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Integrated transcriptomics and metabolomics reveal cadmium uptake and stress response mechanisms in *Coptis chinensis* Franch

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Introduction: *Coptis chinensis* Franch. is a medicinally important plant of global significance. However, its clinical application safety is undermined by excessive cadmium (Cd) accumulation.
Methods: In this study, we determined Cd levels in different tissues of *C. chinensis* and integrated metabolomic and transcriptomic approaches to elucidate the mechanisms underlying Cd uptake and stress response.
Results: That roots act as the primary organ for Cd uptake and accumulation. Metabolomic analysis revealed that Cd stress disrupted flavonoid biosynthesis, cofactor biosynthesis, and DNA-related metabolic pathways. Transcriptomic profiling identified a large set of differentially expressed genes (DEGs), which were predominantly enriched in biological processes including metal ion transport, $Cd2^{+}$ chelation, cell wall metabolism, antioxidant activity, and signal transduction. Integrated multi-omics analysis further confirmed that amino acid biosynthesis pathways are involved in the Cd stress response, with the phenylalanine and lysine biosynthesis pathways being particularly prominent. Notably, two novel genes, *novel.5228* and *novel.14850*, which directly regulate pyruvate synthesis, were found to play critical roles in these regulatory mechanisms.
Discussion: This study provides novel insights into the molecular mechanisms governing Cd uptake and stress adaptation in *C. chinensis*, laying a solid foundation for the breeding of low-Cd-accumulating varieties of this medicinal plant.

KEYWORDS
cadmium (Cd), *Coptis chinensis Franch.*, metabolome, molecular mechanism, stress response, transcriptome

1 Introduction

In recent years, the accelerated industrialization process has led to the widespread and severe heavy metal (HM) contamination of agricultural soils globally (Hou et al., 2025). Among various HMs, cadmium (Cd) is one of the most hazardous pollutants to both plants and humans (Genchi et al., 2020; Huang et al., 2022b). Cd can be released into the natural environment through diverse anthropogenic industrial processes, including electroplating, smelting, mining, plastic stabilizer manufacturing, pigment production, and nickel–cadmium battery fabrication (Wei et al., 2023). Characterized by high solubility and mobility, as well as chemical similarity to numerous nutrient elements, Cd is easily taken up by plants (Wei et al., 2023). In plants, Cd exerts profound adverse impacts on a range of physiological processes, such as compromising normal leaf morphogenesis and plant metabolism, exacerbating oxidative stress, interfering with mineral nutrient uptake, eliciting genotoxic effects, and disrupting key physiological activities including respiration, photosynthesis, nitrogen metabolism, and enzymatic activity (Andresen and Küpper, 2013; Fishbein, 1981; Genchi et al., 2020; Ismael et al., 2019). As Cd accumulates in plant edible tissues, it ultimately translocates into the human body through the food chain, posing significant risks to human health and contributing to the incidence of various diseases (Sikakwe et al., 2024).

Similarly to plant nutrients, HMs enter plant cells via simple diffusion, passive transport, and active transport (Zhang et al., 2020). However, as a non-essential nutrient for plants, Cd^{2+} lacks a specific carrier evolved during the evolutionary process (Lai et al., 2021). Instead, its entry into plants and its translocation to other tissues primarily rely on specific essential nutrient channels and divalent cation transporters in plants (Llugany et al., 2012; Panda et al., 2025). In response to HM stress, plants have developed a variety of defense and detoxification mechanisms. The plant root serves as the first physical barrier against Cd stress, with the root epidermis, root hairs, and cuticle functioning as protective tissues to sequester Cd (Feng et al., 2021). Roots can secrete organic acids, such as citric acid and malic acid, which form complexes with Cd, thereby inhibiting its transmembrane transport. They also alter the rhizosphere pH, reducing Cd migration from the soil into the root system (Li et al., 2025). Polysaccharides and proteins in the root cell wall can bind to Cd, sequestering it outside the root cells to prevent its influx (Wei et al., 2023). Furthermore, thiol-containing chelating compounds, including metallothioneins (MTs), glutathione (GSH), and phytochelatins (PCs), can form Cd complexes to alleviate its toxicity to cellular activities (Cobbett, 2000; Hossain et al., 2012; Jasinski et al., 2008; Park et al., 2012). Once inside the cells, Cd is translocated to tissues with low metabolic activity, such as vacuoles. This compartmentalization has been proven to be an efficient detoxification mechanism (Xu et al., 2022). In addition, Cd can induce the production of reactive oxygen species (ROS), and plants will activate their antioxidant defense system. Antioxidant enzymes and non-enzymatic antioxidants can scavenge excess ROS, thereby mitigating oxidative stress-induced plant toxicity (He et al., 2011; Li et al., 2020; Lu et al., 2018).

With the rapid development and widespread application of metabolomics and transcriptomics over the past few years, significant advances have been made in the understanding of the molecular mechanisms underlying Cd uptake, transport, and detoxification in plants. At the molecular level, it is well established that plants regulate their metabolic pathways by activating molecular signaling networks, thereby participating in the Cd detoxification process (Wei et al., 2023). In wheat, the accumulation of several key components involved in pectin and cellulose synthesis was enhanced, and multiple amino acid biosynthesis and metabolism pathways were upregulated. These changes play crucial roles in Cd binding and tolerance (Lu et al., 2021). Galactose and lipid metabolism is the main pathway in the response to Cd stress in *Fagopyrum tataricum* (Huo et al., 2023).

In recent decades, a large number of genes involved in Cd transmembrane transporters have been discovered and functionally verified, including the heavy metal ATPases (HMAs), ATP-binding cassette (ABC) transporters, natural resistance As-associated macrophage proteins (NRAMPs), zinc- and iron-regulated transporter proteins (ZIPs), cation/hydrogen exchange (CAX) proteins, plant cadmium resistance (PCR) proteins, low-affinity cation transporters (LCTs), iron-regulated transporters (IRTs), cation diffusion facilitators (CDFs), metal tolerance proteins (MTPs), and yellow stripe-like (YSL) transporters (Das et al., 2016; Guo et al., 2022; He, 2021; Lin et al., 2020; Uraguchi et al., 2011; Yan et al., 2024). The zinc transporter ZNT1 is responsible for Zn^{2+} uptake while also possessing the capacity to mediate Cd^{2+} influx, with such influx increasing linearly as the Cd^{2+} concentration rises. Both ZIP and NRAMP transporters can transport Cd^{2+}. The iron transporter BcIRT1 has been identified as the major Cd transporter in *Brassica juncea* (Bozzi et al., 2016; Pence et al., 2000; Wu et al., 2021). CAX proteins have been shown to mediate Cd uptake in rice root systems (Liu et al., 2025). Under Cd stress, the upregulation of the ABC transporters and genes associated with the GSH metabolic pathway in *Celosia argentea* suggests their crucial roles in Cd detoxification in this species (Yu et al., 2023).

The complex signal regulatory network in plants also serves as a crucial pathway for plant tolerance to HMs. Specific transcription factors (TFs) play a prominent role in this regulatory network, regulating the expression of numerous development- and defense-related genes. Studies have demonstrated that, under HM stress, the MAPK signaling pathway activates downstream TFs, including *MYB*, *WRKY*, *ZAT*, *AP2*, *bZIP*, *ERF*, and *DRFB*. Under Cd stress, many TFs, including *MYB*, *AP2*, *DREB*, *WRKY*, and *NAC*, are upregulated in rice (Chen et al., 2006). Specifically, *WRKY51* regulates the expression of UCL23 in rice under Cd stress, thereby affecting Cd uptake and tolerance (Tan et al., 2025). A total of 774 TFs from 33 different families were identified in *Pistia stratiotes* under Cd stress, with the top 10 differentially expressed TFs belonging to families including MYB, AP2/ERF, NAC, C2C2, bHLH, bZIP, WRKY, B3, and LOB (Wei et al., 2023). Thus, the use of metabolomic and transcriptomic techniques to investigate changes in the metabolites and genes in plants is an ideal approach for comprehensively elucidating the molecular mechanisms underlying plant responses to Cd stress.

