Plant Growth Regulation
https://doi.org/10.1007/s10725-019-00547-6

ORIGINAL PAPER

The effect of cadmium on the microRNAome, degradome and transcriptome of rice seedlings

Min Zhong[1,2] · Fenglin Huang[3] · Rongjian Luo[2] · Yusong Lv[2] · Umed Ali[2] · Zhonghua Sheng[2] · Shaoqing Tang[2] · Xiangjin Wei[2] · Peisong Hu[1,2]

Received: 15 May 2019 / Accepted: 7 October 2019

Abstract

The damage induced by the uptake of cadmium (Cd) into the rice plant is of growing concern. Although many micro-RNAs (miRNAs) and their target genes have been identified in experiments designed to elucidate the molecular impact of exposure to Cd, as yet there has been no attempt to integrate data from sequencing the microRNAome, the degradome and the transcriptome of rice plants exposed to Cd. Here, the abundance of 40 miRNAs was shown to be substantially altered as response to Cd exposure. Of those, 38 (belonging to 22 known miRNA families) were already documented in rice and two (PC-3p-38247_129 and PC-3p-102187_26) are novel. The abundance of 18 genes differentially transcribed as a result of Cd exposure was found to be inversely correlated to that of 18 of the Cd-responsive miRNAs. The majority of the target genes encoded transcription factors, including *ARF13*, *SCL6*, various *SPLs*, *NFYA6*, *GAMYB,* and various *NACs* which encode proteins that participate in signal transduction and abiotic stress resistance. In all, the present study established a fundamental basis for evaluating the regulatory role of miRNA and their targets in plant exposure to Cd stress.

Keywords Cadmium (Cd) · miRNA · Degradome · Transcriptome · Integrated analysis · Rice (*Oryza sativa* L.)

Introduction

Contamination of soils by cadmium (Cd) as a result of industrial activity is a growing problem (Gallego et al. 2012; Du et al. 2013). The rice plant readily takes up the Cd^{2+} ion from the soil (Das et al. 1997; Hernandez et al. 1996) and translocates much of it into the grain.As a result, the consumption of rice, a staple across large parts of the world (Kosolsaksakul et al. 2014; Meharg et al. 2013), represents a major source of Cd in the human diet (Yu et al. 2006; Uraguchi et al. 2009). Therefore, reducing Cd accumulation in crops has become an important task. Although the mechanisms whereby Cd is up-taken, translocated and distributed by rice plants are well understood, little study has been performed to understand regulatory networks and targets involved in response to Cd in plants.

Micro-RNAs (miRNAs), a class of endogenous and non-coding small RNA with the length of 20–24 nt, are known to be involved in regulating the expression of a wide range of genes, a process achieved by their binding to a target mRNA, resulting in either the mRNA's degradation or blocking of its translation (Bartel 2004; Carrington and Ambros 2003). It is well known that miRNA take an important role in regulation of plant growth, development, and adaptation to various adverse environment including Cd stress (Chuck et al. 2009; Jones-Rhoades et al. 2006; Reinhart et al. 2002; Zhang et al. 2013; Ding et al. 2013; Zhou et al. 2007). In rice, *miR390*, *miR167*, *miR164*, *miR160*, *miR156*, *miR268* and *miRNA166* were responsive to Cd stress in rice based

Min Zhong and Fenglin Huang have contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10725-019-00547-6) contains supplementary material, which is available to authorized users.

✉ Xiangjin Wei
weixiangjin@caas.cn

✉ Peisong Hu
hupeisong@caas.cn; peisonghu@126.com

1 College of Agronomy, Jiangxi Agricultural University, Nanchang 330045, China

2 State Key Lab of Rice Biology, China National Rice Research Institute, Hangzhou 311400, China

3 Hunan Rice Research Institute, Hunan Academy of Agricultural Science, Changsha 410125, China

Published online: 19 October 2019

on microarray analysis (Ding et al. 2011, 2018) and high-throughput sequencing (Yu et al. 2019; Kang et al. 2017; Wang et al. 2019; Ding et al. 2017). The *Arabidopsis thaliana* plant responds to an excessive presence of Cd by altering the abundance of 13 miRNAs (Gielen et al. 2016), while a comparison drawn between two cultivars of *Brassica parachinensis* (one of which accumulates Cd inefficiently and the other efficiently) detailed 166 miRNAs as being present in significantly different abundance (Zhou et al. 2017). Understanding the biological significance of an individual miRNA requires the recognition of its target gene(s). Degradome sequencing is a high-throughput approach to validate target transcripts spliced by miRNA (Addo-Quaye et al. 2008). The degradome sequencing has been applied with some success in rice (Li et al. 2010), *A. thaliana* (Addo-Quaye et al. 2008) and canola (*B. napus*) (Xu et al. 2012). *miRNA164* targeted-transcript factor NAC was responsive to Cd stress in *Solanum torvum* (Kang et al. 2017) and maize (Wang et al. 2019) based on high-throughput degradome sequencing. The expression level of copper/zinc superoxide dismutase (CSD) was down-regulated by *miR398* under Cd stress in wheat (Qiu et al. 2016) and *Medicago truncatula* (Zhou et al. 2008).

Experiments have shown that the transcriptomic response of the rice plant to exposure to Cd involves over 2500 genes encoding, among others, glutathione s-transferases, sulfate-assimilation pathway enzymes, heat-shock proteins, transcription factors, and sulfate transporters (Tang et al. 2014). Comparative analysis of root transcriptome profiles between low-Cd-accumulating cultivar and high-Cd-accumulating cultivar of *Brassica parachinensis* revealed that the pathways involved in photosynthesis and cell growth were activated to mitigate Cd-induced damages (Zhou et al. 2019). Among 24,414 differentially expressed genes (DEGs), most of genes encoding important transcription factors such as auxin/indole-3-acetic acid (AUX/IAA), basic helix-loop-helix (bHLH) and MYBs, other involved in plant hormone signal transduction, and oxidative phosphorylation, were substantially upregulated under Cd stress in wild paper mulberry (Xu et al. 2019). According to the combined analysis based on the sequencing of microRNAome, the degradome and the transcriptome in the two Cd hyper-accumulating species of *Sedum alfredii*, 754 target genes of 194 miRNAs were identified to respond to Cd stress, such as ARF4 (auxin response factor 4), AAP3 (amino acid permease 3) and genes associated with auxin, redox-related secondary metabolism and metal transport pathways (Han et al. 2016). 18 and 71 targets for 28 novel and 109 known miRNAs, respectively were found to respond to Cd stress in radish (*Raphanus sativus*) based on a combined analysis of transcriptome, miRNAome and degradome (Xu et al. 2013). This approach has yet to be applied to rice. The objective of the present study was to characterize the miRNA-mediated regulation of the response of rice seedlings to Cd exposure, using such combined approach. The aim was to identify which genes were the targets of differentially abundant miRNAs, whereby gaining further insight on the molecular basis of Cd response.

Materials and methods

Plant materials and Cd exposure

The rice cultivar used in the experiments was 'Zhong Jiazao-17' from China National Rice Research Institute (CNRRI), an *indica* type cultivar known to accumulate rather low levels of Cd in its grain. Grains were surface-sterilized by immersion in 5% sodium hypochlorite for 5 min, rinsed three times in sterile distilled water, imbibed for 24 h, then held at 30 °C for 24 h. Germinating seedlings were transplanted to a hydroponics solution composed of Hoagland's solution (pH 5.5), following the suggestion of Wang (1996). Once the third leaf had fully expanded, the solution was either altered to include 0.1 mM $CdCl_2 \cdot 2.5H_2O$ (Cd treatment) or was not altered (Control), and the plants were left to grow for 12 days. Each treatment was represented by three replicates, each of which comprised a set of 24 seedlings of uniform sized. At the end of the treatment period, the chlorophyll content of the youngest expanded leaf was measured using a SPAD (soil and plant analyzer development)-5 chlorophyll meter (Konika Minolta, Tokyo, Japan). A portion of the seedlings was snap-frozen in liquid nitrogen for the purpose of transcriptomic (including microRNAome and the degradome) analysis, and the remaining portion dried at 72 °C for 48 h in order to determine both shoot dry weight and Cd content which was determined as previously described (Gong et al. 2003).

