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ORIGINAL ARTICLE

RNA-sequencing analysis reveals transcriptional changes in the roots of low-cadmium-accumulating winter wheat under cadmium stress

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Abstract

Wheat is the second most important food crop worldwide, and thus, it is important to protect both its yield and quality. Cadmium (Cd) is a heavy metal with strong soil–plant mobility and biological toxicity. Thus, screening low-Cd-accumulating genotypes to elucidate the underlying molecular regulatory mechanisms and gene networks related to Cd stress may be useful to reduce dietary exposure to this heavy metal. Here, the root transcriptomes of the low-Cd-accumulating genotype YM16 treated (YT) or not treated (YC) with Cd were analyzed using the Illumina Hiseq4000 data. The gene expression analysis of YT and YC revealed 1427 differentially expressed genes (DEGs; 429 upregulated and 998 downregulated). Of these, 298 upregulated DEGs were assigned to 41 gene ontology (GO) terms, whereas 537 downregulated DEGs were assigned to 61 GO terms. The pathway enrichment analysis showed that phenylpropanoid biosynthesis, glutathione metabolism, sulfur metabolism, and nitrogen metabolism were significantly enriched in upregulated DEGs, which are involved in defense and detoxification in response to Cd stress. The expression pattern of eight selected DEGs was also analyzed by quantitative real-time polymerase chain reaction, and the results were consistent with those obtained by RNA-sequencing, confirming data reliability. Overall, our study might increase the understanding of complex molecular mechanisms that regulate the responses of plants to Cd stress, and help develop new improved wheat varieties with low Cd content in the grain.

Keywords Winter wheat · Cadmium · Low-Cd-accumulating genotype · RNA-sequencing

Introduction

Cadmium (Cd) is a heavy metal with strong biological toxicity and mobility in the ecosystem (Lima et al. 2006; Ali et al. 2013). Owing to rapid industrial development, high concentrations of Cd in wastewater, scrap, and exhaust fumes have caused large-scale soil pollution. Controlling Cd in the food chain is considered essential to protect human health, and consequently, phytoremediation has attracted attention worldwide due to the low repair cost, low soil disturbance, and complete remediation of heavy metals (Ali et al. 2013; Thakur et al. 2016). However, phytoremediation also has some limitations, such as few effective species, selectivity for heavy metal ions, low survival rate of introduced plants, loss of remediation ability by generation advancement, low or no economic value, and slow remediation rate (Ali et al. 2013). Therefore, other methods that will offer short-term solutions are needed to protect food quality.

Previous studies have identified low-Cd-accumulating genotypes of rice (Liu et al. 2005), pepper (Huang et al. 2015a), sweet potato (Huang et al. 2015b), and Chinese

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cabbage (Liu et al. 2010), which can grow in heavily contaminated sites and yield agricultural products with low Cd levels. Thus, the use of such genotypes is an effective and feasible measure to decrease dietary Cd exposure (Liu et al. 2005). Cd uptake, accumulation, translocation, and detoxification have been suggested to be genotype-dependent processes (Greger and Löfstedt 2004; Zhang and Duan 2008; Gill et al. 2014). However, the underlying molecular mechanisms remain unclear and might be elucidated by screening low-Cd-accumulating genotypes.

Wheat (*Triticum aestivum* L.) is the second most important food crop worldwide, and thus, it is crucial to protect both its yield and quality. Direct root-soil contact allows the plant to take up water and nutrients, which is necessary for all physiological and metabolic activities. Therefore, the root is the first organ that senses and responds to biotic and abiotic stresses. Although numerous studies have been conducted to investigate the molecular changes in the roots under abiotic stress conditions, little is known about the stress response mechanisms of low-Cd-accumulating winter wheat genotypes.

Transcriptome studies link genomic information and biological function, which helps to identify key targets of gene regulation related to growth, development, stress adaptation, and evolution (Pandey and Somssich 2009). High-throughput transcriptome techniques have been successfully applied to study different response mechanisms to abiotic stresses, including Cd stress, in many plant species, but not in winter wheat. Therefore, in the present study, root transcriptomic analysis of Cd-treated or untreated low-Cd accumulating winter wheat was carried out to identify the genes and metabolic pathways involved in Cd uptake, translocation and detoxification. Our data might be useful to further explore the molecular mechanisms related to low Cd accumulation in winter wheat.

Materials and methods

Plant material and treatments

In the present study, we used the low-Cd-accumulating genotype Yaomai16 (YM; 0.066 mg kg^{-1} dry grain weight), which was selected from a field screening trial conducted in Henan Province, China. The screening trial included 79 winter wheat genotypes collected from major wheat-growing areas in China (Henan Province, Hebei Province, Shandong Province, Shanxi Province, and the northern areas of Anhui Province), which were evaluated in Henan Province for 2 consecutive years. The climate in the area is warm, continental, monsoon climate, with an average annual temperature of 14.1 °C, an average annual rainfall of 588.7 mm, a frost-free period of 210 days, and an average annual sunshine duration of 2398.8 h. The physical and chemical properties of the soil are shown in Table S1. The concentration of Cd in YM grains (0.066 mg kg^{-1}, dry weight) was significantly lower than that of other genotypes (Fig. S1).

