

# Transcriptome analysis reveals the roles of stem nodes in cadmium transport to rice grain

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## Research article

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# Abstract

**Background:** Node is the central organ of xylem to phloem transfer of nutrients and ions in plants. Cadmium (Cd)-induced crop pollution threatens food safety. Breeding cultivar with low Cd accumulation is a chance to resolve this universal problem. This study was performed to identify tissue specific genes involved in Cd accumulation in different rice stem nodes. Panicle node and the first node under panicle (node I) were sampled in two rice cultivars: Xiangwanxian No. 12 with low Cd accumulation and Yuzhenxiang with high Cd accumulation in the grains. RNA-seq analysis was performed to identify differentially expressed genes (DEGs) and microRNAs.

**Results:** Xiangwanxian No. 12 had lower Cd concentration in panicle node, node I and grain compared with Yuzhenxiang, and node I had the highest Cd concentration in the two cultivars. RNA seq analysis identified 4,535 differentially expressed genes and 70 miRNAs between the two cultivars. Most genes (OsIRT1, OsNramp5, OsVIT2, OsNRT1.5A, and OsABCC1) related to the “transporter activity” blocked the transport of Cd up to panicle and accumulation in grains of low Cd-accumulative cultivar. Among the genes related to “response to stimulus”, we identified OsHSP70 and OsHSFA2d/B2c in “X”, but not in “Y”, were all down-regulated by Cd stimulus. The up-regulation of miRNAs (osa-miR528 and osa-miR408) played a potent role in lowering Cd accumulation via down regulation of genes, such as bZIP, ERF, MYB, SnRK1 and HSPs in Xiangwanxian No. 12 cultivar.

**Conclusions:** Both panicle node and node I of Xiangwanxian No. 12 played a key role in blocking the upward transportation of Cd, while node I played a critical role in Yuzhenxiang. Distinct expression patterns of various transporter genes such as OsNRT1.5A, OsNramp5, OsIRT1, OsVIT2 and OsABCC1 resulted in differential Cd accumulation in different nodes. Likewise, distinct expression patterns of these transporter genes are likely responsible for the low Cd accumulation in Xiangwanxian No. 12 cultivar. MiRNAs drove multiple transcription factors, such as OsbZIPs, OsERFs, OsMYBs, to play a role in stress response, which contribute to the response to Cd stress in rice.

## Background

Rice (*Oryza sativa*) is one of the largest food crops in China, accounting for 60% of basic food supply. In recent years, an increasing area of rice fields in the farmland ecosystem in China has been contaminated by heavy metal cadmium (Cd). In 2010s, the reduction of annual grain production by heavy metal pollution is about 100 billion tons [1]. The Cd pollution has caused an irreversible and difficult problem in rice production in China, especially in the southern regions. Physical, chemical and phytoremediation strategies have been widely used to treat Cd-contaminated soils, but little was recovered due to technical difficulties or high costs. Therefore, it remains an urgent issue in solving the problem of Cd pollution.

Plants have evolved a plethora of genetic and metabolic mechanisms against Cd stresses. The Cd accumulation capacity in different rice varieties varies greatly [2, 3]. One of the possible solutions for alleviating Cd contamination in rice is to cultivate varieties with less Cd transported to the grains, while

retaining most Cd in the shoot or root. The use of molecular and transgenic technologies coupled with next-generation sequencing (NGS) could facilitate the identification of genes and mechanisms potentially involved in the translocation, detoxification, immobilization, and allocation of Cd in different species and cultivars [4–7]. Recently, some genes involved in Cd uptake, transport and accumulation have been identified and used as targets of genetic manipulation [8–10]. Zhang et al. (2010) found that the *BanCn.ABCC3* played a pivotal role in Cd resistance in rapeseed by blocking Cd transport to seeds and retaining Cd in the root pectin and shoot vacuoles [5]. Luo et al. (2019) found that the loss-of-function mutation of *Arabidopsis PLANT DEFENSIN 2 (AtPDF2.5)* reduced Cd accumulation and enhanced Cd resistance in *Arabidopsis* root by chelating Cd [4]. In addition, *CAL 1* also plays a role in Cd transport, by chelating Cd in the cytoplasm and facilitating Cd secretion to extracellular space [11]. However, the limited number of genes is insufficient to fully understand the biological processes of Cd transport and accumulation in plants.

Studies have shown that node is a pivotal location for nutrient distribution in graminaceous plants [12]. Both root and node are key barriers to Cd transport into rice grains [13]. Node is a central organ for xylem-to-phloem transfer of nutrients, ions, and metabolites [12]. It plays a vital role in Cd transfer from soil to grains [14]. Genetic manipulation of the transporters in stem-portion might prevent the distribution of toxic heavy metals like Cd into grains [15]. Feng et al. (2017) reported that the Cd concentration and transcriptome profiles in node I (first node under panicle) and panicle node were distinct [13]. They showed that node I had higher capacity in Cd sequestration and detoxification, and node I had higher expression of genes associated with glycolysis and detoxification. Fujimaki et al. (2010) found that Cd accumulated most intensively in rice nodes [14]. These results indicate multifaceted roles of plant nodes in Cd accumulation and detoxification. However, very little is known about how differentially expressed genes control the accumulation and transport of Cd in rice nodes.

A rice variety “Xiangwanxian No. 12” with low Cd-accumulation and “Yuzhenxiang” with high Cd accumulation in the grains were identified in our previous work. In this study, we performed deep sequencing to identify differentially expressed genes (DEGs) and miRNAs (DEmiRNAs) between node I and panicle node from the two cultivars with and without-Cd stress. Through bioinformatics analysis, key candidate genes, miRNAs, and biological processes in response to Cd stress were deciphered. These results are useful in the future elucidation of the molecular mechanisms of Cd-accumulation and transport to rice grains.

## Results

# Cd accumulation during Cd-stress

Under the control condition, node I (as “N”) in the two cultivars (21.05 mg/kg DW in “Yuzhenxiang” as “y” and 10.25 mg/kg DW in “Xiangwanxian No. 12” as “X”) had higher Cd accumulation compared with panicle node (as “P”) (2.17 mg/kg DW in “y” and 1.40 mg/kg DW in “X”;  $p < 0.01$ , Fig. 1). The Cd treatment increased the Cd accumulation in all tissues, especially that in the node I (56.43 mg/kg DW in “y” and

44.25 mg/kg DW in “X”). Grains of “X” cultivar had lower Cd accumulation (both in control and Cd treatment) than that in “y” cultivar. These data confirmed that “y” was a high Cd accumulation cultivar, and node I had higher capacity in Cd sequestration.

## Summary of the mRNA-seq and miRNA-seq

Twenty-four cDNA libraries were constructed and a total of 1111.34 M clean reads, with 89.80% average mapping rate (76.45%–92.37%), to the rice reference genome (Additional file 2: Table S1). Principle component analysis (PCA) (Fig. 2A) and sample-to-sample clustering analysis (Fig. 2B) showed that the samples of the same tissue (“P” or “N”) from the same cultivar of control (“C”) and Cd treatment (“T”) were clustered together. Twenty-four small cRNA libraries generated total 350.83 M clean reads (112.57 M unique reads) with an averaged mapping rate of 82.01% to the *O. sativa* reference miRNA in miRbase ([http://www.mirbase.org/cgi-bin/mirna\\_summary.pl?org=osa](http://www.mirbase.org/cgi-bin/mirna_summary.pl?org=osa)). The alignment rate in miRBase of each sample sequenced ranged from 0.62% to 2.37% (average 1.49%, Additional file 2: Table S1). PCA (Fig. 2C) and sample-to-sample clustering analysis (Fig. 2D) showed that samples of the same tissue (“P” or “N”) were grouped together.

## DEGs between high and low Cd accumulation cultivars

A total of 4,535 DEGs were identified by pairwise comparison of Cd-treated (T) vs. untreated control (C) of node I (N) and panicle node (P) in “X” and “y” cultivar, respectively (Fig. 3A-B and Table 1). The results showed that the number of down-regulated genes in “X” cultivar was greater than that in “y” cultivar. GO and KEGG enrichment based on these down regulated genes were performed to identify the main biological processes. The results showed that GO terms of “transporter activity” and “response to stimulus” were significantly enriched in “X”. By contrast, only a few genes were differentially expressed in these two GO terms in “y” cultivar (Fig. 4A). Results of hierarchical clustering analysis of the 84 common DEGs, including 69 genes down regulated after Cd treatment in “X”, showed an enrichment in “transporter activity” (Fig. 4B and Additional file 3: Table S2), whereas another 74 common DEGs, including 62 genes down regulated after Cd treatment in “X”, showed an enrichment related to “response to stimulus” (Fig. 4C and Additional file 4: Table S3). Notably, most of the DEGs in “X” were down-regulated by Cd treatment, and most down-regulated genes in “X” by Cd treatment were unchanged in “y”, especially for the DEGs associated with “response to stimulus” (Fig. 4C).

Among all the DEGs related to “transporter activity”, the iron-regulated transporter 1 (*IRT1*, OS03G0667500) and metal transporter Nramp5 (natural resistance-associated macrophage protein 5, Mg<sup>2+</sup> and Cd transporter, Mn and Cd uptake protein, OS07G0257200) were noteworthy for they had higher expression in the panicle node compared with node I (Additional file 3: Table S2), which indicated that Cd treatment increased *OsIRT1* and *OsNramp5* in the panicle node of the two cultivar. For node I,

*OsIRT1* and *OsNramp5* increased in “X”, while decreased in “y” cultivar (Additional file 1: Fig. S1 A). Although the expression of *OsIRT1* and *OsNramp5* increased after Cd treatment, overall it remained at a low level in “X” cultivar.

*OsNRT1.5A* (OS02G0689900, nitrate transporter 1.5A) and *OsVIT2* (OS09G0396900, Vacuolar Iron Transporter 2) played a key role in the upward transport of Cd. *OsNRT1.5A* showed a higher expression level in node I than panicle node in both cultivars. In “X” cultivar with Cd treatment, *OsNRT1.5A* was up- and down-regulated in node I and panicle node, respectively. For the expression of *OsNRT1.5A* in “y” cultivar, it was up regulated in panicle node. *OsVIT2* was up regulated following Cd treatment in node I in both cultivars, but it was down regulated in panicle node in the “X” cultivar. Cd stress had little effects on the expression level of *OsVIT2* in panicle node in “y” cultivar (Additional file 1: Fig. S1A and Additional file 3: Table S2). With Cd treatment, 6 aquaporin genes (PIPs) (OS02G0629200, OS04G0233400, OS02G0666200, OS04G0559700, OS07G0448100 and OS07G0448800) showed similar expression profiles (Additional file 1: Fig. S1B). The expression of 6 *PIPs* members was higher in “X” than in “y” cultivar under control condition, and down regulated after Cd treatment in “X”, but with little changes in “y”. The expression of *PIPs* did not show significant difference between node I and panicle node in two rice cultivars.

Among the DEGs related to “response to stimulus”, heat shock transcription factor (*HSF*) *A2d/B2c* genes (including OS03G0161900/OS09G0526600), light-harvesting chlorophyll a-b binding protein (*LHC-II*) genes (e.g. OS02G0197600), and genes encoding heat shock protein (*HSP71.1/70/20*; including OS03G0276500, OS01G0840100 and OS06G0253100) showed similar changes in the panicle node and node I of the two rice cultivars (Additional file 4: Table S3). Higher expression levels of aforementioned genes were found in “X” than in “y” cultivar (Additional file 1: Fig. S1C). Cd treatment decreased the expression of all these genes in the “X” cultivar, but increased *OsHSF-A2d/B2c*, *OsLHC-II* and *OsHSP71.1* in the panicle node of “y” cultivar. The distinct expression profiles and responses of aforementioned genes to Cd treatment between the two rice cultivars are likely in part account for the differential Cd accumulation.

