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EDITED BY

Juan Han,
Chinese Academy of Agricultural Sciences,
China

REVIEWED BY

Sheng Qian,
Huazhong Agricultural University, China
Long Zhao,
Hainan University, China

*CORRESPONDENCE

Shan Fu

✉ shanfu77@hbnu.edu.cn

Xuan Wu

✉ wuxuan585@163.com

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Gene identification and transcriptome analysis of cadmium stress in tomato

Liang Chen^{1,2}, Man Wu¹, Wei Jin¹, Ting Lei¹, Ying Li¹, Xuan Wu^{3*} and Shan Fu^{1,2*}

¹College of Life Science, Hubei Normal University, Huangshi, China, ²Hubei Engineering Research Center of Special Wild Vegetables Breeding and Comprehensive Utilization Technology, Huangshi, China, ³Agricultural Technology Promotion Center Agricultural Environmental Protection Station, Huanggang, China

Cadmium (Cd) is a highly toxic heavy metal that can severely hinder plant growth and development. Tomato is one of the most important economical crops in the world, and its quality and safety are closely related to human health. Therefore, it is important to elucidate the molecular mechanisms involved in tomato plant responses to Cd stress. In this study, tomato plants were treated with or without 100 μM Cd²⁺ in hydroponic culture for 3 days. Transcriptional changes in tomato roots and shoots were examined by transcriptome sequencing techniques. A total of 1,123 differentially expressed genes (DEGs) were identified in roots and 159 DEGs were identified in shoots after Cd treatment, including 15 DEGs were upregulated and 24 DEGs were downregulated in both roots and shoots. KEGG enrichment analysis showed that DEGs in the roots and shoots under Cd stress were significantly enriched in the glutathione metabolism pathway, sulfur metabolism pathway, phenylpropanoid biosynthesis, plant-pathogen interaction cutin pathway, suberine and wax biosynthesis pathway, and photosynthesis-antenna proteins pathway. 15 DEGs were further validated by quantitative real-time RT-PCR, including ABC transporter genes, WRKY transcription factors, and NAC transcription factors, among others. This study will provide a theoretical basis for further research on the molecular mechanisms involved in tomato responses to Cd stress, and genetic improvement of Cd tolerance.

KEYWORDS

tomato, cadmium stress, transcriptome, qRT-PCR, transcription factor

1. Introduction

With the rapid development of industrial and agricultural production in recent years, abiotic stresses such as drought, cold, high temperatures, high salt, and heavy metals have become increasingly serious environmental problems that restrict crop yields all over the world (Ashraf and Foolad, 2007; Lata and Shivhare, 2021). Cadmium (Cd) is a highly toxic heavy metal element that is harmful to plant growth and development, and is increasingly causing grain production losses and economic losses (Li et al., 2023). Cd concentrations in the natural environment are usually low, but the expansion of human activities such as mineral mining, smelting, and the use of phosphate fertilizers containing Cd has led to the increase Cd pollution in soil. Plants growing in soils with excessive Cd exhibit obvious symptoms of damage such as yellowing, root growth inhibition, and impaired photosynthesis (Alcántara et al., 1994; Zhang et al., 2014, 2015; Chiao et al., 2020; Zhao et al., 2021). Understanding the process of plant Cd

accumulation or molecular mechanisms is critical importance for plant growth and crop yield.

Many studies have investigated the physiological mechanisms involved in Cd tolerance in plants, and a large number of Cd tolerance genes have been cloned and identified, including complex transporters, metallothionein synthase, and transcription factors (Zhang et al., 2022). Cd is a non-essential element in plants. Cd enters root cells mainly through transporters/channels of essential elements (such as iron, manganese, zinc, and calcium), such as zinc/iron-regulated transporter-like proteins (ZIPs), and natural resistance-associated macrophage protein (Nramp) transporter (Asgher et al., 2015). For example, OsNramp5 was localized in rice root cell membrane, and the Cd content in plants and grains was significantly decreased after *OsNramp5* deletion (Tang et al., 2022). Cd or Cd complexes that enter root cells can be excreted from the cell via Cd transporters on the cell membrane. ATP-binding cassette (ABC) transporters are one of the largest protein families. They use the energy generated by ATP hydrolysis to transport various solutes and catalyze the input of many primary metabolites (Kang et al., 2011; Orelle et al., 2018). Kim et al. (2007) reported that ABC transporter *AtABCG36/AtPDR8* is related to plant Cd tolerance in addition to pathogen defense. Overexpression of *AtABCG36* in *Arabidopsis* improved Cd tolerance by excretion of Cd or Cd complexes (Bovet et al., 2003; Kim et al., 2007). Some transporters are evidently involved in the transport of Cd from roots to shoots. OsHMA2 was localized in rice sheath cells, and overexpression of *OsHMA2* reduced Cd content in rice grains. In addition, vacuole isolation plays an important role in Cd detoxification. For example, both OsHMA3 and OsABCC9 were localized in the vacuolar membrane of root cells, and through sequestration of Cd into the root vacuoles involved in rice cadmium tolerance (Sasaki et al., 2014; Yang et al., 2021). The migration of Cd in cells promotes the detoxification of Cd in roots, which improves the tolerance of plants response to Cd toxicity (Bali et al., 2020).

Transcriptional regulation of the defense gene expression is a key step in plants resistance to abiotic stress, such as NAC, MYB, and WRKY family has been reported play an important role in plant resistance to abiotic stress. For example, the MYB transcription factor *OsMYB45* was localized in the nucleus, and mutation of *OsMYB45* leads to decreased expression of *OsCATA* and *OsCATC*, decreased catalase activity in plants, and increased sensitivity of rice to Cd (Hu et al., 2017). Overexpression *PyWRKY75* improved Cd tolerance and enhanced the accumulation of Cd in transgenic poplars (Wu et al., 2022). Rice *OsNAC15* regulates zinc and Cd tolerance by binding to the ZDRE motif in the promoters of *OsZIP7* and *OsZIP10*, and inhibiting their transcription (Zhan et al., 2022). However, little is known about the role of transcription factors in tomato response to Cd stress.

