Genome-wide analysis of long non-coding RNAs affecting roots development at an early stage in the rice response to cadmium stress

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Abstract

Background: Long non-coding RNAs (lncRNAs) have been found to play a vital role in several gene regulatory networks involved in the various biological processes in plants related to stress response. However, systematic analyses of lncRNAs expressed in rice Cadmium (Cd) stress are seldom studied. Thus, we presented the characterization and expression of lncRNAs in rice root development at an early stage in response to Cd stress.

Results: The lncRNA deep sequencing revealed differentially expressed lncRNAs among Cd stress and normal condition. In the Cd stress group, 69 lncRNAs were up-regulated and 75 lncRNAs were down-regulated. Furthermore, 386 matched lncRNA-mRNA pairs were detected for 120 differentially expressed lncRNAs and 362 differentially expressed genes in cis, and target gene-related pathway analyses exhibited significant variations in cysteine and methionine metabolism pathway-related genes. For the genes in trans, overall, 28,276 interaction relationships for 144 lncRNAs and differentially expressed protein-coding genes were detected. The pathway analyses found that secondary metabolites, such as phenylpropanoids and phenylalanine, and photosynthesis pathway-related genes were significantly altered by Cd stress.

All of these results indicate that lncRNAs may regulate genes of cysteine-rich peptide metabolism in cis, as well as secondary metabolites and photosynthesis in trans, to activate various physiological and biochemical reactions to respond to excessive Cd.

Conclusion: The present study could provide a valuable resource for lncRNA studies in response to Cd treatment in rice. It also expands our knowledge about lncRNA biological function and contributes to the annotation of the rice genome.

Keywords: Rice, Cd stress, lncRNA, mRNA, Cis, Trans
noncoding RNAs, also called ASCO-IncRNAs, can interfere with AtNSRs in alternative splicing of auxin-responsive genes downstream and affect the growth of lateral roots [11]. In plants, numerous molecular functions and biological processes have been determined by IncRNAs; for example, vernalization, photomorphogenesis, fertility, protein re-localization, alternative splicing, phosphate homeostasis, modulation of chromatin loop dynamics etc. [12] Over the last decade, the role and function of small non-coding RNAs in plants have been widely studied [13]. However, the functional mechanisms of IncRNAs in several plant species remain unexplored and only a few of IncRNAs have been fully characterized until now. In Arabidopsis, IncRNAs such as cold-assisted intronic noncoding RNA (COLDAIR) and cold induced long antisense intragenic RNA (COOLAIR) have been confirmed to facilitate chromatin altering activities in transcriptional silencing of FLC during vernalization [10, 14]. LncRNAs have been recognized to play an important role in many gene regulatory networks tangled in several biological processes of plant stress responses [2]. Moreover, a large number of putative stress IncRNAs have been categorized and characterized in Arabidopsis [15], maize [16], wheat [17], Populus trichocarpa [18], cucumber [19], tomato [20], cotton [21] and other plant species [22–24]. Recently, thousands of IncRNAs associated with rice development have been identified [25, 26], and could provide a guidance that rice IncRNAs have been fully described or understood. However, the elusive role of IncRNAs in rice stress responses is still not fully described or understood. Therefore, it is necessary to investigate the function of lncRNAs in rice stress responses.

Cadmium (Cd) is known to be an unnecessary metal element for plants and extensively spread Cd pollution has significantly affected human health in terms of its direct effects on crop production and its high increase in the edible part of crops such as rice [27]. When IncRNA function is well understood, scientists may realize that Cd-regulated IncRNAs may be involved in heavy metal stress responses, and some Cd-regulated IncRNAs have recently been detected [28]. Although previous studies have provided useful information on the mechanisms for rice IncRNA in Cd stress response, the regulatory mechanisms involved are mostly unknown. To improve our understanding of the likely functional roles of IncRNAs in rice Cd stress response, further studies are necessary to understand the functional genetics of IncRNAs in detail, and to determine which specific IncRNAs target selective sites for interaction in the rice genome.

