibited by external As, then *OsMYB30*, *OS05T0545400*, *OsJ_29018* and *OS10T0133600* were up-regulated to enhance P translocation and maintain P homeostasis in the rice shoots, resulting in significantly lower P contents in the roots from Group As1 and As5 than those from Group CK (Fig. 1d). Up-regulation of *OsMyb4* was to counteract the effect of *OsMYB30* on cold tolerance. OsJ_25242 co-expressed with OsMYB30 is an EF-Hand type domain containing protein with Ca ion binding site. Up-regulation of *OsJ_25242* might be due to decreased average Ca concentrations in the rice shoots under As(III) stress.

Five proteins OsWRKY69, OsWRKY21, SNAC1, ONAC039 and OsMyb4 co-expressed together were highly correlated with stress responses. OsWRKY21 and OsWRKY69 are two WRKY transcription

factors. The *WRKY* genes as the regulators in rice plants were demonstrated to mediate hormones such as abscisic acid and jasmonic acid in response to biotic and abiotic stresses (Jeyasri et al. 2021; Xu et al. 2016). The *OsWRKY28* gene was found to be involved in regulation of P uptake and As(V) tolerance in rice seedlings under As(III) or As(V) stress (Chakrabarty et al. 2009). SNAC1 and ONAC093 belonged to NAC transcription factors were widely investigated due to their connections with plant growth against environmental stresses (Singh et al. 2021). Two proteins PoxA and PoxN co-expressed at high confidence level were identified as peroxidases which are involved in response to oxidative stress. Exposure to As has been found to induce the production of reactive oxygen species (ROS) in rice plants (Kalita et al. 2018). Expression of peroxidase genes *poxA* and *poxN* could scavenge ROS and reduce As toxicity. OsJ_10523, OS01T0121500-01, OsRhmbd17, OS01T0254850-00 and OsJ_27726 proteins associated with membrane and cell wall were expressed to defense against abiotic stresses such as As. There was no direct evidence to establish connection between ionomes and stress response genes. However, up-regulation of these genes in the rice shoots was the response to external As(III) stress and could enhance As tolerance in the rice plants.
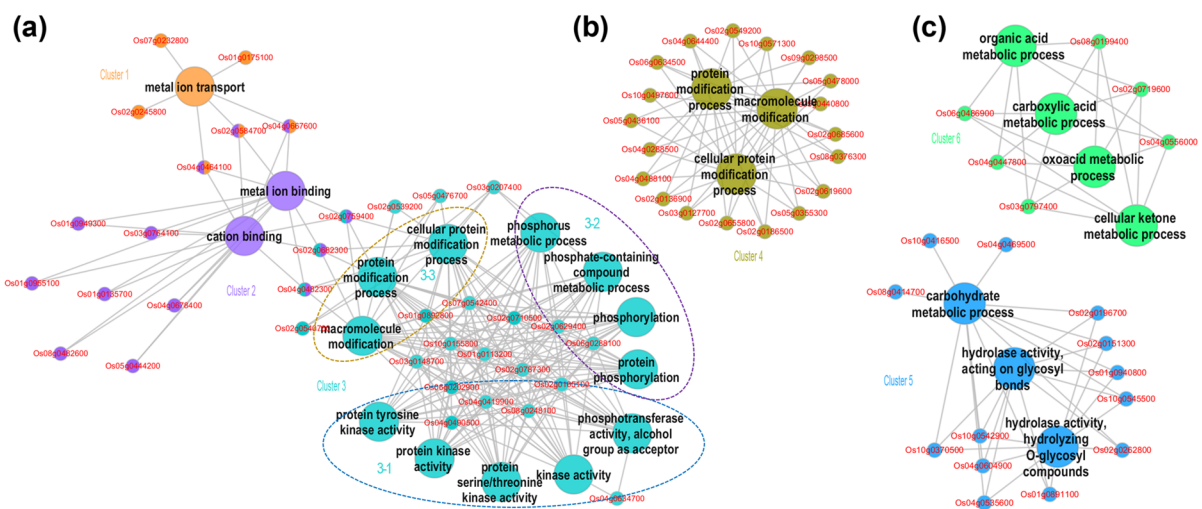
Enrichment of As-responsive genes