## Expression of known Cd-responsive genes

In order to decipher the Cd-stress responses in panicle node and node I, we analyzed the expression profiles of 52 Cd-responsive genes previously reported in the literature. Among these genes associated with Cd-stress in rice, including metallothionein 1 (*OsMT1*) [16], cadmium tolerant 1 (*OsCDT1*), *OsCDT2* [17, 18], *OsMTP1* [7], cation diffusion facilitator (*OsCDF1*), ATP-binding cassette transporter multidrug resistance protein 1 (*OsMRP1/ABCC1*) [19, 20] showed higher expression levels in the two rice cultivars. Furthermore, they expressed higher in the panicle node of the two cultivars compared with node I (Fig. 5). Cd enhanced the expression of *OsCDT1* and *OsABCC1* in the node I of “X” cultivar. Another gene serine hydroxymethyltransferase 1 (*OsSHM1*), which showed relatively higher expression level, was down regulated only in the panicle node of “X” cultivar by Cd stimulus (Fig. 5). In addition, the nicotinamine

synthase 3 (*OsNAS3*) gene and dense and erect panicle 1 (*OsDEP1*) genes, which had low expression levels, showed relatively high expression in node I of the two cultivars. Cd treatment enhanced the expression of *OsNAS3* in node I and that of *OsMT1d* in both nodes (Fig. 5). Other Cd-responsive genes including *OsMT1f*, *OsYSL15*, *OsIRT2*, and *OsGST4* had low expression levels in the two cultivars and were found Cd unresponsive in our study.

## Identification of differentially expressed DE miRNAs

A total of 70 non-overlapping DE miRNAs were identified from panicle node and node I in the two rice cultivars (Fig. 6A and Additional file 5: Table S4). Most DE miRNAs were up-regulated by Cd treatment. There were 12 common DE miRNAs among all the pairwise comparisons (Fig. 6B), of which six miRNAs (*osa-miR398b*, *osa-miR408-3p*, *osa-miR408-5p*, *osa-miR528-5p*, *osa-miR528-3p* and *novel50\_mature*) were up-regulated by Cd treatment (Fig. 6C). In addition, only one common DE miRNA (*osa-miR528-3p*) was identified as Cd up-regulated in the panicle node of both cultivars. There were six common DE miRNAs (*osa-miR408-3p*, *osa-miR408-5p*, *osa-miR528-5p*, *osa-miR398b*, *osa-miR166-5p* and *novel50\_mature*) in node I of both cultivars, among which, only *osa-miR166-5p* showed a different expression pattern in node I of “X” cultivar and panicle node of “y” cultivar. In order to reveal the differential expression profiles of microRNAs more comprehensively, we screened the other miRNA family members (how you select these members) (Fig. 6D). *Osa-miR398b*, *osa-miR408-3p*, *osa-miR528-3p* and *osa-miR528-5p* in panicle node showed a higher expression level than node I with Cd treatment. We then constructed the DE miRNA-mRNA regulatory network (Fig. 7) based on the 15 miRNA (12 common and 3 family members) and the targets among the down-regulated DEGs. In the regulatory network, a HSP member (OS06G0253100) was regulated by two miRNAs including *osa-miR528-5p* and *osa-miR5493*. *OsMYB5P* (OS02G0624300), *OsbZIP18* (Os02g0203000), and *OsERF141* (Os02g0638650) were regulated by *osa-miR528-5p* and *novel13\_mature*, indirectly. Another bZIP member *OsbZIP23* (Os02g0766700) was the target gene of *osa-miR1846a/b/c-5p*; SNF1-related protein kinase 1 subfamily protein (SnRK) gene (OS02G0178000, *OsSnRK1*) was regulated by *osa-miR528-3p*. In addition, *OsAAE3* (OS04G0683700) was regulated by both *osa-miR408-3p* and *novel50\_mature* (Fig. 7 and Additional file 1: Fig. S1D).

## qRT-PCR verification of RNA-seq data

A total of 14 genes were selected for qRT-PCR analysis. The fold-changes found in qRT-PCR and RNA-seq were highly consistent, and the correlation coefficient was 0.6 (Fig. 8).

## Discussion

Cd has serious influences on photosynthesis [21, 22], respiration [23], nutrient metabolism, distribution and ion transport in plants [8, 24–26]. Identification of rice cultivars with low Cd accumulation in the

grains is of highly theoretical and practical significant in rice breeding. Our present study confirmed that the node I of rice plant had a high capacity in Cd sequestration and accumulation. Results of our RNA-seq analyses indicated that different capacities in Cd accumulation between node I and panicle node were mediated by differential gene expression in different rice cultivars.

## Barring Cd transport into rice grains

The high Cd accumulation in the nodes and roots of rice has been reported by Feng et al [13]. Node is the central organ of xylem to phloem transport of nutrients, ions, and metabolites [12]. It plays a vital role in Cd transport from soil to grains [14]. Previous reports showed that the accumulation of heavy metals gradually decreased in successive nodes [13, 27–29]. Cd is transported upward and accumulated in nodes, distributed in the xylem elliptical vascular bundles and the surrounding parenchyma cell bridges [14, 27]. In the present study, the accumulation of Cd in nodes is obvious, consistent with a previous report [14]. But the content of Cd in different nodes is different, so the roles of different nodes in blocking the upward transport of Cd are distinct. The high Cd content in node I indicated that most Cd was blocked in node I during upward transport. The Cd transport was subsequently blocked in panicle node, although to a lesser extent. Therefore, it appears that the upward transport of Cd decreases step by step from node I to panicle node, so the concerted effect of the two nodes is important for the interception of Cd in rice stem.

## Key genes mediating the Cd transport and accumulation in rice node I and panicle node

Using the transcriptome data, we identified several key genes might be responsible for the Cd accumulation in node I and panicle node of rice plant. For panicle node, compared with the high Cd-accumulation cultivar “y”, low Cd-accumulation cultivar “X” had lower expression of *OsIRT1* and *OsNramp5*, but higher expression of *OsVIT2* and *OsNRT1.5A*. *Nramp5* is an Mg and Cd transporter as well as Mn and Cd uptake protein. The uptake of Cd into the root cells is primarily mediated by *OsNramp5*, which showed higher transport activity than its counterpart in wheat or maize [30–32]. The knockout or loss-of-function mutation of *OsNramp5* dramatically reduced the accumulation of Cd and Mn without compromising yield [33, 34]. Compared with *OsNramp5*, *OsIRT1* has a relatively small contribution to Cd uptake [35]. Cd stress reduced the expression of *OsNramp5* and *OsIRT1* in node I of “y”, which indicated that the Cd intake capacity faded. As for “X”, *OsNramp5* and *OsIRT1* were up-regulated in both node I and panicle node after Cd treatment, of which the expression still at a low level, although they had increased. This indicated that the response patterns of *OsNramp5* and *OsIRT1* to Cd stress were distinct between “X” and “y”, especially in node I. It is likely that “X” cultivar reduces Cd intake by maintaining a low expression level of *OsNramp5* and *OsIRT1* in both node I and panicle node, while “y” cultivar blocked Cd uptake mainly by reducing *OsNramp5* expression in node I.

*VIT2* regulates metal sequestration into vacuoles. *VIT2* is up-regulated when excessive metals are available in the environment. The enhanced *VIT2* expression consequently leads to higher vacuolar sequestration capacity and metal accumulation in vacuoles [36–38]. Similar to *OsVIT2*, *OsABCC1* can concentrate heavy metal ions in vacuoles and prevent it from upward transport to the grains [5, 20, 39]. Our transcriptome data showed that *OsVIT2* was up- and down-regulated in node I and panicle node in “X” cultivar, respectively. As for “y”, there were little changes in the expression of *OsVIT2*. In particular, the expression of *OsVIT2* in node I of “y” maintained a higher level than “X”, which might explain the high content of Cd in “yN”. In addition, the expression of *OsABCC1* was induced by Cd treatment in “X”, but not in “y” cultivar (Fig. 5). The enhanced expression of *OsABCC1* might contribute to the relatively low Cd accumulation in grains of “X” cultivar.

*NRT1.5A* mediated nitrate distribution plays a role in plant tolerance to Cd stress [40]. The  $\text{NO}_3^-$  uptake negatively affects Cd uptake in plant roots [41, 42]. In our results, the expression pattern of *OsNRT1.5A* was completely different in different nodes. The expression of *OsNRT1.5A* in node I was several times higher than that in panicle node, which implicated that *OsNRT1.5A* mainly played a role in node I. Cd treatment enhanced *OsNRT1.5A* expression in node I in “X” cultivar, but not in “y” cultivar, which revealed a differential response in the two rice cultivars. Because NRT1 negatively regulates the uptake of Cd and other cations by simultaneously uptake of  $\text{NO}_3^-$  in Arabidopsis [41], our results could be explained by the same inference, as high expression of *OsNRT1.5A* would suppress Cd uptake in node I in “X” cultivar.

The concerted expression of aforementioned genes is likely to have a large impact on Cd accumulation in rice nodes and grains. The proposed mechanism is shown in Fig. 9.

## Key genes responded to the Cd stimulus in node I and panicle node

Among the DEGs related to “response to stimulus”, genes encoding *OsHSFA2d/B2c*, *OsLHC-II*, and *OsHSPs* (including *OsHSP70* and *OsHSP20.0*) showed higher expression in the low Cd-accumulation “X” compared with high Cd-accumulation “y” cultivar. The expression of all these genes was significantly reduced by Cd treatment in “X”, but showed small or undetectable changes in “y” cultivar, indicating a relative insensitivity of “y” cultivar to Cd stress. HSF and HSPs (large or small) play critical roles in plant immunity, growth, defense, and stress responses [43–46]. In rice, Cd-stress induces *HSPs* expression, in turns it inhibits Cd-induced damage in plant cells [47]. It has been reported that overexpression of a small HSP gene enhanced abiotic stress tolerance to heat, drought, abscisic acid, salinity and cold in rice [48]. Cai et al. (year) showed that silencing of *HSFA1a* in tomato plants blocked Cd stress induced *HSP* expression, while *HSFA1a* overexpression promoted *HSP* expression [46]. The unchanged expression of *HSFs* and *HSPs* indicated that “y” cultivar was insensitive to Cd stimulus. Likewise, the unchanged expression of *OsLHC-II* might indicate more stable photosynthesis, growth, and development of “y” cultivar under Cd stimulus. In addition, 6 aquaporin genes (*PIPs*) were expressed at higher levels in “X”

than that in “y” cultivar, and down regulated by Cd treatment only in “X”, but not in “y” cultivar. Heavy metal can cause water deficit in plants, which greatly affects plant growth and productivity. The expression of *PIPs* is closely related to heavy metal stress, with distinct expression patterns in different plant species [49–53]. Different reports have shown that heavy metals can trigger the closure of aquaporins due to their abilities to react with the S-H group of the protein [54, 55]. In “X” cultivar, the expression of aquaporin genes was repressed by Cd stress, but no significant changes in “y” cultivar. The decreased expression of aquaporin genes triggered by heavy metals was similar to what Kholodova et al. (2011) reported [51]. The decreased activities of aquaporins lead to low transpiration rate, under which the essential mass apoplastic water flow, determined mainly by the rate of transpiration, was replaced by predominantly cell-to-cell symplastic flow [56], which is regulated at the level of membrane water channels. Our results also indicated that there were significant differences between the two rice cultivars in response to Cd stress.