Tomato is one of the most largest horticultural crop in the world. Many studies investigating tomato response to salt, drought, and heat stress have been reported. However, the transcriptional response of tomato to Cd accumulation is still unclear. Transcriptome sequencing is an effective technique for examining molecular mechanisms of gene expression networks and complex biological pathways by systematically studying gene transcription at the entire transcriptional level. In order to investigate potential mechanisms involved in tomato response to Cd stress, comparative transcriptome analysis of tomato roots and shoots under Cd stress was conducted in the current study. Differentially expressed genes (DEGs) were then further verified by

qRT-PCR. The results of the present study will accelerate research-investigating responses of tomato to Cd stress, have practical reference significance in the field of tomato Cd tolerance breeding, and promote the effective utilization of tomato resources for human life.

2. Materials and methods

2.1. Plant materials and cadmium treatment

Seeds of *Solanum lycopersicum* Mill. cv. *Ailsa Craig* were surface sterilized using 75% ethanol and 1% sodium hypochlorite, then rinsed thoroughly with distilled H₂O. They were then germinated on 1/2 MS medium and cultivated under controlled conditions (16-h photoperiod at 28±2°C) until two true leaves had grown. The resulting seedlings were carefully uprooted, washed thoroughly with ddH₂O, and then transferred to 1/2 Hoagland nutrient solution (pH 5.8) which was replaced every 4 days. 30-day-old seedlings were then divided into two groups treated with 0 or 100 µM Cd²⁺. After 72 h roots and shoots were sampled and recorded as CK_R (roots of the control group), CK_S (shoots of the control group), Cd_R (roots of the Cd treatment group), and Cd_S (shoots of the Cd treatment group). Each group was composed of three biological replicates. The samples were rapidly frozen in liquid nitrogen and stored at -80°C. All the samples were sent to Shanghai Majorbio for transcriptome sequencing.

2.2. RNA extraction, cDNA library construction, and Illumina sequencing

Total RNA was extracted from samples using TRIzol reagent (Amboin, United States) in accordance with the manufacturer's instructions. The purity and concentration of total RNA in each sample was detected by Nanodrop spectrophotometer (Termo Scientific). cDNA library construction and RNA-seq were performed by Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China). The main cDNA library processes were as follows: (1) mRNA was enriched with Oligo (dT) magnetic beads. (2) mRNA was randomly broken into short fragments with fragmentation buffer. (3) The first cDNA strand was synthesized using random hexamer primers, then the second cDNA strand was synthesized using DNA polymerase I, dNTPs, and RNase H. The double-stranded cDNA was purified with AMPure XP beads. (4) The purified double-stranded cDNA was subjected to end reparation, including addition of an "A" tail and ligation to the sequencing adaptors, then AMPure XP beads were used for fragment size selection. (5) The purified cDNA template was enriched via PCR amplification. (6) Twelve cDNA libraries were constructed and sequenced using the Illumina HiSeq platform.

For Illumina RNA-seq, the raw RNA-seq reads were trimmed and quality controlled by fastp.¹ Then clean reads were separately aligned to reference genome of *Solanum lycopersicum*² with orientation mode using Hisat2³ software. The mapped reads of each sample were

1 <https://github.com/OpenGene/fastp>

2 https://solgenomics.net/organism/Solanum_lycopersicum/genome/

3 <http://ccb.jhu.edu/software/hisat2/index.shtml>

TABLE 1 Summary of tomato sequencing results.

Sample	Total base number	Clean read number	Total mapped	Q30 (%)	GC content (%)
CK_R1	50,313,098	49,992,716	46,644,780(93.3%)	93.56	43.27
CK_R2	49,292,068	48,985,316	45,972,445(93.85%)	93.79	43.29
CK_R3	51,416,376	51,079,616	46,867,237(91.75%)	93.86	43.42
CK_S1	54,815,434	54,460,586	52,875,628(97.09%)	93.76	43.36
CK_S2	45,254,792	44,966,640	43,651,845(97.08%)	93.71	43.24
CK_S3	52,756,274	52,435,302	50,714,388(96.72%)	93.85	43.52
Cd_R1	54,056,002	53,746,538	49,251,407(91.64%)	93.86	43.45
Cd_R2	46,989,802	46,605,950	42,108,494(90.35%)	93.74	43.55
Cd_R3	52,365,930	51,986,470	47,970,646(92.28%)	93.73	43.52
Cd_S1	47,539,718	47,182,408	45,800,166(97.07%)	93.66	43.27
Cd_S2	55,575,264	55,195,938	53,427,223(96.8%)	93.57	43.3
Cd_S3	49,793,298	49,503,068	48,142,583(97.25%)	93.98	43.18

The roots (CK_R) and shoots (CK_S) of the control group and the roots (Cd_R) and shoots (Cd_S) of the 100 $\mu\text{mol/L}$ Cd²⁺ treatment group. Q30: The percentage of bases with a clean data is equal to or greater than 30.

assembled by StringTie⁴ software. GO was annotated using Blast2GO⁵ software. KOBAS 2.1.1 software⁶ was used to obtain unigene Genes and Genomes orthology results in Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis. The predicted amino acid sequences of unigenes were aligned with the Protein Family (Pfam) database using HMMER 3.2.1 software (<https://selab.janelia.org/pub/software/hmmer3/3.0/hmmer-3.0.tar.gz>; E value $<10^{-10}$) to acquire unigene annotation information.

2.3. Differentially expressed genes and protein–protein interactions network analysis

DESeq2⁷ software was used for the screening of DEGs. Based on the alignment results, gene expression levels were normalized using fragments per kilobase of transcript per million mapped reads (FPKM) values. In statistical analysis, the corrected p value, i.e., false discovery rate (FDR), was used as the key factor for DEG screening. An $FDR < 0.05$ and a fold change (FC) ≥ 2 were set as threshold parameters in the screening process. Volcano plots combining the statistical significance of the FDR with FC were generated. The abscissa was represented by \log_2 FC, and the ordinate was represented by $-\log_{10}$ FDR. Genes in the upper left and upper right parts of the volcano plot represent the most statistically significant DEGs with the largest FCs. Goatools⁸ software was used to analyze the functional GO enrichment of DEGs. KEGG pathway enrichment analysis of DEGs was conducted using Python scipy⁹ software. The enrichment degree of KEGG pathways was analyzed using the enrichment factor, and the significance of enrichment was calculated via Fisher's exact test.