There were 547 up-regulated and 717 down-regulated genes assigned as low-As-responsive genes. The numbers of up- and down-regulated genes were 397 and 133, respectively for high-As-responsive genes. GO enrichment analysis was performed by ClueGO in Cytoscape to reveal the biological functions of these genes. The results of GO enrichment are demonstrated in Fig. 5. All the As-responsive genes were enriched in 25 GO terms which could be classified into 5 GO clusters. The detailed information on GO terms and correlated genes is listed in Table S4. Gene annotations are summarized in Table S5. The up-regulated low-As responsive genes were enriched into 3 GO clusters consisting of 15 GO terms, while the down-regulated low-As responsive genes were only enriched into 1 GO cluster including 3 GO terms. The up-regulated high-As-responsive genes were enriched into 2 GO clusters consisting of 7 GO terms. There was no GO term obtained from enrichment analysis of down-regulated high-As-responsive genes.

As shown in Fig. 5, GO cluster 1 containing 1 GO term (GO:0030001) was correlated with ion transport. Three *HMA* genes (*Os04g0464100*, *Os04g0667600* and *Os02g0584700*), 2 potassium channel (*KAT*) genes (*KAT3* and *Os01g0175100*) and 1 zinc transporter gene (*OsZIP8*) were involved in "metal ion transport". GO cluster 2 contained 2 GO terms (GO:0043169 and GO:0046872) associated with biological process of ion binding. There were 4 *ZFP* genes (*OsRING-1*, *WZF1*, *OsDof17* and *Os05g0444200*) and 2 calcium-binding protein genes (*OsCML31* and *OsCML16*) involved in "cation binding" and "metal ion binding". Heavy metals such as Cd, Pb and As are non-essential elements to plants and hinder plant growth at very low level. The rice plants had to restrict uptake and translocation of As through chelation and transport by various HMAs, resulting in up-regulation of *HMA* genes in the shoots (Li et al. 2020). K, Ca and Zn are essential elements for plant growth and production. Most of the ion binding and transport genes were correlated with K, Zn and Ca. As mentioned before, translocations of Zn and Ca from the roots to shoots were retarded by As(III) stress (Fig. 1). The homeostatic mechanism stimulated the rice plants to express more Zn^{2+} and Ca^{2+} binding and transport genes to sustain essential metabolic pathways. Increased expression of *KAT* genes were also detected in both rice roots and shoots under abiotic stresses (Musavizadeh et al. 2021). PPI network revealed the connection between K^+ channel proteins and several metabolic pathways such as carbohydrate metabolism and glycerophospholipid metabolism (Musavizadeh et al. 2021). It implied that external As(III) disturbed biological processes and

Fig. 5 GO terms and correlated As-responsive genes enriched by GO analysis, **a** up-regulated low-As-responsive genes, **b** down-regulated low-As-responsive genes, and **c** up-regulated high-As-responsive genes. Large circles represent GO terms, and small circles represent enriched genes

upregulated expression of *KAT3* and *Os01g0175100* in the rice plants, leading to decreased K concentrations in the rice roots as shown in Fig. 1e.