Coptis chinensis Franch. has a long medicinal history and contains berberine-based alkaloids as its main active ingredient. It has diverse pharmacological effects, including antiviral,

antibacterial, anti-inflammatory, and antitumor properties (Fu et al., 2021; Wang et al., 2019). However, *C. chinensis* easily accumulates Cd, which negatively impacts its clinical safety (Zhou et al., 2018). The Cd content in *C. chinensis* is often beyond its limit standard of ≤0.3 mg/kg stated in the *Green Industry Standard for Import and Export of Medicinal Plants and Preparations* and the World Health Organization (WHO) (Huang et al., 2019). To regulate and reduce Cd uptake in *C. chinensis*, it is essential to clarify the molecular mechanisms underlying its uptake, transport, and accumulation. To date, genome-wide identification and transcriptomic analyses of the MYB genes in *C. chinensis* have been performed, revealing that *CcMYB21*, *CcMYB40*, *CcMYB105*, and *CcMYB116* are highly expressed in the stems. Notably, the expression of *CcMYB94* is significantly upregulated under Cd stress, suggesting a potential role in stress regulation (Yang et al., 2025). The MTP family, which functions as a divalent cation transporter in plants, plays a crucial role in maintaining HM homeostasis and tolerance. Researchers have conducted a comprehensive identification and characterization of the MTP family genes in *C. chinensis*, identifying 25 CcMTP genes, which are classified into Zn-MTPs, Zn/Fe-MTPs, and Mn-MTPs based on their primary substrate specificity. Among them, *CcMTP11*, *CcMTP16*, and *CcMTP24* were highly expressed in the roots, rhizomes, and leaves of *C. chinensis* and exhibited enhanced tolerance to multiple HMs, including Mn, Fe, and Zn. Importantly, *CcMTP24* was found to improve Cd stress resistance in *C. chinensis* (Zhang et al., 2025). In a study by Li et al., ultra-performance liquid chromatography (UPLC) was used to quantify the isoquinoline alkaloid content in *C. chinensis* after Cd exposure. The results showed increased levels of four major alkaloids: berberine, coptisine, palmatine, and epiberberine (Li et al., 2024). Although some studies have explored the Cd stress response mechanisms in *C. chinensis*, comparisons with research on other plant species revealed several knowledge gaps. For instance, do the NRAMP and ABC family genes also participate in Cd absorption and transport in *C. chinensis*, and do they exhibit temporal specificity under stress conditions? Under Cd stress, can *C. chinensis* mediate Cd^{2+} chelation by activating substances such as GST, GSH, and PCs? Furthermore, does Cd affect the structural composition of the cell wall (e.g., through pectin degradation), and how does the plant trigger corresponding adaptive responses to such changes? Therefore, this study employs an integrated transcriptomic and metabolomic approach to gain deeper insights into the mechanisms of metal transport, detoxification, and metabolic regulation in *C. chinensis* in response to Cd exposure and to identify key responsive candidate genes that regulate and reduce Cd uptake in *C. chinensis*.

2 Materials and methods

2.1 Plant materials and Cd treatments

Plant samples were collected from Zhenping, Ankang City, Shaanxi Province, China (31.783955° N, 109.400025° E; 1,433 m.a.s.l.), which is one of the main production areas of *C. chinensis* in China. The plants were hydroponically pre-cultured in 1/2 Hoagland's nutrient solution (the components are shown in Supplementary Table 1) for 7 days. Black hydroponic pots were used to shield the plant roots from light, and an air pump was operated continuously to maintain aeration in the hydroponic tanks. The plants were grown under natural light conditions, and the pH of the nutrient solution was adjusted daily with 0.1 mol/L HCl or 0.1 mol/L NaOH to maintain it at approximately 5.6. The nutrient solution was replaced every 3 days. After pre-culture, all plants were transferred into fresh 1/2 Hoagland's nutritional solution supplemented with 25, 50, 100, or 200 μM $CdCl_2$ and cultured for 24 h (designated as groups H25, H50, H100, and H200, respectively) or 10 days (designated as groups D25, D50, D100, and D200, respectively). Allowing 24 h or 10 days of cultivation, all plants were then harvested, rinsed three times with ultrapure water, and blotted dry with absorbent paper. Samples treated with 0 μM Cd were collected as the control (CK) group. The plants were separated into leaves, rhizomes, and roots. All collected samples were immediately snap-frozen in liquid nitrogen and stored at −80° C until subsequent analysis.

2.2 Cd concentration in plants

The separated plant samples (roots, rhizomes, and leaves) were dried in an oven at 105°C for 12 h. After drying, the samples were ground to a particle size <0.02 mm. A 0.5-g aliquot of each plant powder was added to 5% HNO_3 solution, placed in a microwave digestion apparatus (LabTech, REVO, Framingham, MA, USA), and subjected to digestion at 120°C, 150 °C, and 190 °C for 5, 10, and 20 min, respectively, following a 1-h pretreatment. Subsequently, the digested samples were diluted with water to a final volume of 25 ml to prepare the test solution. The Cd content in the plant samples was determined using inductively coupled plasma mass spectrometry (ICP-MS) (ICP-MS-2030; Shimadzu, Tokyo, Japan).

2.3 Transcriptomic analysis

2.3.1 RNA extraction and sequencing

Total RNAs were isolated from root samples using the RNAprep Pure Plant Plus Kit for Illumina® (Tiangen, Beijing, China) in strict accordance with the manufacturer's instructions. The RNA purity, concentration, and integrity were evaluated using a NanoPhotometer® spectrophotometer (Implen, Westlake Village, CA, USA), the Qubit® RNA Assay Kit with a Qubit®2.0 Flurometer (Life Technologies, Carlsbad, CA, USA), and the RNA 6000 Nano Assay Kit of the Bioanalyzer 2100 System (Agilent Technologies, Carlsbad, CA, USA), respectively. A cDNA library was constructed using 1 mg of high-quality RNA per sample, and sequencing was performed on the Illumina Hi-Seq platform by Metware (Metware Biotechnology, Wuhan, China). Raw data were filtered and trimmed using fastp software (v0.19.3) to obtain high-quality clean reads, which were then mapped to the *C. chinensis* reference genome (GCA_015680905.1_ASM1568090v1_genomic.fna.gz) using HISAT software (v2.1.0).

2.3.2 Screening and analysis of the differentially expressed genes

The expression abundance of the sequencing reads was quantified using the fragments per kilobase of transcript per million mapped reads (FPKM) metric. Differentially expressed genes (DEGs) between the control group and each treatment group were screened using the DESeq2 software (version 1.22.1, developed by Ross Ihaka from the University of Auckland, New Zealand) with a threshold of $|\text{Log}_2\text{Fold Change}| \geq 1$ and false discovery rate (FDR) <0.05. Functional annotation of the DEGs was performed via the free online platform Metware Cloud (https://cloud.metware.cn), including annotation against the SwissProt Protein Sequence Database (SwissProt), Gene Ontology (GO) classification, and enrichment analysis in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

2.3.3 RT-qPCR validation of the transcriptome sequencing results

Template cDNA was synthesized from the RNA extracted from *C. chinensis* roots using real-time quantitative PCR (RT-qPCR). Subsequently, 10 genes were randomly selected for RT-qPCR analysis, with *β-actin* as the internal reference gene to determine the relative expression levels of the target genes in *C. chinensis* roots under Cd stress. Total RNA was extracted using a Plant RNA Extraction Kit (B001008022; Beijing Beitech Biotechnology Co., Ltd., Beijing, China), and the RNA quality was evaluated using gel electrophoresis and a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). cDNA was synthesized using the PrimeScript™ RT Master Mix kit (RR036A; Takara, Bao Bioengineering Co., Ltd., Dalian, China). The reaction system and the procedure for RNA reverse transcription are presented in Supplementary Tables 2 and S3. RT-qPCR was performed on a Bio-Rad CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) using a fluorescent quantitative gradient qPCR instrument (qTOWER2.0; Jena Analytik GmbH, Jena, Germany). The reaction system and the RT-qPCR procedure are shown in Supplementary Tables 4 and S5. Primers were designed using the NCBI Primer-BLAST tool (https://ncbi.nlm.nih.gov/tools/primer-blast/) and synthesized by Shanghai Sangon Biological Engineering Co., Ltd. The primer sequences are listed in Supplementary Table 6. The relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ data analysis method.

