Title: Ionomic and transcriptomic analysis provides new insight into the distribution and transport of cadmium and arsenic in rice

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Abstract: To identify the key barrier parts and relevant elements during Cd/As transport into brown rice, 16 elements were measured in 14 different parts of 21 rice genotypes; moreover, transcriptomic of different nodes was analyzed. Cd/As contents in root and nodes were significantly higher than those other parts. Node I had the highest Cd content among nodes, leading an increase in gene expressions involved in glycolytic and Cd detoxification. The Cu/Zn/Co distribution and transport to various parts was similar to that of Cd, and Fe/Sb distribution and transport to various parts was similar to that of As. Moreover, Cu/Zn/Co/Mg was correlated with Cd in root and nodes, as well as Fe with As. Besides, the ionomic profile showed the different parts of an organ are closely related, and the spatial distribution of different organs is consistent with the growth morphology of rice. Therefore, root and nodes are two key barriers to Cd/As transport into brown rice. Moreover, Node I has the highest Cd accumulation capacities among nodes. The ionomic profile reflect relationships among plant parts and correlations between the elements, suggesting that nodes are hubs for element distribution, as well as the correlation between Cd with Zn/Cu/Co/Mg, between Fe with As.

Keywords: cadmium and arsenic; transport; ionomic; transcriptomic; rice

1. Introduction

Ore mining, heavy emissions of wastes (waste gas, wastewater, and industrial residues), sewage irrigation, and continuous use of pesticides and fertilizers containing heavy metals may cause serious heavy metal contamination of farmlands [1–3]. Rice (*Oryza sativa* L.), a staple food in many Asian countries is a crop with high accumulation of Cd and As, which are two highly toxic and non-essential elements for human body [4,5]; Cd and As accumulations directly affect food security and threaten human health [6,7]. However, Cd and As distribution and transport mechanisms in rice and their interactions with other elements are unclear, thereby impeding the restoration and use of Cd- and As-contaminated paddy fields [8–11]. Cd and As are initially absorbed from the soil through the roots, then portions of Cd and As are entrapped in root vacuoles [12,13]; the other Cd and As portions enter the shoot via the xylem [14,15]. Cd and As in the xylem of the stem are transported to the phloem and finally enter the grain [16–18]. Moreover, Cd and As in the leaves may be transported to the grain via the phloem in the stem [19,20]. The distribution and transport mechanisms of Cd and As in both stem and leaves have been studied as a whole organ [21], but studies on various nodes, internodes, and leaves are few; studying these should provide many details in Cd and As transport and accumulation mechanisms. Rice nodes currently are being studied and showed that nodes are barriers to the transport of Cd, As, and other mineral elements [22,23], and a hub for the preferential distribution of these mineral elements [24–26]. However, these studies focused on one node or considered all nodes as a part, but few analyzed the differences between the distribution and transport of Cd

and As, as well as the gene expressions in response to Cd and As among various nodes. Moreover, results were acquired primarily through several rice varieties (for example, wild and mutant types). Few studies analyzed the distribution and transport of Cd and As in different nodes, internodes, and leaves of numerous rice genotypes.

Mineral elements often interact with one another because of their similarities in chemical properties and interrelated metabolic pathways [27]. Therefore, foliar application of silica sols in rice can effectively decrease Cd and As concentration in grains [28,29]; moreover, Se^{VI} supplementation can minimize As accumulation in rice [30], providing the possibility of regulating Cd and As transport into brown rice through elemental interactions. However, designing a method that uses elemental interactions to regulate heavy metal uptake is difficult, because molecular mechanisms that regulate the balance of various elements in plants are highly complex and transporters have multi-elemental specificities [31–33]. Developing ionomics primarily in plants has provided compelling evidence for exploring the interactions between mineral elements [34–36]. Ionomics evaluates the adsorption and accumulation mechanisms of mineral nutrients and heavy metal pollutants in plants mainly through high-throughput elemental analysis [35]. For example, to clarify the translocation of Cs in *Amaranthus*, ionomic was performed to analyze the interactions between Cs and other mineral elements [36]. Moreover, the link between different chemical elements in the absorption and transport of *Lotus corniculatus* was explored based on the changes of ionomics induced by adding a certain element exogenously [37]. Chen et al. [38] proposed that studying the interactions between heavy metal elements and those