## Identification of miRNAs involved in Cd accumulation

Among the DEmiRNA related to Cd accumulation in rice node I, we identified that the *osa-miR408-3p*, *osa-miR528-3p* *osa-miR528-5p* were commonly up-regulated by Cd in both nodes of the two cultivars. The effect of *osa-miR528-3p* has not been studied till now. Both *osa-miR528* and *osa-miR408* family members were differentially expressed in response to abiotic stresses, such as drought [57], low temperature [58], heavy metal [59] and plant defense responses (the reference is about drought) [60]. Cheah et al. showed that several miRNAs including *osa-miR398*, *osa-miR397*, *osa-miR408-5p* and *osa-miR528-5p* were up-regulated in the drought-susceptible rice variety [57]. This was also true for in cool-tolerant rice (Hitomebore) under cool-temperature [58]. Our study showed that 4 transcription factors (TFs), *OsZIP18*, *OsZIP23*, *OsMYB5P* and *OsERF141*, were potential target genes of aforementioned miRNAs. WRKY, bZIP, ERP and MYB proteins play an important role in controlling the expression of their downstream genes in response to Cd stress[61]. The down regulation of *OsERFs* and *OsZIPs* was also found in another rice variety after Cd stress [62]. The unchanged expression of these four TFs further demonstrated that “y” cultivar was insensitive to Cd stress. SnRKs play an important role in plant biotic interaction in *Arabidopsis thaliana*. SnRK1 overexpression plants displayed enhanced resistance to geminivirus, while *SnRK1* silenced plants were more susceptible than the wild-type plants [63, 64]. The higher expression of *OsSnRK1* in the panicle node suggests that panicle node is the key part of “X” cultivar in response to Cd stress. In addition, *SnRKs* are the upstream regulatory genes of *HSPs* in ABA signal transduction pathway [65, 66]. Therefore, the expression changes of *HSPs* in panicle node of “X” cultivar may be due to the action of *OsSnRK1*.

## Conclusions

In this study, we have demonstrated that distinct Cd accumulation in the panicle node and node I in two different rice cultivars was mediated by differential gene expression. Both panicle node and node I of “X” cultivar played a key role in blocking the upward transportation of Cd, while only node I played a critical role in “y” cultivar. We have identified a cluster of candidate genes which might be responsible for Cd accumulation in panicle node and node I. Most of these genes (*OsIRT1*, *OsNramp5*, *OsVIT2*, *OsNRT1.5A*, and *OsABCC1*) are related to the “transporter activity”. The concerted action of these transporters could block the transport of Cd up to panicle and accumulation in the grains of low Cd-accumulation cultivar. Among the DEGs related to “response to stimulus”, we identified *OsHSP70* and *OsHSFA2d/B2c* down regulated by Cd in “X”, but not in “y” cultivar. MiRNAs including *osa-miR528* and *osa-miR408* family members which were up-regulated by Cd, showed potential roles in lowering Cd accumulation via regulating genes like *bZIP*, *ERF*, *MYB*, *SnRK1* and *HSPs*. The differential gene expression is likely responsible for the low Cd accumulation in “X” cultivar. These findings have provided novel insights into breeding new rice varieties with low Cd accumulation.

## Materials And Methods

### Plant materials and treatment

The seeds of two Chinese rice (*O. sativa*) cultivars “Xiangwanxian No. 12” (low Cd accumulation, “X” for short) and “Yuzhenxiang” (high Cd accumulation, “y” for short) were obtained from the germplasm resources bank of Hunan rice research institute. All seeds were sterilized with prochloraz, soaked in deionized water at room temperature (RT) for 48 hours, and germinated at 30°C for 24 hours, then sowed into the fields with normal standard conditions (15–22 °C for nighttime and 20–25 °C for daytime, without Cd stress) on 30<sup>th</sup> June in 2017. Seedlings with three true leaves were transplanted into pots (54 cm×41 cm×23 cm) with the density of 8 plants/4 holes/pot. Rice cultivars were sowed into the experimental pots full of muddy water without (control, “C” for short) or with CdCl<sub>2</sub>·2.5H<sub>2</sub>O (15 mg/Kg; treatment, “T” for short) [67]. Each cultivar was planted in 20 replications. Plants were managed with standard fertilization (compound fertilizer N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O 15:8:12, 3g/pot) was applied on July 5, 2017; urea was applied on August 16, 2017, 0.6g/pot) The panicle node (“P” for short) and node I (“N” for short) were collected at grain-filling stage on November 9, 2017. According to the treatment strategies and sampling positions, the group samples were named as XPC, XPT, yPC, yPT, XNC, XNT, yNC and yNT, where “X” notes “Xiangwanxian No. 12”, “y” notes “Yuzhenxiang”, “P” indicates panicle node, “N” indicates node I, “C” represents control and “T” represents Cd treatment. Liquid nitrogen flash frozen samples were stored at –80°C before the extraction of RNA. Each sample was pooled by tissues from 3–5 individuals, and three repetitive samples were prepared for RNA isolation.

### Determination of Cd concentration

The accumulation of Cd in rice “P” and “N” tissues and grain were determined by atomic absorption spectrophotometer (PerkinElmer PinAAcle 900T, USA). Tissues were drying in a drying box (105°C for 30 min and at 80°C to constant weight). 50 mg powder samples were immersed into 1 mL HNO<sub>3</sub> and digested to transparent solutions. Cooled samples were then diluted into water to a final volume of 13.5 mL. Standard Cd solution was used as quality control samples.

## RNA extraction and libraries construction

Total RNA was extracted from collected samples from two cultivars using TRIzol (Invitrogen, USA). The purification, qualification and quantification were conducted (DNase I, Invitrogen) by Agilent 2100 Bioanalyzer (Agilent Technologies, USA). For the preparation of the miRNA-seq, total RNA fragmentation (16–30 nt) was performed using fragmentation buffer (Ambion, USA), followed with purification, enrichment, ligation (with 3′ and 5′ RNA adapters) and PCR amplification. The finally purified amplification products were regarded small RNA libraries (n = 24) used for miRNA-seq. For the preparation of the mRNA-seq, total RNA was reverse transcribed to the first strand cDNA using SuperScript III reverse transcriptase (Invitrogen) with 6-base random primers. The DNA samples were used for the mRNA-seq library (n = 24) construction following the instruction from the mRNA-Seq Sample Preparation Kit (Illumina, USA). Illumina HiSeq 4000 sequencing platforms (pair-end 2 ×150 bp for mRNA-seq, and single-end 50 bp for miRNA-seq) were used for the sequencing analysis.

## Data processing

The raw sequencing data in the format of FastQ were quality-controlled using the FastQC (version 0.11.5, <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) by removing the low quality reads and adaptor reads.

## mRNA profiling

The clean reads assembled and aligned to the reference genome sequence ([http://rice.plantbiology.msu.edu/pub/data/Eukaryotic\\_Projects/o\\_sativa/](http://rice.plantbiology.msu.edu/pub/data/Eukaryotic_Projects/o_sativa/); IRGSP–1.0.28) was conducted using hisat2 [68]. The reads numbers were counted by htseq-count [69]. Cufflinks (version 2.2.1) [70] was used for the quantitative analysis of the reads by calculating the FPKM values (expected number of Fragments Per kb per Millions reads) of reads in each sample. Principal component analysis (PCA) and sample-to-sample clustering analysis were performed based on the FPKM of reads. The differentially expressed genes (DEGs) were identified using DESeq (<http://bioconductor.org/packages/release/bioc/html/DESeq.html>) [71] by pairwise comparison. DEGs were identified using the Negative binomial distribution test with the criteria of p value <0.05 and  $|\log_2(\text{Fold Change, FC})| \geq 1$ . Up- and down-regulated DEGs were identified as  $\log_2\text{FC} > 1$  and  $\log_2\text{FC} < -1$ ,

respectively. The expression profiles of DEGs were presented using hierarchical clustering by heatmap (version 1.0.10; <https://cran.r-project.org/web/packages/heatmap/index.html>).

## miRNA profile analysis

The clean reads generated by miRNA-seq were aligned with the reference genome sequence (*O. sativa*; [http://www.mirbase.org/cgi-bin/mirna\\_summary.pl?org=osa](http://www.mirbase.org/cgi-bin/mirna_summary.pl?org=osa)). Small RNA annotation and assignment (rRNA, snRNA, snoRNA and tRNA) were conducted in Rfam [72], cDNA sequencing, species repeat library [73] and miRBase [74, 75]. Small RNA sequences (>26 nt) were removed using Bowtie [76]. Sequences in the length of 15–26 nt and those unable to match the mRNA transcripts were used for the identification of known and novel miRNAs after removing the repetitive sequences. Novel miRNAs were identified using Mirdeep2 software [77] and RNAfold [78]. The expression of miRNAs were calculated using transcript per million (TPM). PCA and sample-to-sample clustering analyses were performed based on the expression levels. Differentially expressed miRNAs (DEmiRNAs) in response to Cd stimulus were identified using DESeq [71] by pairwise comparison methods, with the threshold of p value <0.05 and  $|\log_2(\text{Fold change, FC})| \geq 0.5$ . Subsequently, the predictive mRNAs of DEmiRNAs were identified using targetfinder [79]. The overlapping DEGs between DEmiRNAs' targets were used for further enrichment analysis.

## Enrichment analysis

The DEGs and overlapping DEGs between DEmiRNAs' targets were separately subjected to the enrichment analysis of Gene Ontology (GO; <http://www.Geneontology.org/>) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways [80]. GO categories (biological processes, BP; molecular functions, MFs; and cellular components, CCs) and KEGG pathways related to the DEGs were identified with the criterion of p <0.05. The miRNA-gene regulatory network was constructed using Cytoscape (version 2.8) [81].

## qRT-PCR verification of RNA-seq data

qRT-PCR analysis was performed on a Light Cycler system (Roche) using a SYBR Green PCR Kit (Qiagen). PCR amplification was performed under the following conditions: 94°C for 5 min, followed by 40 cycles of 94°C for 15 s, 58°C for 15 s, and 72°C for 20 s and a final extension at 72°C for 5 min. Quantification of gene expression was performed by the comparative  $2^{-\Delta\Delta C_T}$  method. The validation analysis was performed with three independent biological replicates. The gene-specific primers for qRT-PCR were designed using Primer Premier 5.0 (<http://www.PremierBiosoft.com>) and were synthesized by Invitrogen (Carlsbad, USA). The correlation analysis of qRT-PCR and RNA-seq were based on Pearson's correlation coefficient.

# Statistical analysis

All experimental data were expressed as mean  $\pm$  SD. Statistical analysis of all data was performed using the GraphPad Prism 6. Differences were analyzed using the unpaired t-test or one-way ANOVA.  $P < 0.05$  and  $p < 0.01$  was considered as significant and very significant difference, respectively.

## Abbreviations

Cd, cadmium; DEGs, differentially expressed genes; NGS, next-generation sequencing; “X”, Xiangwanxian No. 12; “y”, Yuzhenxiang; “N”, node I; “P”, panicle node; PCA, principle component analysis; C, control; T, Cd treatment; TFs, transcription factors; TPM, transcript per million; GO, Gene Ontology; BP, biological processes; MF, molecular functions; CC, cellular components; KEGG, Kyoto Encyclopedia of Genes and Genomes.

## Declarations

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## Authors' contributions

ZBZ planted rice materials, treated and sampled. YKY performed Cd concentration analysis. ALL designed the experiments, analyzed the results and wrote the manuscript. GHC guided this research. All authors approved the manuscript.

## Availability of data and materials

The datasets for this study can be found in the BIG SUB database (<https://bigd.big.ac.cn/gsub/>) with the access No. CRA001894 and CRA001895.

# Competing interests

The authors declare that they have no competing interests.

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## Additional files

*Additional file 1: Figure S1.* The expression level of candidate genes related to Cd transport. T, Cd-treatment; C, control; N, node I; P, panicle node; X, *Xiangwanxian No. 12*; y, *Yuzhenxiang*.