2.4 Non-targeted metabonomic analysis

C. chinensis roots were freeze-dried using a vacuum freeze dryer (Scientz-100F; Ningbo Scientz Biotechnology, Ningbo, China). The freeze-dried samples were ground in a mixer mill (Retsch-MM 400; Retsch GmbH, Haan, Germany) with zirconia beads at 30 Hz for 1.5 min. Of the lyophilized powder, 50 mg was dissolved in 1.2 ml of 70% methanol and vortexed for 30 s every 30 min for a total of six cycles. After centrifugation at 12,000 rpm for 3 min, the supernatant was filtered through a 0.22-μm membrane filter. Analysis was performed using an ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) system, with detailed parameters provided in Supplementary Information. Multivariate analysis was conducted on the data, including principal component analysis (PCA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA). OPLS-DA analysis was further used to calculate the variable importance in the projection (VIP) values. Differentially expressed metabolites (DEMs) were screened based on the criteria of VIP > 1, fold change (FC) ≥2 or ≤0.5, and *p*-value <0.05. The KEGG database was employed for metabolic pathway enrichment analysis.

2.5 Statistical analysis

Metabolomic and transcriptomic analyses were conducted using Metware Cloud, a free online data analysis platform (https://cloud.metware.cn). The Cd concentrations are expressed as the mean ± standard deviation (SD), and the data were analyzed using one-way analysis of variance (ANOVA) in SPSS Statistics 26.0, with the significance level set at $p < 0.05$. Graphical visualization and plotting were performed using Origin 2021 software (OriginLab, Northampton, MA, USA).

3 Results

3.1 Determination of the Cd content in *C. chinensis*

C. chinensis seedlings were cultured in solutions supplemented with 25, 50, 100, or 200 μM $CdCl_2$ for 24 h or 10 days, and the Cd accumulation in different tissues was determined by ICP-MS. Regardless of treatment duration, Cd accumulation was highest in the roots, moderate in rhizomes, and lowest in the leaves, indicating the roots as the predominant tissue for Cd accumulation in *C. chinensis*. As the Cd treatment concentration increased, the Cd accumulation in all tissues also increased, demonstrating that *C. chinensis* possesses a strong Cd absorption capacity and had not reached the accumulation peak even at 200 μM Cd. Compared with the 24-h treatment, the Cd accumulation in the roots was significantly higher after 10 days of treatment, indicating that the roots exhibit not only strong Cd absorption capacity but also a robust Cd storage capacity (Figure 1). Therefore, the roots were selected as the experimental material for the transcriptomic and metabolomic analyses to elucidate the response of seedlings to high-concentration Cd and their potential adaptation mechanisms.

3.2 Transcriptomic analysis

3.2.1 Transcriptome sequencing and DEG identification

Samples from eight treatment groups and one control group (three replicates per group) were subjected to sequencing and data filtering, yielding a total of 191.2 Gb clean bases. The statistical results of the transcriptome sequencing data and GC contents for each group are presented in Supplementary Table 7. The results indicated that the proportion of bases with quality values above Q20

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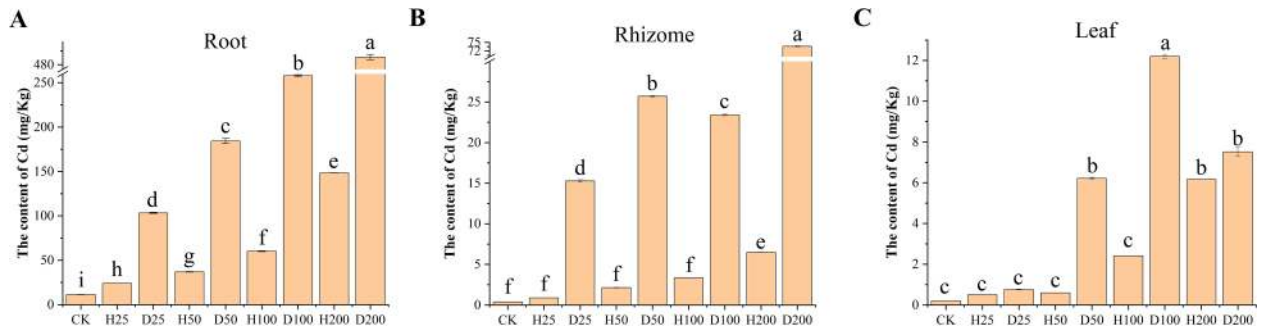

FIGURE 1
The Cd contents in the roots, rhizomes, and leaves of *Coptis chinensis* under different Cd stress concentrations and times. **(A)** The roots. **(B)** The rhizomes. **(C)** The leaves. Different letters indicate significant differences ($p < 0.01$). The figure is made by Origin 2021 (OriginLab, Northampton, MA, USA) software.

exceeded 97% in all samples, and those above Q30 surpassed 91%, demonstrating high sequencing quality across all samples that met the requirements for subsequent analyses.

In this study, the DEGs were categorized into eight comparison groups. The comparison between short-term treatments (24 h) and the control showed a rapid response to Cd stress. In contrast, those between long-term treatments (10 days) and the control may offer insights into the adaptation mechanisms under continuous Cd stress.

The DEGs were screened using the criteria of $|\text{Log}_2\text{Fold Change}| \geq 1$ and FDR < 0.05. The distributions of the DEGs in the 24-h and 10-day comparison groups are presented in Figure 2. A total of 6,132, 5,614, 3,049, and 2,794 DEGs were identified in the short-term treatment comparison groups (Figure 2A), while 1,703, 4,773, 3,145, and 6,288 DEGs were detected in the long-term treatment comparison groups (Figure 2B). With the increase in Cd concentration, the number of DEGs in the short-term treatment groups exhibited a decreasing trend, whereas that in the long-term treatment groups showed an increasing trend. This indicates that both the Cd concentration and the treatment duration can significantly alter the gene expression profiles in *C. chinensis* roots.

To further identify the core genes responsive to Cd stress at different time points, Venn diagram analysis was performed to determine the common DEGs between the short-term and long-term comparison groups. A total of 470 co-expressed DEGs were identified in the short-term treatment comparison groups (Figure 2C) and 483 identified in the long-term treatment comparison groups (Figure 2D). However, only 101 DEGs were shared across all treatment comparison groups (Figure 2E), suggesting that the response patterns of *C. chinensis* to Cd stress differed significantly between the two time spans. These shared DEGs were selected for subsequent research on the response of the roots to Cd stress.

3.2.2 GO and KEGG enrichment analyses of the DEGs

GO and KEGG enrichment analyses were conducted to further clarify the functions and associated biological processes of the DEGs. The GO enrichment results were classified into three categories: molecular function, biological process, and cellular component. The 470 common DEGs in all short-term treatment comparison groups were mainly involved in glucosyltransferases, various oxidoreductases, numerous binding proteins, and a variety of transporters. In addition, a large number of genes that participate in aging, senescence, toxin response, and other stress-responsive processes were included in these DEGs (Figure 3A). In the 483 common DEGs in all long-term treatment comparison groups, numerous transporter genes were included, and multiple genes related to glucan and terpenoid synthesis were involved as well (Figure 3B).

KEGG pathway analysis revealed that the metabolite biosynthesis pathways, particularly the secondary metabolite biosynthesis pathways, accounted for the most significant proportion of shared DEGs across all comparison groups. In the short-term comparison groups, these metabolites mainly included various monosaccharides and polysaccharides; several lipids, alkaloids, terpenoids, and thiamine; and some amino acids (Figure 4A). For the long-term comparison groups, the metabolite pathways were similar, with the addition of the flavonoid biosynthesis pathway (Figure 4B). However, specific differences were observed between the short-term and long-term comparison groups, primarily in the signal transduction pathways: the plant hormone signal transduction pathway was involved in the short-term groups. In contrast, the MAPK signal transduction pathway was involved in the long-term groups.