between heavy metals and mineral nutrients via ionomics is significant when studying the migration rules for heavy metals and regulating the accumulation of heavy metals by correlated elements in plants. Moreover, Baxter et al. [39] found that ionomes are under tight genetic control and vary in different tissues. However, simultaneous ionomic studies on roots, nodes, internodes, leaves, husk, or brown rice have been rare, thereby hindering research on regulating Cd and As absorption and transport via elemental interactions. Furthermore, environments, populations, and species can change the multi-element phenotypes of the ionome; conversely, ionomics has been used to find genes of novel function, lines, or environments that produce foods with altered nutritional profiles; or define gene by their environmental effects with which they regulate elemental accumulation [40]. Significant differences in ionomes of the different plant parts have been observed [39], but few ionomic studies have been done to reflect tissue specificity and relationships among various plant parts.

In the present study, 16 elements were investigated in 14 parts of 21 rice genotypes grown in Cd/As compound contaminated soils using inductively coupled plasma mass spectroscopy (ICP-MS), and digital gene expression profiling (DGE) was used to compare different nodes. The accumulation of the mineral elements and their correlations in different plant parts were analyzed. Moreover, ionomic profiles and the relationship between different parts were investigated using PCA, to study the distribution and transport mechanisms of Cd/As in different plant parts, as well as the relationship between Cd/As and other elements. Finally, we studied the key barriers to the transport of Cd and As into brown rice and the correlated elements with Cd and As.

2. Material and methods

2.1. Site description

The trial field was located in Du'an county of Guangxi province, China (N: 24°24'04.64", E: 107°58'03.26"). The experiment was performed in an alluvial soil that has been used to cultivate paddy-rice for many years. Due to the immersion of flood containing wastewater from mines, the soil has been contaminated by Cd and As. The basic chemical properties of the topsoil (0–20 cm) at the onset of the experiment are listed in Table 1. The mean annual temperature of Du'an county is 19.6 ° to 21.6 °C and the total annual precipitation is 1726 mm.

2.2. Plant materials and experimental design

A 62 plots random experimental design was employed in this study. Seeds of 21 rice (*O. sativa* L.) cultivars (C1 Yliangyou 087, C2 Yliangyou 696, C3 Teyou 524, C4 Yexiangyou 2, C5 Dxiang 287, C6 Shiyu 838, C7 Luoyou 8, C8 Chuanyou 673, C9 Jingfuyou 270, C10 Liufeng 1004, C11 Teyou 233, C12 Hliangyou 6839, C13 Zhongzheyu 8, C14 Fyou 498, C15 Teyouyu 1, C16 Yixiang 2239, C17 Hongxiang 110, C18 Zhongzheyu 634, C19 Luxiang 658, C20 Yliangyou 5867, and C21 Shenliangyou 5814) were obtained from rice seed companies of Guangxi Province. Each cultivar was planted in three plots (area 20 m²). Seeds of the 21 rice cultivars were sowed in non-contaminated soil of the nursery site on February 25, 2014. Rice seedlings were transplanted into the plots on April 5th. Before transplanting rice seedlings, NPK compound fertilizer (N:P₂O₅:K₂O = 15:13:12, 600 kg ha⁻¹) was used as a base fertilizer. Urea (150 kg ha⁻¹) at tillering stage and urea (150 kg ha⁻¹) and

potassium fertilizer (K_2SO_4 , 150 kg ha⁻¹) at heading stage were used as topdressing, respectively. The field was managed according to conventional operating practices.

2.3. Sampling and measurement

The rice was sampled in mid-July 2014. In each plot, three plants were sampled and thoroughly rinsed with tap water and de-ionized water. Because only the upper four or five internodes on the main culm below the panicle were elongated during the reproductive growth stage [41], each plant was divided into 14 parts: Brown rice (BR), Husk (H), Panicle node (PN), Node I (NI), Node II (NII), Node III (NIII), Internode I (INI), Internode II (INII), Internode III (INIII), Internode IV (INIV), Flag leaf (LI), 2nd leaf (LII), 3rd leaf (LIII), and Root (R) (Fig. 1); and the same parts of three plants from the same plot were mixed and designated as the plant sample of that plot. The plant parts were dried in an oven at 75 °C for 72 h. Dry samples were ground to homogeneous powder using a stainless steel cutter blender (T250D, IKA, Germany) to analyze the elements concentration.