The seeds of YM were immersed in 75% (v/v) ethanol solution for 5 min and then rinsed 5 times with deionized water. The soaked seeds were placed on a sterilized gauze for germination at 28 °C. After full emergence, the seeds were transplanted into pots (38 × 30 × 12 cm) containing well-washed and sterilized sand (particle size of 0.5–0.8 mm). At the stage of 2 leaves and 1 bud, 10 seedlings of uniform size were transplanted into a single polyethylene pot filled with 2 L Hoagland's solution (pH 6.5), which was replaced every 3 days, and placed in climate-controlled chamber (12 h photoperiod/27 °C, 12 h dark period/18 °C; light intensity of 1000 μmol m^{-2} s^{-1}; Blum and Sullivan 1997). The wall and bottom of the container were covered with black plastic to stimulate root growth. After 10 days, $CdCl_2$ (100 μmol Cd L^{-1}; YT) was added in the nutrient solution. In YC, no $CdCl_2$ was added. At 48 h after Cd exposure (Peng et al. 2015), fresh roots were collected, snap frozen in liquid nitrogen, and stored at − 80 °C until further analyses. Each treatment was repeated in triplicate.

RNA isolation, library construction, and Illumina sequencing

Total RNA was isolated from untreated YM roots and those treated with Cd, using the Plant RNA Kit (Omega, USA). The purity of RNA was checked using the NanoPhotometer spectrophotometer (IMPLEN, CA, USA), and the integrity was assessed using the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA). The mRNA was isolated using poly-T oligo-attached magnetic beads and fragmented into short segments using the RNA Fragmentation Kit (Ambion). Double-stranded cDNA was synthesized by reverse transcription using random hexamer primers, followed by the addition of buffers, dNTPs, and DNA polymerase I. The cDNA was then purified using AMPure XP beads and modified by end-repair, poly(A) addition, and adaptor ligation. The fragment size of the double-stranded cDNA was selected using AMPure XP beads, and the cDNA library was constructed by polymerase chain reaction (PCR) amplification. cDNA libraries were sequenced using Illumina Hiseq4000 to obtain 150 bp paired-end reads. All the Illumina reads have been submitted to the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/sra) with the accession number SRP125276.

Read processing and identification of differentially expressed genes

The original image data files obtained by RNA sequencing (RNA-seq) were transformed into raw reads by CASAVA. Subsequently, the raw reads were filtered to exclude reads containing adaptors, reads with poly-N ($N > 10\%$), and low-quality reads ($Q \leq 5$) using in-house perl scripts. The Q20, Q30, and GC content of clean data were calculated to check the quality of reads. The reference genome and gene model annotation files were downloaded from the NCBI (https://www.ncbi.nlm.nih.gov/genome/11). The reference genome index was built using Bowtie 2.0.6 (Langmead and Salzberg 2012), and paired-end clean reads were aligned to the reference genome using TopHat2 2.1.0 (mismatch parameter, 2; all other parameters, default values) (Kim et al. 2013). The number of reads mapped to each gene was calculated by HTSeq 0.5.4 p3, and the gene expression levels were calculated by the fragments per kilobase of exon model per million mapped reads (FPKM; Mortazavi et al. 2008) method. The aligned reads were analyzed using Cufflinks, and the assembled transcripts were further filtered using the FPKM value > 0. To test the repeatability and reliability of the results, the relation of expression patterns among YT and YC replicates was tested by Pearson's correlation test. Differential expression analysis (3threebiological replicates per treatment) was performed using the "DESeq" package in R 1.10.1. The *P* values were adjusted to control false discovery rate (Benjamini and Hochberg 1995) using DEGsEq. The genes with an adjusted *P* value of < 0.05 were assigned as differentially expressed genes (DEGs).

Gene ontology classification and pathway enrichment analysis

Gene ontology (GO) classification is a standard system that consists of three main ontologies: molecular function, biological process, and cellular component. The GO classification of the DEGs was performed using the "GOseq" package in the R environment, and the gene length bias was corrected. The GO terms with corrected *P* values of < 0.05 were considered significantly enriched. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of DEGs was performed using KOBAS (Mao et al. 2005).

Quantitative real-time PCR analysis

The total RNA was isolated from YM roots, and the purity and integrity were checked following the method described above. Eight DEGs were selected to validate RNA-seq data by quantitative real-time PCR (q-PCR) using actin as a housekeeping gene. All primers were designed by Invitrogen (Carlsbad, CA, USA) and are listed in Table S2. The cDNA was prepared using the PrimeScript RT reagent kit with gDNA Eraser (TaKaRa, Tokyo, Japan) according to the instruction of manufacturer. The q-PCR was performed using an optical 96-well plate with the ABI 7500 Real-Time System (Applied Biosystems, Foster City, CA, USA). The thermal cycling conditions were as follows: 40 cycles at 95 °C for 30 s, 95 °C for 5 s, and 60 °C. The relative quantitative method ($2^{-\Delta\Delta CT}$) was used to calculate the fold change in the expression levels of the selected DEGs. All reactions were performed in three technical replicates using one biological sample. For the functional annotation of the eight selected DEGs, their sequences were compared with homologous sequences in the non-redundant (NR) database.