Inclusive identification of plant IncRNAs at the genomic level is mainly dependent on and determined by advances in technical platforms [2]. Genome-wide screening by high-throughput RNA sequencing and computational applications for inclusive identification of IncRNAs would be a better choice for detailed mapping and structural studies to understand the RNA-protein and RNA-DNA interactions [29]. In plants, genome-wide analysis of IncRNAs with RNA sequencing transcriptomic data has been executed in only a limited plant species such as Arabidopsis thaliana [30–32], Triticum aestivum [22], Oryza sativa [25], tomato [33] and Zea mays [16, 34]. In the current study, we used a deep RNA sequencing strategy to clarify the IncRNAs profiled associated with Cd stress using the Cd response rice genotype which could provide more insights into the regulatory role of more IncRNAs in the rice Cd stress response. The results obtained in our study provided a valuable resource to study the IncRNAs involved in Cd stress response and will increase the knowledge for a better understanding of the biological processes of rice stress response.

Methods

Plant material and growth conditions

One rice genotype (DX142) was selected from 82 different screened rice genotypes to measure phenotypic traits and for gene expression in this study. DX142 is a pure line and shows the highest sensitivity to Cd stress.

Full seeds of DX142 were surface sterilized with 0.5% NaClO solution for 30 min, rinsed five times with distilled water and maintained for 2 days at 25–30 °C in the dark, thereby inducing germination. Seedlings were then exposed to treatments without 100 mg/L CdCl₂ (as a control) and with 100 mg/L CdCl₂. Growth conditions were as follows: 16/8 h day/night photoperiod under 28/ 25 °C day/night temperatures. Roots were collected for gene expression analysis on the 5th day after treatments.

RNA extraction, sequencing and database access

Total RNA was extracted from 5-day-old rice roots with Trizol reagent (Invitrogen, Carlsbad, CA, USA). Six samples (three replicated samples for each treatment; CK_R1, CK_R2 and CK_R3 for the normal condition; SY_R1, SY_R2 and SY_R3 for the stress condition) were used for sequencing, and differentially expressed IncRNAs and mRNAs were obtained.

RNA isolation, quantification and library preparation for IncRNA sequencing, clustering and sequencing and quality control were analysed as described by Ren et al. (2016) [35]. After these analyses, the purified data with high quality were mapped to the reference genome of Oryza sativa variety Nipponbare [36] using Bowtie v2.0.6 and TopHat v2.0.9 software [37]. The clean data were uploaded into the NCBI Sequence Read Archive under the accession number SRP099996. The mapped reads of each sample were assembled by both Scripture (beta2) [38] and Cufflinks (v2.1.1) [39] in a reference-based approach. Cuffdiff (v2.1.1) was used to calculate FPKMs (fragments per kb per million reads) of IncRNAs in each
sample [39]. Furthermore, the negative binomial distribution method was used to detect the IncRNAs obtained from the three biological replicates of each treatment. Finally, the IncRNAs developed were referred to as Cd stress (SY_R) and control (CK_R)-related IncRNAs.

**IncRNA identification pipeline**

To attain putative IncRNAs, we initially filtered the transcripts according to the class code annotated by Cuffcompare; only the transcripts that occurred in at least two samples were retained. To acquire IncRNAs, we only retained novel (not overlapping with known genes in sense), large (longer than 200 nucleotides), expressed (for multiple-exon transcripts FPKM ≥0.5, for single-exon transcripts FPKM ≥2) transcripts. Then, to obtain high-quality data, CPC (0.9-r2) [40] and Pfam-scan [41] were used to identify the candidate IncRNAs. Transcripts with coding potential predicted by any of the two tools previously described were filtered out, and those without coding potential were retained. Finally, we selected those shared by the two tools as the final candidate IncRNAs and used them for further analysis.

**Identification of differentially expressed IncRNAs**

The fragment per kb per million reads (FPKM) for IncRNAs was calculated by using Cuffdiff (v2.1.1) software [42]. For biological replicates, transcripts or genes with an adjusted \( p<0.05 \) (\( q<0.05 \)) were designated differentially expressed among the two groups of rice roots.

**IncRNAs target gene prediction and functional enrichment analysis**

We searched coding genes 10 kb/100 kb upstream and downstream of IncRNAs as the cis target gene, and then analysed their function. The trans role of IncRNAs was identified by the expression level. We calculated the expressed correlation between IncRNAs and coding genes with custom scripts; further, we clustered the genes from different samples with WGCNA [43] to search for common expression modules and then analyzed their function through functional enrichment analysis.

To understand the functional roles of the target genes of IncRNAs, we used the Gene Ontology (GO) seq [44] R package to implement enrichment analysis, in which gene length bias was corrected. In addition, Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis on target genes was performed on KOBAS software [45] using a hypergeometric test. GO terms and KEGG pathways with a corrected \( p<0.05 \) (\( q<0.05 \)) were considered significantly enriched.