2.4. Determination of elements in plant tissues

The pulverized plant parts (0.2500 g) were digested using concentrated HNO_3 and 30% H_2O_2 (V:V was 7:1) using a microwave digestion instrument (MARS, CEM, USA). The total Cd, As, Na, Mg, K, Ca, Cr, Fe, Mn, Co, Cu, Zn, Se, Mo, Sb, and Pb in the different plant parts were determined using ICP-MS (THERMO, USA) at the Central Laboratory of the Tianjin Academy of Agricultural Sciences of China. The accuracy of the analysis followed the standard reference material (GBW07603, GSV-2) from the Centre for Standard Reference of China.

2.5. Illumina Sequencing and real-time quantitative PCR (qRT-PCR) analysis

The nodes of Teyou 524 were frozen in liquid nitrogen and conserved under -80°C . Samples of PN, NI and NII were chosen for RNA isolation. Three libraries were constructed and used for sequencing in the Novogene Company. Sequencing libraries were generated from 3 μg of total RNA using NEBNext Ultra RNA Library Prep Kit (NEB, USA) and sequenced on an IlluminaHiseq 4000 platform and 150 bp paired-end reads were generated. Differential expression analysis was implemented using the DEGSeq R package (1.20.0) [42]. Corrected P-value of 0.005 and $\log_2(\text{Fold change})$ of 1 were set as the thresholds for a significantly differential expression. To validate the expression profiles obtained through DGE analysis, the expression levels of 12 differentially expressed genes (DEGs) were confirmed by qRT-PCR. RNA was reverse-transcribed using Trans PCR SuperMix (Beijing CoWin Biotech Co., Ltd., Beijing, China) for qPCR. qPCR mixtures (20 μL of total volume) were composed of 10 μL of UltraSYBR Mixture (Beijing CoWin Biotech), 1.0 μL of cDNA template (10 ng), 0.5 μL of each of forward and reverse primers (0.25 mM), and 8 μL of RNase-free water. PCR was performed using a LineGene 9600 plus PCR system (Bioer Technology Co., Ltd., Hangzhou, Zhejiang, China). Melting curves were analyzed immediately to confirm product specificity. The mean Ct value of each gene was obtained from three independent PCR experiments. A stable internal reference gene was amplified using the following primers: forward primer 50-TTTCACCTCTTGGTGTGAAGCAGAT-30 and reverse primer 50-GACTTCCTT CACGATTTCATCGTAA-30. The relative expression levels of genes were calculated using the $2^{-\Delta\Delta\text{CT}}$ method [43]. These genes

are shown in Fig. S2.

2.6. Statistical Analysis

Statistical differences in the ion concentrations in rice tissues were assessed by variance (ANOVA) and means were compared using Duncan's Multiple Range Test. A significance level of $P < 0.05$ was applied. Pearson's correlation analyses of 16 mineral concentrations in different tissues were performed using a significance level of $P < 0.05$. PCA was performed to visualize the ionic profile of 21 rice genotypes and the relationship between different parts. All statistical calculations were performed using SAS 9.1.

3. Results

3.1. Distributions of Cd, As, and other mineral elements in different plant parts

The contents of Cd or As in the brown rice, husk, nodes, internodes, leaves, and roots of the 21 rice genotypes were significantly different. (Fig. 2A,B). The contents of Cd and As in the roots and nodes were significantly higher than those in internodes and leaves, and those in husk and brown rice were the lowest. The contents of Cd or As in internodes decreased from the bottom to the top, with insignificant differences from INI compared with the brown rice or husk. Moreover, no significant differences in Cd or As contents occurred among leaves. Notably, Cd distribution was different from that of As in different nodes. The Cd content in NI was significantly higher than other nodes, and no significant difference existed in other nodes. However, As content in the node appreciably decreased from bottom to the top.

Similar to Cd and As distribution in different parts of rice plants, Fe, Cu, Zn, Sb,

Se, Mo, Pb, and Co contents (except for the husk, which had the highest Co content) the root and node of rice plants were significantly higher than those in other parts (Fig. 2C,P); Unlike Cd and As distributions, K and Na contents in the node and internode were the highest; moreover, the Cr content in the husk was the highest. The Mg, Ca, Mn contents in the leaves were the highest, followed by those in the nodes. The changing trends of Mg and Ca in the nodes were similar to that of Cd.