Results

Transcriptome assembly and quality assessment

RNA-seq yielded 52,421,235 raw reads for YC and 53,332,260 raw reads for YT, whereas 51,349,956 and 52,125,145 clean reads were obtained, respectively, after filtering. The GC content of clean reads was almost identical between YC and YT (Table S3). Of all the clean reads, 93.92% had a quality score of 20; 62.3–69.39% were mapped to the wheat reference genome in YT; 53.69–57.41% were mapped to the wheat reference genome in YC; 48.4–62.47% were uniquely mapped reads in YT or YC; and 5.3–6.62% were multiple aligned reads (Table 1).

Analysis of differential transcript expression

Differential transcript expression was analyzed based on the read count data obtained from the analysis of gene expression levels. The RNA-seq correlation coefficients of FPKM among YC and YT replicates showed that the gene expression patterns were similar (Fig. 1a), confirming the repeatability and reliability of evaluation results. Using FPKM > 1 as the criterion for gene expression, we found that 45,984 and 43,553 genes were expressed in the roots of YC and YT, respectively. Of these, 41,633 genes were co-expressed in YC and YT, whereas 4351 and 1920 genes were specifically expressed in YT and YC, respectively (Fig. 1b). A total

Table 1 Root transcriptome assembly of Yaomai16 seedlings treated (YT) or not treated (YC) with Cd

Sample name	YC	YT
Total reads	51,349,956	52,125,145
Total mapped reads	28,439,714 (55.42%)	34,481,400 (66.15%)
Multiple mapped reads	2,860,716 (5.57%)	3,405,550 (6.53%)
Uniquely mapped reads	25,578,998 (49.84%)	31,075,850 (59.62%)

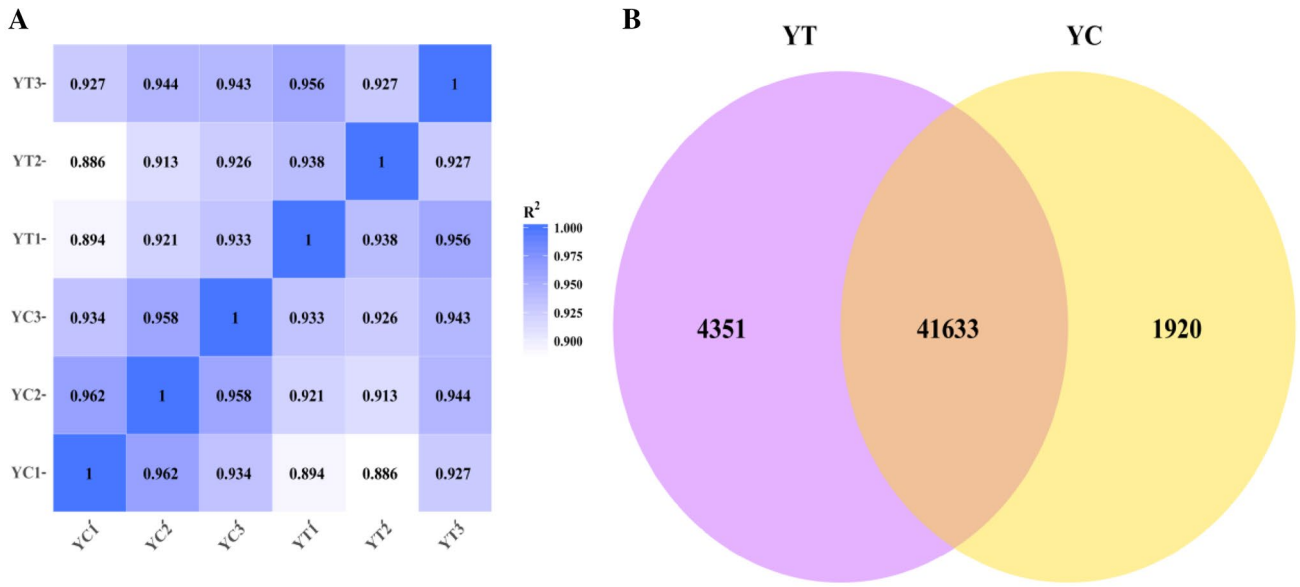

Fig. 1 Root transcriptome analysis of Yaomai16 seedlings treated (YT) or not treated (YC) with Cd. **a** Correlation coefficients of fragments per kilobase of exon model per million mapped reads among YC and YT replicates. The R^2 values indicate the correlation coefficients between samples. **b** Co-expressed and specifically expressed genes in YC and YT root transcriptome