The protein–protein interaction (PPI) network of DEGs was constructed by Cytoscape (v3.9.1) using the STRING online database.

2.4. Quantitation real-time PCR verification

To verify the reliability of the sequencing results of DEGs, 15 DEGs from roots were detected by quantitative real-time RT-PCR (qRT-PCR). Total RNA (1 μg) was reverse transcribed using a reverse transcriptase. First-strand cDNA was synthesized using HiScriptII 1st Strand cDNA Synthesis Kit (+gDNA), HiScriptII Q RT SuperMix for qPCR (Vazyme), and Q RT SuperMix for qPCR (Vazyme). qRT-PCR experiments were analyzed using the ChamQ SYBR qPCR Master Mix (Vazyme) in accordance with the manufacturer's instructions. The specific primers used in the qRT-PCR analysis were designed using Primer 5.0 software (Premier Biosof International) as shown in Supplementary Table 1. *SICAC* was used as an internal gene. Three technical replicates were performed for each gene. Expression levels were calculated by $2^{-\Delta\Delta Ct}$ method.

3. Results

3.1. Transcriptome sequencing and differentially expressed genes analysis

The transcriptomes of 12 samples were sequenced via the Illumina HiSeq high throughput-sequencing platform, and a total of 89.54 Gb of clean data were obtained. At least 6.66 Gb of clean data were obtained per sample, the average GC content was 43.36%, and the ratio of Q30 was $>93.56\%$. All clean reads were mapped to the tomato reference transcriptome. The percentages of mapped reads were similar among the 12 libraries (90.35–97.25%; Table 1).

The DEGs were identified by pairwise comparisons of libraries from Cd stress and the control in roots and shoots. A total of 1,123 DEGs were detected in the roots (CK_R vs. Cd_R), of which 554 DEGs were upregulated and 569 DEGs were downregulated (Figure 1A). 159 DEGs were detected in the shoots (CK_S vs. Cd_S),

4 <http://ccb.jhu.edu/software/stringtie/>

5 <https://www.blast2go.com/>

6 <http://kobas.cbi.pku.edu.cn/download.php>

7 <http://bioconductor.org/packages/stats/bioc/DESeq2>

8 <https://github.com/tanghaibao/GOatools>

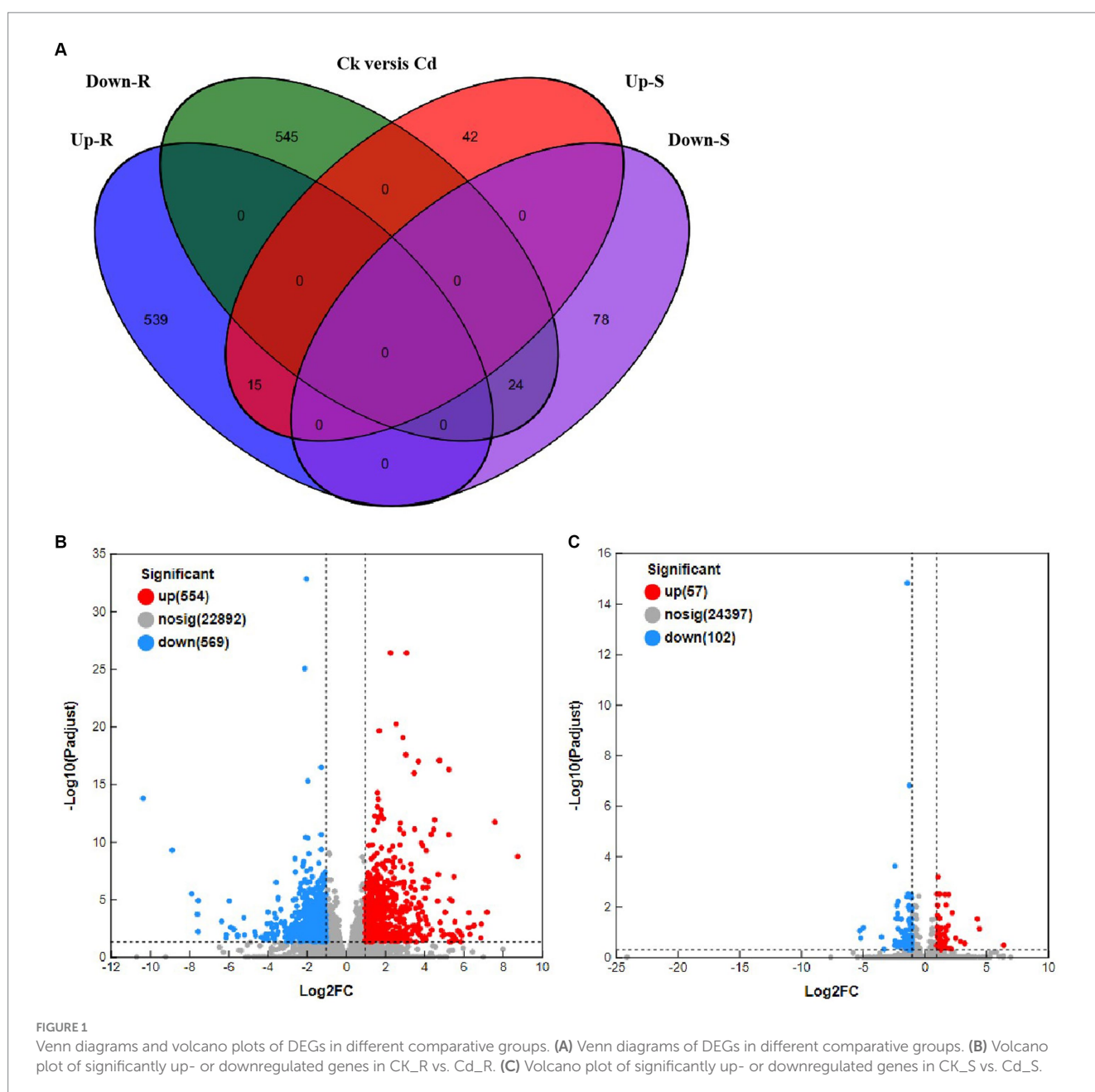
9 <https://scipy.org/install1>

of which 57 DEGs were upregulated and 102 DEGs were downregulated. Among them, there were 15 DEGs that were upregulated and 24 DEGs that were downregulated in both roots and shoots (Supplementary Table 2). In intra-group comparisons of different group, the number of DEGs that were downregulated in shoots was higher than that of upregulated, and that trend was the same in roots (Figures 1B,C).