Construction, sequencing and analysis of the transcriptome library

Total RNA was extracted from frozen shoot tissue using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. The quantity and integrity of the resulting RNA were measured using a Bioanalyzer 2100 and an RNA 6000 Nano LabChip kit (Agilent, Santa Clara, CA, USA): samples were only accepted if the RIN number was > 7.0. Poly(A) mRNA was isolated from ~ 10 µg of total RNA representing a specific adipose type by the addition of magnetic beads coated in poly(T) (Invitrogen). The resulting mRNAs were fragmented using divalent cation under elevated temperature, then reverse-transcribed into cDNA, using the protocol supplied with an mRNA-Seq sample preparation kit (Illumina, San Diego, CA, USA). The average insert size was 300 bp (± 50 bp). Paired-end sequencing

was performed on an Hiseq 2500 device (Illumina) following the supplier's protocol. Prior to sequence assembly, all low-quality reads (those containing adaptor or primer sequence, along with those having a quality score < 20) were discarded. The edited set of reads was aligned and mapped onto the rice reference genome sequence (phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org Osativa) using TopHat software (Trapnell et al. 2009). Relative abundances of each transcript (measured using the FPKM parameter) were derived using Cufflinks software (Trapnell et al. 2012). Only transcripts having a q value < 0.05 and a status marked as "OK" in the Cuff-diff output were classified as differentially expressed genes (DEGs).

Small RNA library construction, sequencing and analysis

An aliquot of ~ 1 µg of the total RNA was used to prepare small RNA library, following the protocol provided with a TruSeq Small RNA Sample Prep kit (Illumina). Single-end sequencing (50 bp) was achieved using an Illumina Hiseq 2500 device at LC-BIO (Hangzhou, China) following a standard protocol. Raw reads were subjected to the Solexa 0.3 pipeline filter (Illumina) and the resulting dataset subjected to the ACGT101-miR program (LC Sciences, Houston, Texas, USA) in order to remove adapter dimers, rRNA, tRNAs, snRNAs, snoRNAs and repeats. Unique sequences in the length range 21–22 nt were matched with precursors listed in miRBase 21.0 (http://www.mirbase.org/) using a BLAST search. Those matching the hairpin region of a mature miRNA were considered as "known" miRNAs, and the remainder as "novel" miRNAs. The remaining sequences were mapped to precursors in miRBase 21.0 using a BLAST search and then their genomic location was obtained from the reference genome sequence. The differential abundance of a miRNA based on its normalized sequencing count was validated using the Students' *t* test, andthe significance threshold was set at 0.05.

Degradome sequencing, target identification and functional analysis

An aliquot of ~ 20 µg of total RNA from experimental samples was used to prepare a degradome library as follows: first ~ 150 ng poly(A) RNA was annealed with biotinylated random primers, and the RNAs were captured on streptavidin-coated beads. A 5′ adaptor was ligated to only those RNAs containing 5′-monophosphate, and the remaining RNA was reverse transcribed and processed by PCR. The resulting library was sequenced using the 5′ adapter only, resulting in the acquisition of the first 36 nucleotides, representing the 5′ end of the original RNA. The sequencing was performed using an Illumina Hiseq 2500 device housed at LC-BIO (Hangzhou, China). The potential target genes identified were subjected to an NCBI search using the BLASTX algorithm, and thereafter to a gene ontology (GO, Harris et al. 2004) and Kyoto Encyclopedia of Genes and Genomes (KEGG, Ogata et al. 1999) analysis. The networks of microRNA and their targeted genes were visualized using Cytoscape v3.0 software (Shannon et al. 2003). The potentially cleaved transcripts were classified into five categories based on the signature abundance at each occupied transcript position (Yang and Chen 2013).

Validation of differential abundance using quantitative real-time PCR (qRT-PCR)

A qRT-PCR assay was used to verify the differential abundance of a selection of the DEGs. The template was that used to derive the sequencing data. The RNA was reverse-transcribed to obtain sscDNA using either a First-Strand cDNA Synthesis kit (Toyobo, Osaka, Japan) or a miRcut Plus miRNA First-Strand cDNA Synthesis kit (Tiangen, Beijing, China). The reverse sequences of the specific miRNAs were used as the forward primers of the respective miRNA. Primers used to amplify DEGs and target genes were designed using the Primer5 program (Lalitha 2000). The rice *ubiquitin* (AF184280) and *5SrRNA* genes (Lang et al. 2011) were used as the reference sequences for measuring the abundance of transcripts and miRNA, respectively. All primer sequences are given in Table S1. The qRT-PCRs were based on either a SYBR qPCR mix (Toyobo) or a miRcute Plus miRNA qPCR Detection kit (Tiangen). Each reaction had three biological and technical replicates.

Results

The phenotypic effect of exposure to Cd stress

Exposure to 0.1 mM Cd^{2+} suppressed seedling growth: the seedlings under Cd stress were significantly shorter in stature than the seedlings of the control group (Fig. 1a). They also accumulated less biomass (Fig. 1b) and their leaves contained less chlorophyll (Fig. 1c). As expected, the Cd content of the shoot was higher under Cd stress than that in shoot tissue of control plants (Fig. 1d).

The transcriptomic response to Cd exposure

A comparison between the transcriptomes of seedlings under Cd treatment and control plants revealed a set of 4223 DEGs, of which 2487 were up-regulated in the seedling exposed to Cd (Fig. 2a). A GO analysis showed that most of the DEGs encode products involved in heme binding, response to stress and oxidoreductase activity

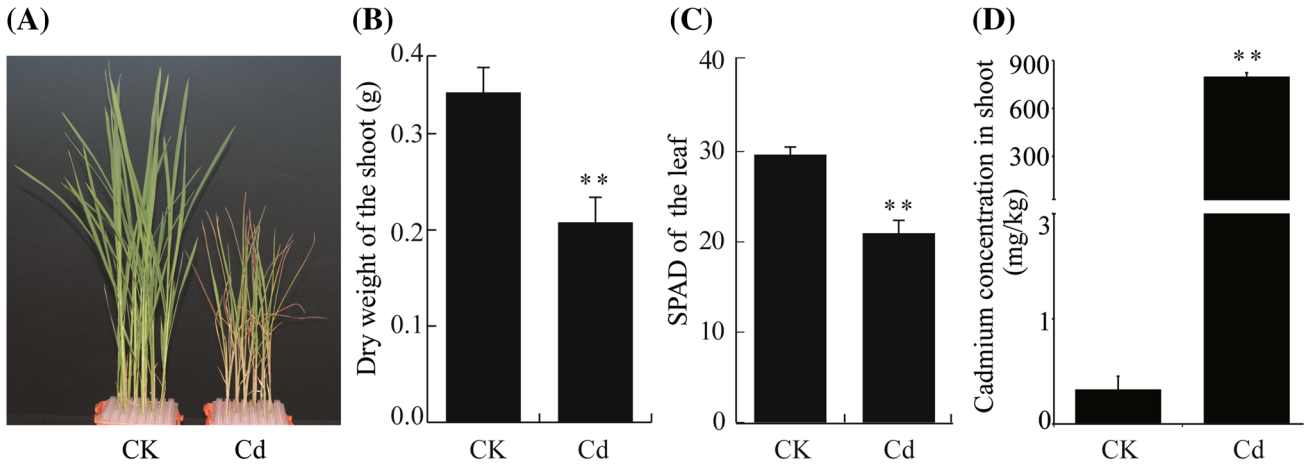

Fig. 1 The morphology and Cd content of hydroponics-grown rice seedlings exposed to Cd. **a** The appearance of seedlings challenged for 12 days with 0.1 mM Cd^{2+}, **b** the effect of Cd exposure on the accumulation of biomass: each value represents the mean of five seedlings, **c** the effect of the treatment on the content of chlorophyll in the leaf, **d** the effect on shoot Cd content. All data shown in the form mean ± SE. ($n = 3$). **Seedling performance means of the seedlings under Cd treatment and the control differ significantly ($P < 0.01$) from one another. Error bars represent SE