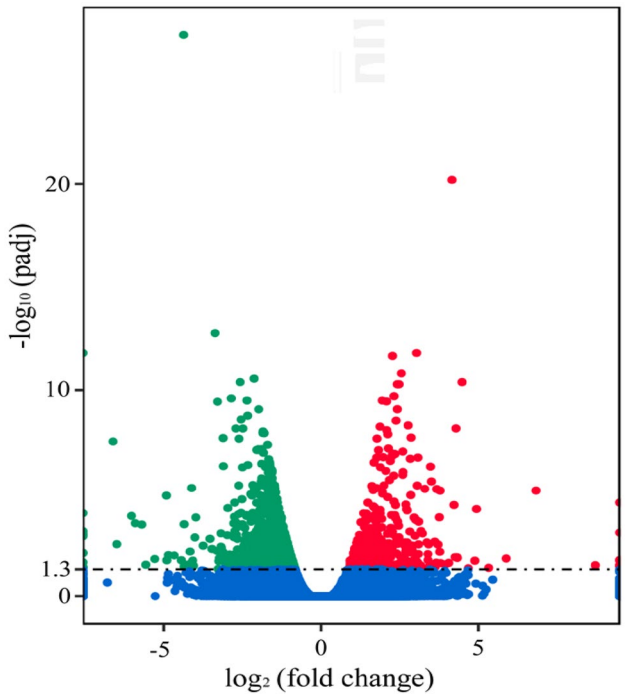

Fig. 2 Differentially expressed genes in the roots of Yaomai16 seedlings treated or not treated with Cd. Red dots, upregulated genes (429); green dots, downregulated genes (998); blue dots, no significant difference (74,074)

of 1,427 DEGs (corrected P value of <0.05) were induced by Cd stress, of which 429 were upregulated and 998 were downregulated (Fig. 2).

GO enrichment analysis

The functional annotation and GO enrichment analysis of upregulated and downregulated DEGs showed that 835 DEGs were annotated, of which 298 were upregulated and 537 were downregulated. The upregulated DEGs were assigned to 41 GO terms (biological processes, 21; molecular functions, 19; and cell components, 1). As shown in Fig. 3, the most enriched GO terms of the upregulated DEGs were associated with membrane (GO: 0016020), transport (GO: 0006810), establishment of localization (GO: 0051234), localization (GO: 0051179), response to oxidative stress (GO: 0006979), response to chemical stimulus (GO: 0042221), sulfur compound metabolic process (GO: 0006790), peroxidase activity (GO: 0004601), oxidoreductase activity acting on peroxide as an acceptor (GO: 0016684), antioxidant activity (GO: 0016209), and inorganic anion transmembrane transporter activity (GO: 0015103).

The downregulated DEGs were assigned to 61 GO terms (biological processes, 23; molecular functions, 20; and cell components, 18). As shown in Fig. 4, the most enriched GO terms of the downregulated DEGs were associated with DNA binding (GO: 0003677), membrane-bounded organelle (GO: 0043227), intracellular membrane-bound organelle (GO: 0043231), nucleus (GO: 0005634), protein complex (GO: 0043234), response to stress (GO: 0006950), RNA metabolic process (GO: 0016070), carbohydrate metabolic process (GO: 0005975), RNA biosynthetic process (GO: 0032774), transcription, DNA-dependent (GO: 0006351), response to oxidative stress (GO: 0006979), response to