*Additional file 2: Table S1.* Statistics of the differentially expressed genes (DEGs) and miRNAs (DEmiRNAs) by different pairwise comparison.

*Additional file 3: Table S2.* The expression profiles of the 84 common differentially expressed genes associated with “transporter activity” in Fig. 4B

*Additional file 4: Table S3.* The list of 74 common differentially expressed genes related to “response to stimulus” in Fig. 4C.

*Additional file 5: Table S4.* The list of the differentially expressed miRNAs.

*Additional file 6: Table S5.* The sequences of primers of genes in qRT-PCR

## References

1. Wang C, Mo Z, Wang H, Wang Z, Cao Z: The transportation, time-dependent distribution of heavy metals in paddy crops. *Chemosphere* 2003, 50(6):717–723.
2. Wang M, Chen A, Wong MH, Qiu R, Cheng H, Ye Z: Cadmium accumulation in and tolerance of rice (*Oryza sativa* L.) varieties with different rates of radial oxygen loss. *Environmental Pollution* 2011, 159(6):1730–1736.
3. Afzal J, Hu C, Imtiaz M, Elyamine A, Rana M, Imran M, Farag M: Cadmium tolerance in rice cultivars associated with antioxidant enzymes activities and Fe/Zn concentrations. *International Journal of*

- Environmental Science and Technology 2018:1–12.
4. Luo JS, Yang Y, Gu T, Wu Z, Zhang Z: The Arabidopsis defensin gene AtPDF2. 5 mediates cadmium tolerance and accumulation. *Plant Cell Environment*. 2019:doi: 10.1111/pce.13592.
  5. Zhang ZH, Zhou T, Tang TL Song HX, Guan CY, Huang JY, Hua YP: Multiomics landscapes uncover the pivotal role of subcellular reallocation of cadmium in regulating rapeseed resistance to cadmium toxicity. *Journal of Experimental Botany* 2019, pii: erz295.
  6. Huang YY, Shen C, Chen JX, He CT, Zhou Q, Tan X, Yuan JG, Yang ZY: Comparative transcriptome analysis of two *Ipomoea aquatica* Forsk. cultivars targeted to explore possible mechanism of genotype-dependent accumulation of cadmium. *Journal of Agricultural and Food Chemistry* 2016, 64(25):5241–5250.
  7. Das N, Bhattacharya S, Maiti MK: Enhanced cadmium accumulation and tolerance in transgenic tobacco overexpressing rice metal tolerance protein gene OsMTP1 is promising for phytoremediation. *Plant Physiology and Biochemistry* 2016, 105:297–309.
  8. Uraguchi S, Fujiwara T: Cadmium transport and tolerance in rice: perspectives for reducing grain cadmium accumulation. *Rice* 2012, 5(1):5.
  9. Chiang HC, Lo JC, Yeh KC: Genes associated with heavy metal tolerance and accumulation in Zn/Cd hyperaccumulator *Arabidopsis halleri*: a genomic survey with cDNA microarray. *Environmental Science and Technology* 2006, 40(21):6792–6798.
  10. Ueno D, Yamaji N, Kono I, Huang CF, Ando T, Yano M, Ma JF: Gene limiting cadmium accumulation in rice. *Proceedings of the National Academy of Sciences of the United States of America* 2010, 107(38):16500–16505.
  11. Luo JS, Huang J, Zeng DL, Peng JS, Zhang GB, Ma HL, Guan Y, Yi HY, Fu YL, Han B, Lin HX, Qian Q, Gong JM: A defensin-like protein drives cadmium efflux and allocation in rice. *Nature Communication* 2018, 9(1):645.
  12. Yamaji N, Ma JF: The node, a hub for mineral nutrient distribution in graminaceous plants. *Trends in Plant Science* 2014, 19(9):556–563.
  13. Feng X, Han L, Chao D, Liu Y, Zhang Y, Wang R, Guo J, Feng R, Xu Y, Ding Y: Ionic and transcriptomic analysis provides new insight into the distribution and transport of cadmium and arsenic in rice. *Journal of Hazardous Materials* 2017, 331:246–256.
  14. Fujimaki S, Suzui N, Ishioka NS, Kawachi N, Ito S, Chino M, Nakamura SI: Tracing cadmium from culture to spikelet: noninvasive imaging and quantitative characterization of absorption, transport, and accumulation of cadmium in an intact rice plant. *Plant Physiology* 2010, 152(4):1796–1806.
  15. Yamaji N, Ma JF: Node-controlled allocation of mineral elements in Poaceae. *Current Opinion in Plant Biology* 2017, 39:18–24.
  16. Shahpiri A, Deljoonia R: Enhancement of catalase and superoxide dismutase activities in transgenic *Escherichia coli* expressing rice metallothionein isoforms. *Journal of BioScience and Biotechnology* 2018, 7(1):5–10.

17. Sebastian A, Prasad M: Exogenous citrate and malate alleviate cadmium stress in *Oryza sativa* L.: Probing role of cadmium localization and iron nutrition. *Ecotoxicology and Environmental Safety* 2018, 166:215–222.
18. Gu C, Liu L, Song A, Liu Z, Zhang Y, Huang S: *Iris lactea* var. *chinensis* (Fisch.) cysteine-rich gene *lICDT1* enhances cadmium tolerance in yeast cells and *Arabidopsis thaliana*. *Ecotoxicology and Environmental Safety* 2018, 157:67–72.
19. Deng F, Yu M, Martinoia E, Song WY: Ideal cereals with lower arsenic and cadmium by accurately enhancing vacuolar sequestration capacity. *Frontier in Genetics* 2019, 10:322.
20. Song WY, Yamaki T, Yamaji N, Ko D, Jung KH, Fujii-Kashino M, An G, Martinoia E, Lee Y, Ma JF: A rice ABC transporter, *OsABCC1*, reduces arsenic accumulation in the grain. *Proceedings of the National Academy of Sciences of the United States of America* 2014, 111(44):15699–15704.
21. Hasan SA, Hayat S, Ahmad A: Brassinosteroids protect photosynthetic machinery against the cadmium induced oxidative stress in two tomato cultivars. *Chemosphere* 2011, 84(10):1446–1451.
22. Farooq M, Ali S, Hameed A, Bharwana S, Rizwan M, Ishaque W, Farid M, Mahmood K, Iqbal Z: Cadmium stress in cotton seedlings: physiological, photosynthesis and oxidative damages alleviated by glycinebetaine. *South African Journal of Botany* 2016, 104:61–68.
23. Jia H, Wang X, Dou Y, Liu D, Si W, Fang H, Zhao C, Chen S, Xi J, Li J: Hydrogen sulfide-cysteine cycle system enhances cadmium tolerance through alleviating cadmium-induced oxidative stress and ion toxicity in *Arabidopsis* roots. *Scientific Report* 2016, 6:39702.
24. Gupta D, Pena LB, Romero-Puertas M, Hernández A, Inouhe M, Sandalio L: NADPH oxidases differentially regulate ROS metabolism and nutrient uptake under cadmium toxicity. *Plant Cell Environment* 2017, 40(4):509–526.
25. Parker SP, Bowden WB, Flinn MB, Giles CD, Arndt KA, Beneš JP, Jent DG: Effect of particle size and heterogeneity on sediment biofilm metabolism and nutrient uptake scaled using two approaches. *Ecosphere*. 2018, 9(3):e02137
26. Uraguchi S, Kamiya T, Sakamoto T, Kasai K, Sato Y, Nagamura Y, Yoshida A, Kyojuka J, Ishikawa S, Fujiwara T: Low-affinity cation transporter (*OsLCT1*) regulates cadmium transport into rice grains. *Proceedings of the National Academy of Sciences of the United States of America* 2011, 108(52):20959–20964.
27. Yamaguchi N, Ishikawa S, Abe T, Baba K, Arai T, Terada Y: Role of the node in controlling traffic of cadmium, zinc, and manganese in rice. *Journal of Experimental Botany* 2012, 63(7):2729–2737.
28. Shu F, Nobuo S, Ishioka NS, Naoki K, Sayuri I, Mitsuo C, Shin-Ichi N: Tracing cadmium from culture to spikelet: noninvasive imaging and quantitative characterization of absorption, transport, and accumulation of cadmium in an intact rice plant. *Plant Physiology* 2010, 152(4):1796–1806.
29. Baxter I: Should we treat the ionome as a combination of individual elements, or should we be deriving novel combined traits? *Journal of Experimental Botany* 2015, 66(8):2127–2131.
30. Tang L, Mao B, Li Y, Lv Q, Zhang LP, Chen C, He H, Wang W, Zeng X, Shao Y: Knockout of *OsNramp5* using the CRISPR/Cas9 system produces low Cd-accumulating indica rice without compromising

- yield. *Scientific Report* 2017, 7(1):14438.
31. Sui FQ, Chang JD, Tang Z, Liu WJ, Huang XY, Zhao FJ: Nramp5 expression and functionality likely explain higher cadmium uptake in rice than in wheat and maize. *Plant Soil* 2018, 433(1–2):377–389.
  32. Takahashi R, Ishimaru Y, Shimo H, Bashir K, Senoura T, Sugimoto K, Ono K, Suzui N, Kawachi N, Ishii S: From laboratory to field: OsNRAMP5-knockdown rice is a promising candidate for Cd phytoremediation in paddy fields. *Plos One* 2014, 9(6):e98816.
  33. Tang L, Mao B, Li Y, Lv Q, Zhang L, Chen C, He H, Wang W, Zeng X, Shao Y: Knockout of OsNramp5 using the CRISPR/Cas9 system produces low Cd-accumulating indica rice without compromising yield. *Scientific Report* 2017, 7(1):14438.
  34. Sasaki A, Yamaji N, Yokosho K, Jian FM: Nramp5 Is a Major Transporter Responsible for Manganese and Cadmium Uptake in Rice. *Plant Cell* 2012, 24(5):2155–2167.
  35. Takahashi R, Ishimaru Y, Senoura T, Shimo H, Ishikawa S, Arao T, Nakanishi H, Nishizawa NK: The OsNRAMP1 iron transporter is involved in Cd accumulation in rice. *Journal of Experimental Botany*. 2011, 62(14):4843–4850.
  36. Zhang Y, Xu YH, Yi H-Y, Gong JM: Vacuolar membrane transporters OsVIT1 and OsVIT2 modulate iron translocation between flag leaves and seeds in rice. *The Plant journal: for cell and molecular biology* 2012,72(3):400–410.
  37. Peng JS, Gong JM: Vacuolar Sequestration Capacity And Long-distance Metal Transport In Plants. *Frontier in Plant Science* 2014, 5:19.
  38. Connorton JM, Jones ER, Rodríguez-Ramiro I, Fairweather-Tait S, Uauy C, Balk J: Altering expression of a vacuolar iron transporter doubles iron content in white wheat flour. *bioRxiv* 2017:131888.
  39. Deng F, Yamaji N, Ma JF, Lee SK, Jeon JS, Martinoia E, Lee Y, Song WY: Engineering rice with lower grain arsenic. *Plant Biotechnology Journal* 2018, 16(10):1691–1699.
  40. Li JY, Fu YL, Pike SM, Bao J, Tian W, Zhang Y, Chen CZ, Zhang Y, Li HM, Huang J: The Arabidopsis Nitrate Transporter NRT1.8 Functions in Nitrate Removal from the Xylem Sap and Mediates Cadmium Tolerance. *Plant Cell*. 2010, 22(5):1633–1646.
  41. Mao QQ, Guan MY, Lu KX, Du ST, Fan SK, Ye YQ, Lin XY, Jin CW: Inhibition of Nitrate Transporter 1.1-Controlled Nitrate Uptake Reduces Cadmium Uptake in Arabidopsis. *Plant physiology* 2014, 166(2):933–934.
  42. Guan MY, Fan SK, Fang XZ, Jin CW: Modification of nitrate uptake pathway in plants affects the cadmium uptake by roots. *Plant Signal Behaviour* 2015, 10:e990794.
  43. Mcloughlin F, Basha E, Fowler ME, Kim M, Bordowitz J, Katiyar-Agarwal S, Vierling E: Class I and II small heat-shock proteins protect protein translation factors during heat stress. *Plant Physiology* 2016, 172(2):pp.00536.02016.
  44. Pandey S, Fartyal D, Agarwal A, Shukla T, James D, Kaul T, Negi YK, Arora S, Reddy MK: Abiotic Stress Tolerance in Plants: Myriad Roles of Ascorbate Peroxidase. *Frontier in Plant Science* 2017, 8.