As shown in Fig. 5a and Table S4, GO cluster 3 containing 12 GO terms could be separated into 3 subgroups according to their molecular functions and biological processes. GO terms "kinase activity" (GO:0016301), "protein kinase activity" (GO:0004672), "protein tyrosine kinase activity" (GO:0004713), "protein serine/threonine kinase activity" (GO:0004674) and "phosphotransferase activity, alcohol group as acceptor" (GO:0016773) in GO cluster 3–1 are associated with catalysis of the phosphorylation in a protein. Meanwhile, GO terms "phosphorylation" (GO:0016310), "protein phosphorylation" (GO:0006468), "phosphorus metabolic process" (GO:0006793) and "phosphate-containing compound metabolic process" (GO:0006796) in GO cluster 3–2 are relevant to phosphate metabolisms. The rest three GO terms "macromolecule modification" (GO:0043412), "protein modification process" (GO: GO:0036211) and "cellular protein modification process" (GO:0006464) in GO cluster 3–3 were also enriched by down-regulated low-As-responsive genes as shown in Fig. 5b. As listed in Table S5, receptor-like cytoplasmic kinases (RLCKs) genes were the dominant genes enriched in GO cluster 3 and 4. RLCKs from plants were proved to participate in development and stress responses (Vij et al. 2008). In this work, expressions of *OsRLCK2*, *OsRLCK63*, *OsRLCK100*, *OsRLCK152* and *OsRLCK203* were enhanced in the rice shoots under As(III) stress, while expressions of *OsRLCK64*, *OsRLCK73* and *OsRLCK308* were limited. Interestingly, upregulation of *OsRLCK2*, *OsRLCK63* and *OsRLCK203* and downregulation of *OsRLCK2308* were also detected in rice plants under cold, dehydration or salt stress, indicating differential expression patterns of *RLCK* genes in response to environmental stress (Vij et al. 2008). Moreover, U-box type ubiquitin ligase (PUB) genes were suggested as regulator to control cellular development and stress responses in rice (Kim et al. 2021). Different from *RLCK*s, *PUB* genes *OsPUB2*, *OsPUB33* and *OsPUB45* differentially expressed in the current study were all up-regulated.

As shown in Fig. 5c, GO cluster 5 including "carbohydrate metabolic process" (GO:0005975), "hydrolase activity, acting on glycosyl bonds" (GO:0016798) and "hydrolase activity, hydrolyzing O-glycosyl compounds" (GO:0004553) 3 GO terms were associated with carbohydrate hydrolysis. A total of 9 genes enriched in this cluster belong to glycosyl hydrolase (GH) family which is sensitive to biotic and abiotic stresses. The *GH* genes were supposed as a role in stress adaptation due to up-regulation of 2/3 *GH* genes in stress treatments (Sharma et al. 2013). It indicated that increased expression of GH genes might be compensation for disturbance of carbohydrate metabolisms by high-level As(III) in this work. GO terms in cluster 6 mainly connected to organic acid (OA) and ketone metabolic processes. OAs in plants play a critical role in cellular metabolism which was approved to promote heavy metal tolerance and essential mineral acquisition (Panchal et al. 2021). Increased expression of OA correlated genes in the rice shoots should be the response to As(III) stress. Moreover, *HMA5* enriched in GO cluster 6 is a copper transporter gene involved in translocation of Cu from roots to shoots. Targeted knockout of *HMA5* gene was reported to decrease Cu content in the rice shoots accompanied with Cu increasing in the roots (Deng et al. 2013). Predictably, upregulation of *HMA5* under high-level As(III) stress eventually caused accumulation of Cu in the rice shoots as shown in Fig. 1n.

Conclusion

The rice ionomes exhibited discriminatory response to environmental disturbances. Compared to non-essential elements, mineral nutrients in the rice plants were more sensitive to external As(III) stress. Uptake of P was hindered under As(III) stress, resulting in decreased P concentrations in the roots. The genes involved in protein kinase activity, phosphorus metabolic process and phosphorylation were up-regulated under As(III) stress to maintain P homeostasis in the shoots for biochemical functions and biophysical balance. Similarly, *KAT* genes co-expressed with carbohydrate metabolic pathways were up-regulated to transport essential K into growing tissues. Translocation of Zn and Ca from roots to shoots was inhibited by excess As, stimulating expression of Zn and Ca binding genes to sustain essential metabolic pathways. The rice plants promoted the expression of *HMA* genes to reduce As toxicity and confer As tolerance. Transport of Cu was simultaneously enhanced

by *HMA* genes, resulting in higher Cu contents in the shoots from Group As5 than other groups. Upregulation of GH and OA correlated genes indicated that carbohydrate metabolic and organic acid metabolic processes in the rice had been seriously disturbed under high-level As(III). The *WRKY*, *NAC* and *PUB* genes were also up-regulated to cope with abiotic stress. Close connection has been established between transcriptomics and ionomics in the rice plants. Solid evidence of the effects of As(III) stress on binding, transport or metabolism of P, K, Ca, Zn and Cu was obtained in this work. The results suggest that macro and essential elements are the dominant composition of plant ionome. Plants were able to regulate the expression of corresponding genes to maintain mineral nutrient homeostasis for essential metabolic processes.