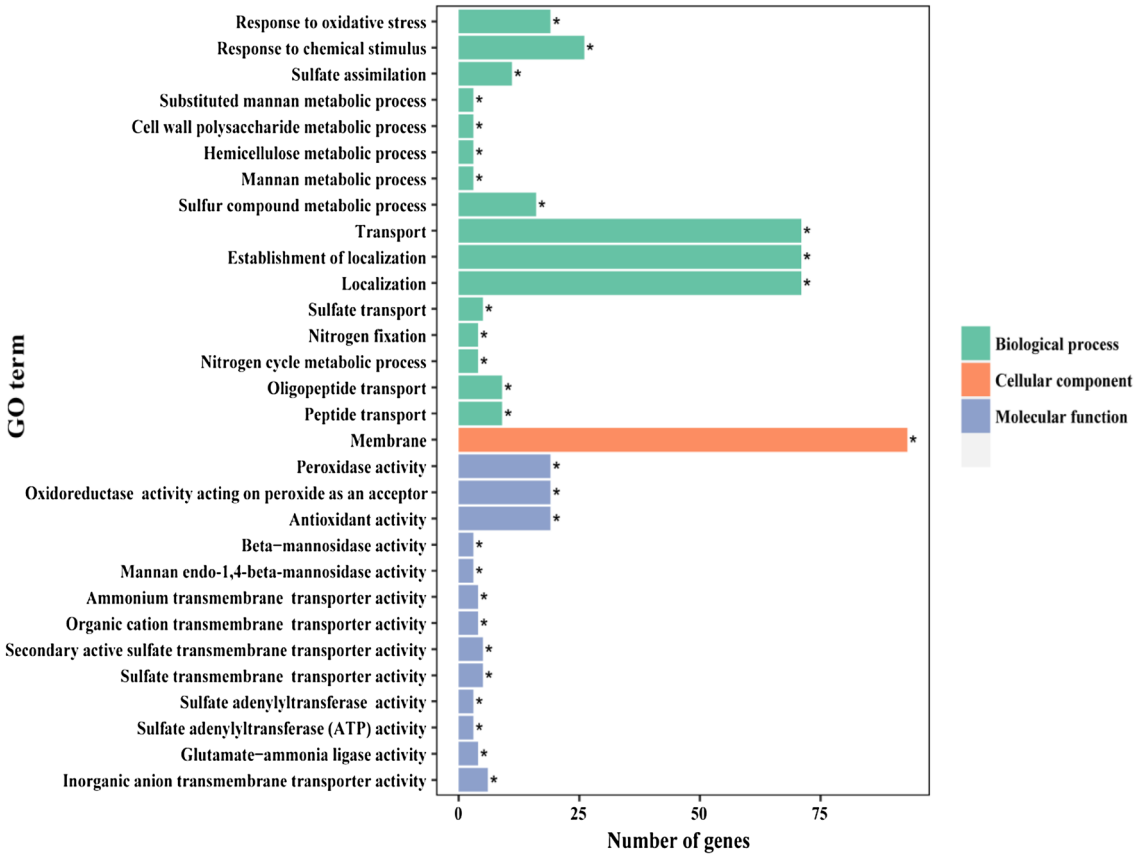

Fig. 3 Gene ontology (GO) classification of the upregulated differentially expressed genes in the root of Yaomai16 seedlings treated with Cd

chemical stimulus (GO: 0042221), and antioxidant activity (GO: 0016209).

KEGG enrichment analysis

Based on the KEGG enrichment analysis, 923 DEGs were assigned to 90 metabolic pathways, of which biosynthesis of secondary metabolites (194 DEGs; 21.02% of all DEGs) and metabolic pathways (122 DEGs; 13.22% of all DEGs) were the most highly enriched, followed by protein processing in the endoplasmic reticulum; phenylpropanoid biosynthesis; glutathione metabolism; galactose metabolism; and valine, leucine, and isoleucine degradation (Fig. 5).

The KEGG enrichment analysis also showed that eight pathways were highly enriched with upregulated DEGs, including phenylpropanoid biosynthesis; glutathione metabolism; sulfur metabolism; ABC transporters; alanine, aspartate, and glutamate metabolism; cysteine and methionine metabolism; nitrogen metabolism; and cyanoamino acid metabolism (Table 2). In addition, five pathways were significantly enriched with downregulated DEGs, including protein processing in the endoplasmic reticulum; phenylpropanoid biosynthesis; valine, leucine, and isoleucine degradation; galactose metabolism; and taurine and hypotaurine metabolism (Table 3). Furthermore, the pathway of phenylpropanoid biosynthesis was significantly enriched in both upregulated and downregulated DEGs (Tables 2, 3).

Validation of RNA-seq by qRT-PCR

To verify the reliability of RNA-seq, the expression level of eight DEGs (six upregulated and two downregulated DEGs), which might be involved in Cd stress response, was determined by q-PCR. The gene function was predicted by homologous alignments with sequences in the NR database. The q-PCR showed that the expression pattern of the eight selected DEGs was similar to those found by RNA-seq (Table 4), confirming the reliability of the sequencing data. Furthermore, the functional annotation of the eight selected DEGs was also obtained by comparing with homologous sequences in the non-redundant (NR) database (Table 4).