45. Park CJ, Seo YS: Heat Shock Proteins: A Review of the Molecular Chaperones for Plant Immunity. *Plant Pathology Journal*. 2015, 31(4):323–333.
46. Cai SY, Zhang Y, Xu YP, Qi ZY, Li MQ, Ahammed GJ, Xia XJ, Shi K, Zhou YH, Reiter RJ: HsfA1a upregulates melatonin biosynthesis to confer cadmium tolerance in tomato plants. *Journal of Pineal Research* 2017, 62(2):e12387.
47. Reddy GN, Prasad M: Cadmium-induced protein phosphorylation changes in rice (*Oryza sativa* L.) seedlings. *Journal of Plant Physiology* 1995, 145(1–2):67–70.
48. Wang A, Yu X, Mao Y, Liu Y, Liu G, Liu Y, Niu X: Overexpression of a small heat-shock-protein gene enhances tolerance to abiotic stresses in rice. *Plant Breeding* 2015, 134(4):384–393.
49. Zhang Y, Wang Z, Chai TY, Zhensong W, Zhang H: Indian Mustard Aquaporin Improves Drought and Heavy-metal Resistance in Tobacco. *Molecular Biotechnology* 2008;40(3):280–292.
50. Przedpeńska-Wąsowicz EM, Wierzbicka M: Gating of aquaporins by heavy metals in *Allium cepa* L. epidermal cells. *Protoplasma* 2011,248(4):663–671.
51. Kholodova V, Volkov K, Abdeyeva A, Kuznetsov V: Water status in *Mesembryanthemum crystallinum* under heavy metal stress. *Environmental and Experimental Botany* 2011, 71(3):382–389.
52. Lin H, Fang C-X, Li Y, Lin W, He J, Lin R, Lin W: Cadmium-stress mitigation through gene expression of rice and silicon addition. *Plant Growth Regulation* 2017, 81(1):91–101.
53. Faraji J, Sepehri A: Titanium Dioxide Nanoparticles and Sodium Nitroprusside Alleviate the Adverse Effects of Cadmium Stress on Germination and Seedling Growth of Wheat (*Triticum aestivum* L.). *Universitas Scientiarum* 2018;23(1):208.
54. Kozono D, Yasui M, S King L, Agre P: Aquaporin water channels: Atomic structure and molecular dynamics meet clinical medicine. *The Journal of clinical investigation*. 2002, 11(109):1395–1399.
55. Wan X, Steudle E, Hartung W: Gating of water channels (aquaporins) in cortical cells of young corn roots by mechanical stimuli (pressure pulses): Effects of ABA and of HgCl<sub>2</sub>. *Journal of Experimental Botany* 2004, 55(396):411–322.
56. Steudle E: The cohesion-tension mechanism and the acquisition of water by plant roots. *Annual Review of Plant Physiology and Plant Molecular Biology* 2001, 52:847–875.
57. Cheah BH, Nadarajah K, Divate MD, Wickneswari R: Identification of four functionally important microRNA families with contrasting differential expression profiles between drought-tolerant and susceptible rice leaf at vegetative stage. *BMC Genomics* 2015, 16(1):692.
58. Maeda S, Sakazono S, Masuko-Suzuki H, Taguchi M, Yamamura K, Nagano K, Endo T, Saeki K, Osaka M, Nabemoto M: Comparative analysis of microRNA profiles of rice anthers between cool-sensitive and cool-tolerant cultivars under cool-temperature stress. *Genes & Genetic System* 2016:15–00056.
59. Sharma D, Tiwari M, Lakhwani D, Tripathi RD, Trivedi PK: Differential expression of microRNAs by arsenate and arsenite stress in natural accessions of rice. *Metallomics* 2015, 7(1):174–187.

60. Wei L, Zhang D, Xiang F, Zhang Z: Differentially expressed miRNAs potentially involved in the regulation of defense mechanism to drought stress in maize seedlings. *International Journal of Plant Sciences* 2009, 170(8):979–989.
61. DalCorso G, Farinati S, Furini A: Regulatory networks of cadmium stress in plants. *Plant Signal Behavior* 2010, 5(6):663–667.
62. Sun L, Wang J, Song K, Sun Y, Qin Q, Xue Y: Transcriptome analysis of rice (*Oryza sativa* L.) shoots responsive to cadmium stress. *Scientific Report* 2019, 9(1):10177.
63. Shen Q, Liu Z, Song F, Xie Q, Hanley-Bowdoin L, Zhou X: Tomato SlSnRK1 Protein Interacts with and Phosphorylates $\beta$ C1, a Pathogenesis Protein Encoded by a Geminivirus  $\beta$ -Satellite. *Plant Physiology*. 2011, 3(157):1394–1406.
64. Hao L, Wang H, Sunter G, Bisaro DM: Geminivirus AL2 and L2 proteins interact with and inactivate SNF1 kinase. *Plant Cell*. 2003, 15(4):1034–1048.
65. Slocombe SP, Beaudoin F, Donaghy PG, Hardie DG, Dickinson JR, Halford NG: SNF1-related protein kinase (snRK1) phosphorylates class I heat shock protein. *Plant Physiology Biochemistry* 2004, 42(2):111–116.
66. Testerink C, Dekker HL, Lim ZY, Johns MK, Holmes AB, Koster CG, Ktistakis NT, Munnik T: Isolation and identification of phosphatidic acid targets from plants. *Plant Journal* 2004, 39(4):527–536.
67. Cheng W, Yao H, Zhang G, Meiling T: Effects of cadmium stress on rice growth and nutrient metabolism. *Chinese Agricultural Sciences* 2005, 38(3):528–537.
68. Kim D, Langmead B, Salzberg SL: HISAT: a fast spliced aligner with low memory requirements. *Nat methods* 2015, 12(4):357.
69. Anders S, Pyl PT, Huber W: HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics* 2015, 31(2):166–169.
70. Roberts A, Trapnell C, Donaghey J, Rinn JL, Pachter L: Improving RNA-Seq expression estimates by correcting for fragment bias. *Genome Biology* 2011, 12(3):R22.
71. Anders S: Analysing RNA-Seq data with the DESeq package. *Molecular Biology* 2010, 43(4):1–17.
72. Griffiths-Jones S, Bateman A, Marshall M, Khanna A, Eddy SR: Rfam: an RNA family database. *Nucleic Acids Research* 2003, 31(1):439–441.
73. Chen N: Using RepeatMasker to identify repetitive elements in genomic sequences. *Current protocols in bioinformatics* 2004, 5(1):4.10. 11–14.10. 14.
74. Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ: miRBase: tools for microRNA genomics. *Nucleic Acids Research* 2007, 36(suppl\_1):D154-D158.
75. Griffiths-Jones S, Grocock RJ, Van Dongen S, Bateman A, Enright AJ: miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Research* 2006, 34(suppl\_1):D140-D144.
76. Langmead B, Salzberg SL: Fast gapped-read alignment with Bowtie 2. *Nat Methods* 2012, 9(4):357.
77. Friedländer MR, Mackowiak SD, Li N, Chen W, Rajewsky N: miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. *Nucleic Acids Research* 2011,

40(1):37–52.

78. Denman RB: Using RNAFOLD to predict the activity of small catalytic RNAs. *Biotechniques* 1993, 15(6):1090–1095.
79. Fahlgren N, Carrington JC: miRNA target prediction in plants. *Plant MicroRNAs* 2009, 592: 51–57.
80. Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, Itoh M, Katayama T, Kawashima S, Okuda S, Tokimatsu T: KEGG for linking genomes to life and the environment. *Nucleic Acids Research* 2007, 36(suppl\_1):D480-D484.
81. Smoot ME, Ono K, Ruscheinski J, Wang P-L, Ideker T: Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics* 2010, 27(3):431–432.

## Tables

**Table 1. Statistics of the differentially expressed genes (DEGs) and miRNAs (DEmiRNAs) by different pairwise comparison.**

Comparison		DEG			DEmiRNAs		
Case	Control	Up	Down	Total	Up	Down	Total
XNT	XNC	759	1006	1765	20	10	30
XPT	XPC	1258	888	2146	11	14	25
yNT	yNC	621	385	1006	15	10	25
yPT	yPC	1033	268	1301	18	8	26

“X” notes low Cd accumulation cultivar “Xiangwanxian No. 12”, “y” notes high Cd accumulation cultivar “Yuzhenxiang”, “P” indicates panicle node, “N” indicates the first node, “C” represents control and “T” represents Cd treatment.