3.3 Metabolomic analysis

3.3.1 Metabolite distribution

Based on the transcriptomic results, Cd accumulation significantly altered the expression levels of the genes involved in metabolism. To further elucidate detailed differences in the metabolic responses after Cd treatments, a non-targeted metabolomics approach was employed to identify stress-associated metabolites. A total of 1,058 metabolites were identified and quantified, including 190 lipids, 174 phenolic acids, 129 amino acids and their derivatives, 129 alkaloids, 111 flavonoids, 88 organic acids, 60 nucleotides and their derivatives, 41 lignans and coumarins, 23 terpenoids, 10 quinones, 2 tannins, and 101 other compounds (Figure 5A). Multivariate PCA and partial least

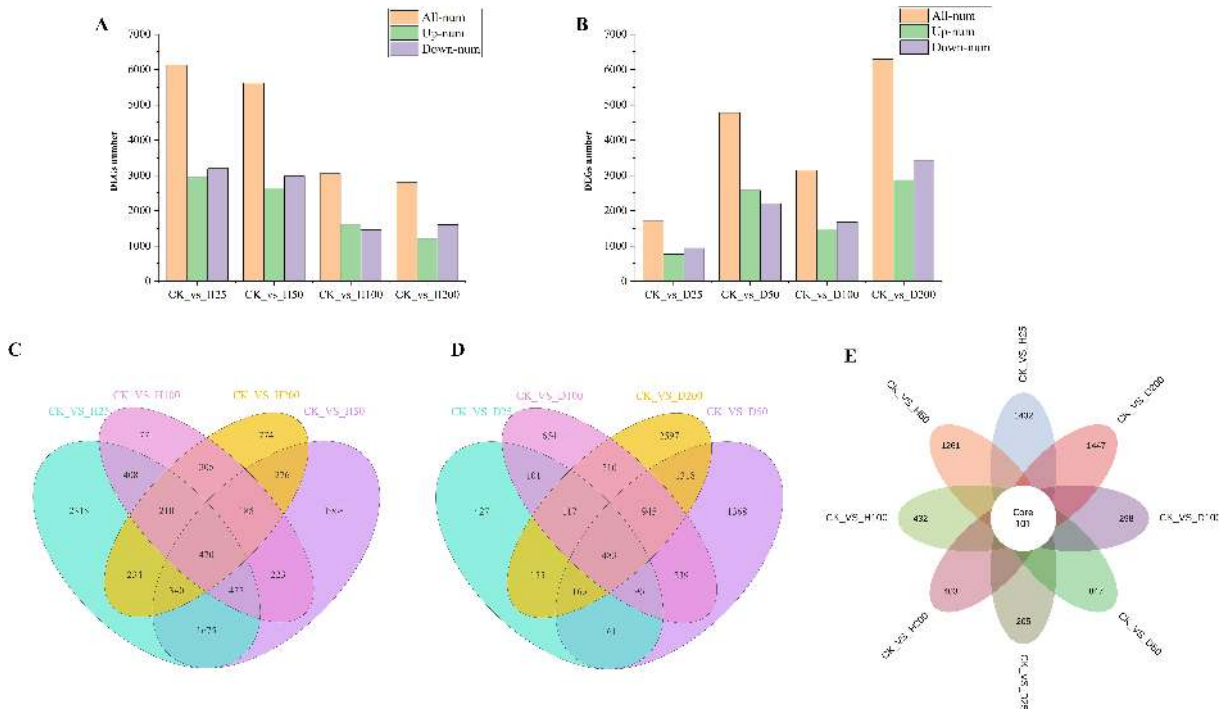

FIGURE 2
Analysis of the differentially expressed genes (DEGs) of *Coptis chinensis* roots under 24-h and 10-day Cd treatments. **(A, B)** Column charts of the upregulated and downregulated DEGs. (**C**–**E**) Comparison of the Venn graph of the number of DEGs in groups pairwise. The overlap represents the number of genes common to each comparison group, and the non-overlap represents the number of genes unique to the comparison group.

squares discriminant analysis (PLS-DA) were performed on the clustering information of the metabolites from the control and the treatment groups (Figures 5B–E). A clear separation was observed between the control group and the treatment groups, indicating that the root metabolite profiles of *C. chinensis* differed significantly across Cd exposure doses and treatment durations.

3.3.2 Effects of Cd stress on root metabolism

The DEMs were screened based on the FC and VIP values using the criteria of FC 2 ≤ FC ≤ 0.5 combined with VIP > 1. The number and the distribution of the DEMs in the short-term and long-term Cd stress comparison groups are shown in Figure 6, along with the common DEMs in each group. There were 79 common DEMs in the short-term comparison groups (Figure 7A). In contrast, only 34 common DEMs were identified in the long-term comparison groups (Figure 7B). When these DEMs were categorized into different types, flavonoids accounted for the most significant proportion under both treatment conditions. Alkaloids, amino acids, and lipids were also the main categories of DEMs, although their quantities varied between the two treatment conditions (Figures 7C, D).

3.3.3 KEGG enrichment analysis of the DEMs

The specific pathways involved in DEMs were identified using KEGG pathway enrichment analysis. The top 20 metabolic pathways with the highest enrichment levels were selected from the KEGG database (Figure 8). Flavonoid biosynthesis, lysine biosynthesis and degradation, oxidative phosphorylation, pyrimidine metabolism, purine metabolism, nucleotide metabolism, and cofactor biosynthesis pathways were enriched in all stress comparison groups. Phenylpropanoid biosynthesis; sphingolipid metabolism; valine, leucine, and isoleucine biosynthesis and degradation; and 2-oxocarboxylic acid metabolism pathways were enriched in the 24-h Cd stress groups. The cofactor biosynthesis and pyruvate metabolism pathways were enriched in the 10-day Cd stress groups. These results indicate that, regardless of short- or long-term treatments, similar metabolic changes occurred, suggesting that these changes may underlie Cd tolerance in *C. chinensis*. On the other hand, differences in the metabolite enrichment pathways between the two time points also explained the underlying mechanism of resistance variation across different treatment durations. Furthermore, these metabolites may play a crucial role in Cd toxicity tolerance in the root system of *C. chinensis*.

3.3.4 Correlation network analysis

As reported, the intermediates derived from amino acids during osmotic regulation and protein synthesis contribute to HM compartmentalization (Wei et al., 2023; Zhao, 2021). ABC transporters mediate the vacuolar sequestration of Cd in plants (Park et al., 2012). Therefore, from the KEGG pathways commonly enriched in both treatment groups, we selected the biosynthesis of amino acid and ABC transporter pathways and performed Pearson's correlation analysis between the DEMs and DEGs enriched in these two pathways. The DEMs and DEGs were