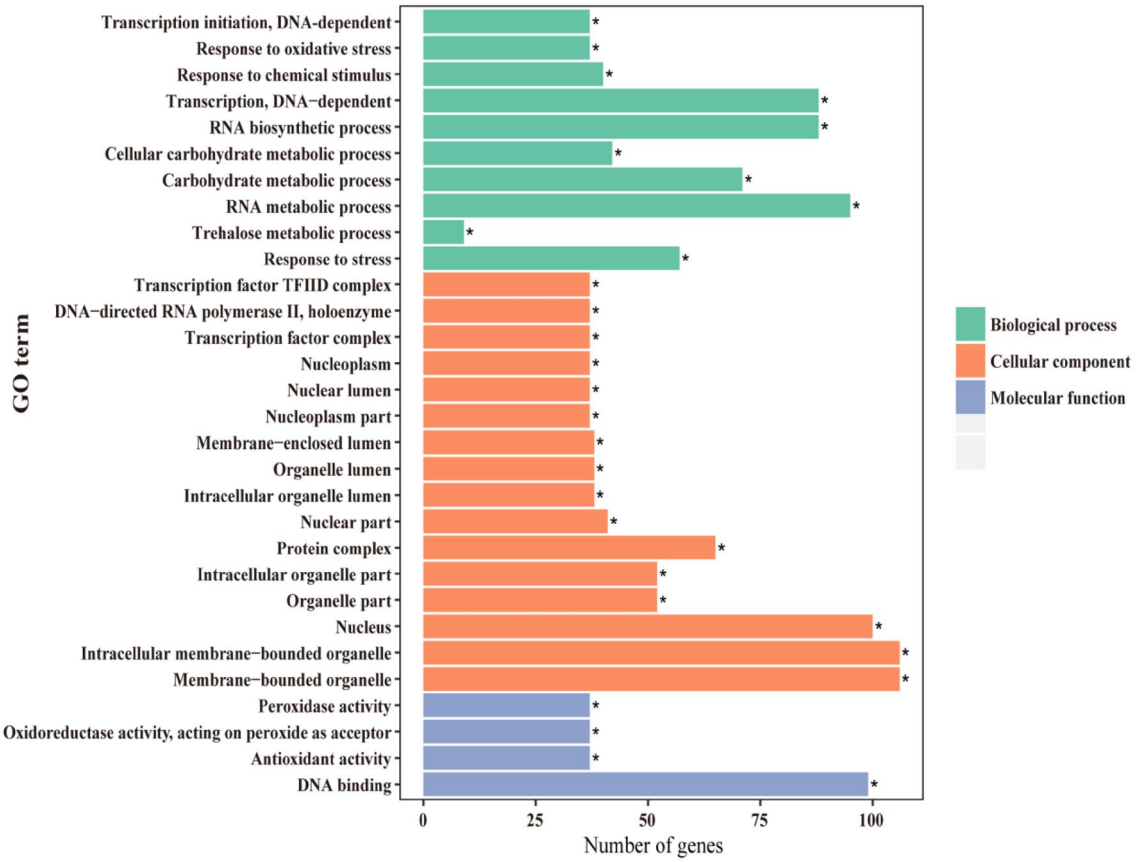

Fig. 4 Gene ontology (GO) classification of the downregulated differentially expressed genes in the root of Yaomai16 seedlings treated with Cd

Discussion

Cd is a non-essential element for plants and animals, and its excessive intake can result in irreparable damage (Santos et al. 2016). In vivo, Cd can replace the metal elements (such as Ca, Fe, and Zn) in the activity center of superoxide dismutase, peroxidase, guaiacol peroxidase, ascorbate peroxidase, glutathione reductase, and catalase, which are important protective enzymes against stress, affecting the metabolism of nitrogen, carbohydrate, nucleic acid, and respiration in plant (Assche and Clijsters 1990; Tran and Popova 2013). Even at low concentrations in the soil, Cd is absorbed by the roots, transported via the xylem and phloem, and accumulated in plant organs (Fujimaki et al. 2010; Fontanili et al. 2016). Cereals are considered as a major source of dietary Cd exposure (Zheng et al. 2007). Therefore, elucidating the underlying mechanisms that regulate plant response to Cd stress might be helpful to develop genotypes with low Cd accumulation in the grain.

Cd as an abiotic stress factor can alter gene expression in plants (Xu 2016). The DEGs are considered useful to discover of potential transcription factors and tolerance genes related to metal transport and signal transduction (Wang et al. 2013). In the present study, we analyzed the Illumina Hiseq4000 data from the roots of YC and YT to identify the DEGs related to Cd stress. A total of 1,427 DEGs was obtained, of which 429 (30% of the total DEGs) were upregulated and 998 (70% of the total DEGs) were downregulated, suggesting that the pattern of gene expression in the root of wheat plant is modified under Cd stress conditions (Fig. 1b). Different genes coordinate their biological functions in organisms (Phillips 2008). Through metabolic pathway enrichment analysis, we can identify the most important biochemical metabolic and signal transduction pathways involved in DEGs. The KEGG pathway enrichment analysis was performed for the upregulated and downregulated DEGs to identify the metabolic pathways that are related to Cd stress in wheat. The pathways of phenylpropanoid biosynthesis (Pawlak-Sprada et al. 2011), glutathione metabolism (Liu et al. 2016), sulfur metabolism (Liang et al. 2016), and ABC transporters (Song et al. 2014) were significantly enriched in the upregulated DEGs (Table 2), which had been improved to be related with Cd uptake, translocation, and detoxification. Sulfur metabolism plays a vital role in plant life, regulating the communication between cells and the environment. It improves plant resistance