3.2. GO functional enrichment of DEGs

Gene ontology functional enrichment analysis was carried out to further characterize the main biological functions of DEGs in tomato roots and shoots under Cd stress. In GO analysis 1,123 DEGs with at least one annotation were obtained in tomato roots and shoots under

Cd stress. These DEGs were classified into three categories, including biological process (BP), cellular component (CC), and molecular function (MF). In tomato roots, a total of 114 GO terms were significant enriched under Cd stress (p adjust < 0.05), including 58 biological process, 13 cellular components, and 43 molecular function terms (Supplementary Table 3). The top 10 GO terms of BP, CC, and MF categories of significantly enriched DEGs in roots were shown in Figure 2A. The biological processes related to ion transport, such as iron ion transport (GO:0006826), metal ion transport (GO:0030001), phosphate ion transport (GO:0006817), and several molecular functions, such as glutathione transferase activity (GO:0004364), ion transmembrane transporter activity (GO:0015075), and manganese ion transmembrane transporter activity (GO:0005384), may play important roles in the tomato resistance to Cd stress. In shoots, a total of 141 GO terms were identified, 29 GO terms were significant



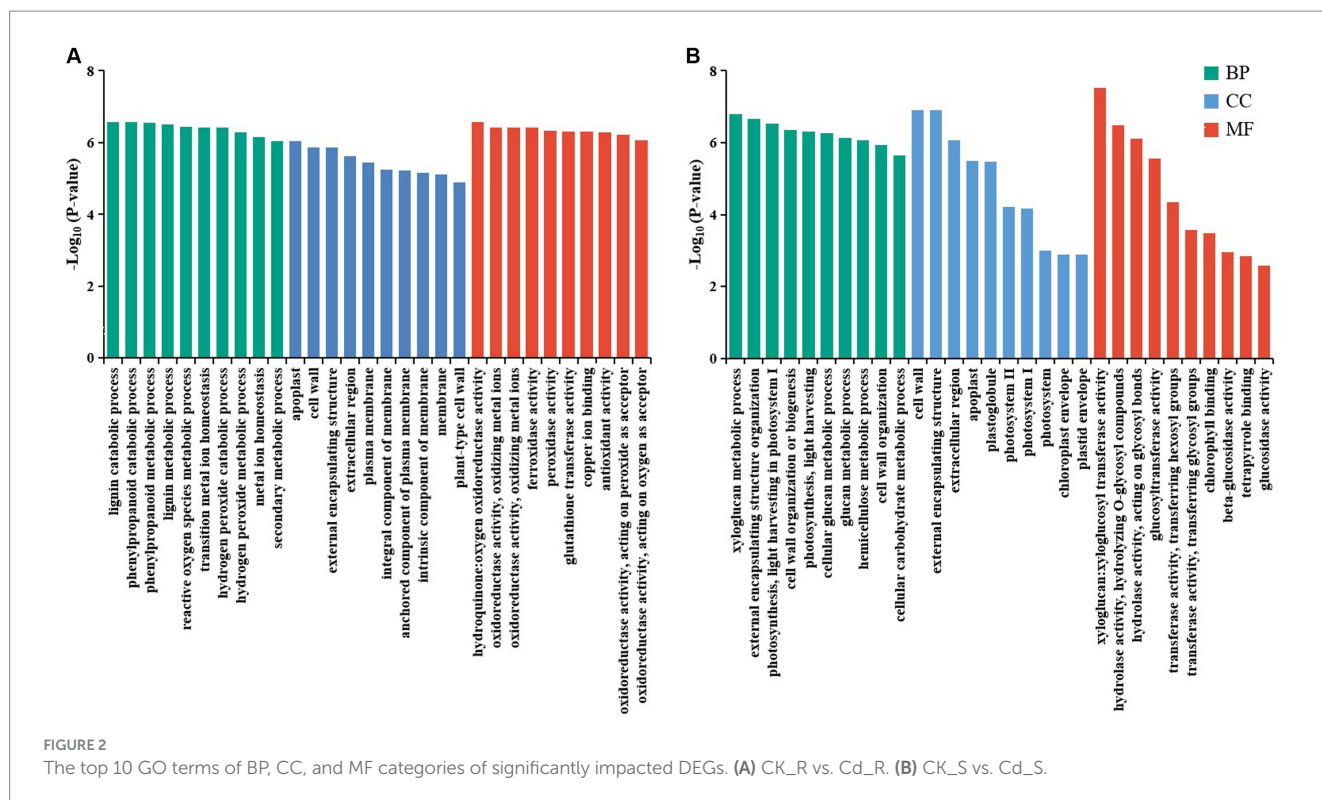


FIGURE 2

The top 10 GO terms of BP, CC, and MF categories of significantly impacted DEGs. (A) CK_R vs. Cd_R. (B) CK_S vs. Cd_S.

enriched in shoots under Cd stress (p adjust < 0.05), including 17 biological process, seven cellular components, and five molecular function terms (Supplementary Table 4). The top 10 GO terms of BP, CC, and MF categories of DEGs in shoots were shown in Figure 2B. In roots and shoots, 13 GO terms were significant enriched in both comparisons (CK_R vs. Cd_R and CK_S vs. Cd_S), such as cell wall organization or biogenesis (GO:0071554), cell wall (GO:0005618), and polysaccharide metabolic process (GO:0005976).