## Figures

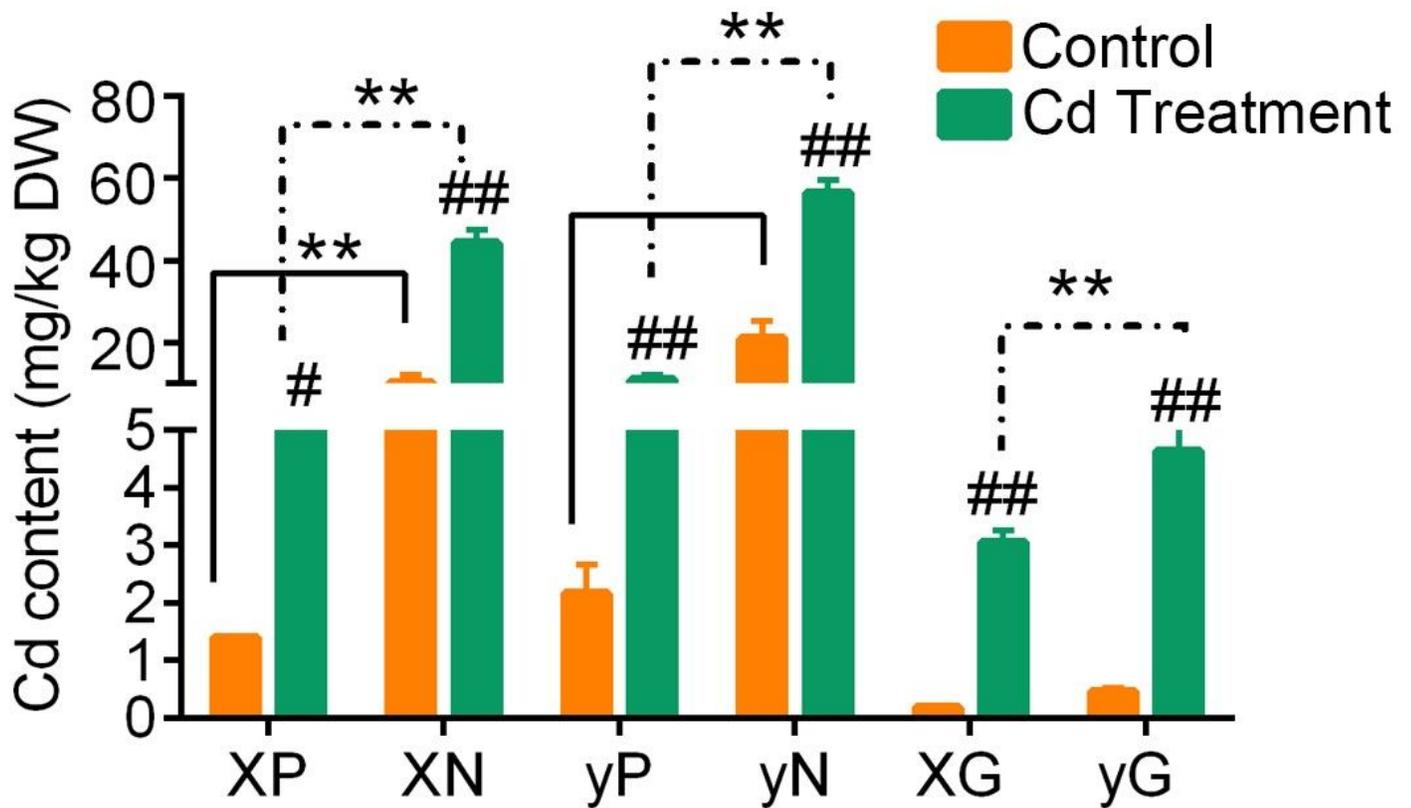
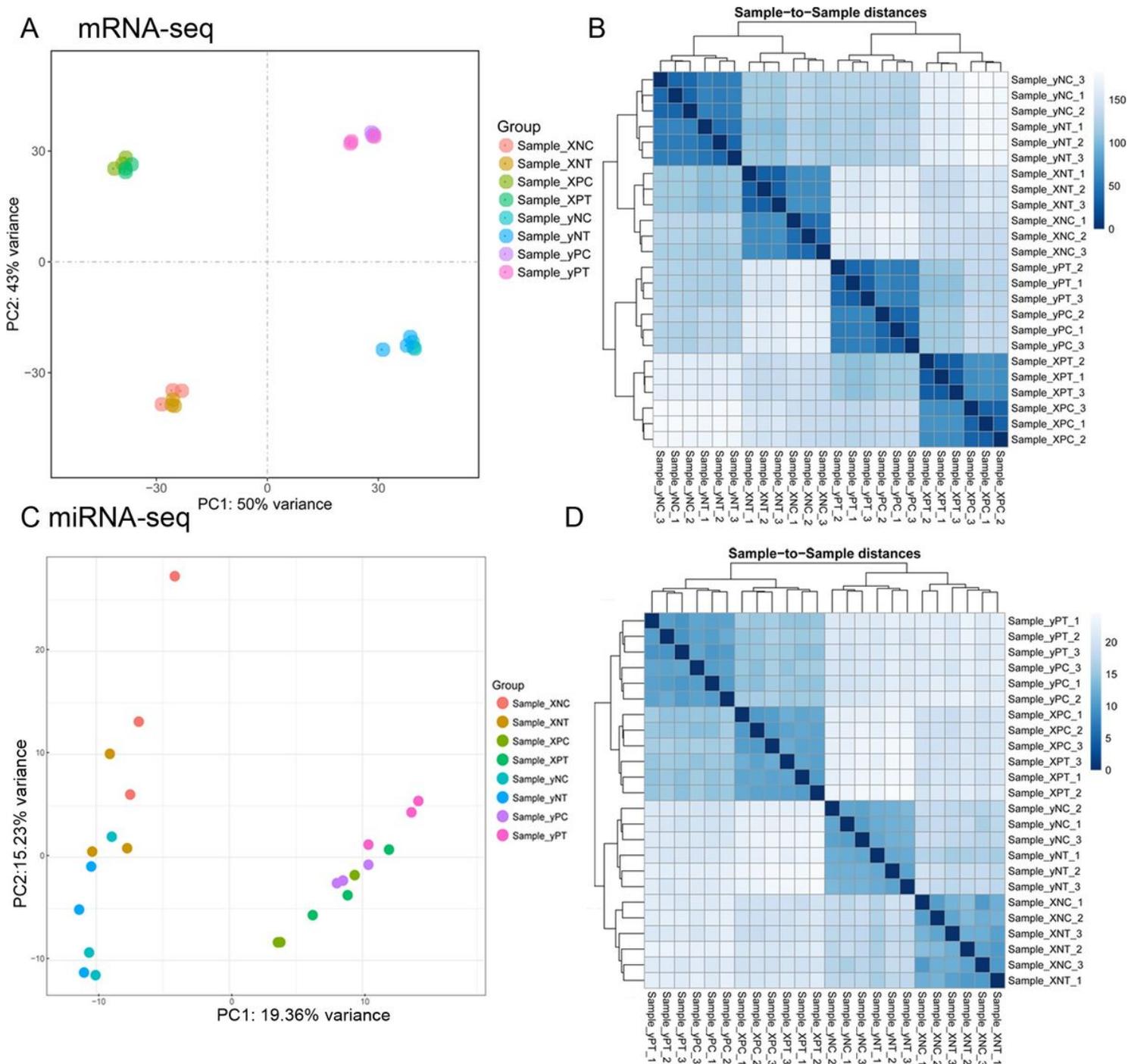


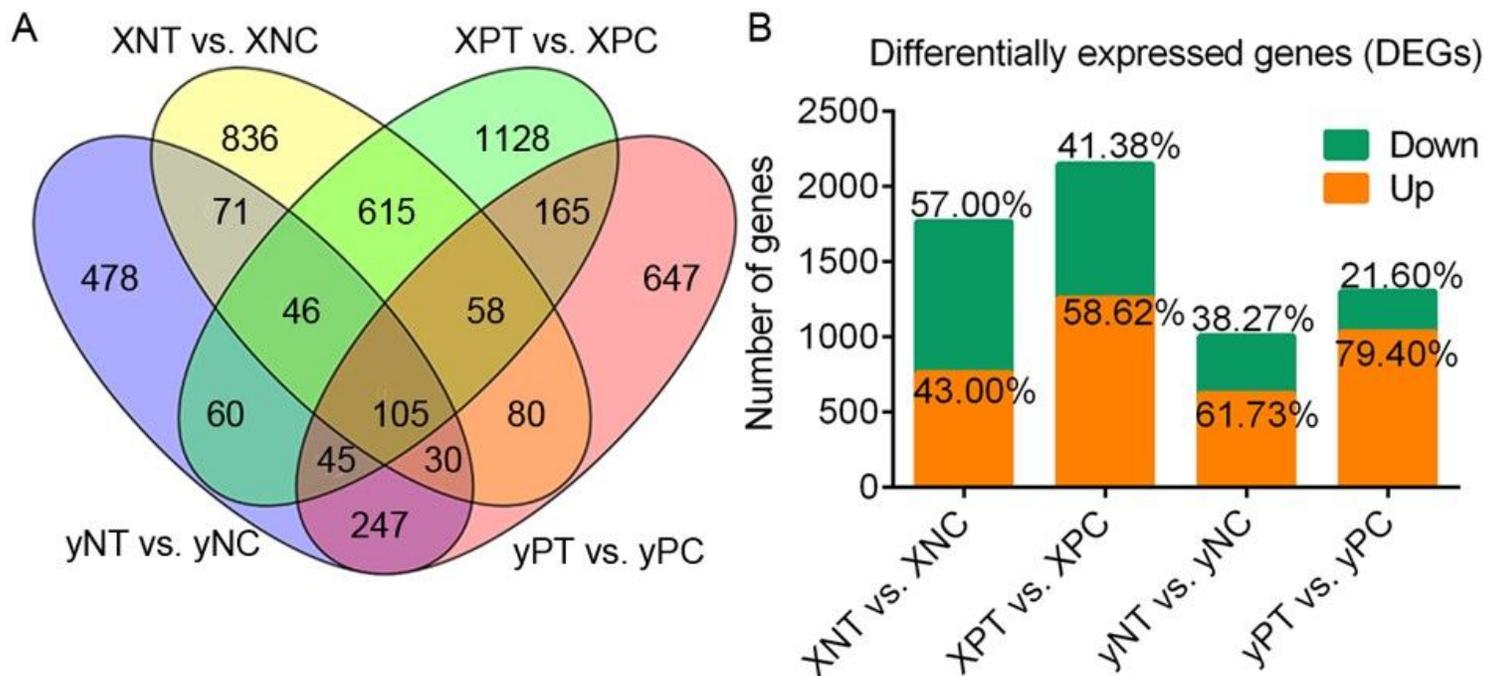
Figure 1

Cd contents in node I, panicle node and grain of different rice varieties. DW, dry weight (kg); XP, panicle node of Xiangwanxian No.12; XN, node I of Xiangwanxian No.12; yP, panicle node of Yuzhenxiang; yN, node I of Yuzhenxiang; XG, grain of Xiangwanxian No.12; yG, grain of Yuzhenxiang. # p<0.05, ## p<0.01 Cd treatment vs. control; \* p<0.01, \*\* p<0.05 y (Yuzhenxiang) vs. X (Xiangwanxian No.12).



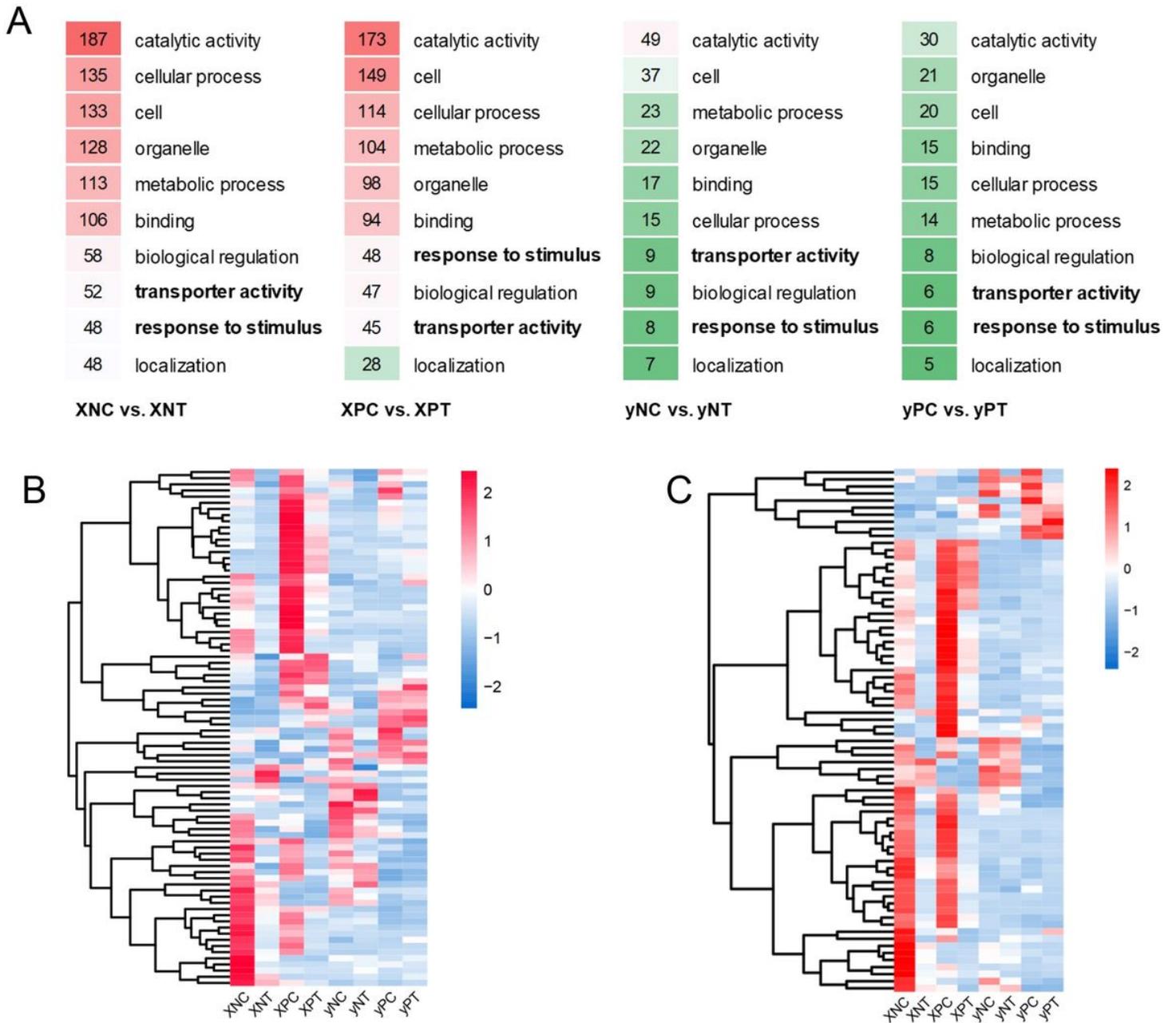
**Figure 2**

Sample clustering and correlation analysis. A. and C. the principal component analysis (PCA) of the samples sequenced using the mRNA and miRNA expression level, respectively. B. and D. the sample-to-sample clustering analysis based on the mRNA and miRNA expression level, respectively. The color depth notes the similarity between samples (0~1). The deeper the color, the higher the similarity.