**Real-time quantitative PCR**

Total RNA from 6 sequenced samples (include 3 biological duplication 2 treatment) were extracted using a PrimeScript™ RT reagent Kit with gDNA Eraser. SYBR-based qRT-PCR reactions (SYBR Green I, Osaka, Japan) were performed on a ABI VIIA@7 using the following reaction conditions: 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s and 60 °C for 30s. All qRT-PCR reactions were performed in triplicate samples, and the results were analyzed with the system’s relative quantification software (ver.1.5) based on the \((\Delta\Delta CT)\) method. The detection of threshold cycle for each reaction was normalized against the expression level of the rice ACTB gene.

**Results**

**Morphology under cd stress and control conditions**

First, 82 different rice genotypes were used in this study to check their Cd stress sensitivity for acquiring additional insights into the rice transcriptomic response to environmental Cd stress. One genotype DX142, showed the highest sensitivity and was used for further analyses.

The average root length of DX142 under Cd stress and control conditions is significantly different. The average root length of DX142 under the control condition was significantly higher than under the Cd stress condition from the second day (Fig. 1a), indicating that the rice root length was obviously inhibited by Cd. Furthermore, the root length of DX142 under control condition increased slowly after six days. Therefore, to obtain as much as IncRNA information as possible regarding rice Cd stress, the roots in five-day-old roots were harvested to detect IncRNAs and for further analysis (Fig. 1b).

**Identification of IncRNAs**

A total of 679,838,650 raw reads were generated using the Illumina HiSeq 2000 platform. Low-quality paired-end sequences and adapter sequences were trimmed off, and 660 million clean reads (99 Gb) were obtained with an average of 110 million reads (16.5 Gb) per sample (Table 1). Subsequently, we mapped the clean reads to the Nipponbare reference genome [36] to identify the transcripts.

Considering the characteristics of IncRNA sequences (≥200 nt) and their differences from other classes of RNA (mRNA, tRNA, rRNA, snRNA, snoRNA, pre-miRNA, and pseudogenes), we used Scripture (beta2) and Cufflinks (v.1.1) software to classify transcripts into different subtypes. Overall, 3558 transcripts out of all the 46,933 identified transcripts were predicted to be IncRNAs. We performed coding potential analysis using the software CPC and Pfam-scan to confirm these 3558 IncRNAs. After screening using harsh criteria and two analytic tools, a total of 2580 IncRNAs from Cd stress and control conditions in rice were identified and subjected for further analysis (Fig. 2).
Identification of differentially expressed IncRNAs

Genome-wide analysis of IncRNA expression under Cd stress and control conditions was performed to profile differentially expressed IncRNAs associated with Cd stress. We first assessed the IncRNA expression profiles in two different conditions (Cd stress vs control condition), and 144 differentially expressed IncRNAs from 143 IncRNA genes were identified between Cd stress (SY_R) and control (CK_R) (Additional file 1: Table S1). The 144 IncRNAs consisted of 120 large intergenic non-coding RNAs (lincRNAs), 1 intronic IncRNA, and 23 anti-sense_lncRNAs (Additional file 1: Table S1). Among them, 69 lncRNAs were up-regulated and 75 lncRNAs were down-regulated (Fig. 3 and Additional file 1: Table S1).

The cis role of differentially expressed IncRNAs in target genes

To examine the IncRNA function, we predicted the potential targets of IncRNAs in cis. We examined protein-coding genes 10 and 100 kb upstream and downstream of the IncRNAs, respectively. In total 386 matched IncRNA-mRNA pairs for 120 differentially expressed IncRNA genes and 362 differentially expressed mRNAs were found (Additional file 2: Table S2).

GO analysis predicted that there was no significant enrichment in GO terms targeted by lncRNAs. The pathway analyses revealed 17 different pathways corresponding to the target genes (Table 2 and Additional file 3: Table S6); one of them is the cysteine and methionine metabolism pathway, which was significant in the Cd stress condition compared with the normal condition with an enrichment ($q < 0.05$, Fig. 4; Table 2).

The trans role of IncRNAs in target genes

On the other hand, we examined the trans role of 143 IncRNAs genes on the basis of its expressed correlation coefficient (Pearson correlation $\geq 0.95$ or $\leq -0.95$). In a total, 28,276 interaction relationships were identified in trans between 144 IncRNAs and the protein-coding genes (Additional file 4: Table S3 and Additional file 5: Table S7).