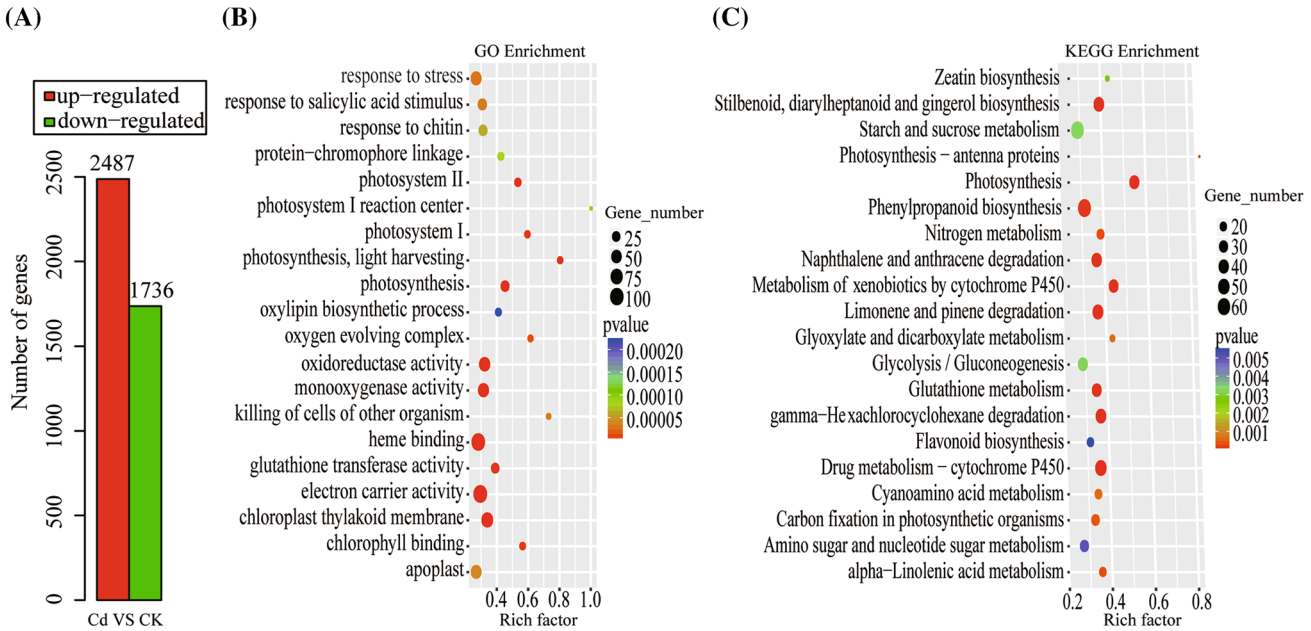

Fig. 2 Differentially transcribed genes (DEGs) and their ontology and pathway enrichment analysis. **a** The number of genes up- and down-regulated by exposure to 0.1 mM Cd. **b** GO enrichment classification of the set of DEGs, **c** KEGG pathway enrichment analysis of the set of DEGs

(Fig. 2b). A KEGG analysis suggested some enrichment for genes encoding proteins participating in photosynthesis-related pathways (such as Metabolism of xenobiotics by cytochrome P450, drug metabolism—cytochrome P450) and in protein degradation (naphthalene and anthracene degradation, limonene and pinene degradation, and γ—hexachlorocyclohexane degradation) (Fig. 2c).

The set of miRNAs responding to Cd exposure

The total numbers of raw reads recovered from leaf RNA of seedlings under Cd treatment and the control were, respectively, 15,561,015 and 18,177,924 (Table S2). Of these, 2,872,995 and 2,826,563, respectively, were deemed to be miRNAs (Table S2). The range in length of the short

RNAs did not differ significantly between the two libraries (Fig. S1). In all, 699 of the presumed miRNAs differed in abundance between the seedlings under Cd stress and the control group. They have been categorized into four different groups based on their abundance and whether they are known or novel miRNAs (Table S3). The known ones, which totaled 634, belong to 65 recognized miRNA families (Fig. S2). Of these, only those represented by at least ten reads in one of the libraries and showing a fold difference in abundance between the two libraries of at least two were analyzed further. Applying these criteria the number of miRNAs was reduced from 699 to 40. Of these 40, 38 were known miRNAs (belonging to 22 families); they included 29 conserved and nine non-conserved sequences (Fig. 3, Tables S4, S5). With the exception of three members of the *miRNA156* family, members belonging to the same family responded similarly to Cd exposure. Thus, 13 of the miRNAs that belong to nine miRNA families were all significantly reduced in abundance by the Cd treatment, while 22 miRNAs that belong to 12 miRNA families, along with the two novel miRNAs (*PC-3p-38247_129* and *PC-3p-102187_26*), were all significantly increased in abundance.

Cd responsive genes targeted by miRNAs identified by degradome analysis

Degradome sequencing was performed to attempt to identify the genes targeted by the differentially abundant miRNAs. The five categories (0 through 4) harbored, respectively, 171, 16, 216, 8 and 346 transcripts (Fig. S3, Table S6). A set of 101 differentially abundant transcripts, generated by 61 genes, was concluded to represent the targets of 23 of the