3.2. The correlations between Cd/As and other elements

The dataset of the 16 elements in all parts was analyzed using PCA to show the relationship between elements (Fig. 3). The cumulative contribution ratio of PCA was 66.8% up to the third principal component (PC3). A collection of many elements exhibited an interesting phenotype. Cd was closely related to Zn, Cu, and Co (Cd is closest to Zn); and As was closely related to Fe and Sb. K, Mg, Ca, and Mn were linked; and Se, Mo and Pb were similar. However, Na and Cr were far from other elements.

The correlation between elements varied in different parts (Figs. 4 and S1). Few element pair correlations appeared consistent in all parts, but many were significantly correlated with each other within specific parts. In the root and nodes, Cd was positively correlated with Cu, Zn, Co, and Mg. Moreover, Cd was positively correlated with Mo, Se, and Pb in the root and Fe in the nodes. As was positively correlated with Fe in root and nodes, and positively correlated with Co and Cu in the nodes. In all rice plant parts, PN exhibited the most dense elemental correlation network, in which Cd was positively correlated with 14 elements, and As was

positively correlated with 9 elements.

3.3. Relationship between different plant parts

To characterize the relationship between different parts, the results of 16 elements in 14 parts were subjected to PCA (Fig. 5a). The cumulative contribution ratio of PCA was 80.2% up to the third principal component (PC3). The parts can be divided into five types by ionome difference, namely, the root, brown rice and husk, internodes, nodes, and leaves, showing that the different parts of an organ are closely related, and that the overall spatial distribution of different organs is basically consistent with the growth morphology of the rice plant. The root and nodes were interconnected, the nodes were connected with the leaves and internodes, and the internodes were connected with brown rice and husk. Different leaves or different internodes were relatively closer, whereas different nodes were distantly seated. Furthermore, the major elements that contributed to the PC1 were Cd, As, Cu, Zn, Co, Fe, Sb, Se, Pb, and Cr, representing 49.9% of mutation; the contribution of elements to the PC2 was dominated by Mg, Mn, and Ca, explaining 16.3% of the variation (Fig. 5b). The Cd and As contents of grain were correlated with those of nodes and internodes, but not with those of root (Fig. S3).

3.4. Transcriptome analysis of different nodes

The number of DEGs in response to As in PN, NI, and NII were not significant, but those in response to Cd were significantly different. The DEGs in response to Cd between NI and NII were low and only had 1 up-regulated gene and 1 down-regulated gene. However, compared with PN and NII, NI had 29 and 30 DEGs in response to

Cd, respectively, and in these DEGs, 12 same genes were up-regulated and 8 same genes were down-regulated. The 12 up-regulated genes consisted of the 7 enzyme genes involved in glycolytic reactions (including triosephosphate isomerase, glyceraldehydes-3-phosphate dehydrogenase and alcohol dehydrogenase) and the 4 genes involved in Cd detoxification (including glutathione-S-transferase (GST), trehalose-phosphatase and cysteine (Cys)) (Table 2). Analysis of the 12 up-regulated genes by qRT-PCR showed that 10 genes were consistent with the DGE results (Fig. S2), and the primers of candidate genes for qRT-PCR are listed in Table S1.

3.5. The ionome difference among different genotypes

The Cd and As difference in brown rice among different genotypes is not remarkably distinguished, but those in nodes and internodes among different genotypes is remarkable (Fig. S4 and S5). The Cd concentrations of nodes and internodes in C3 and C9 are higher than those in C15 and C16 (Fig. S4). The As concentration of nodes and internodes in C11 is higher than those in C4 and C16.

The PCA analysis of ion abundance in brown rice and NI showed the ionome difference in brown rice and NI among different genotypes was distinguished. Furthermore, in these two parts, the major elements that contributed to the PC1 were Cd, As, Co, and Mg, representing 34.6 and 25.6% of mutation (Fig. S6 and S7). The PCA analysis of ion abundance in other parts was not shown.