Declarations

Competing interests The authors declare that they have no competing interests.

References

Banerjee A, Singh A, Sudarshan M, Roychoudhury A (2021) Silicon nanoparticle-pulsing mitigates fluoride stress in rice by fine-tuning the ionomic and metabolomic balance and refining agronomic traits. Chemosphere 262:127826

Baxter I (2015) Should we treat the ionome as a combination of individual elements, or should we be deriving novel combined traits? J Exp Bot 66(8):2127–2131

Baxter I, Dilkes BP (2012) Elemental profiles reflect plant adaptations to the environment. Science 336(6089):1661–1663

Baxter IR, Vitek O, Lahner B, Muthukumar B, Borghi M, Morrissey J, Guerinot ML, Salt DE (2008) The leaf ionome as a multivariable system to detect a plant's physiological status. Proc Natl Acad Sci USA 105(33):12081–12086

Cao Y, Sun D, Ai H, Mei H, Liu X, Sun S, Xu G, Liu Y, Chen Y, Ma LQ (2017) Knocking out *OsPT4* gene decreases arsenate uptake by rice plants and inorganic arsenic accumulation in rice grains. Environ Sci Technol 51(21):12131–12138

Chakrabarty D, Trivedi PK, Misra P, Tiwari M, Shri M, Shukla D, Kumar S, Rai A, Pandey A, Nigam D, Tripathi RD, Tuli R (2009) Comparative transcriptome analysis of arsenate and arsenite stresses in rice seedlings. Chemosphere 74(5):688–702

Chen H, Liang X, Gong X, Reinfelder JR, Chen H, Sun C, Liu X, Zhang S, Li F, Liu C, Zhao J, Yi J (2021) Comparative physiological and transcriptomic analyses illuminate common mechanisms by which silicon alleviates cadmium and arsenic toxicity in rice seedlings. J Environ Sci 109:88–101

Chutia R, Abel S, Ziegler J (2019) Iron and phosphate deficiency regulators concertedly control coumarin profiles in *Arabidopsis thaliana* roots during iron, phosphate, and combined deficiencies. Front Plant Sci 10:113

Cong W, Miao Y, Xu L, Zhang Y, Yuan C, Wang J, Zhuang T, Lin X, Jiang L, Wang N, Ma J, Sanguinet KA, Liu B, Rustgi S, Ou X (2019) Transgenerational memory of gene expression changes induced by heavy metal stress in rice (*Oryza sativa* L.). BMC Plant Biol 19(1):282

Cubadda F, Jackson BP, Cottingham KL, Van Horne YO, Kurzius-Spencer M (2017) Human exposure to dietary inorganic arsenic and other arsenic species: state of knowledge, gaps and uncertainties. Sci Total Environ 579:1228–1239

Deng F, Yamaji N, Xia J, Ma JF (2013) A member of the heavy metal P-type ATPase OsHMA5 is involved in xylem loading of copper in rice. Plant Physiol 163(3):1353–1362

Di X, Zheng F, Norton GJ, Beesley L, Zhang Z, Lin H, Zhi S, Liu X, Ding Y (2021) Physiological responses and transcriptome analyses of upland rice following exposure to arsenite and arsenate. Environ Exp Bot 183:104366

Du F, Yang Z, Liu P, Wang L (2018) Accumulation, translocation, and assessment of heavy metals in the soil-rice systems near a mine-impacted region. Environ Sci Pollut Res 25(32):32221–32230

Du F, Liu P, Wang K, Yang Z, Wang L (2019) Influence of different arsenic species on uptake, speciation and efflux of arsenic in hydroponic rice plants. Ecotoxicol Environ Saf 186:109791