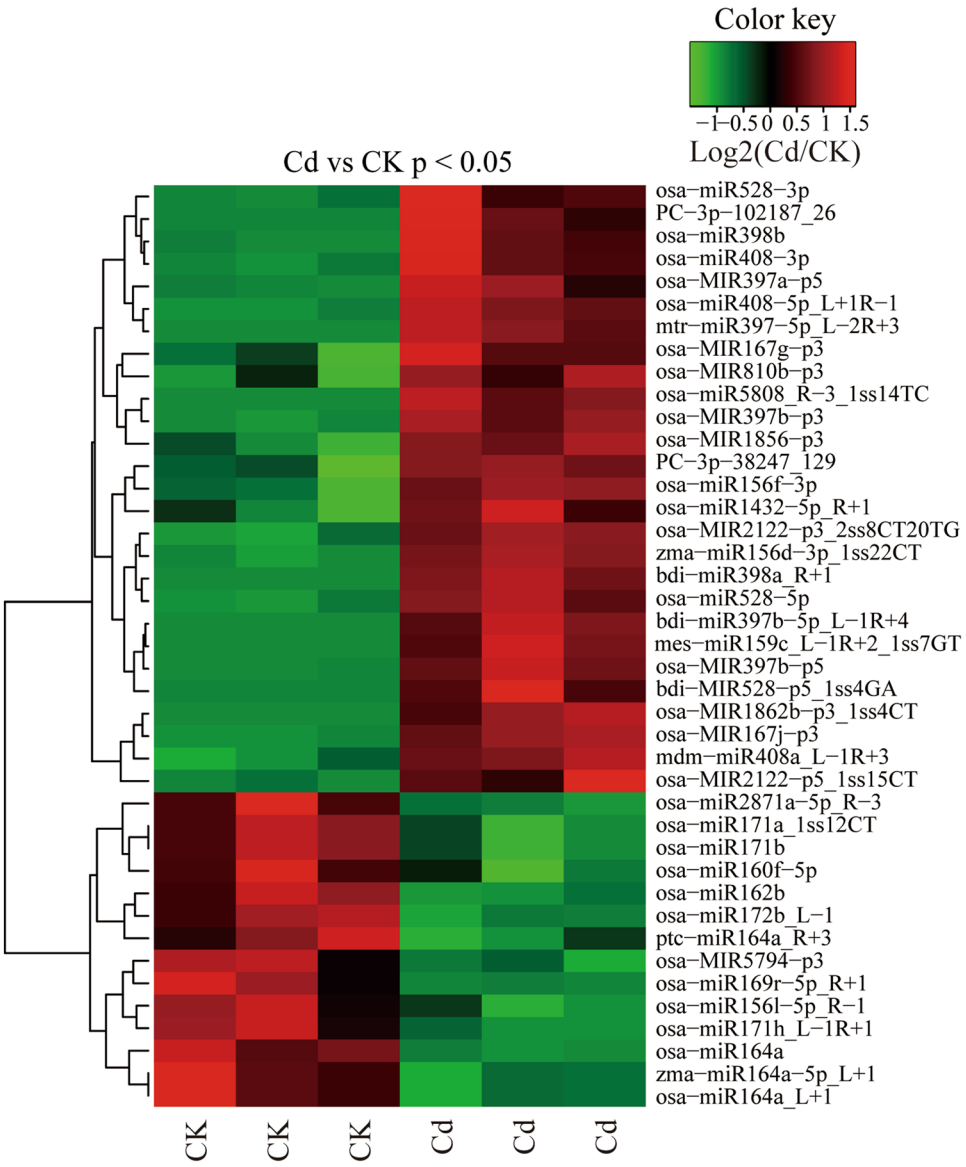

Fig. 3 A hierarchical clustering of differentially abundant miRNAs. Differentially abundant miRNAs identified in the comparison between Cd-treated and CK-treated seedlings. Cells colored in red indicate an enhanced abundance of the relevant miRNA and those colored in green indicate a lower abundance. Raw abundances were normalized using Z-score normalization. The absolute signal intensity ranged from − 1 to + 1.5

Cd-responsive miRNAs (Table S7). Of these 61, 15 genes were negatively regulated by 10 Cd-responsive miRNAs (Table 1).

GO and KEGG analyses of the Cd-responsive genes targeted by miRNAs are shown in Fig. 4. The former analysis indicated enrichment for genes involved in transcription factor activity (GO:0016602, CCAAT-binding factor complex, GO:0006355, regulation of transcription, DNA-dependent and GO:0003700, sequence-specific DNA binding transcription factor activity) and auxin-mediated signaling (GO:0009734) (Fig. 4a). The output of the KEGG analysis implied that most of the target genes encoded products involved in nitrogen metabolism (ko00910), ascorbate and aldarate metabolism (ko00053), and protein degradation (ko04612: antigen processing and presentation, ko03050: proteasome and ko03040: spliceosome) (Fig. 4b).

Integrated analysis of the transcriptome, the miRNAome and the degradome

An integrated analysis of the transcriptome, the miRNAome and the degradome was then performed in an attempt to elucidate the regulatory network operating in the plant response to Cd exposure. This analysis suggested that 227 target genes interacted with 250 miRNAs (Fig. S4, Table S8). Of this gene set, 18 were down-regulated as a result of an interaction with 18 miRNAs (Fig. 5a, Table S9). Most of the targets were regulated by just one miRNA, but the gene *OMTN3* interacted with 13 miRNAs belonging to eight miRNA families, indicating that this target gene plays a key role in response to cadmium stress in the seedling. And most of miRNAs targeted at least three genes: for instance, *osa-miRNA156I-5p_R-1* interacted with six targets and *osa-miRNA164a* with four. A qRT-PCR assay of 16 of the miRNA-target pairs (Fig. 5b) and of 23 of the DEGs encoding products involved in either photosynthesis or protein degradation (Fig. S5), indicated that the sequence-based identification of differential abundance for miRNA and genes were reliable (Table S8–9).

Based on a functional analysis of miRNA targets and the DEGs, Cd responsive miRNA targets and the DEGs were involved in regulating transcription, signaling transduction or protein degradation (Tables 2, S9). For instance, transcription factors, such as SCL6, various SPLs and NAC, were separately targeted by *osa-miR171, osa-miR156I-5p_R-1* and miRNAs (*osa-miR156I-5p_R-1*, *osa-miR160f-5p*, *osa-miR162b* and *osa-miR164a*). According to both, sequencing data (Tables 2, S9) and qRT-PCR (Figs. 5B, S5), SPL2 and SPL16 were induced by Cd exposure and both were negatively regulated by *osa-miR156I-5p_R-1*. The signaling-related genes MPK8, GAMYB and ARF13 were negatively-regulated by *osa-miR160f-5p* and *cmes-miR159c_L-1R + 2_1ss7GT* in response to Cd exposure, respectively. Additionally, the stress up-regulated genes NFYA6 and DTGP10, which interacted with the miRNAs *osa-miR169r-5p_R + 1* and *osa-miR171a_1ss12CT*, respectively (Fig. 4), encoded products that participate in protein degradation, a pathway also featured by products of five of the DEGs, encoding calreticulin family proteins, DnaK family proteins and heat shock proteins (Fig. S5; Table S9). In conclusion, our results show that the response of rice to Cd exposure involves multiple pathways, including at least transcription modification, signaling transduction and protein degradation.

Discussion

Plants, including rice, have evolved various strategies to cope with excessive levels of Cd in the soil; some of these involve post-transcriptional regulation mediated by miRNAs (Lu et al. 2005; Jones-Rhoades and Bartel 2004). Analysis of the response to Cd exposure shown by the Cd hyperaccumulator species *S. alfredii* (Han et al. 2016) and radish (Xu et al. 2013) has led to the identification of a number of Cd-responsive miRNAs and their target genes. Both RNA sequencing (Li et al. 2013; Huang et al. 2009) and microarray analysis (Ding et al. 2011, 2013) have revealed numerous rice miRNAs as responsive to Cd exposure, but the current study represents the first attempt to combine microRNAome, degradome and transcriptome sequencing for this purpose. As a result, 40 miRNAs, of which two have not been documented to date as being present in rice, are classified as being Cd-responsive (Fig. 3, Table S4), while 18 genes, encoding various transcription factors, signaling proteins and abiotic stress-related proteins are identified as targets (Table 2).

The Cd-responsive population of miRNAs in the rice seedling

A number of miRNAs have been reported to be highly conserved across plant species, whereas a much smaller number appears to be species-specific (Martinez et al. 2011; Chen et al. 2012). Here, consistent with findings in radish (Xu et al. 2013), 29 of the 40 Cd-responsive miRNAs were classified as being highly conserved, about three times the number of those considered as non-conserved (Fig. 3; Tables S4, S5). The participation of miRNAs in regulating the response to Cd exposure has been demonstrated in rice (Li et al. 2013; Ding et al. 2011), canola (Zhou et al. 2012) and radish (Xu et al. 2013). Here, members of some miRNA families (*miRNA156*, *miRNA162* and *miRNA171*) were all reduced in abundance in the seedlings under Cd treatment compared to control plants, while for other families (*miR528* and *miRNA1432*) their abundance was increased, consistent

Table 1 Differentially abundant targets negatively regulated by Cd responsive miRNAs