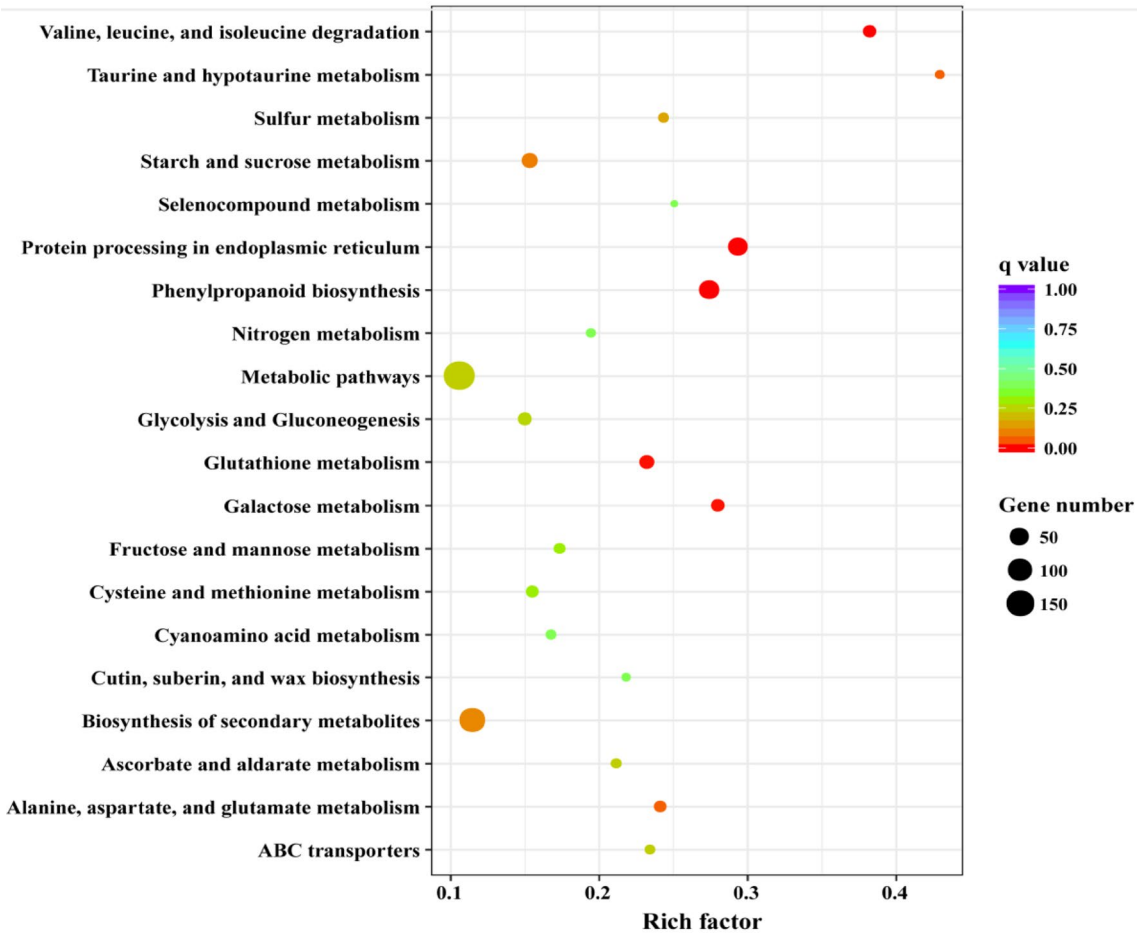

Fig. 5 Most highly enriched pathways in the differentially expressed genes in the root of Yaomai16 seedlings treated with Cd

Table 2 KEGG pathways significantly enriched in the upregulated differentially expressed genes (DEGs) in the root of Yaomai16 seedlings treated with Cd

Pathway ID	Pathway	Number of DEGs with pathway annotation	Background number	*P* value	Corrected *P* value
bdi00940	Phenylpropanoid biosynthesis	25	224	2.96E−07	1.63E−05
bdi00480	Glutathione metabolism	16	104	1.06E−06	2.92E−05
bdi00920	Sulfur metabolism	8	33	3.47E−05	0.000637
bdi02010	ABC transporters	7	30	0.000133	0.001829
bdi00250	Alanine, aspartate, and glutamate metabolism	8	50	0.000437	0.004807
bdi00270	Cysteine and methionine metabolism	9	91	0.004254	0.038997
bdi00910	Nitrogen metabolism	5	31	0.00512	0.040226
bdi00460	Cyanoamino acid metabolism	6	48	0.006807	0.046799

to Cd stress, as it increases sulfate uptake to enhance the synthesis rate of cysteine, glutathione, and phytochelatins (PCs), thus alleviating Cd toxicity (Rother et al. 2006; Gill et al. 2012; Ferri et al. 2017). In this process, PCs, with glutathione as the precursor, are one of the most important chelating agents in plants (Pereira et al. 2002). It can form non-toxic, low molecular weight Cd-S-PC compounds with Cd, and enter the vacuole through the Cd/H^+ reverse transport protein of the vacuolar membrane and the ATP binding cassette (ABC), forming a high-molecular mass compound for storage (Hassan et al. 2005). This not only alleviates the toxicity of Cd, but also blocks the migration of Cd to

Table 3 KEGG pathways significantly enriched in the downregulated differentially expressed genes (DEGs) in the roots of Yaomai16 seedlings treated with Cd