Gene ontology enrichment was also conducted to analysis the 15 DEGs that were upregulated and 24 DEGs that were downregulated in both roots and shoots. A total of 21 GO terms were identified in 15 DEGs and 53 GO terms were identified in 24 DEGs, respectively. In upregulated DEGs, the top three GO terms were “response to stimulus (GO:0050896),” “response to stress (GO:0006950),” and “response to oxidative stress (GO:0006979)” (Supplementary Figure 1A). In downregulated DEGs, the top three GO terms were “glucosidase activity (GO:0015926),” “cell wall (GO:0005618),” and “external encapsulating structure (GO:0030312)” (Supplementary Figure 1B).

3.3. KEGG enrichment and protein–protein interactions networks analysis of DEGs

Kyoto encyclopedia of genes and genomes pathway enrichment analysis was performed to estimate the number of DEGs contained at different levels. A total of 79 and 26 KEGG pathways were identified in roots and shoots DEGs analysis, of which 21 pathways were both exist in roots and shoots (Supplementary Tables 5, 6). In roots, five KEGG pathways were significantly enriched under Cd stress (adjusted $p < 0.05$), including phenylpropanoid biosynthesis, glutathione (GSH) metabolism, plant-pathogen interaction, sulfur metabolism, and

cutin, suberine, and wax biosynthesis (Figure 3A). In the shoots, two KEGG pathways were significantly enriched under Cd stress (adjusted $p < 0.05$), including photosynthesis-antenna proteins and phenylpropanoid biosynthesis (Figure 3B; Table 2).

A PPI network based on STRING database was constructed by Cytoscape. After filtering out the non-interacting proteins, we were left with a PPI network with 245 nodes and 300 edges for the DEGs from the CK_R vs. Cd_R comparison, and the top five sub-network were shown in Figure 4A. In shoots, a PPI network was analyzed with 46 nodes and 32 edges for the DEGs from the CK_S vs. Cd_S comparison, and the top three sub-network were shown in Figure 4B.

3.4. Transcription factor family analysis of DEGs

When plants are subjected to Cd stress, transcription factors (TFs) combine with Cd-related genes to regulate expression of related genes at the transcriptional level. In the roots, the 1,123 DEGs included 20 TF families, and the distribution of these TFs was shown in Figure 5. The top 5 TF families were MYB (21.54%), WRKY (16.92%), ERF (10.77%), NAC (12.31%), and bHLH (9.23%) families (Figure 6). In shoots under Cd stress, 159 DEGs included three ERF transcription factors (*Solyc08g082210.4*, *Solyc12g009240.1*, and *Solyc11g012980.1*), one bHLH transcription factor (*Solyc01g106460.4*), one HSF transcription factor (*Solyc04g016000.3*), one Trihelix transcription factor (*Solyc09g005560.4*), and one GATA transcription factor (*Solyc04g015360.3*). The results indicated that TF families such as NAC, WRKY, bHLH, and MYB superfamily might play important roles in the regulation of tomato response to Cd stress. Differential expression levels of TFs in the same family demonstrated that different

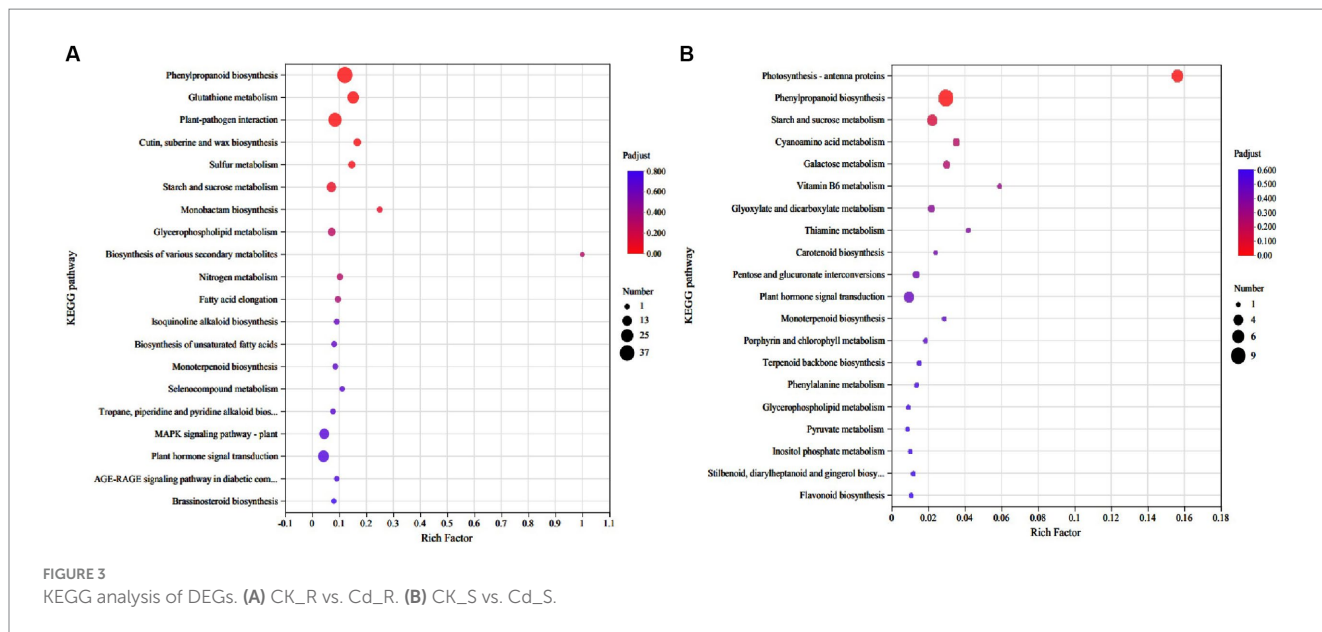


TABLE 2 Summary of significantly enriched KEGG pathways in roots and shoots.