Small RNA	log2 (Cd/CK)	P value	Transcript	Symbol	log2 (Cd/CK)	P value	Transcript annotation	Degradome detection	Alignment score
osa-miR171a_1ss12CT	−0.81	0.007	LOC_Os02g44370	SCL6	2.63	1.15E−83	Scarecrow-like protein 6	Y	1
osa-miR171a_1ss12CT	−0.81	0.007	LOC_Os05g34460	DEGP10	inf	1.264E−56	Protease Do-like 10, mitochondrial	Y	4
osa-miR171b	−0.81	0.007	LOC_Os02g44370	SCL6	2.63	1.145E−83	Scarecrow-like protein 6	Y	1
osa-miR171b	−0.81	0.007	LOC_Os05g34460	DEGP10	inf	1.264E−56	Protease Do-like 10, mitochondrial	Y	4
osa-miR164a	−1.94	0.014	LOC_Os02g36880	NAC100	1.92	5.93E−67	NAC domain-containing protein 100	Y	3
osa-miR164a	−1.94	0.014	LOC_Os06g46270	NAC021	0.49	0	NAC domain-containing protein 21/22	Y	2
osa-miR164a	−1.94	0.014	LOC_Os12g41680	NAC021	1.13	0	NAC domain-containing protein 21/22	Y	2
osa-miR164a	−1.94	0.014	LOC_Os12g05260	PSK5	0.82	1.551E−20	Phytosulfokines 5	Y	3
osa-miR164a_L+1	−1.57	0.029	LOC_Os02g36880	NAC100	inf	4.709E−06	NAC domain-containing protein 100	Y	3
osa-miR164a_L+1	−1.57	0.029	LOC_Os06g46270	NAC021	1.40	4.628E−20	NAC domain-containing protein 21/22	Y	3
zma-miR164a-5p_L+1	−1.57	0.029	LOC_Os02g36880	NAC100	inf	4.709E−06	NAC domain-containing protein 100	Y	3
zma-miR164a-5p_L+1	−1.57	0.029	LOC_Os06g46270	NAC021	1.40	4.628E−20	NAC domain-containing protein 21/22	Y	3
ptc-miR164a_R+3	−1.33	0.015	LOC_Os12g41680	NAC021	1.13	0	NAC domain-containing protein 21/22	Y	3
ptc-miR164a_R+3	−1.33	0.015	LOC_Os06g46270	NAC021	0.49	0	NAC domain-containing protein 21/22	Y	4
ptc-miR164a_R+3	−1.33	0.015	LOC_Os12g05260	PSK5	0.82	1.551E−20	Phytosulfokines 5	Y	4
mes-miR159c_L-1R+2_1ss7GT	inf	0.019	LOC_Os01g47530	MPK8	−inf	3.656E−24	Mitogen-activated protein kinase 8	Y	3.5
mes-miR159c_L-1R+2_1ss7GT	inf	0.019	LOC_Os01g59660	GAM1	−inf	3.477E−05	Transcription factor GAMYB	Y	3.5
osa-miR156 l-5p_R-1	−1.31	0.023	LOC_Os02g07780	SPL4	1.40	8.83E−11	Squamosa promoter-binding-like protein 4	Y	2.5
osa-miR156 l-5p_R-1	−1.31	0.023	LOC_Os02g04680	SPL3	0.04	0.033208	Squamosa promoter-binding-like protein 3	Y	2

Table 1 (continued)

Small RNA	log2 (Cd/CK)	P value	Transcript	Symbol	log2 (Cd/CK)	P value	Transcript annotation	Degradome detection	Alignment score
osa-miR156 l-5p_R-1	− 1.31	0.023	LOC_Os08g41940	SPL16	inf	2.448E−38	Squamosa promoter-binding-like protein 16	Y	2.5
osa-miR156 l-5p_R-1	− 1.31	0.023	LOC_Os01g69830	SPL2	0.37	1.653E−24	Squamosa promoter-binding-like protein 2	Y	2.5
osa-miR156 l-5p_R-1	− 1.31	0.023	LOC_Os09g32944	SPL18	2.40	8.544E−67	Squamosa promoter-binding-like protein 18	Y	2.5
osa-miR160f-5p	− 0.82	0.033	LOC_Os04g59430	ARF13	inf	0	Auxin response factor 13	Y	1
osa-miR169r-5p_R+1	− 3.41	0.049	LOC_Os03g07880	NFYA6	0.74	2.419E−19	Nuclear transcription factor Y subunit A-6	Y	3

Alignment Score: The prediction of target genes in cDNA libraries and small RNA libraries of sequenced species was analyzed by targetfinder, and the predicted results were scored. The scoring rules are as follows: (1) Mismatches: minus 1 penalty; (2) G: U pairing: penalty point − 0.5; (3) if the above two conditions occur in the 5′-terminal small RNA sequences at bits 2–13, the penalty points will be doubled. The penalty is not more than 4 point

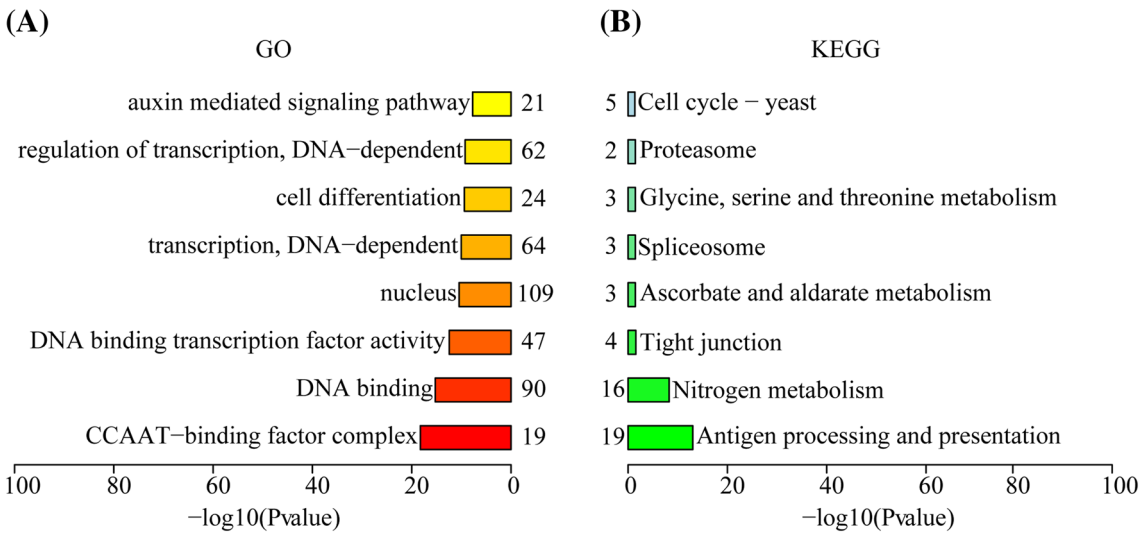

Fig. 4 Functional analysis of the set of putative target genes of the Cd responsive miRNAs. **a** GO enrichment classification, **b** KEGG pathway enrichment analysis of target genes. The data besides the bar plot represent count of genes participating in the corresponding GO or KEGG terms

with conclusions derived from both a microarray-based experiment (Ding et al. 2011) and a combined miRNAs and mRNAs analysis (Li et al. 2013). In canola, the abundance of *miRNA167*, *miRNA397* and *miRNA408* was reduced by exposure to Cd, while that of *miR172* was increased (Zhou et al. 2012). In radish, meanwhile, the abundance of *miRNA167*, *miRNA397* and *miRNA408* was enhanced by the stress and that of *miR172* was reduced (Xu et al. 2013). The species differences described above may be a genuine species effect, but they may also reflect differences in the severity of the stress treatment imposed in the various experiments. Of the 40 induced miRNAs, two (*PC-3p-38247_129* and