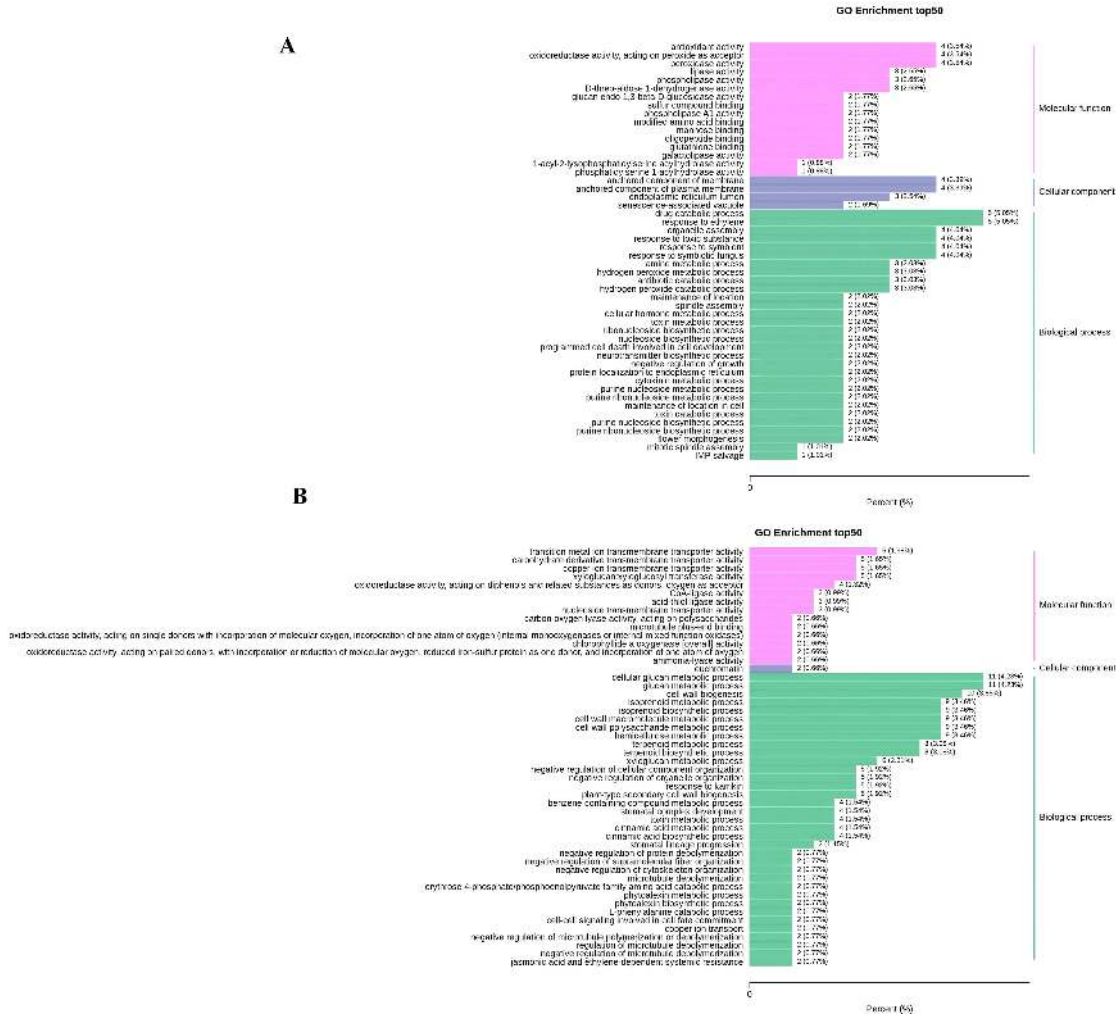

FIGURE 3
Gene ontology (GO) enrichment analysis of the root of *Coptis chinensis* under 24-h and 10-day Cd treatments. **(A, B)** The top 50 GO functional pathways, with DEGs classified into three ontologies [biological processes (BPs), molecular functions (MFs), and cellular components (CCs)] for the three comparison groups. **(A)** The 24-h Cd treatments. **(B)** The 10-day Cd treatments.

screened with a Pearson's correlation coefficient >0.8 and a *p*-value <0.05. In the amino acid biosynthesis pathway, under short-term Cd stress in *C. chinensis*, the core regulatory genes *IFM89_030093* and *IFM89_018981* co-regulated four differential metabolites through positive correlations, suggesting that these two genes play a dominant regulatory role in this pathway. Furthermore, *IFM89_006144* synergistically regulated DL-2-aminoadipic acid (mws1346), 3-methyl-2-oxobutanoic acid (mws0823), and allysine (6-oxo DL-norleucine) (pme3351) together with the above two core genes, while *IFM89_012195* independently regulated DL-2-aminoadipic acid through a negative correlation. This "synergy +antagonism" regulatory pattern implies that DL-2-aminoadipic acid may serve as a key downstream metabolite in this module. Under long-term Cd stress, allysine (6-oxo DL-norleucine) emerged as the core metabolic hub, interacting with all gene nodes. All genes exhibited positive regulatory effects on this metabolite, except for *novel.14850*, which exerted negative regulation, reflecting a finely balanced regulatory mechanism of *C. chinensis* toward this metabolite under Cd stress. Notably, *IFM89_030093* and pme3351 were present in both the short-term and long-term stress groups, indicating that they may not exhibit obvious time dependence in mediating the Cd stress response of *C. chinensis* (Figure 9).

In the ABC transport pathway, under short-term Cd stress in *C. chinensis*, *IFM89_033292* and *IFM89_014398* co-regulated two metabolites: 2′-deoxycytidine (pme1194) and 2′-deoxyguanosine (pme1184). Furthermore, *IFM89_014398* independently regulated Raffinose* (pme2125) through a positive correlation. Under long-term Cd stress, 2′-deoxyguanosine was positively regulated by both *IFM89_033292* and *novel.3783*, indicating that 2′-deoxyguanosine can be regulated by different genes under varying stress durations. Notably, *IFM89_033292* and pme1184 were present in both comparison groups, suggesting that they may also lack an obvious time dependence in mediating the Cd stress response of *C. chinensis* (Figure 10).

3.3.5 RT-qPCR validated transcriptome data

To validate the reliability of the RNA sequencing (RNA-seq) data, nine genes were selected for qPCR verification, including

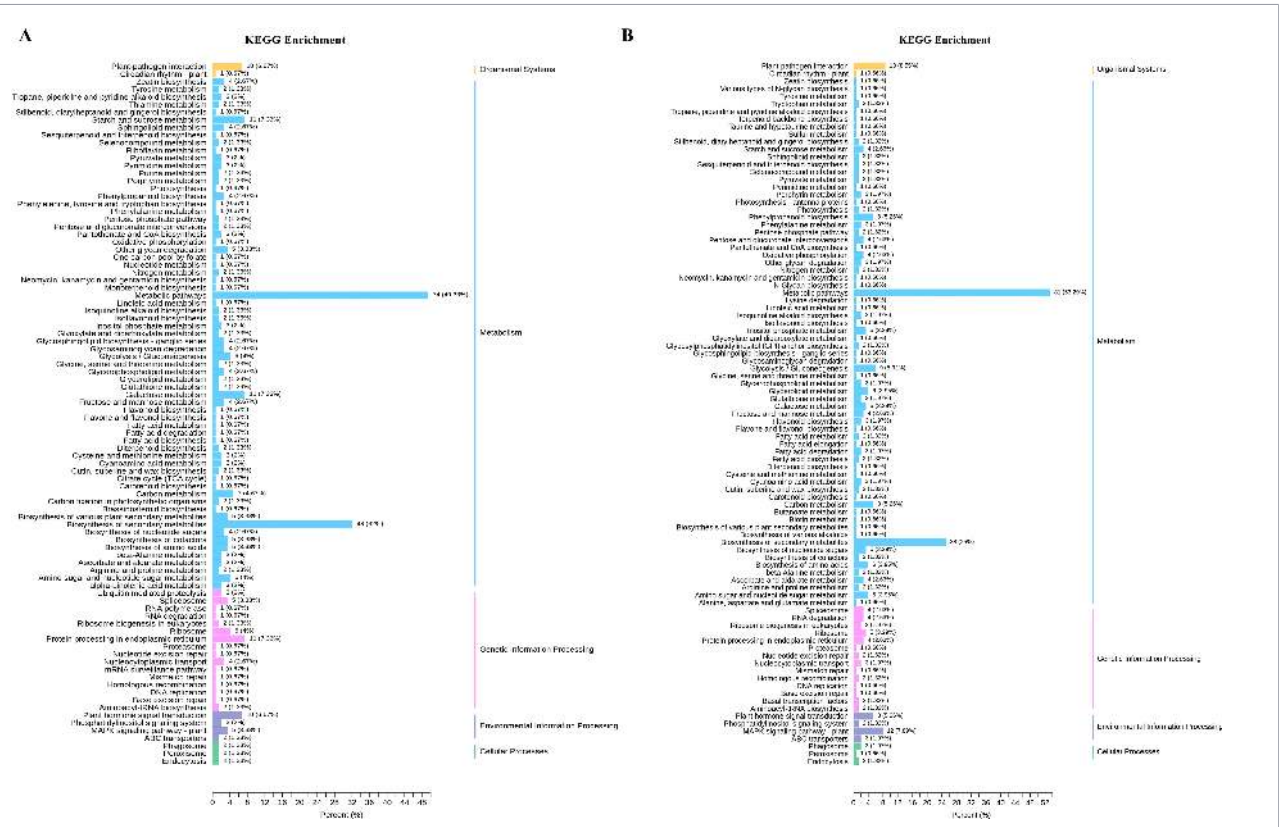

FIGURE 4
Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis in the root of *Coptis chinensis* under 24-h and 10-day Cd treatments. **(A)** The 24-h Cd treatments. **(B)** The 10-day Cd treatments.