4. Discussion

4.1. The key barrier parts to the transport of Cd and As into brown rice

The present study showed that Cd and As accumulations in the roots and nodes

of rice plant were significantly higher than those in other parts. This indicated that the accumulation and distribution properties of Cd and As in different parts of rice plants were tissue-specific, and roots and nodes are two key barriers to Cd and As transport into brown rice. The results coincide with other studies. Zheng et al. [44] showed that the As content in the root accounts for 55% of the total As, thereby limiting As transport to the shoot. Miyadate et al. [12] found that OsHMA3 promotes Cd being sealed in the root vacuoles, thereby limiting Cd transport to the shoot. Chen et al. [26] found that the node, as a filter, limits As transport to the grain. Yamaguchi et al. [45] indicated that NI was a barrier to Cd and Zn transport.

However, the accumulation and sequestration capacity of Cd and As in different nodes have been rarely studied, and systematic results are absent. In our experiment, the Cd content in NI was significantly higher than that in the other nodes, and the As content apparently decreased from bottom to the top in the nodes, indicating the significant differences among nodes in the accumulation and barrier capacity of Cd and As. Moreover, the accumulation and barrier capacity of Cd in NI were the highest, whereas that of As gradually decreased from the lower node to the upper node. Zheng et al. [40] found via synchrotron-based X-ray fluorescence that 40% of As was transported from nodes to their connected leaves, and the remaining 60% was transported to the upper portion of the shoot, indirectly indicating that As accumulation in different nodes gradually decreases from the bottom to the top. However, the Cd distribution and transport in different nodes have not been investigated.

To maintain correct cell functioning under Cd stress, plants need to increase the non-protein thiol materials (Cys, glutathione and phytochelatins) and antioxidant enzymes (GST), and demand for adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (NADH), and nicotinamide adenine dinucleotide phosphate (NADPH) by inducing increases in genes expression of molecules and enzymes involved in energy provision (glycolysis, and tricarboxylic acid cycle) and Cd detoxification [46,47]. Zhang et al. [48] found that the GSH content and GST activities are significantly related to Cd content. The present study showed that NI involves a total of 12 same up-regulated genes and 8 same down-regulated genes compared with PN and NII, and the 12 up-regulated genes consist of the 7 enzyme gene involved in glycolytic reactions and the 4 genes involved in Cd detoxification. Moreover, this experiment showed the Cd content at NI was significantly higher than that in PN and NII. Therefore, it can be inferred that the highest Cd content in NI induced an increase in genes expressions of molecules and enzymes involved in glycolytic reactions and Cd detoxification. However, the expression levels of genes in response to As were insignificantly different in three nodes, indicating that the gradual decrease in As accumulation in the nodes from the bottom to the top does not change the gene expression in response to As.

4.2. The correlated elements with Cd and As

Baxter et al. [40] indicated that the ionome is not simply a collection of elements, and there are clear relationships between the elements. The present study found that the distributions of Fe, Cu, Zn, Sb, Se, Mo, Pb, and Co are similar to those of Cd and

As in different parts of rice plants. For the PCA on the datasets of the 16 elements in all plant parts, a collection of many elements exhibited an interesting phenotype. Cd was closely related to Zn, Cu, and Co (especially Cd was the most closely related to Zn), and As was closely linked with Fe and Sb. This finding indicated that in different parts, ranging from the roots, nodes, and internodes to the grain, the Cd distribution and transport are similar to those of Zn, Cu, and Co (especially Zn), and As distribution and transport are similar to those of Fe and Sb. Similar studies found that Zn is positively correlated with Cd, and Zn and Cd have similar transporters in rice plants [49,50]. Cu was positively correlated with Cd in the stem [51], and the absence of Cu transporter *copt5* decrease Cd transport to the shoot [52]. Co and Cd have a similar transporter *AtHMA4* in *A. thaliana* [53]. Moreover, As is positively correlated with Fe in the grain [54], and As/Fe have similar transporters. Root plasma membrane transporters for iron take up arsenate [55]. Shaibur et al. [56] suggested that As decreases Fe translocations in rice xylem. Tiwari et al. [57] first demonstrated that iron transporter *OsNRAMP1* in the xylem mediates loading and enhanced accumulation of As. Wang et al. [58] found that mitochondrial iron transporter (mitoferrin-2) participates in As_2O_3 transport. Sb and As are analogs, and are similar in chemical properties and transport mechanisms [59]. These findings further confirmed that, although the transporters of multi-elements are unknown, the interactions between Cd/As and other elements can be elucidated using ionomics.