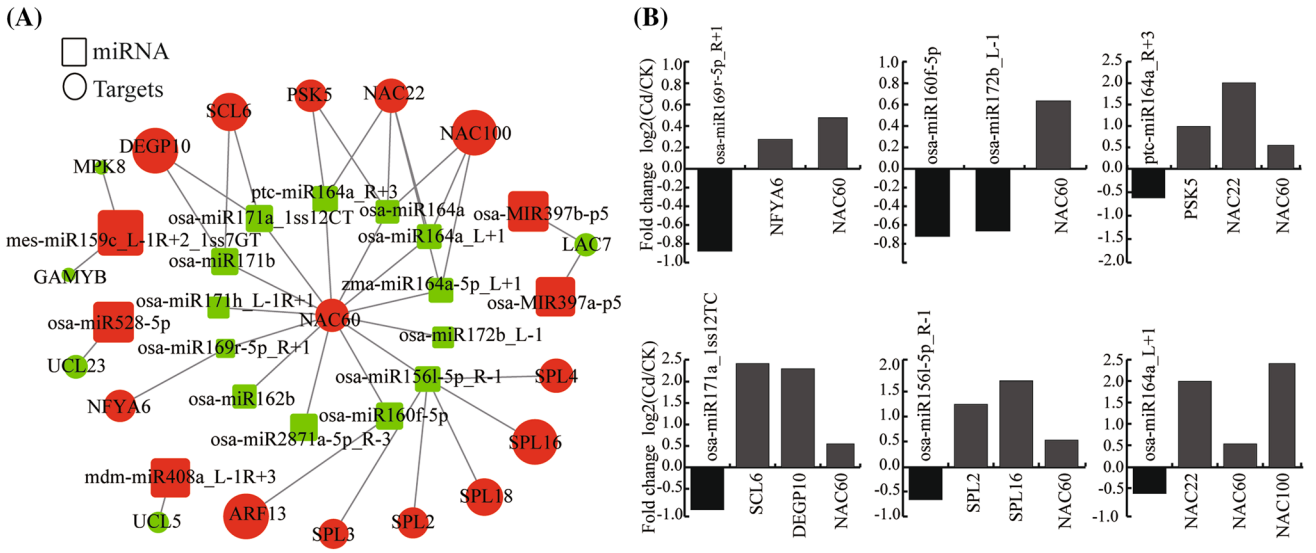

Fig. 5 Network of Cd-responsive miRNAs and their target genes and RT-PCR validation. **a** Network of Cd-responsive miRNAs and their target genes as displayed by Cytoscape software. Red lettering implies an increased abundance and green lettering a reduced abundance. Full forms of the abbreviated ID's are given in Table S8; **b** Confirmation of the differential abundance of miRNAs and target genes obtained by qRT-PCR analysis. The rice genes *Ubquitin* and *5SrRNA* were used as the reference sequences. The abundance of each miRNA and mRNA in rice seedlings exposed to Cd were normalized by comparison with their abundance in seedlings not exposed to Cd (CK). The black and gray bars indicate the abundance of, respectively, the various miRNAs and target genes

PC-3p-102187_26) have not been documented to date, while seven appear to be rice-specific (*miRNA810*, *miRNA818*, *miRNA1856*, *miRNA2122*, *miRNA2871*, *miRNA5794* and *miRNA5808*). Nevertheless, no target gene was identified for these Cd-responsive miRNAs. The precise regulatory activity of these miRNAs during the response to Cd exposure remains to be experimentally determined.

miRNA-mediated response to Cd exposure

Although numerous genes targeted by both conserved and non-conserved miRNAs have been proposed to participate in the response of rice to Cd exposure, only a few have been tested experimentally (Li et al. 2013; Huang et al. 2009; Ding et al. 2011). Here, 18 DEGs, associated with specific Cd-responsive miRNAs, have been described (Table 2). As pointed out elsewhere (Bonnet et al. 2004), most of the targets of Cd-responsive miRNAs have proven to be transcription factors, including the products of *ARF13*, *SCL6*, various *SPLs*, *NFYA6*, *GAMYB* and *NAC*. In the present study, five Cd induced *SPL* genes were identified to be up-regulated by *osa-miR156I-5p_R-1*, such as *OsSPL2*, *OsSPL3*, *OsSPL4*, *OsSPL16* and *OsSPL18*, which was consistent with the Cd-induced SPLs down-regulation by *miRNA156* in maize (Wang et al. 2019), barley (Yu et al. 2019) and rice (Ding et al. 2011). In addition, *DEGP10* gene which were up-regulated by *osa-miR171a_1ss12CT* in response to Cd exposure, is involved in the degradation of damaged proteins (Kato et al. 2012), suggesting that *DEGP10*/*osa-miR171a_1ss12CT* interactions may also respond to Cd stress through degradation of damaged proteins. This conclusion was in agreement with the result from transcriptomic analysis that DTGs, encoding calreticulin family proteins, DnaK family proteins and heat shock proteins (Table S9), were induced by Cd exposure. It is likely that many of the miRNA-target gene interactions respond to Cd stress through abscisic acid (*ABA*) and/or auxin signal cascades, which is also a mechanism involved in the *miR165/166*-mediated regulation of abiotic stress response exerted by *ABI4* and *BG1* (Yan et al. 2016). In the present study the Cd-responsive gene *ARF13* interacts with the miRNA *osa-miR160f-5p*. The signaling-related genes *MPK8* and *GAMYB*, which were both targeted by cmes-*miR159c_L-1R+2_1ss7GT*, were down-regulated by Cd exposure, which implies that they were regulated by this miRNA as a result of Cd exposure. We postulate that a number of miRNA/transcription factor interactions induce signaling pathways which are switched on as a result of the rice seedling's exposure to Cd. In summary, miRNA-mediated response to Cd exposure were involved in regulating transcription, signaling and protein degradation. Clearly, this hypothesis requires to be tested experimentally.

Table 2 Cd responsive targets identified by integrated analysis of the transcriptome, miRNAome and degradome

Small RNA	log_2(Cd/CK)	Target	log_2(Cd/CK)	Symbol	GO_Term
mdm-miR408a_L-1R+3	2.98	LOC_Os02g49850	− 2.70	UCL5	GO:0005507 (copper ion binding); GO:0009055 (electron carrier activity)
mes-miR159c_L-1R+2_1ss7GT	inf	LOC_Os01g47530	− inf	MAPK8	GO:0005524 (ATP binding); GO:0004707 (MAP kinase activity)
mes-miR159c_L-1R+2_1ss7GT	inf	LOC_Os01g59660	− inf	GAMYB	GO:0003677 (DNA binding); GO:0006355 (regulation of transcription, DNA-dependent);
osa-miR156I-5p_R-1	− 1.31	LOC_Os02g07780	1.40	SPL4	GO:0046872 (metal ion binding); GO:0006355 (regulation of transcription, DNA-dependent)
osa-miR156I-5p_R-1	− 1.31	LOC_Os02g04680	0.04	SPL3	GO:0046872 (metal ion binding); GO:0006355 (regulation of transcription, DNA-dependent)
osa-miR156I-5p_R-1	− 1.31	LOC_Os08g41940	inf	SPL16	GO:0046872 (metal ion binding); GO:0006355 (regulation of transcription, DNA-dependent)
osa-miR156I-5p_R-1	− 1.31	LOC_Os01g69830	0.37	SPL2	GO:0046872 (metal ion binding); GO:0006355 (regulation of transcription, DNA-dependent)
osa-miR156I-5p_R-1	− 1.31	LOC_Os09g32944	2.40	SPL18	GO:0046872 (metal ion binding); GO:0006355 (regulation of transcription, DNA-dependent)
osa-miR156I-5p_R-1	− 1.31	LOC_Os12g41680	1.09	NAC60	GO:0003700 (transcription factor activity); GO:0009734 (auxin mediated signaling pathway)
osa-miR160f-5p	− 0.82	LOC_Os04g59430	inf	ARF13	GO:0009734 (auxin mediated signaling pathway); GO:0006355 (regulation of transcription, DNA-dependent)
osa-miR160f-5p	− 0.82	LOC_Os12g41680	1.09	NAC60	GO:0003700 (transcription factor activity); GO:0009734 (auxin mediated signaling pathway)
osa-miR162b	− 1.77	LOC_Os12g41680	1.09	NAC60	GO:0003700 (transcription factor activity); GO:0009734 (auxin mediated signaling pathway)
osa-miR164a	− 1.94	LOC_Os02g36880	1.92	NAC100	GO:0006355 (regulation of transcription)
osa-miR164a	− 1.94	LOC_Os06g46270	0.49	NAC22	GO:0003700 (transcription factor activity); GO:0009734 (auxin mediated signaling pathway)
osa-miR164a	− 1.94	LOC_Os12g41680	1.13	NAC60	GO:0003700 (transcription factor activity); GO:0009734 (auxin mediated signaling pathway)
osa-miR164a	− 1.94	LOC_Os12g05260	0.82	PSK5	GO:0008283 (cell proliferation); GO:0007275 (multicellular organismal development)
osa-miR164a_L+1	− 1.57	LOC_Os02g36880	inf	NAC100	GO:0006355 (regulation of transcription, DNA-dependent)
osa-miR164a_L+1	− 1.57	LOC_Os06g46270	1.40	NAC22	GO:0003700 (transcription factor activity); GO:0009734 (auxin mediated signaling pathway)
osa-miR164a_L+1	− 1.57	LOC_Os12g41680	1.09	NAC60	GO:0003700 (transcription factor activity); GO:0009734 (auxin mediated signaling pathway)
osa-miR169r-5p_R+1	− 3.41	LOC_Os03g07880	0.74	NFYA6	GO:0016602 (CCAAT-binding factor complex)
osa-miR169r-5p_R+1	− 3.41	LOC_Os12g41680	1.09	NAC60	GO:0003700 (transcription factor activity); GO:0009734 (auxin mediated signaling pathway)
osa-miR171a_1ss12CT	− 0.81	LOC_Os02g44370	2.63	SCL6	GO:0006355 (regulation of transcription, DNA-dependent)
osa-miR171a_1ss12CT	− 0.81	LOC_Os05g34460	inf	DEGP10	GO:0004252 (serine-type endopeptidase activity); GO:0006508 (proteolysis)
osa-miR171a_1ss12CT	− 0.81	LOC_Os12g41680	1.09	NAC60	GO:0003700 (transcription factor activity); GO:0009734 (auxin mediated signaling pathway)