Tissue	Pathway ID	Ratio	Description	p adjust
Roots	map00940	37/438	Phenylpropanoid biosynthesis	2.94E-10
Roots	map00480	20/438	Glutathione metabolism	3.19E-07
Roots	map04626	28/438	Plant-pathogen interaction	9.17E-05
Roots	map00073	7/438	Cutin, suberine, and wax biosynthesis	6.95E-03
Roots	map00920	6/438	Sulfur metabolism	2.97E-02
Shoots	map00196	5/68	Photosynthesis—antenna proteins	1.28E-05
Shoots	map00940	9/68	Phenylpropanoid biosynthesis	2.66E-04

members might have distinct mechanisms in plant responses to Cd stress, and appear to exhibit different biological functions.

3.5. Validation of RNA-Seq data by qRT-PCR

To further verify the reliability of gene changes identified in comparative transcriptome analyses, several key genes with significant differences were analyzed via qRT-PCR. Expression levels under Cd treatment and the control condition were shown in Figure 6, including 10 transcription factors (Figures 6A–J) and five transporter genes (Figures 6K–O). Expression levels of these genes were all consistent with the transcriptome analysis (Supplementary Table 7), indicating that the DEGs identified via comparative transcriptome analysis were reliable.

4. Discussion

Tomato is a highly nutritious fruit and vegetables that is widely consumed in the world. Fruit quality and metabolite biosynthesis of tomato are affected by adverse plant growth conditions, including water scarcity, soil salinization, and other abiotic stresses such as Cd

(Alyemini et al., 2018; Diouf et al., 2018). Tomato is a vegetable that accumulates Cd readily in the fruit, and the Cd concentration in tomato after Cd treatment is two or even three times the limit of edible concentration in vegetables (Hussain et al., 2015; Xu Z. M. et al., 2018). Tomato yields and fruit quality decrease after Cd stress, substantially reducing global yields (Hussain et al., 2017). Currently most research on tomato is focused on domestication, breeding, fruit development and ripening, and defense regulation against biological and abiotic stress (Xie et al., 2021; Wei et al., 2022). However, there were few studies on the genes and molecular mechanisms of tomato response to cadmium stress. In tomato, Cd toxicity markedly inhibits its growth and development (Hussain et al., 2019). Reducing Cd accumulation elucidating the mechanism of tomato in Cd stress are of great significance for improving yield and quality and food safety.

In recent years, various omics technologies have emerged and been widely used in plant science, life science and other fields. Among them, transcriptomics is considered one of the most widely used techniques to study the adaptation mechanism of plants to stress and the mining of stress-response genes. It has been reported that RNAseq-based methods were used to detect arsenic-responsive miRNAs in rice roots, and found that miRNAs were involved in the responses of rice to arsenic (Liu and Zhang, 2012). In the current study transcriptome analysis identified 1,123 DEGs in roots, of these, 554 genes were upregulated and 569 were downregulated. In

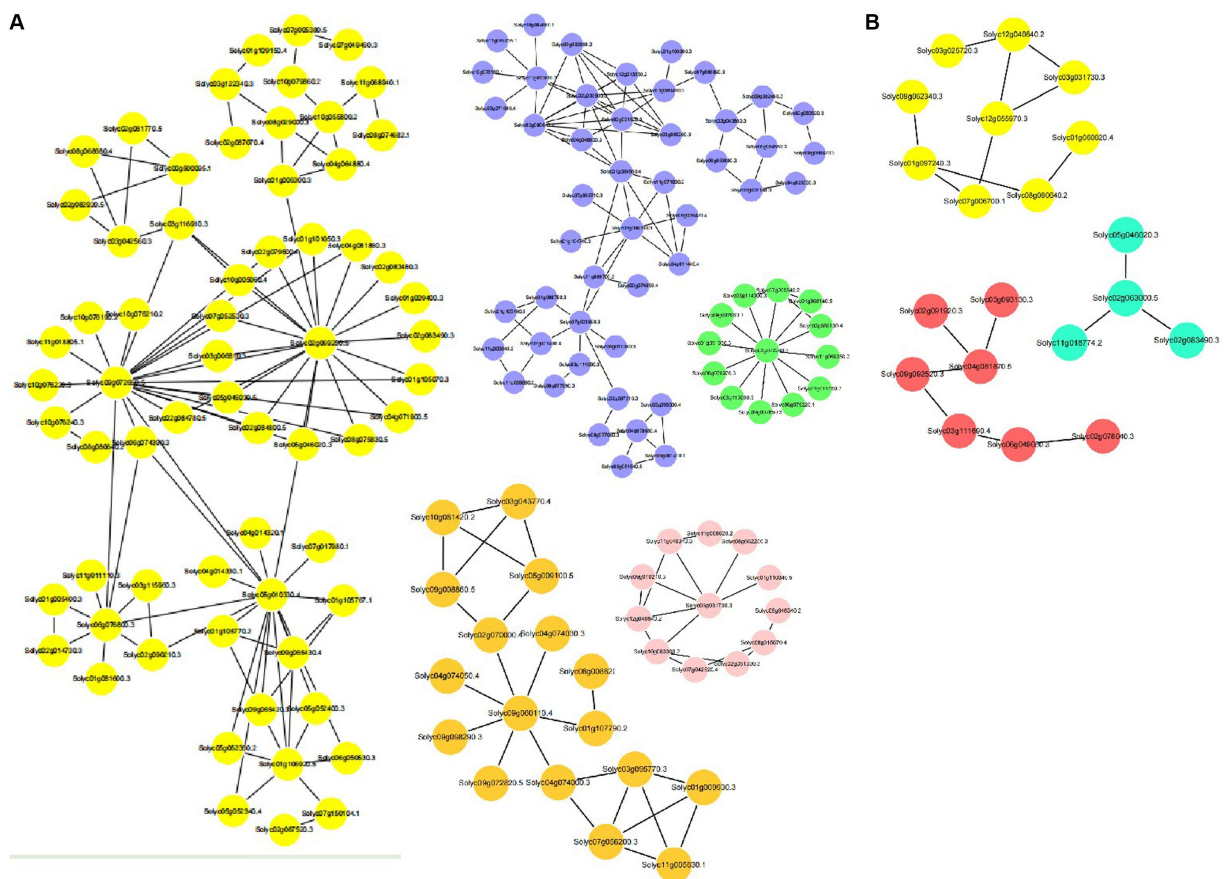


FIGURE 4 PPI network analysis. (A) The top five modules of differentially expressed genes of CK_R vs. Cd_R. (B) The top three modules of differentially expressed genes of CK_S vs. Cd_S.