Previous studies showed similarities between Cd and Zn, Co and Cu, as well as As and Fe. Mg generally mediates the Cd transport by regulating Zn and Fe [60,61].

However, the element correlations have been intensively studied in the shoot, stem, and xylem, with a few studies on various nodes, internodes and leaves. Baxter et al. [39] found that the ionomes of different plant tissue are variable, and suggested that researchers should focus on profiling the ionomics in the tissue of interest owing to the lack of tissue correlations. Roots and nodes are important barriers to Cd and As transport into the grain. Elements related to Cd and As in the roots and nodes were analyzed in detail. Results showed that Cd is closely related to Zn, Co, Cu and Mg; and As is correlated with Fe in the roots and nodes. The results indicated that the transport mechanisms of Cd were similar to those of Cu, Zn, Co and Mg, and the transport mechanisms of As were similar to those of Fe in the roots and nodes.

To summarize, the plant ionome provided detailed information of element interactions. Plant ionome also revealed that Cd is closely correlated with Zn/Cu/Co/Mg; and Fe correlated with As.

4.3. Ionomics analysis can reflect the relationship of different plant parts

The significant differences in ionome of various plant parts were observed in this study and similar results have been found as well by Baxter et al. [39]. In addition, the PCA results of the distribution of all the elements in 14 parts showed that the different parts of an organ were closely related. The overall spatial distribution of different organs was basically consistent with the growth morphology of the rice plant (Fig. 5a). Thus, ionomics can reflect tissue specificity and correlation between different parts of plants. Elements were absorbed into the aboveground parts from the roots, and first went through the nodes and were redistributed. Then a portion entered into the

internodes and the husk and brown rice, whereas a portion entered into the leaves. The results suggested that the nodes did not only act as key barrier of element transport but also might play a role in elemental distribution. Yamaji et al. [25,62,63] indicated that the node was a hub for preferential distribution of mineral elements. The results of factor loading showed that factor 1 contains 10 elements, namely, Cd, As, Cu, Zn, Co, Fe, Sb, Se, Pb, and Cr accounting for 49.9% of mutation (Fig. 5b). The results further indicated that the node was a hub of the distribution and transport of Cd and As, as well as the elements related to Cd and As. To find out the correlation between the Cd/As content of grain and that of other plant parts, the correlation between them was analyzed (Fig. S3). The results showed that the Cd/As content of grain was correlated with that of nodes and internodes, but not correlated with that of root.

This study also showed that different leaves or different internodes were relatively close, whereas different nodes were distantly seated (Fig. 5a). This finding indicated that differences of the ionomic among the nodes were more remarkable than those among the internodes or leaves. Thus, further study on ionomic of different nodes is highly significant. PN exhibited the most dense elemental correlation network in all parts of the rice plant (Fig. 4). The reason is probably because PN is a join node connecting the stem and panicle.

In addition, the Cd and As difference in brown rice among different genotypes is not remarkably distinguished probably as a result of heavily genetic structure of the lines we tested. C3 and C9 may be used to remediate Cd contaminated soil because of higher Cd concentration in stem than other rice genotypes. And C11 may be used to

remediate As contaminated soil. The ionome difference in brown rice and NI among different genotypes indicated that the ionome in a part of rice may be used to characterize a genotype.

5. Conclusions

The roots and nodes were key barriers to Cd and As transport into brown rice. Significant differences were found between nodes in the accumulation and barrier capacities of Cd and As. Particularly, the highest content of Cd in NI compared with other nodes induced an increase in gene expression of molecules and enzymes involved in glycolytic reactions and Cd detoxification. Ionomic profile revealed that Cd might be closely linked with Cu, Zn, Co and Mg, and As might be closely linked with Fe in uptake or translocation mechanism. Ionomics can also be used to reflect tissue specificity and correlation between different plant parts, as well as to verify that nodes are a hub for the distribution and transport of Cd, As, and most of other elements.

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Figure captions

Figure. 1. Images showing the positions of samples in a rice plant collected for mineral concentration analysis.