Du F, Liu P, Wang K, Yang Z, Wang L (2020) Ionomic responses of rice plants to the stresses of different arsenic species in hydroponics. Chemosphere 243:125398

Gindri RG, Navarro BB, da Cruz Dias PV, Tarouco CP, Nicoloso FT, Brunetto G, Berghetti ÁLP, da Silva LOS, Fett JP, Menguer PK, Ricachenevsky FK (2020) Physiological responses of rice (*Oryza sativa* L.) oszip7 loss-of-function plants exposed to varying Zn concentrations. Physiol Mol Biol Plants 26(7):1349–1359

Gu M, Zhang J, Li H, Meng D, Li R, Dai X, Wang S, Liu W, Qu H, Xu G (2017) Maintenance of phosphate homeostasis and root development are coordinately regulated by MYB1, an R2R3-type MYB transcription factor in rice. J Exp Bot 68(13):3603–3615

Guan S, Xu Q, Ma D, Zhang W, Xu Z, Zhao M, Guo Z (2019) Transcriptomics profiling in response to cold stress in cultivated rice and weedy rice. Gene 685:96–105

Hussain MM, Bibi I, Niazi NK, Shahid M, Iqbal J, Shakoor MB, Ahmad A, Shah NS, Bhattacharya P, Mao K, Bundschuh J, Ok YS, Zhang H (2021) Arsenic biogeochemical cycling in paddy soil-rice system: interaction with various factors, amendments and mineral nutrients. Sci Total Environ 773:145040

Jeyasingh PD, Goos JM, Thompson SK, Godwin CM, Cotner JB (2017) Ecological stoichiometry beyond redfield : an ionomic perspective on elemental homeostasis. Front Microbiol 8:722

Jeyasri R, Muthuramalingam P, Satish L, Adarshan S, Lakshmi MA, Pandian SK, Chen J-T, Ahmar S, Wang X, Mora-Poblete F, Ramesh M (2021) The role of *OsWRKY* genes in rice when faced with single and multiple abiotic stresses. Agronomy 11(7):1301

Kalita J, Pradhan AK, Shandilya ZM, Tanti B (2018) Arsenic stress responses and tolerance in rice: physiological cellular and molecular approaches rice. Science 25(5):235–249

Karley AJ, White PJ (2009) Moving cationic minerals to edible tissues: potassium, magnesium, calcium. Curr Opin Plant Biol 12(3):291–298

Khan E, Gupta M (2018) Arsenic–silicon priming of rice (*Oryza sativa* L.) seeds influence mineral nutrient uptake and biochemical responses through modulation of Lsi-1, Lsi-2, Lsi-6 and nutrient transporter genes. Sci Rep 8(1):10301

Kim M-S, Kang K-K, Cho Y-G (2021) Molecular and functional analysis of U-box E3 ubiquitin ligase gene family in rice (*Oryza sativa*). Int J Mol Sci 22(21):12088

Kumari P, Rastogi A, Shukla A, Srivastava S, Yadav S (2018) Prospects of genetic engineering utilizing potential genes for regulating arsenic accumulation in plants. Chemosphere 211:397–406

Li N, Liu H, Sun J, Zheng H, Wang J, Yang L, Zhao H, Zou D (2018) Transcriptome analysis of two contrasting rice cultivars during alkaline stress. Sci Rep 8:1–16

Li J, Zhang M, Sun J, Mao X, Wang J, Liu H, Zheng H, Li X, Zhao H, Zou D (2020) Heavy metal stress-associated proteins in rice and arabidopsis: genome-wide identification, phylogenetics, duplication, and expression profiles analysis. Front Genet 11:477

Lin G, He X, Zeng J, Yang Z, Wang L (2023) Ionome profiling and arsenic speciation provide evidence of arsenite detoxification in rice by phosphate and arsenite-oxidizing bacteria. J Environ Sci 128:129–138