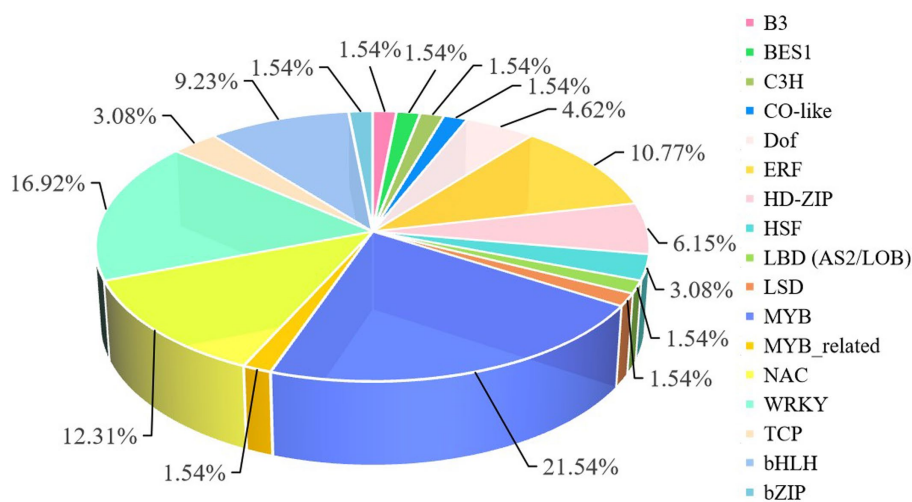


FIGURE 5 Distribution of different TF families identified in roots of DEGs.

shoots under Cd, 159 DEGs were identified, of which 57 genes were upregulated and 102 were downregulated in shoots (Figure 1). Among them, there were 15 DEGs that were upregulated and 24 DEGs that were downregulated in both roots and shoots under Cd

stress (Supplementary Table 2). It is suggested that these genes may play an important role in tomato response to cadmium stress. However, the mechanisms of these DEGs under Cd stress in tomato require further investigation.

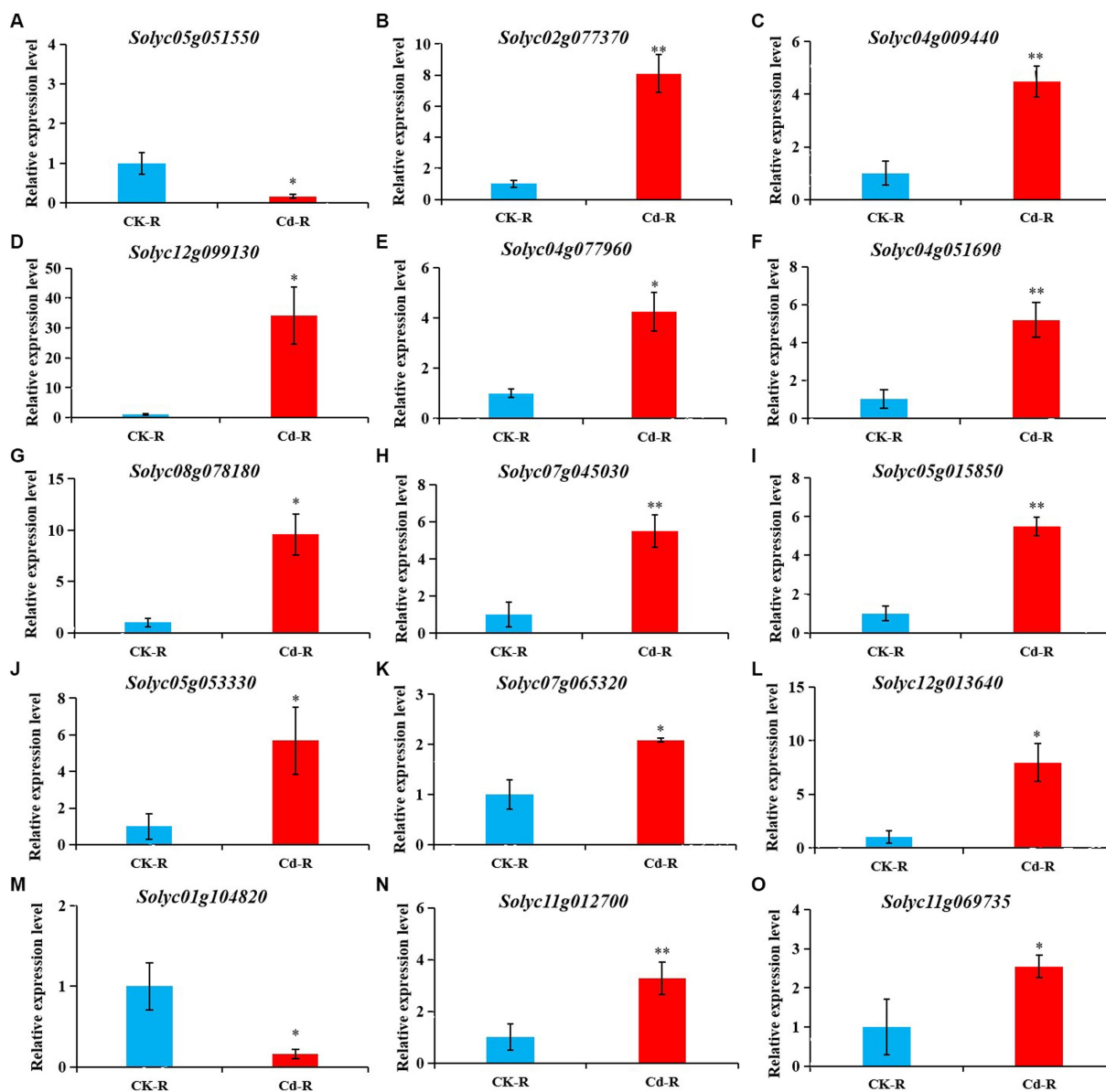


FIGURE 6

The expression pattern of transcription factor and transporter genes in the root of tomato after Cd treatment. Transcription levels were verified by qRT-PCR with SICAC as an internal control. (A–J) The relative gene expression of transcription factor genes examined by qRT-PCR. (K–O) The relative gene expression of transporter genes examined by qRT-PCR.