Figure. 2. Ionomic profile in different tissues of 21 Chinese rice lines subjected to variable cadmium (Cd), arsenic (As), sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), iron (Fe), manganese (Mn), chromium (Cr), cobalt (Co), copper (Cu), zinc (Zn), selenium (Se), molybdenum (Mo), antimony (Sb), lead (Pb) concentrations. Cd (a), As (b), Na (c), Mg (d), K (e), Ca (f), Cr (g), Fe (h), Mn (i), Co (j), Cu (k), Zn (l), Se (m), Mo (n), Sb (o), Pb (p) concentrations are expressed per unit dry weight of tissue in brown rice (BR), husk (H), panicle node (PN), node I (NI), node II (NII), node III (NIII), inter node I (INI), inter node II (INII), inter node III (INIII), inter node

IV (INIV), flag leaf (LI), 2nd leaf (LII), 3rd leaf (LIII) and root (R). The different letters denote values significantly different at $P < 0.05$ by Duncan's test.

Figure. 3. Principal component analysis results from the ionomes of 21 rice genotypes. Cadmium and arsenic-related groups of elements (circled and denoted I and II) showed strong positive correlations.

Figure. 4. Mineral correlation analyses. Pearson's correlation analysis of 16 mineral concentrations in PN (a), NI (b), NII (c), NIII (d) and R (e) of all genotypes of rice. Only significant correlations are displayed in the correlations wheels ($P < 0.05$). Positive correlations are denoted by black lines, negative correlations are denoted by red lines. Thick solid lines indicate $R > 0.7$, thin solid lines indicate $0.4 \leq R \leq 0.7$, dotted lines indicate $R < 0.4$.

Figure. 5. Principal component analysis of element concentrations in various organs of rice (a) and the loadings of elements to the first principal component (PC1) and the second principal component (PC2) (b).

Figure. S1. Mineral correlation analyses. Pearson's correlation analysis of 16 mineral concentrations in BR (f), H (g), INI (h), INII (i), INIII (j), INIV (k), LI (l), LII (m), LIII (n) of all cultivars of rice. Correlations wheels only display significant correlations ($P < 0.05$). Positive correlations are denoted by black lines, negative correlations are denoted by red lines. Thick solid line indicate $R > 0.7$, thin solid line indicate $0.4 \leq R \leq 0.7$, dotted line indicate $R < 0.4$.

Figure. S2. Validation of the differentially expressed genes by qRT-PCR.

Figure. S3 The relationship of different plant parts. Pearson's correlation analysis of

14 plant parts in Cd and As, respectively. Only significant correlations are displayed in the correlations wheels ($P<0.05$). Positive correlations are denoted by black lines, negative correlations are denoted by red lines. Thick solid lines indicate $R>0.7$, thin solid lines indicate $0.4\leq R\leq 0.7$, dotted lines indicate $R<0.4$.

Figure. S4 Cd concentration and principal component analysis of 21 rice genotypes in different plant parts.

Figure. S5 As concentration and principal component analysis of 21 rice genotypes in different plant parts.

Figure. S6 In Brown Rice, the ion concentrations and principal component analysis of 21 rice genotypes.

Figure. S7 In Node I, the ion concentrations and principal component analysis of 21 rice genotypes.

Table 1 The basic chemical properties of the topsoil (0-20 cm) at the onset of the experiment.

Soil property	
Total Cd (mg kg^{-1})	1.45
Total As (mg kg^{-1})	53.46

Total Cr (mg kg ⁻¹)	22.64
Total Ni (mg kg ⁻¹)	9.72
Total Cu (mg kg ⁻¹)	12.34
Total Zn (mg kg ⁻¹)	95.57
Total Pb (mg kg ⁻¹)	80.36
Total N (g kg ⁻¹)	0.96
Total P (g kg ⁻¹)	0.58
Total K (g kg ⁻¹)	19.60(4.3)
Organic matter (g kg ⁻¹)	34.57
Cation exchange capacity (CEC) (cmol kg ⁻¹)	14.85
pH (H ₂ O)	6.16
Particle size distribution ^a (wt %)	
Clay (%)	19.15
Silt (%)	18.32
Sand (%)	62.53
Soil texture	Sandy silty loam

^a Sand, 0.02-2 mm; silt, 0.002-0.02 mm; clay, <0.002 mm.

Table 2 List of differentially expressed genes that in response to cadmium and arsenic among the PN, NI and NII.