GO analysis showed that the highly enriched GO terms targeted by IncRNAs are single-organism metabolic process, photosynthesis and response to stimulus (Fig. 5). The pathway analyses revealed 118 different pathways corresponding to the target genes, and four of them are biosynthesis of secondary metabolites, phenylpropanoid biosynthesis, phenylalanine metabolism and photosynthesis signalling pathways in the Cd stress condition.

Table 1 Statistics of the read alignments in the RNA-Seq study

| Sample name | Raw reads | Clean reads | clean bases | Error rate (%) | Q20(%) | Q30(%) | GC content(%) |
|-------------|-----------|-------------|-------------|----------------|--------|--------|---------------|
| CK_R1       | 102,609,352 | 100,129,870 | 15.02G      | 0.01           | 97.57  | 93.83  | 52.22         |
| CK_R2       | 102,837,492 | 100,135,724 | 15.02G      | 0.01           | 97.59  | 93.82  | 52.55         |
| CK_R3       | 119,750,698 | 116,752,212 | 17.51G      | 0.01           | 97.66  | 93.98  | 52.72         |
| SY_R1       | 108,004,232 | 104,861,548 | 15.73G      | 0.01           | 97.56  | 93.79  | 52.69         |
| SY_R2       | 138,500,332 | 133,956,886 | 20.09G      | 0.01           | 97.65  | 93.97  | 52.72         |
| SY_R3       | 108,136,544 | 104,207,142 | 15.63G      | 0.01           | 97.62  | 93.89  | 53.65         |
compared with the normal condition \((q < 0.05)\) (Additional file 6: Figure S1 and Table 3).

**Validation of gene expression by quantitative real-time PCR**

To validate the findings from sequencing data, 10 genes correlated with mRNAs, including lncRNAs were selected randomly and analyzed by quantitative real-time polymerase chain reaction (qRT-PCR) (Fig. 6). We randomly selected 10 genes in the lncRNA-seq data. According to the random criteria, we have selected genes that were significantly different and genes that were not significantly different to enhance the reliability of the results by lncRNA-seq. The primer sequences are listed in Additional file 7: Table S4. The results showed that the expression trends were consistent for all transcripts in both analyses, with a correlation coefficient of \(R^2\) of 0.9643 (Additional file 8: Figure S2).

**Discussion**

Although various examples of recently characterized lncRNAs [46–49] support a functional relationship between lncRNAs and their related-protein coding gene(s). The study of differentially expressed lncRNAs controlling gene expression in the rice stress response at the whole transcriptome level especially in Cd stress, is reported to be limited. Thus, our data systemically predict the lncRNAs at the whole transcriptome level and showed that which specific lncRNAs seek out selective sites in the genome for interaction in rice Cd stress. Compared to the previous studies, which were based on two or three replicated samples for each treatment for RNA sequencing, our sequencing data were obtained from three individually replicated samples for each treatment. Then, the negative binomial distribution method was used to detect the RNA information for each treatment, which could minimize the experimental error. Thus, our results could provide more comprehensive information.

Here, 143 lncRNA genes were detected that showed differential expression in the Cd stress response in the root, and 83.9 and 100% of them were identified that contained at least one differentially expressed mRNA in cis and trans, respectively, which suggests that these lncRNAs may play an important role in the rice Cd stress response. Furthermore, lncRNAs showed a higher ratio than those of the other two subtypes of all the differentially expressed transcription factors under Cd treatment compared with control in rice roots.
lncRNAs, indicating that lncRNAs may be the main form of lncRNAs in the Cd stress response in rice. Previous studies confirmed that altered splicing of lncRNA genes could quantitatively modulate, gene expression in development and other physiological processes through co-transcriptional coupling mechanisms [50–52], but there is less information available on the effects of heavy metal stress on altered splicing regulation particularly when global lncRNA profiles are induced by treatment with certain heavy metals. In this study, 144 lncRNA transcripts from 143 lncRNA genes were identified, only one lncRNA coding gene (XLOC_057621) was alternatively spliced and yielded two different lncRNAs transcripts, and therefore it can be predicted that alternative splicing might not be the major form of regulation in the Cd stress process for rice. In addition, we compared the lncRNA data with the study of Fei et al. [28] and found that XLOC_003991 and XLOC_044902 shared the same changed pattern (Additional file 1: Table S1).