MDR, *ASO*, *METE1*, and *ZFP*. These genes are involved in metal ion absorption and transport, metal chelate formation, antioxidant pathways, and signal transduction processes (Kaushik et al., 2024; Zulfiqar et al., 2022; Park et al., 2012; Jasinski et al., 2008; Lee et al., 2004; Li et al., 2022; Zhang et al., 2020). The results of the RNA-seq and qPCR analyses were highly consistent, exhibiting a consistent overall expression trend with a correlation coefficient exceeding 90% (Figure 11).

4 Discussion

The heavy metal Cd is absorbed mainly through some ion channels and transporter proteins. When Cd enters plants, it triggers oxidative stress in these plants and accumulates ROS. Excess ROS can destroy nucleic acids, proteins, and lipids. A huge amount of GSH is depleted, which leads to growth inhibition and cell death. However, plants have evolved a set of defense systems to reduce the Cd-induced damage, such as an increase of the chelating agent biosynthesis, activation of some transporters for Cd isolation, elevation of the antioxidant enzyme contents, and upregulation of the signal transduction factors. These pathways, to a great extent, help plants improve their HM detoxification ability and increase their tolerance. In order to further clarify the mechanism of Cd transportation and detoxification, we compared the DEGs and DEMs between the control and the Cd treatment samples.

4.1 Distribution and accumulation of Cd in *C. chinensis*

The root tip is considered the primary entry point for Cd. For instance, the root cap zone and the meristem zone adjacent to the root tip have been identified as the primary Cd-absorbing sites in *Morus alba* (Guo et al., 2021b). Once inside the plant root system, Cd is immobilized in the roots by forming metal chelates with chelators, sequestering in root vacuoles, or binding to the cell walls; only a small amount of Cd translocates into the xylem via the apoplastic or the symplastic pathway (Dong et al., 2019; Tian et al., 2011). Consequently, Cd is typically distributed in the order root > stem > leaf > fruit > seed (Jia et al., 2020).

In this study, consistent with the majority of plants, *C. chinensis* exhibited a Cd distribution pattern of root > rhizome > leaf. Furthermore, for both the 24-h and 10-day treatment groups, the Cd content in the roots increased significantly with the increase of Cd stress concentration. Under the same Cd concentration, the root Cd content in the 10-day stress groups was considerably higher than that in the 24-h groups. Thus, we conclude that the root is the primary site for Cd uptake and accumulation in *C. chinensis*.

4.2 *C. chinensis* genes associated with Cd chelation

When HMs enter the plant root system, non-accumulating plants immobilize them in the roots as chelates, reducing their

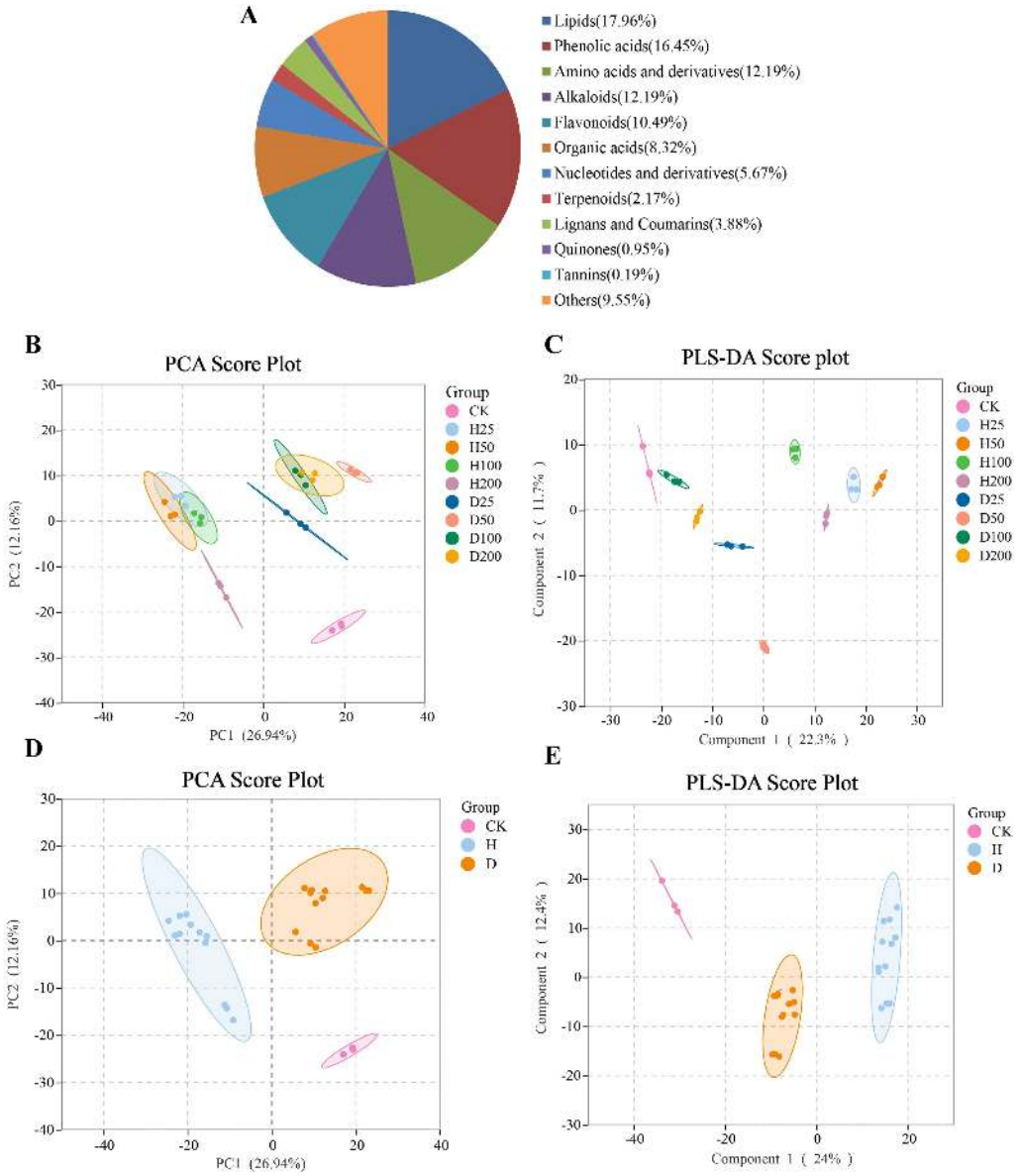

FIGURE 5
Quality control of the metabolite data and classification. **(A)** Results of the classification of metabolites. **(B, D)** Principal component analysis (PCA) of the differentially expressed metabolites (DEMs). **(C, E)** Analysis of the DEMs based on the partial least squares discriminant analysis (PLS-DA) scores.

translocation to the aboveground parts and alleviating their toxic effects on the plant. Thiol-containing chelating compounds such as MTs, GSH, and PCs are the key components involved in Cd chelation in plant roots (Jia et al., 2020). MTs are characterized by a primary structure rich in highly conserved cysteine residues. These residues possess a remarkable ability to bind large quantities of HM ions, forming non-toxic or low-toxic compounds—particularly for Cu, Zn, and Cd (Lee et al., 2004; Li et al., 2022). MT2, identified in *Coptis japonica*, contains two cysteine-rich regions with eight and six cysteine residues, respectively, which can bind up to four Cd^{2+} (Singh et al., 2019). PCs are small metal-binding peptides in plants that chelate HMs *in vivo* to form non-toxic complexes. Their general structure is rich in cysteine, glycine, alanine, and serine, with the thiol group serving as the primary site for Cd^{2+} binding. PC2 in *Brassica chinensis* can chelate Cd^{2+} at a 2:1 ratio to form the $Cd(PC2)_2$ complex, thereby reducing Cd toxicity (Zhao et al., 2021). The biosynthesis of valine, leucine, and isoleucine; the metabolism of alanine, aspartate, and glutamate; and the metabolism of arginine and proline all play crucial roles in PC synthesis. Aspartic acid, glutamic acid, and glutamate synthetase can promote the metabolism of alanine, aspartate, and glutamate (Xie et al., 2019). As a precursor of PCs, GSH also chelates with Cd^{2+} to form the GSH–Cd complex and participates in Cd detoxification (Huang et al., 2022a).