Gene ID	Gene Description	Fold change		
		NI vs PN	NI vs NII	PN vs NII
Response to cadmium ion				
BGIOSGA000301	Aldolase-type TIM barrel	2.24	2.32	—
BGIOSGA004775	Aldolase-type TIM barrel, Triosephosphate isomerase	2.33	3.59	—
BGIOSGA006005	Myb domain	4.58	9.30	—
BGIOSGA011768	Glyceraldehyde 3-phosphate dehydrogenase, NAD(P)-binding domain	2.09	4.16	—
BGIOSGA012770	Trehalose-phosphatase	5.14	7.06	—
BGIOSGA020330	Aldolase-type TIM barrel, Glutamate synthase, NADH/NADPH, small subunit 1, Alpha-helical ferredoxin	7.34	30.38	—
BGIOSGA021949	Cysteine-rich transmembrane CYSTM domain	2.06	2.15	—
BGIOSGA025216	Glutathione S-transferase	2.60	2.76	—
BGIOSGA033319	Glutathione S-transferase	2.00	3.05	—
BGIOSGA034309	Alcohol dehydrogenase, NAD(P)-binding domain	4.76	5.52	—
BGIOSGA034312	Alcohol dehydrogenase, NAD(P)-binding domain	3.81	4.35	—
BGIOSGA034421	Aldolase-type TIM barrel, Fructose-bisphosphate aldolase class-I active site	2.89	2.86	—
BGIOSGA011066	Protein kinase, ATP binding site	2.03	—	—
BGIOSGA028653	Trehalose-phosphatase	2.17	—	—
BGIOSGA031524	Phospholipase D, C2 calcium-dependent membrane targeting	2.30	—	—
BGIOSGA005446	Leucine aminopeptidase	—	2.04	—
BGIOSGA008564	Glyceraldehyde 3-phosphate dehydrogenase, NAD(P)-binding domain	—	2.14	—
BGIOSGA009095	Metalloenzyme	—	2.46	—
BGIOSGA012837	Thioredoxin-like fold	—	2.07	—
BGIOSGA019844	Aldolase-type TIM barrel	—	2.17	—
BGIOSGA031510	Glutathione S-transferase	—	32.10	—

BGIOGA020330	Aldolase-type TIM barrel, Glutamate synthase, NADH/NADPH, small subunit 1, Alpha-helical ferredoxin	—	—	4.11
BGIOGA005809	Proton-dependent oligopeptide transporter family	-34.75	-24.48	—
BGIOGA009847	Cystathionine beta-synthase	-2.12	-2.00	—
BGIOGA019170	Sucrose-6-phosphate phosphohydrolase	-3.48	-5.40	—
BGIOGA020897	Pyridoxal phosphate-dependent transferase	-2.83	-2.06	—
BGIOGA021074	Pyridoxal phosphate-dependent transferase	-2.34	-2.35	—
BGIOGA023965	Translation elongation factor	-2.01	-2.36	—
BGIOGA025754	unknown	-2.36	-2.20	—
BGIOGA033338	Glutathione S-transferase	-6.10	-5.32	—
BGIOGA002548	1-deoxy-D-xylulose 5-phosphate reductoisomerase	-2.02	—	—
BGIOGA004222	ATPase domain	-2.26	—	—
BGIOGA004313	Pyridoxal phosphate-dependent transferase	-2.10	—	—
BGIOGA010570	Sucrose synthase	-2.50	—	—
BGIOGA011282	Serine acetyltransferase	-3.78	—	—
BGIOGA012836	Thioredoxin-like fold	-2.38	—	—
BGIOGA015619	Cysteine synthase	—	-2.80	—
BGIOGA016314	Aminoacyl-tRNA synthetase	—	-2.21	—
BGIOGA019833	Chitin-binding, type 1, Glycoside hydrolase, family 19	—	-26.40	—
BGIOGA023636	Catalase, mono-functional, haem-containing, Catalase core domain	—	-2.25	—
BGIOGA006504	Butirosin biosynthesis	—	—	-2.14
Response to arsenic-containing substance				
BGIOGA031561	Major intrinsic protein, Aquaporin-like	-6.18	-5.19	—
BGIOGA009906	Signal transduction histidine kinase	-2.61	-2.95	—

* Corrected *P*-value of 0.005 and log₂ (Fold change) of 1 were set as the threshold for significantly differential expression. “—” stands for not

reached differentially expres

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