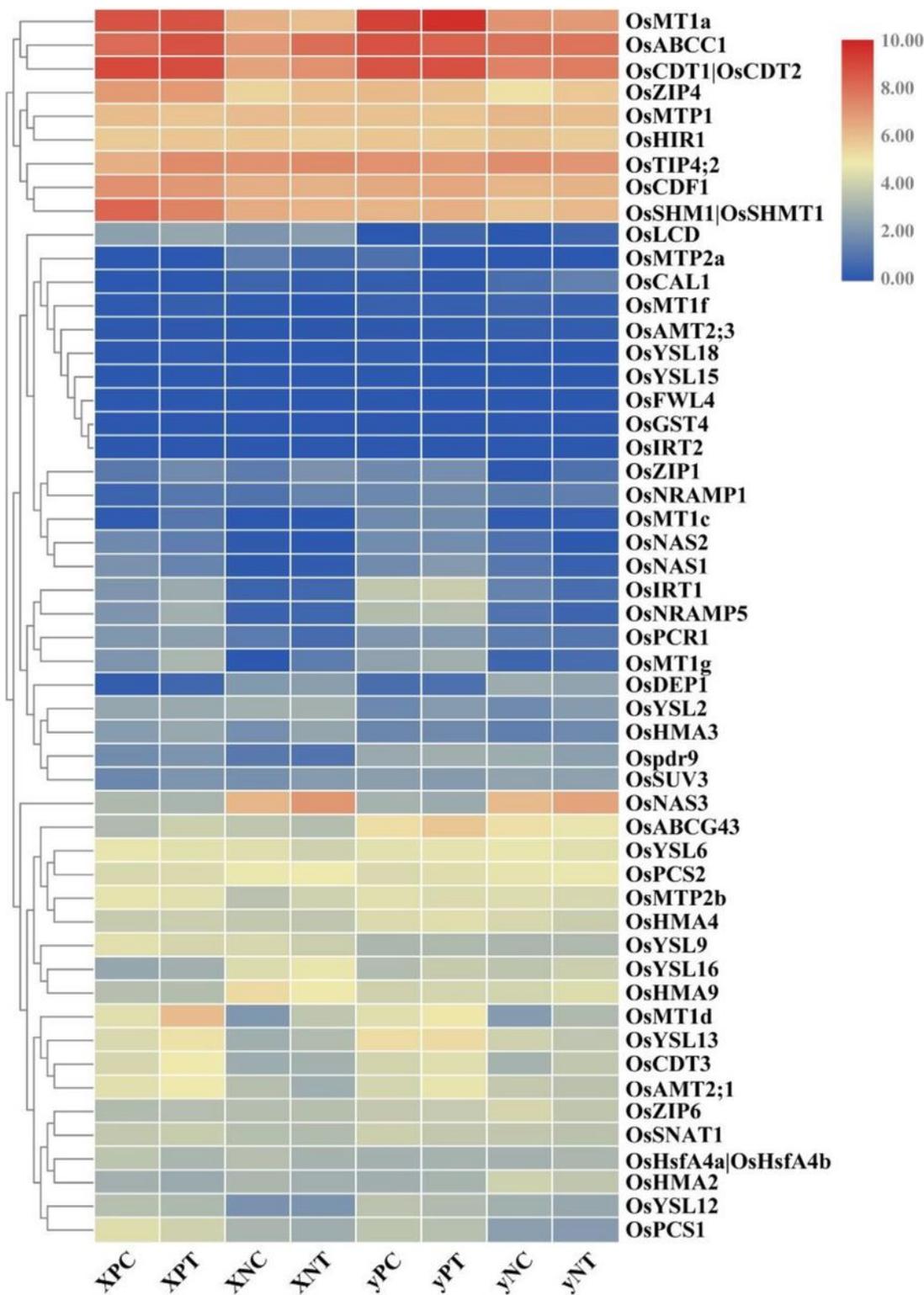
**Figure 3**

Summary of differentially expressed genes (DEGs). A. The Venn Fig.s of the DEGs in pairwise comparison of Cd-treated (T) vs. untreated control (C) of node I (N) and panicle node (P) in “X” and “y” cultivar, respectively. B. the statistics of the up- and down-regulated DEGs. The number on the column indicates the percent of the up and down-regulated DEGs by each comparison.



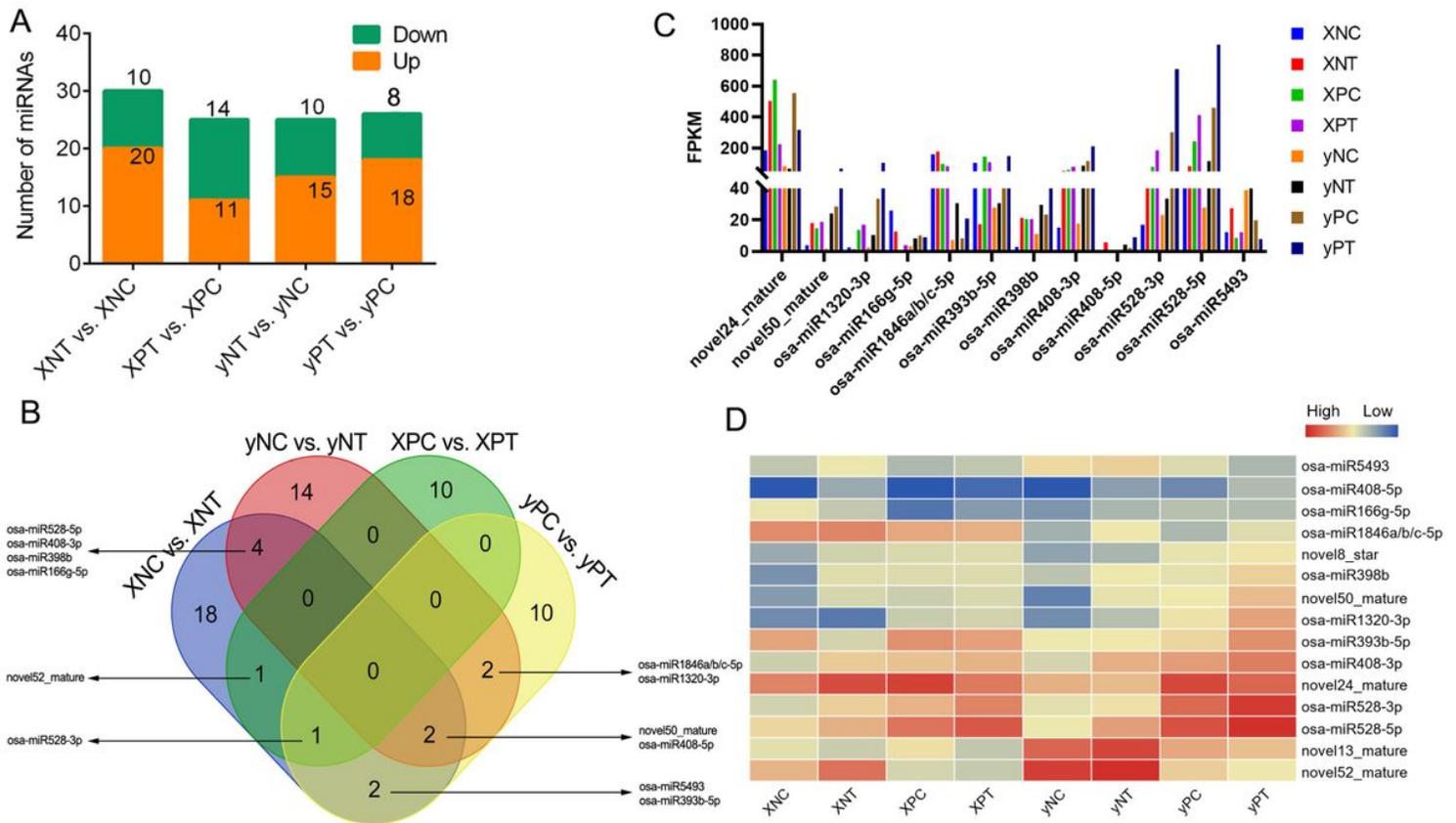
**Figure 4**

Differentially expressed genes (DEGs) between high and low Cd accumulation cultivars. A. Gene Ontology (GO) and KEGG pathway enrichment analyses of down-regulated DEGs in pairwise comparison of Cd-treated (T) vs. untreated control (C) of node I (N) and panicle node (P) in “X” and “y” cultivar, respectively. The redder the color, the more significantly enriched DEGs there were, the greener the color, the less. B. and C. Hierarchical clustering analysis of the DEGs involved in “transporter activity” (B) and “response to stimulus” (C), respectively.