In the current study, five and three DEGs involved in Cd chelation were identified in the 24-h and 10-day stress comparison groups, respectively (Supplementary Table 8). Cluster analysis was performed on the variations in the expression levels. Upregulation of the GST genes was observed in the 24-h stress group (Figure 12A), providing direct evidence of the crucial role of GSH and its associated metabolism in early Cd chelation and detoxification, consistent with the perspective of Huang et al.

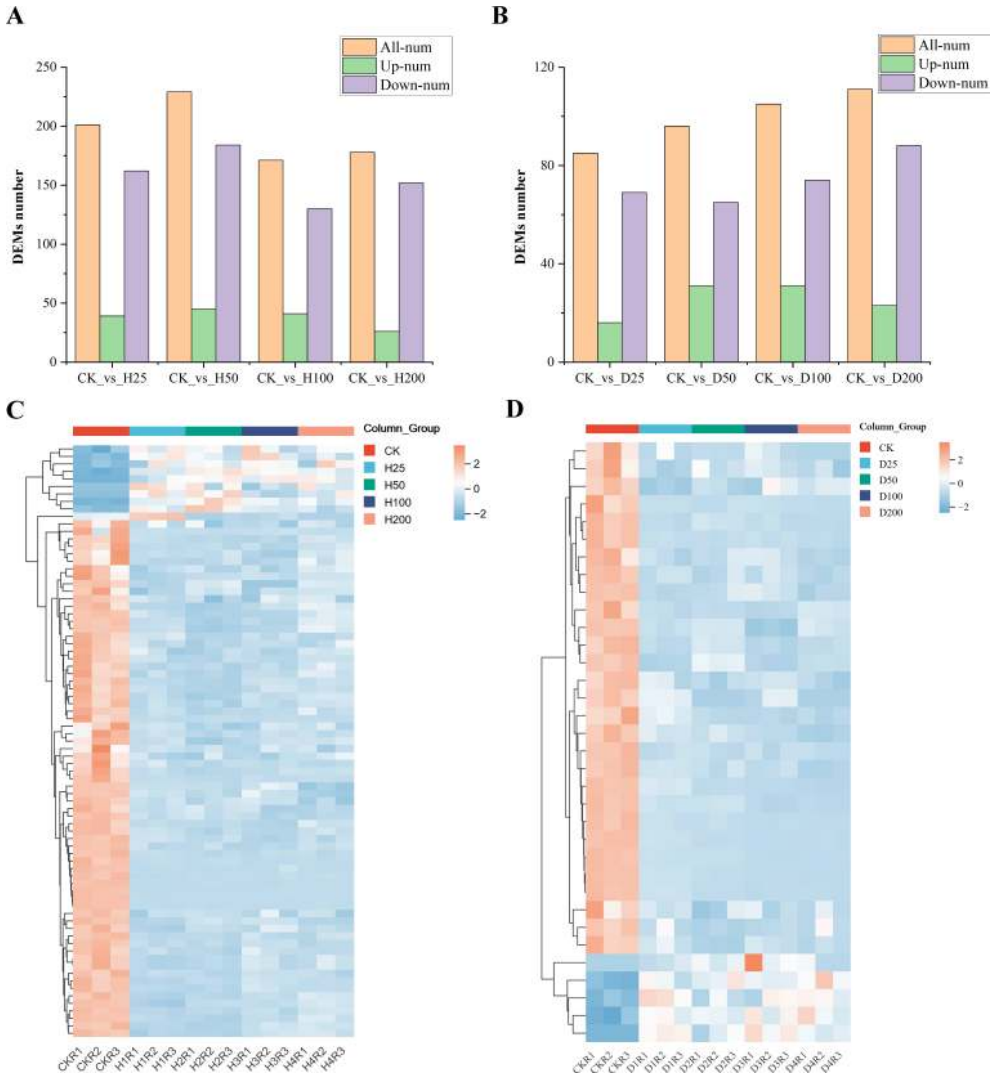

FIGURE 6
Analysis of the differentially expressed metabolites (DEMs) of *Coptis chinensis* roots under 24-h and 10-day Cd treatments. **(A, B)** Column charts of the upregulated and downregulated DEMs. **(C, D)** Heatmaps of the DEMs compared between different groups.

(2022a). GSTs not only participate in the formation of GSH–Cd complexes but also initiate PC synthesis via their isomerase activity. Furthermore, genes involved in cysteine metabolism are also implicated, which are closely linked to the supply of the precursors for PC synthesis (Xie et al., 2019). This underscores, at the genetic level, that sulfur-containing amino acid metabolic pathways are central to the Cd stress response. In contrast, genes associated with glutamate biosynthesis, particularly ferredoxin–nitrite reductase, were significantly downregulated under 24-h stress (Figure 12A). In the 10-day stress group, all three identified DEGs are related to glutamate (Figure 12B) and exhibited significant downregulation. Given that glutamate serves as the direct precursor of PC synthesis, its downregulation appears contradictory to studies suggesting that glutamate enhances PC production. We speculated that *C. chinensis* may enhance the Cd chelation capacity mainly by upregulating the GST activity and downregulating the glutamate receptor and glutamate decarboxylase activities, thereby reducing Cd toxicity in plants.

4.3 *C. chinensis* genes related to cell wall Cd binding

The cell wall is another critical site for HM ion immobilization, and pectin is the primary binding site for metal ions due to its high ion exchange activity (Guo et al., 2021a). The Golgi apparatus secretes highly methylated pectin, which is demethylated by pectin methylesterase (PME) to reduce the degree of methylation, releasing a large number of free carboxyl groups and thereby enhancing its binding capacity for metal ions. Pectin demethylation is associated with ROS signaling molecules, which play a vital role in regulating pectin biosynthesis, PME activity, and pectin demethylation (Xiong et al., 2015). Pectinase, an enzyme capable of decomposing pectin, has been shown to break down pectin in the surface layer of cotton fibers (Xu et al., 2023). The plant cell walls are mainly composed of polysaccharides, and pectate lyase specifically cleaves non-methylated substrates, including unmethylated polygalacturonic acid, thereby affecting the plant cell wall structure (Lagaert et al., 2009).

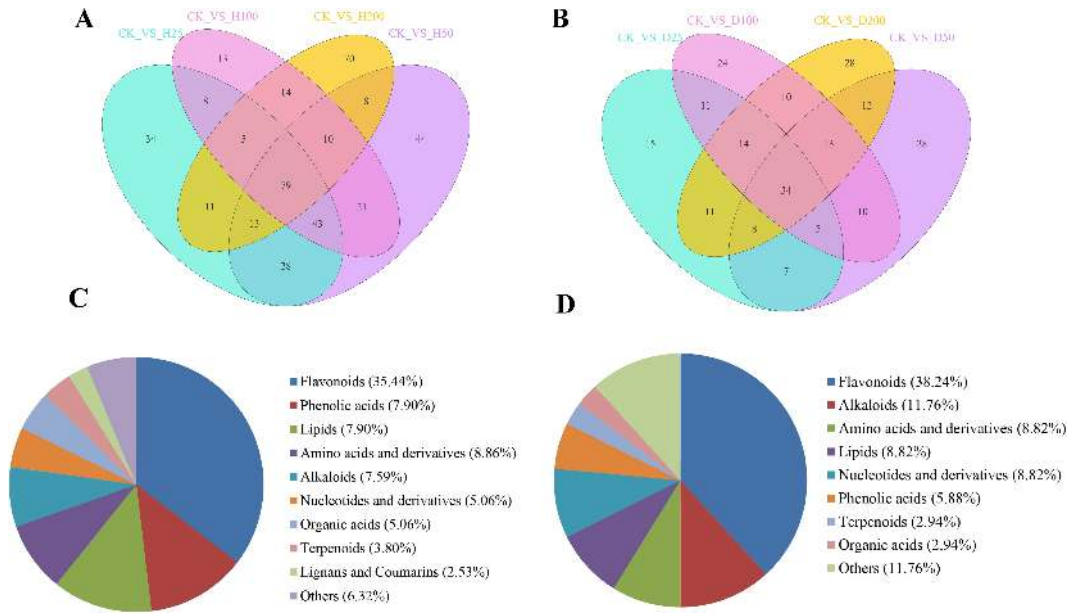

FIGURE 7
Differentially expressed metabolite (DEM) selection and classification of *Coptis chinensis* roots under 24-h and 10-day Cd treatments. **(A, B)** Comparison of the Venn graph of the number of DEMs in groups pairwise. The overlap represents the number of metabolites common to each comparison group, and the non-overlap represents the number of metabolites specific to the comparison group. **(C, D)** Results of the classification of metabolites.