Table 2 (continued)

Small RNA	log_2(Cd/CK)	Target	log_2(Cd/CK)	Symbol	GO_Term
osa-miR171b	− 0.81	LOC_Os02g44370	2.63	SCL6	GO:0006355 (regulation of transcription, DNA-dependent)
osa-miR171b	− 0.81	LOC_Os05g34460	inf	DEGP10	GO:0004252 (serine-type endopeptidase activity); GO:0006508 (proteolysis)
osa-miR171b	− 0.81	LOC_Os12g41680	1.09	NAC60	GO:0003700 (transcription factor activity); GO:0009734 (auxin mediated signaling pathway)
osa-miR171h_L-1R+1	− 2.46	LOC_Os12g41680	1.09	NAC60	GO:0003700 (transcription factor activity); GO:0009734 (auxin mediated signaling pathway)
osa-miR172b_L-1	− 2.55	LOC_Os12g41680	1.09	NAC60	GO:0003700 (transcription factor activity); GO:0009734 (auxin mediated signaling pathway)
osa-miR2871a-5p_R-3	− 0.72	LOC_Os12g41680	1.09	NAC60	GO:0003700 (transcription factor activity); GO:0009734 (auxin mediated signaling pathway)
osa-MIR397a-p5	3.03	LOC_Os01g63190	− 2.09	LAC7	GO:0005507 (copper ion binding); GO:0008471 (laccase activity)
osa-MIR397b-p5	3.37	LOC_Os01g63190	− 2.09	LAC7	GO:0005507 (copper ion binding); GO:0052716 (hydroquinone:oxygen oxidoreductase activity)
osa-miR528-5p	3.47	LOC_Os08g04310	− 1.91	UCL23	GO:0005507 (copper ion binding); GO:0009055 (electron carrier activity)
ptc-miR164a_R+3	− 1.33	LOC_Os12g41680	1.13	NAC60	GO:0003700 (transcription factor activity); GO:0009734 (auxin mediated signaling pathway)
ptc-miR164a_R+3	− 1.33	LOC_Os06g46270	0.49	NAC22	GO:0003700 (transcription factor activity); GO:0009734 (auxin mediated signaling pathway)
ptc-miR164a_R+3	− 1.33	LOC_Os12g05260	0.82	PSK5	GO:0030154 (cell differentiation); GO:0008283 (cell proliferation)
zma-miR164a-5p_L+1	− 1.57	LOC_Os02g36880	inf	NAC100	GO:0006355 (regulation of transcription, DNA-dependent)
zma-miR164a-5p_L+1	− 1.57	LOC_Os06g46270	1.40	NAC22	GO:0003700 (transcription factor activity); GO:0009734 (auxin mediated signaling pathway)
zma-miR164a-5p_L+1	− 1.57	LOC_Os12g41680	1.09	NAC60	GO:0003700 (transcription factor activity); GO:0009734 (auxin mediated signaling pathway)

Acknowledgements This research was supported by the National Key Research and Development Program of China (2017YFD0100300), Agricultural Sciences and Technologies Innovation Program of Chinese Academy of Agricultural Sciences (CAAS) and by the Central Level, Non-Profit, Scientific Research Institutes Basic R & D Operations Special Fund (Y2017PT46; 2017RG002-1).

Author contributions MZ, FH, RL and YL performed the experiments. MZ and XW analyzed the data. PH and XW conceived the project. MZ, ZS and UA drafted the manuscript. PH, XW and ST critically revised the article. All authors have read and approved the final version of the manuscript.

Compliance with ethical standards

Conflicts of interest The authors declare no conflict of interest.

References

Addo-Quaye C, Eshoo TW, Bartel DP, Axtell MJ (2008) Endogenous siRNA and miRNA targets identified by sequencing of the *Arabidopsis* degradome. Curr Biol 18(10):758–762

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116(2):281–297

Bonnet E, Wuyts J, Rouze P, Van de Peer Y (2004) Detection of 91 potential conserved plant microRNAs in *Arabidopsis thaliana* and *Oryza sativa* identifies important target genes. Proc Natl Acad Sci USA 101(31):11511–11516

Carrington JC, Ambros V (2003) Role of microRNAs in plant and animal development. Science 301(5631):336–338

Chen L, Ren Y, Zhang Y, Xu J, Sun F, Zhang Z, Wang Y (2012) Genome-wide identification and expression analysis of heat-responsive and novel microRNAs in *Populus tomentosa*. Gene 504(2):160–165