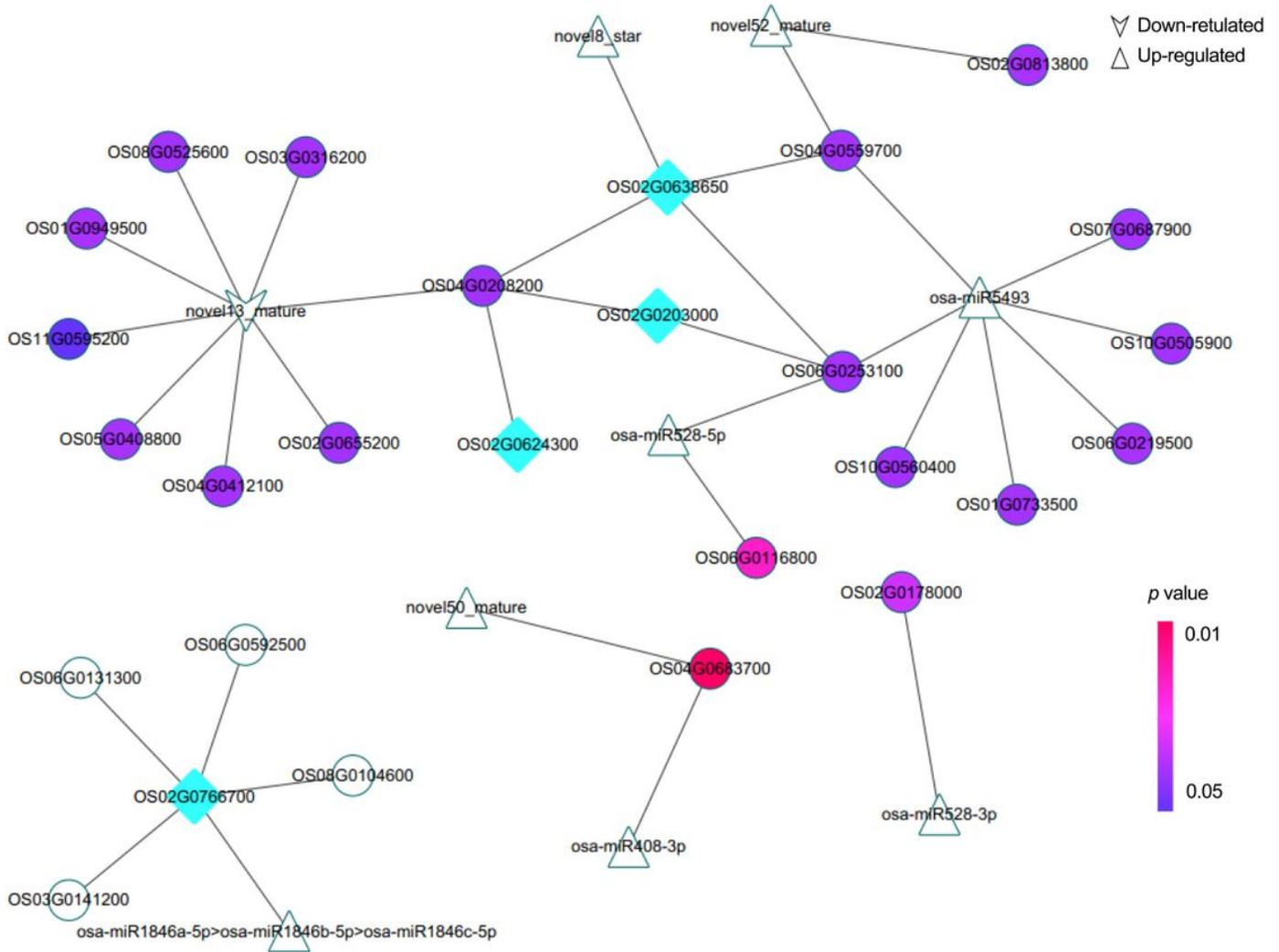
**Figure 5**

Heatmap of known 52 Cd-responsive genes. These Cd-responsive genes were collected from previous literatures. The change of color from blue to red indicated that gene expression level was low to high.



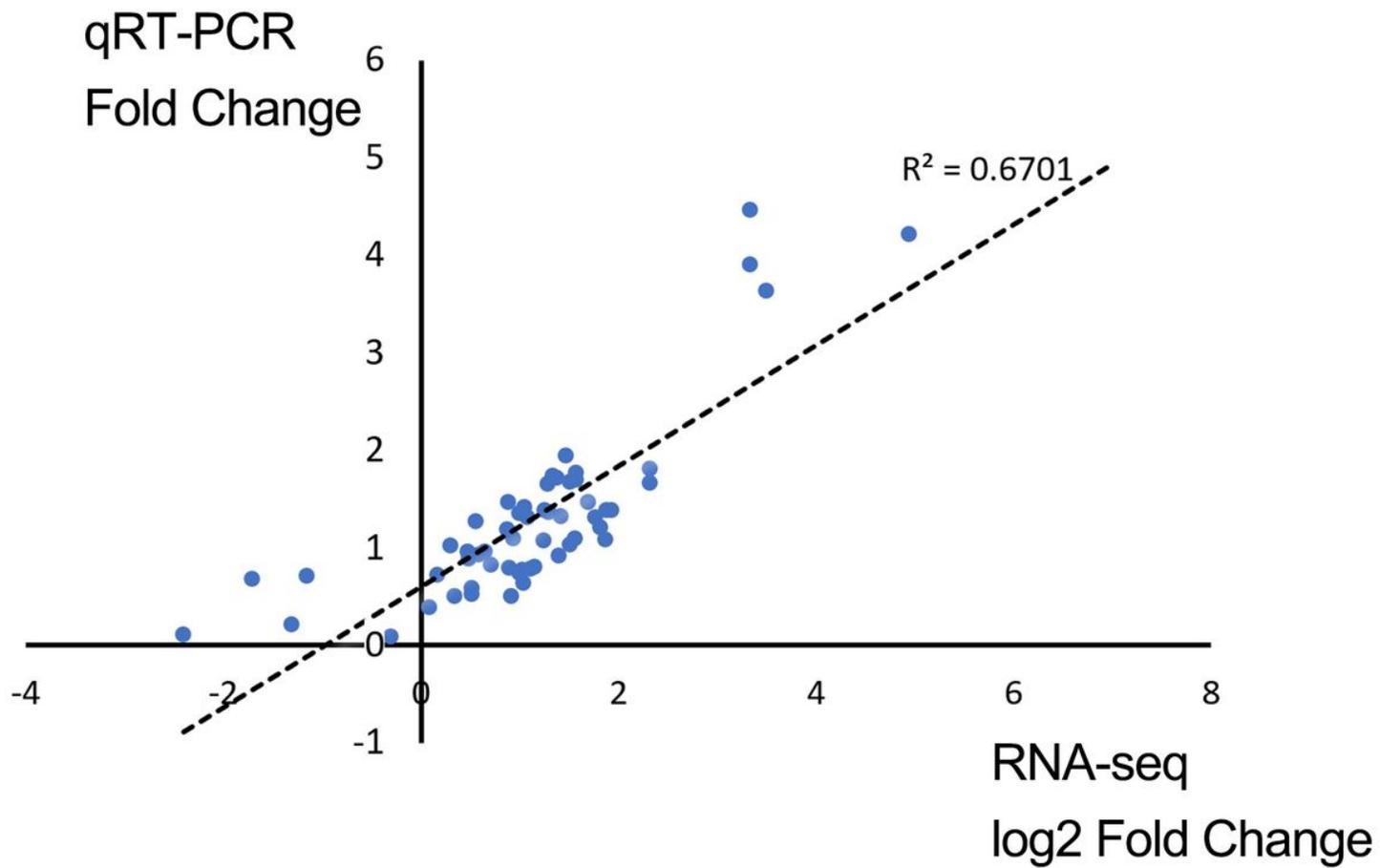
**Figure 6**

Differentially expressed miRNAs (DEmiRNAs) responsible for low Cd accumulation. A. The statistics of the up- and down-regulated DEmiRNAs. The number on the column indicates the number of the up and down-regulated DEGs by each comparison; B. The Venn Fig.s of the of DEmiRNAs in Node I DEGs in pairwise comparison. The common DEmiRNAs were pointed out by the arrow; C. Expression of the common DEmiRNAs in Cd-treated (T) vs. untreated control (C) of node I (N) and panicle node (P) in “X” and “y” cultivar, respectively; D. Heatmap of the candidate miRNAs and their differentially expressed family members. The change of color from blue to red indicated that gene expression level was low to high.



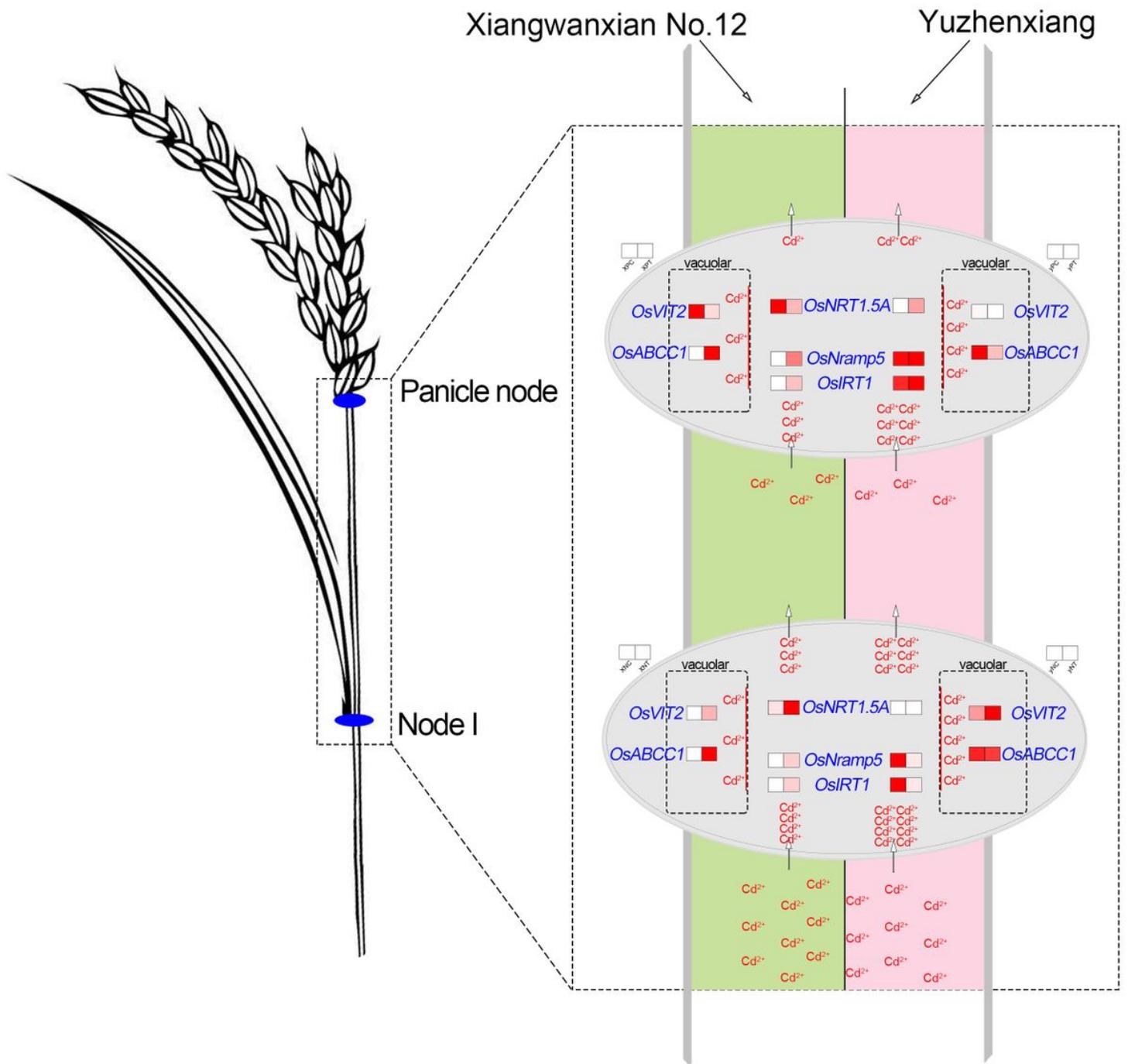
**Figure 7**

Regulatory network of candidate miRNAs and target genes. ▽ represented down-regulated differentially expressed miRNAs (DEmiRNAs) after Cd treatment; ▴ represented up-regulated DEmiRNAs after Cd treatment; ○ represented the target genes. Different p values are expressed in different colors, as shown in the Fig.. □ indicated that the target gene was a transcription factor. Different p values are expressed in different colors, as shown in the Fig..



**Figure 8**

Consistency results of qRT-PCR and RNA-seq. The Fig. was based on the fold change of qRT-PCR and log2 fold change of RNA-seq. R2 represented the correlation coefficient between qRT-PCR and RNA-seq.



**Figure 9**

Schematic maps of upward transport mechanism of blocking Cd in different rice varieties. Panicle node (P) and node I (N) were marked in blue on the rice sketch. The schematic map (the right of the Fig.) showed the upward transport mechanism of blocking Cd in Xiangwanxian No.12 (pale green) and Yuzhenxiang (pale pink). The ellipse at top and bottom represented panicle node and node I, respectively. The expression of key genes was showed by heatmap (box on left and right represented control and Cd treatment, respectively), the redder the color, the higher the expression. The direction of Cd transportation was indicated by arrows and the content of Cd was expressed by the number of "Cd<sup>2+</sup>".

## Supplementary Files

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