The role of lncRNAs in stress processes creates an insistence to understand the mechanisms by which these RNAs seek their targets [15–20, 22–24]. To improve the accuracy of target prediction, a detailed co-expression network between differentially expressed mRNA and differentially expressed lncRNAs was constructed, which showed that one lncRNA could target one or more coding genes. The result indicated that the regulation of mRNA by lncRNAs is involved in Cd-induced root development. Therefore, lncRNAs should be given more attention in heavy metal stress response studies in the future.

To gain more insight into the function of targets of lncRNAs, GO term and KEGG pathway annotations were applied to their target gene pool. KEGG analysis showed a significant change in the cysteine and methionine metabolism pathway in the Cd stress compared to normal condition. Plants produce cysteine-rich (Cys-rich) peptides that

### Table 2

| Pathway term                                      | q-value   | Gene number |
|---------------------------------------------------|-----------|-------------|
| Cysteine and methionine metabolism                | 0.047189  | 7           |

Fig. 4 Scatter plot of KEGG pathway enrichment statistics for differentially expressed target genes in cis in rice roots. Rich Factor is the ratio of differentially expressed gene numbers annotated in this pathway term to all gene numbers annotated in this pathway term. Greater Rich Factor means greater intensiveness. q-value is corrected p-value ranging from 0~1, and its less value means greater intensiveness. We displayed KEGG pathways significantly enriched due to exposure to Cd in our experiments.
chelate Cd to procedure non-toxic complexes which are then sequestered into the vacuole to avoid high levels of free cytotoxic Cd in the cytosol in response to Cd stress [53]. In this study, we observed that OS03G0196600, which is involved in the cysteine and methionine metabolism pathways, was clearly up-regulated (Additional file 9: Table S5) and might contribute to the production of cysteine-rich (Cys-rich) peptides. It is interesting that XLOC_086307 (the lncRNA targeted OS03G0196600 in cis) was also up-regulated significantly, which suggests that XLOC_086307 likely participates in Cd response processes in rice by regulating the cysteine-rich peptide metabolism-related gene OS03G0196600. Previous studies showed that exposure to Cd stress will lead to impairment of the photosynthetic function in many plant species. Both chlorophylls and carotenoid contents decrease when exposed to Cd [54–56]. It was noticed that OS03G0184000 (the target of XLOC_086119 and XLOC_066284 in cis), which is involved in carotenoid biosynthesis, is up-regulated (Additional file 9: Table S5), which may result in increased carotene content. Oxidative cleavage of carotenoids will produce apocarotenoids, while the phytohormone ABA is an apocarotenoid derivative [57]. With the increase in apocarotenoids, ABA content was increased, and the signalling pathway was activated. Furthermore, ABA was found to be involved in the regulation of antioxidative defence systems and Cd-induced oxidative stress in mung bean seedlings [58]. Therefore, XLOC_086119 and XLOC_066284 might be involved in carotenoid biosynthesis associated with cadmium stress in rice.

In trans, GO and pathway analyses predicted that the regulated transcripts of lncRNAs are mainly associated with metabolic process (ontology: biological process), intracellular part (ontology: cellular component) and catalytic activity (ontology: molecular function), which are associated with four significant gene pathways that correspond to the transcripts. Among these pathways, we found that secondary metabolites such as phenylpropanoids and phenylalanine-related genes were significantly altered by Cd stress, and our results were consistent with a previous study [53]. In the current study, the trans role of lncRNAs, including XLOC_058523, XLOC_104363 and XLOC_059778, targeted phenylpropanoids and the phenylalanine related-gene OS11G0552000, which indicated that lncRNAs may regulate the genes of the secondary metabolites in far distance and then activate the various transporters to successively guide removal of excessive Cd from the cell. Furthermore, our analysis revealed that differentially expressed mRNA OS07G0148900 (Additional file 9: Table S5) with the trans targets of differentially expressed lncRNAs in trans such as XLOC_122123, XLOC_125848 and XLOC_098316, is highly enriched in photosynthesis. The results are consistent with the fact that Cd can damage the photosynthetic apparatus and lead to impairment of photosynthetic function [59, 60]. Because our samples were

### Table 3

Significant pathways and proportions after KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis of differentially expressed target genes in trans in the root. We displayed KEGG pathways significantly enriched due to exposure to Cd in our experiments.