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. Methods 25(4):402–408

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15(12):1–21

Lv Y, Yang M, Hu D, Yang Z, Ma S, Li X, Xiong L (2017) The OsMYB30 transcription factor suppresses cold tolerance by interacting with a JAZ protein and suppressing β-amylase expression. Plant Physiol 173(2):1475–1491

Ma JF, Yamaji N, Mitani N, Xu X-Y, Su Y-H, McGrath SP, Zhao F-J (2008) Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. Proc Natl Acad Sci 105(29):9931–9935

Mukhopadhyay A, Vij S, Tyagi AK (2004) Overexpression of a zinc-finger protein gene from rice confers tolerance to cold, dehydration, and salt stress in transgenic tobacco. Proc Natl Acad Sci 101(16):6309–6314

Musavizadeh Z, Najafi-Zarrini H, Kazemitabar SK, Hashemi SH, Faraji S, Barcaccia G, Heidari P (2021) Genome-wide analysis of *Potassium channel* genes in rice: expression of the *OsAKT* and *OsKAT* genes under salt stress. Genes 12(5):784

Nocito FF, Lancilli C, Dendena B, Lucchini G, Sacchi GA (2011) Cadmium retention in rice roots is influenced by cadmium availability, chelation and translocation. Plant Cell Environ 34(6):994–1008

Oono Y, Yazawa T, Kanamori H, Sasaki H, Mori S, Matsumoto T (2017) Genome-wide analysis of rice *cis*-natural antisense transcription under cadmium exposure using strand-specific RNA-Seq. BMC Genomics 18(1):761

Panchal P, Miller AJ, Giri J (2021) Organic acids: versatile stress-response roles in plants. J Exp Bot 72(11):4038–4052

Shaibur MR, Sera K, Kawai S (2016) Effect of arsenic on concentrations and translocations of mineral elements in the xylem of rice. J Plant Nutr 39(3):365–376

Sharma R, Cao P, Jung K-H, Sharma M, Ronald P (2013) Construction of a rice glycoside hydrolase phylogenomic database and identification of targets for biofuel research. Front Plant Sci 4:330

Singh S, Koyama H, Bhati KK, Alok A (2021) The biotechnological importance of the plant-specific NAC transcription factor family in crop improvement. J Plant Res 134(3):475–495

Stone R (2008) Food safety—arsenic and paddy rice: a neglected cancer risk? Science 321(5886):184–185

Sun J, Sun Y, Ahmed RI, Ren A, Xie M (2019) Research progress on plant RING-finger proteins. Genes 10(12):973

Vannini C, Locatelli F, Bracale M, Magnani E, Marsoni M, Osnato M, Mattana M, Baldoni E, Coraggio I (2004) Overexpression of the rice *Osmyb4* gene increases chilling and freezing tolerance of *Arabidopsis thaliana* plants. Plant J 37(1):115–127

Vij S, Giri J, Dansana PK, Kapoor S, Tyagi AK (2008) The receptor-like cytoplasmic kinase (*OsRLCK*) gene family in rice: organization, phylogenetic relationship, and expression during development and stress. Mol Plant 1(5):732–750

Wickham H (2011) ggplot2. Wires Comput Stat 3(2):180–185

Wu C, Zou Q, Xue SG, Pan WS, Huang L, Hartley W, Mo JY, Wong MH (2016) The effect of silicon on iron plaque formation and arsenic accumulation in rice genotypes with different radial oxygen loss (ROL). Environ Pollut 212:27–33

Xiao W, Liu P, Wang K, Yang Z, Wang L (2021) Relationship between ionomics and transcriptomics of rice plant in response to arsenite stress. Environ Exp Bot 189:104565

Xu H, Watanabe KA, Zhang L, Shen QJ (2016) WRKY transcription factor genes in wild rice *Oryza nivara*. DNA Res 23(4):311–323

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 Phytol 195(1):97–112

Zhao R, Xie C-T, Xu Y, Ji D-H, Chen C-S, Ye J, Xue X-M, Wang W-L (2020) The response of *Pyropia haitanensis* to inorganic arsenic under laboratory culture. Chemosphere 261:128160