Pathway ID	Pathway	Number of DEGs with pathway annotation	Background number	*P* value	Corrected *P* value
bdi04141	Protein processing in the endoplasmic reticulum	53	192	1.96E−18	1.49E−16
bdi00940	Phenylpropanoid biosynthesis	36	224	2.39E−07	9.10E−06
bdi00280	Valine, leucine, and isoleucine degradation	13	42	6.09E−06	1.54E−04
bdi00052	Galactose metabolism	13	61	0.000168	3.20E−03
bdi00430	Taurine and hypotaurine metabolism	5	14	0.002977	4.52E−02

Table 4 Functional annotation and expression level (RNA-seq vs. q-PCR) of the eight selected differentially expressed genes (DEGs) in the root of Yaomai16 seedlings treated with Cd

Gene	Functional annotation	RNA-seq	q-PCR
Traes_2AS_36F44A53A	APRL1—*Zea mays* adenosine 5′-phosphosulfate reductase-like [*Zea mays*]	2.31	2.22
Traes_4BS_6EC31E8D4	Indole-3-acetic acid-amido synthetase GH3.8 [*Zea mays*]	−2.64	−1.76
Traes_4DL_469179461	TPA: IN2-1 protein [*Zea mays*]	1.65	1.11
Traes_2BL_1AED96909	Auxin response factor 6 [*Zea mays*]	1.27	1.72
Traes_2BS_DDCEEE1DE	TPA: hypothetical protein ZEAMMB73_191811 [*Zea mays*]	3.07	4.00
Traes_5BL_9A790E8CF	Pathogenesis-related protein PRMS [*Triticum urartu*]	−0.55	−0.11
Traes_6BL_986D595B9	PREDICTED: aldehyde dehydrogenase family 3 member F1-like [*Setaria italica*]	3.05	2.63
TRAES3BF003100040CFD_g	Glutathione-*S*-transferase 1 [*Eragrostis tef*]	0.71	0.84

Negative numbers indicate downregulation

other organelles. This complex coping mechanism of Cd ions in root cells plays an important role in reducing the upward transport of Cd ions or grain accumulation (Sarwar et al. 2015; Ferri et al. 2017). In addition, coordination of S assimilation with N and C metabolism also plays a crucial role in abiotic stress tolerance (Gill and Tuteja 2011; Khan et al. 2016). In the present study, the pathways of plant hormone signal transduction, seleno-compound metabolism, protein export, and histidine metabolism were also enriched in the upregulated DEGs, whereas the pathways of endocytosis, glutathione metabolism, peroxisome, phagosome, plant hormone signal transduction, protein processing in the endoplasmic reticulum, and seleno-compound metabolism were enriched in the downregulated DEGs. The genes involved in these processes also play an important role in the normal metabolism of cells. The downregulation of DEGs revealed that Cd stress might have no significant effect on plant growth during the early stages; however, Cd exhibited a significant negative effect on plant cell metabolism at the cellular level. On the other hand, eight DEGs from the glutathione metabolism (Traes_4DL_469179461, TRAES3BF003100040CFD_g), sulfur metabolism (Traes_2AS_36F44A53A, Traes_2BS_DDCEEE1DE), plant hormone signal transduction (Traes_4BS_6EC31E8D4, Traes_2BL_1AED96909, Traes_5BL_9A790E8CF) and histidine metabolism (Traes_6BL_986D595B9) were evaluated by q-PCR. Although the detection range and sensitivity of RNA-seq and q-PCR analyses are different, both can detect changes in the expression pattern of DEGs (Table 4). Thus, the reliability of the results is further verified.

In summary, the sequencing of root transcriptome of a low-Cd-accumulating winter wheat genotype enabled the identification of numerous genes and metabolic pathways related to growth, metabolism, and environmental stimulus response, which are involved in plant response to Cd stress. Our results shed light on the effects of stress due to heavy metal pollution on the growth, development, and metabolism of wheat roots, and showed the complexity of the underlying molecular mechanisms. Further research is needed on the role of key genes that will help improve the effectiveness of phytoremediation through genetic engineering.

Conclusions

In the present study, we used RNA-seq technology to elucidate the transcriptional response to Cd stress in the roots of a low-Cd-accumulating wheat genotype. We obtained a large volume of high-quality data and transcript information that we used for the DEG identification, GO and KEGG enrichment analysis, and functional annotation. The pathways of phenylpropanoid biosynthesis, glutathione metabolism,

sulfur metabolism, and nitrogen metabolism were significantly enriched by the DEGs, explaining the low Cd accumulation in the grain. Overall, our data might be useful to elucidate the underlying molecular mechanisms that regulate the response to Cd stress in wheat and to develop new improved varieties with low heavy metal content in the grain.

Author contribution statement YX performed the experiments and drafted the manuscript. ZD and XQ have contributed in the conception and provided some guidance, and HW, WG, and ZZ have contributed in the analysis of the data and further modifications of the manuscript. All authors read and approved the final version of the manuscript.

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