| Pathway term                                      | q-value   | Gene number |
|---------------------------------------------------|-----------|-------------|
| Biosynthesis of secondary metabolites             | 0.00098479| 231         |
| Phenylpropanoid biosynthesis                       | 0.00176395| 54          |
| Phenylalanine metabolism                           | 0.02025548| 41          |
| Photosynthesis                                     | 0.02612353| 34          |
| Photosynthesis - antenna proteins                  | 0.0715502 | 11          |
| Phenylalanine, tyrosine and tryptophan biosynthesis| 0.07830629| 20          |
| Glutathione metabolism                             | 0.07830629| 31          |
| Valine, leucine and isoleucine biosynthesis        | 0.07830629| 10          |

![Fig. 5](image_url)
grown in solution, the roots may have been passively exposed to light, which could strongly activate photosynthesis in root tissues. A similar result can be seen in the study of Zhai et al. [61]. These results suggest that the photosynthesis pathway-related genes are involved in the Cd stress response in rice and may also be regulated by lncRNAs in trans. In addition, the further experiments of the interaction among these lncRNAs and their targets both in cis and trans are underway.

Conclusions
Taken all together, our study systematically determines the genome-wide lncRNA expression profile in Cd-induced rice roots by deep sequencing. Our results showed that some lncRNAs are aberrantly expressed in Cd-treated rice roots when compared with untreated roots. In addition, after pathway analyses of the target genes of these differentially expressed lncRNAs, cysteine and methionine metabolism pathway, carotenoid biosynthesis, ABA signalling pathway (in cis), and secondary metabolites and photosynthesis (in trans) were enriched, which indicated that lncRNAs may play an important role in these pathways in response to Cd stress. Therefore, further studies are necessary needed to fully understand these lncRNAs to effectively control rice Cd pollution in the future.

Additional files

**Additional file 1:** Table S1. Differentially expressed lncRNAs in response to Cd stress in root libraries. The log2(foldchange) value is positive and native mean that the gene is up-regulated and down-regulated in the CK sample, respectively. (XLSX 23 kb)

**Additional file 2:** Table S2. The interaction information of rice root differential genes and co-expressed lncRNAs in cis. (XLSX 15 kb)

**Additional file 3:** Table S6. Pathways and proportions after KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis of differentially expressed target genes in cis in the root. KEGG pathways significantly enriched in our experiments were displayed. (XLS 14 kb)

**Additional file 4:** Table S3. The interaction information of rice root differential genes and co-expressed lncRNAs in trans. (XLSX 870 kb)

**Additional file 5:** Table S7. Pathways and proportions after KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis of differentially expressed target genes in trans in the root. KEGG pathways significantly enriched in our experiments were displayed. (XLS 14 kb)

**Additional file 6:** Figure S1. Scatter plot of KEGG pathway enrichment statistics. Rich Factor is the ratio of differentially expressed gene numbers annotated in this pathway term to all gene numbers annotated in this pathway term. Greater Rich Factor means greater intensiveness. We just display the top 20 pathway terms enriched by KEGG database. (TIFF 646 kb)

**Additional file 7:** Table S4. The qPCR used transcripts and their primers. We made a random selection of 10 genes to determine by quantitative real-time polymerase chain reaction. The y-axis and x-axis indicate Log2(Fold Change) and the name of the genes, respectively. XLOC_033045 represent one of the whole lncRNAs. The Fold Change stands for the fold change between FPKM of SY and the FPKM of CK.

**Additional file 8:** Figure S2. Comparison of the log2 (FC) of 10 selected transcripts using RNA-Seq and qRT-PCR. (TIFF 15 kb)

**Additional file 9:** Table S5. The differentially expressed mRNAs by lncRNA-seq. (XLS 831 kb)

Abbreviations
Cd: Cadmium; CK_R: The rice root in normal condition; FPKMs: Fragments per kb for a million reads; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; lncRNAs: Long non-coding RNAs; SY_R: The rice root in Cd stress.
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Availability of data and materials
The data supporting the conclusions of this article are included within the article and its additional files. The sequence data generated during the current study are available in the NCBI Sequence Read Archive under the accession number of SRP099996 (http://www.ncbi.nlm.nih.gov/srar/SRP099996).

Authors’ contributions
HH conceived and designed the experiments. JB conceived and designed the experiments, and wrote the manuscript. LC, SS, and NJ performed the experiments. CZ, XF, QY, XH, JF, XC, LX, LO, XS, and JX analyzed the data. HK and GMW revised the manuscript for the language. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The rice line DX142 (including the seeds) obtained from the native cultival in China, Nanchang, China. 3College of Agronomy, Jiangxi Agricultural University, Nanchang 330045, China.

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

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