Chuck G, Candela H, Hake S (2009) Big impacts by small RNAs in plant development. Curr Opin Plant Biol 12:81–86
Das P, Samantaray S, Rout GR (1997) Studies on cadmium toxicity in plants: a review. Environ Pollut 98(1):29–36
Ding Y, Chen Z, Zhu C (2011) Microarray-based analysis of cadmium-responsive microRNAs in rice (*Oryza sativa*). J Exp Bot 62(10):3563–3573
Ding Y, Qu A, Gong S, Huang S, Lv B, Zhu C (2013) Molecular identification and analysis of cd-responsive microRNAs in rice. J Agric Food Chem 61(47):11668–11675
Ding Y, Wang Y, Jiang Z et al (2017) MicroRNA268 overexpression affects rice seedling growth under cadmium stress. J Agric Food Chem 65(29):5860–5867
Ding Y, Gong S, Wang Y et al (2018) MicroRNA166 modulates cadmium tolerance and accumulation in rice. Plant Physiol 177(4):1691–1703
Du Y, Hu XF, Wu XH, Shu Y, Jiang Y, Yan XJ (2013) Effects of mining activities on Cd pollution to the paddy soils and rice grain in Hunan province. Central South China. Environ Monit Assess. 185(12):9843–9856
Gallego SM, Pena LB, Barcia RA, Azpilicueta CE, Iannone MF, Rosales EP, Zawoznik MS, Groppa MD, Benavides MP (2012) Unravelling cadmium toxicity and tolerance in plants: insight into regulatory mechanisms. Environ Exp Bot 83(Supplement C):33–46
Gielen H, Remans T, Vangronsveld J, Cuypers A (2016) Toxicity responses of Cu and Cd: the involvement of miRNAs and the transcription factor SPL7. BMC Plant Biol 16:145
Gong JM, Lee DA, Schroeder JI (2003) Long-distanc root-to-shoot transport of phytochelatins and cadmium in *Arabidopsis*. Proc Natl Acad Sci USA 100:10118–10123
Han X, Yin H, Song X, Zhang Y, Liu M, Sang J, Jiang J, Li J, Zhuo R (2016) Integration of small RNAs, degradome and transcriptome sequencing in hyperaccumulator uncovers a complex regulatory network and provides insights into cadmium phytoremediation. Plant Biotech J 14(6):1470–1483
Harris MA, Clark J, Ireland A et al (2004) The Gene Ontology (GO) database and informatics resource. Nucleic Acids Res 32(Database issue):258–261
Hernandez LE, CarpenaRuiz R, Garate A (1996) Alterations in the mineral nutrition of pea seedlings exposed to cadmium. J Plant Nutr 19(12):1581–1598
Huang SQ, Peng J, Qiu CX, Yang ZM (2009) Heavy metal-regulated new microRNAs from rice. J Inorg Biochem 103(2):282–287
Jones-Rhoades MW, Bartel DP (2004) Computational identification of plant MicroRNAs and their targets, including a stress-induced miRNA. Mol Cell 14(6):787–799
Jones-Rhoades MW, Bartel DP, Bartel B (2006) MicroRNAs and their regulatory roles in plants. Annu Rev Plant Biol 57:19–53
Kang XP, Gao JP, Zhao JJ et al (2017) Identification of cadmium-responsive microRNAs in *Solanum torvum* by high-throughput sequencing. Russ J Plant Physiol 64(2):283–300
Kato Y, Sun X, Zhang L, Sakamoto W (2012) Cooperative D1 degradation in the photosystem II repair mediated by chloroplastic proteases in *Arabidopsis*. Plant Physiol 159(4):1428–1439
Kosolsaksakul P, Farmer JG, Oliver IW, Graham MC (2014) Geochemical associations and availability of cadmium (Cd) in a paddy field system, northwestern Thailand. Environ Pollut 187:153–161
Lalitha S (2000) Primer Premier 5. Biotech Software & Internet Report 1(6):270–272
Lang QL, Jin CZ, Lai LY, Feng JL, Chen SN, Chen JS (2011) Tobacco microRNAs prediction and their expression infected with Cucumber mosaic virus and Potato virus X. Mol Biol Rep 38(3):1523–1531
Li YF, Zheng Y, Addo-Quaye C, Zhang L, Saini A, Jagadeeswaran G, Axtell MJ, Zhang W, Sunkar R (2010) Transcriptome-wide identification of microRNA targets in rice. Plant J. 62(5):742–759
Li BS, Duan H, Li JG, Deng XW, Yin WL, Xia XL (2013) Global identification of miRNAs and targets in *Populus euphratica* under salt stress. Plant Mol Biol 81(6):525–539
Lu SF, Sun YH, Shi R, Clark C, Li LG, Chiang VL (2005) Novel and mechanical stress-responsive microRNAs in *Populus trichocarpa* that are absent from Arabidopsis. Plant Cell. 17(8):2186–2203
Martinez G, Forment J, Llave C, Pallas V, Gomez G (2011) High-throughput sequencing, characterization and detection of new and conserved cucumber miRNAs. PLoS ONE. https://doi.org/10.1371/journal.pone.0019523
Meharg AA, Norton G, Deacon C, Williams P, Adomako EE, Price A, Zhu YG, Li G, Zhao FJ, McGrath S et al (2013) Variation in rice cadmium related to human exposure. Environ Sci Technol 47(11):5613–5618
Ogata H, Goto S, Sato K et al (1999) KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 27(1):29–34
Qiu Z, Hai B, Guo J et al (2016) Characterization of wheat miRNAs and their target genes responsive to cadmium stress. Plant Physiol Biochem 101:60–67
Reinhart BJ, Weinstein EG, Rhoades MW, Bartel B, Bartel DP (2002) MicroRNAs in plants. Genes Dev 16:1616–1626
Shannon P, Markiel A, Ozier O et al (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13(11):2498–2504
Tang MF, Mao DH, Xu LW, Li DY, Song SH, Chen CY (2014) Integrated analysis of miRNA and mRNA expression profiles in response to Cd exposure in rice seedlings. BMC Genom. https://doi.org/10.1186/1471-2164-15-835
Trapnell C, Pachte L, Salzberg SL (2009) TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25(9):1105–1111
Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, Pimentel H, Salzberg SL, Rinn JL, Pachter L (2012) Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc 7(3):562–578
Uraguchi S, Mori S, Kuramata M, Kawasaki A, Arao T, Ishikawa S (2009) Root-to-shoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice. J Exp Bot 60(9):2677–2688
Wang K (1996) Comparative study on Cd phytotoxicity to different genes of rice. Rural Eco-Environ 12(3):18–23
Wang B, Cheng D, Chen Z, Zhang M, Zhang G, Jiang M, Tan M (2019) Bioinformatic exploration of the targets of Xylem Sap miRNAs in Maize under Cadmium Stress. Int J Mol Sci. https://doi.org/10.3390/ijms20061474
Xu MY, Dong Y, Zhang QX, Zhang L, Luo YZ, Sun J, Fan YL, Wang L (2012) Identification of miRNAs and their targets from Brassica napus by high-throughput sequencing and degradome analysis. BMC Genom. https://doi.org/10.1186/1471-2164-13-421
Xu L, Wang Y, Zhai L, Xu Y, Wang L, Zhu X, Gong Y, Yu R, Limera C, Liu L (2013) Genome-wide identification and characterization of cadmium-responsive microRNAs and their target genes in radish (*Raphanus sativus* L.) roots. J Exp Bot 64(14):4271–4287
Xu Z, Dong M, Peng X et al (2019) New insight into the molecular basis of cadmium stress responses of wild paper mulberry plant by transcriptome analysis. Ecotoxicol Environ Saf 171:301–312
Yan J, Zhao C, Zhou J, Yang Y, Wang P, Zhu X, Tang G, Bressan R, Zhu J (2016) The miR165/166 mediated regulatory module plays critical roles in ABA homeostasis and response in *Arabidopsis thaliana*. PLoS Genet 12(11):e1006416
Yang ZM, Chen J (2013) A potential role of microRNAs in plant response to metal toxicity. Metallomics 5(9):1184–1190

Yu H, Wang JL, Fang W, Yuan JG, Yang ZY (2006) Cadmium accumulation in different rice cultivars and screening for pollution-safe cultivars of rice. Sci Total Environ 370(2–3):302–309

Yu J, Wu L, Fu L et al (2019) Genotypic difference of cadmium tolerance and the associated microRNAs in wild and cultivated barley. Plant Growth Regul 87(3):389–401

Zhang Q et al (2013) Genome-wide identification of *Thellungiella salsuginea* microRNAs with putative roles in the salt stress response. BMC Plant Biol 13:180

Zhou GK, Kubo M, Zhong RQ, Demura T, Ye ZH (2007) Overexpression of miR165 affects apical meristem formation, organ polarity establishment and vascular development in *Arabidopsis*. Plant Cell Physiol 48(3):391–404

Zhou ZS, Huang SQ, Yang ZM (2008) Bioinformatic identification and expression analysis of new microRNAs from *Medicago truncatula*. Biochem Biophys Res Commun 374(3):538–542

Zhou ZS, Song JB, Yang ZM (2012) Genome-wide identification of Brassica napus microRNAs and their targets in response to cadmium. J Exp Bot 63(12):4597–4613

Zhou Q, Yang YC, Shen C et al (2017) Comparative analysis between low- and high-cadmium-accumulating cultivars of *Brassica parachinensis* to identify difference of cadmium-induced microRNA and their targets. Plant Soil 420(1–2):223–237

Zhou Q, Yang Y, Yang Z (2019) Molecular dissection of cadmium-responsive transcriptome profile in a low cadmium-accumulating cultivar of *Brassica parachinensis*. Ecotoxicol Environ Saf 176:85–94