Plants have evolved well-developed mechanisms in response to Cd stress, such as cell wall binding, redistribution of transporters, compartmentalization in vacuoles, chelation with phytochelatins, recovery of damaged proteins, and antioxidant defense (Xu et al., 2017; Rui et al., 2018; Sidhu et al., 2019; Szerement and Szatanik-Kloc, 2022). In plants Cd absorption and distribution are related to many metal-regulated transporters, including heavy metal ATPase, ABC transporter, NRAMP, and ZIP family, which are important to metal homeostasis (Yu et al., 2017; Zhao et al., 2018). For example, ABC transporters AtABCC33 is considered a key factor in plant chelatin-mediated Cd tolerance in *Arabidopsis* (Brunetti et al., 2015). To further clarify gene functions in tomato under Cd stress in the current study, identified DEGs were analyzed by GO enrichment (Figure 3). Based on

GO enrichment analysis, all of these DEGs in roots and shoots were distributed in molecular function, biological process, and cellular component under Cd stress. The GO terms related to ion transport, such as iron ion transport, metal ion transport in biological processes, and glutathione transferase activity, ion transmembrane transporter activity, and manganese ion transmembrane transporter activity in molecular functions were analyzed in roots. This indicated that tomato initiated complex responsive approaches to adapt to external Cd stress. In addition, five DEGs annotated as transporter genes were also verified by qRT-PCR. Four genes encoding ABC transporter (*Solyc12g013640* and *Solyc07g065320*), oligo peptide transporter (*Solyc11g012700*), and high-affinity nitrate transporter (*Solyc11g069735*) were found to be upregulated and one gene

encoding vacuolar iron transporter (*Solyc01g104820*) was downregulated under Cd stress (Figure 6), which indicate that transporters may be directly involved in Cd response in tomato plants.

Glutathione can act as a substrate of GSH S-transferase, participating in the reduction of hydroperoxide, and its synthesis in plants also depends on sulfur assimilation (Edwards et al., 2000; Hernández et al., 2017). Cd often induces the production of toxic reactive oxygen species such as H₂O₂, -OH, and O²⁻, then induces oxidation of biological macromolecules including proteins, lipids, and nucleic acids, resulting in enzyme inactivation, lipid peroxidation, and membrane damage (Nagajyoti et al., 2010; Daud et al., 2016). Plants have evolved antioxidant defense mechanisms to maintain normal function, including superoxide dismutase, peroxidase, and GSH, to avoid the toxic effects of reactive oxygen species (Wang et al., 2020). In current study, KEGG enrichment analysis showed five KEGG pathways and two KEGG pathways were significantly enriched in roots and shoots under Cd stress, respectively (Figure 3). In roots, the top three KEGG pathways were phenylpropanoid biosynthesis, glutathione (GSH) metabolism, and plant-pathogen interaction. It can be speculated that tomato plants may adapting to a high Cd environment by promoting GSH accumulation, or upregulate GSH synthesis.

Transcription factors, such as WRKY, bZIP, NAC, and MYB proteins plays an important role in response to Cd stress by controlling the expression of their specific related genes (DalCorso et al., 2010; Tokumoto et al., 2019). It has been reported that *JrVHAG1* participated in Cd stress by MYB transcription regulatory network and Abscisic acid (ABA) signaling pathway (Xu Z. et al., 2018). Overexpression *AemNAC2* increased the cadmium tolerance of transgenic wheat (Du et al., 2020). In current study, transcriptome sequencing indicated that DEGs induced by Cd stress in tomato roots and shoots belong to multiple transcription factor families, such as MYB, WRKY, ERF, NAC, and bHLH (Figure 5). Given this and the above-described findings, it is speculated that these DEGs may improve the tolerance of tomato plants to Cd by promoting GSH accumulation, or participating in regulatory networks such as the ethylene signaling pathway. Combined with functional annotation, 10 DEGs annotated as transcription factors were verified by qRT-PCR. Nine genes encoding ERF, MYB, WRKY, and bHLH in the pathway of signal transduction were found to be upregulated and one gene encoding MYB (*Solyc05g051550*) was downregulated under Cd stress (Figure 6), which indicate that TFs may be directly involved in Cd response in tomato plants. Further study is required to determine the key genes and regulatory pathways of tomato response under cadmium stress. In conclusion, through this study, we hope to enhance the understanding of the mechanism involved in tomato plants response to Cd stress, provide reference for further gene function research, and provide reference for further research on the response genes and molecular mechanisms of tomato under Cd stress.

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Data availability statement

The original contributions presented in the study are publicly available. This data can be found at: <https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA1030794>, PRJNA1030794.

Author contributions

LC: Methodology, Validation, Writing – original draft. MW: Data curation, Writing – original draft, Writing – review & editing. WJ: Data curation, Formal Analysis, Writing – original draft. TL: Data curation, Formal Analysis, Writing – original draft. YL: Writing – original draft, Data curation, Methodology. XW: Supervision, Validation, Writing – original draft. SF: Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2023.1303753/full#supplementary-material>

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Glossary

ABA	Abcisic acid
ABC	ATP-binding cassette
BP	Biological process
bZIP	Basic (region-leucine) zipper
CC	Cellular component
Cd	Cadmium
DEG	Differentially expressed genes
FC	Fold change
FDR	False discovery rate
FPKM	Fragments per kilobase of transcript per million mapped reads
GO	Gene ontology
GSH	Glutathione
KEGG	Kyoto encyclopedia of genes and genomes
MF	Molecular function
NRAMP	Natural resistance-associated macrophage protein
Pfam	Protein Family
TF	Transcription factors
ZIP	Zinc/iron-regulated transporter-like protein