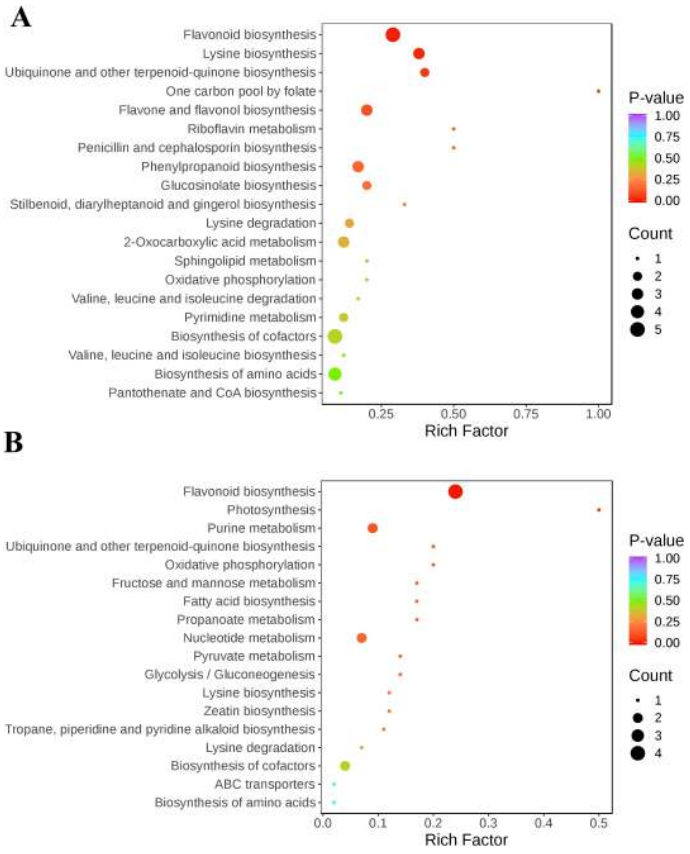

FIGURE 8
Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis in the root of *Coptis chinensis* under 24-h and 10-day Cd treatments. The top 20 pathways of the significant upregulated and downregulated differentially expressed metabolites (DEMs) in KEGG. The *X*-axis represents the rich factor, while the *Y*-axis represents the pathway name. The *bubble size* denotes the number of DEMs involved. The *bubble color* indicates the enrichment degree of the pathway. **(A)** Results of CK_vs_H (*H* indicates Cd stress for 24 h). **(B)** Results of CK_vs_D (*D* indicates Cd stress for 10 days).

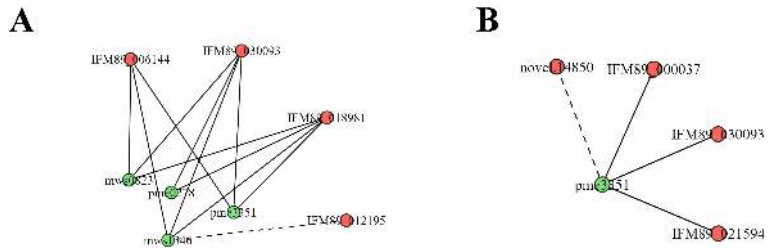

FIGURE 9
Correlation analysis of the differentially expressed metabolites (DEMs) and differentially expressed genes (DEGs) in the biosynthesis of amino acid pathway. *Green* and *red circles* represent the DEMs and DEGs, respectively. *Solid lines* represent positive correlations, and *dashed lines* represent negative correlations. **(A)** The 24-h Cd treatments. **(B)** The 10-day Cd treatments.

One PME gene was found to be upregulated in the roots of *C. chinensis* under short-term Cd stress. In this study, the upregulation of a PME gene in *C. chinensis* roots under 24-h Cd exposure suggests that pectin demethylation catalyzed by PME may also enhance the cell wall Cd-binding capacity via the generation of free carboxyl groups. However, whether this pectin demethylation is associated with ROS signaling molecules remains unclear. Previous research has shown that the PME activity in rice roots decreased after H_2O_2 removal, while exogenous H_2O_2 addition increased the maximum Cd adsorption capacity of the rice root cell wall (Wang et al., 2022). Thus, it is hypothesized that H_2O_2 can enhance the PME activity and thereby improve the Cd adsorption in the plant root cell wall. A key focus of future research will be to determine whether enhancing the Cd adsorption capacity of the *C. chinensis* root cell wall can be achieved by modulating the H_2O_2 levels to increase PME activity. Under long-term Cd stress, one pectinesterase inhibitor gene and one pectate lyase gene were downregulated in *C. chinensis* roots; interestingly, one pectinase gene was upregulated (Supplementary Table 9). Pectate lyase specifically acts on demethylated pectin (Lagaert et al., 2009). We hypothesize that this downregulation may serve to prevent excessive degradation of the demethylated pectin—resulting from the increased PME activity—by pectate lyase, thereby maintaining the structural integrity of the cell wall. This fine-tuned balance between "enhanced immobilization" and "prevention of excessive structural disassembly" has rarely been reported in other plants under Cd stress and may represent a unique adaptation strategy of *C. chinensis* to prolonged Cd exposure.

In this study, the genes involved in the binding capacity of metal ions were enriched in the GO entries, and the biosynthetic pathway of glycosylphosphatidylinositol (GPI) anchor, an important component of cell membranes with functions affecting cell wall biomechanics, was enriched in the KEGG analysis. GPI anchor proteins (GAPs) modulate the biomechanical properties of plant cell walls by participating in the early synthesis and late remodeling of xyloglucan and cellulose, engaging in pectin degradation, and regulating pectin organization (Yeats et al., 2018). Existing studies have shown that BC1, a rice GAP, can interact with cellulose and alter microfibril crystallinity to regulate cellulose assembly, thereby influencing rice stem brittleness and mechanical strength (Liu et al., 2013). The integrity of GPI anchor biosynthesis directly determines the GAP targeting efficiency. When the key genes involved in *Arabidopsis* GPI biosynthesis (e.g., *SETH1* and *SETH2*) are mutated, GAPs fail to anchor to the plasma membrane, usually leading to disordered cell wall synthesis and impaired pollen tube growth (Lalanne et al., 2004). These studies further confirm that the GPI anchor pathway indirectly modulates the structural and mechanical properties of the cell walls by regulating the functions of GAPs. Beyond directly affecting cell wall biomechanics, the GPI anchor pathway also plays a crucial role in metal ion transport. For instance, LYM2, a GAP in *Arabidopsis*, can regulate plasmodesmal permeability by sensing chitin signals from fungal pathogens, thereby indirectly modulating intercellular metal ion transport (Faulkner et al., 2013). Under Cd stress, the *Arabidopsis* p24 protein regulates the delivery of GAPs to the plasma membrane and cell wall, thereby affecting the Cd^{2+} chelating capacity and the

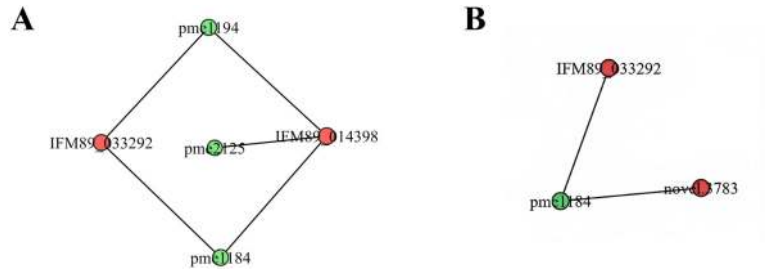

FIGURE 10
Correlation analysis of the differentially expressed metabolites (DEMs) and differentially expressed genes (DEGs) in the ABC transporter pathway. *Green* and *red circles* represent the DEMs and DEGs, respectively. Solid lines represent positive correlations, and dashed lines represent negative correlations. **(A)** The 24-h Cd treatments. **(B)** The 10-day Cd treatments.

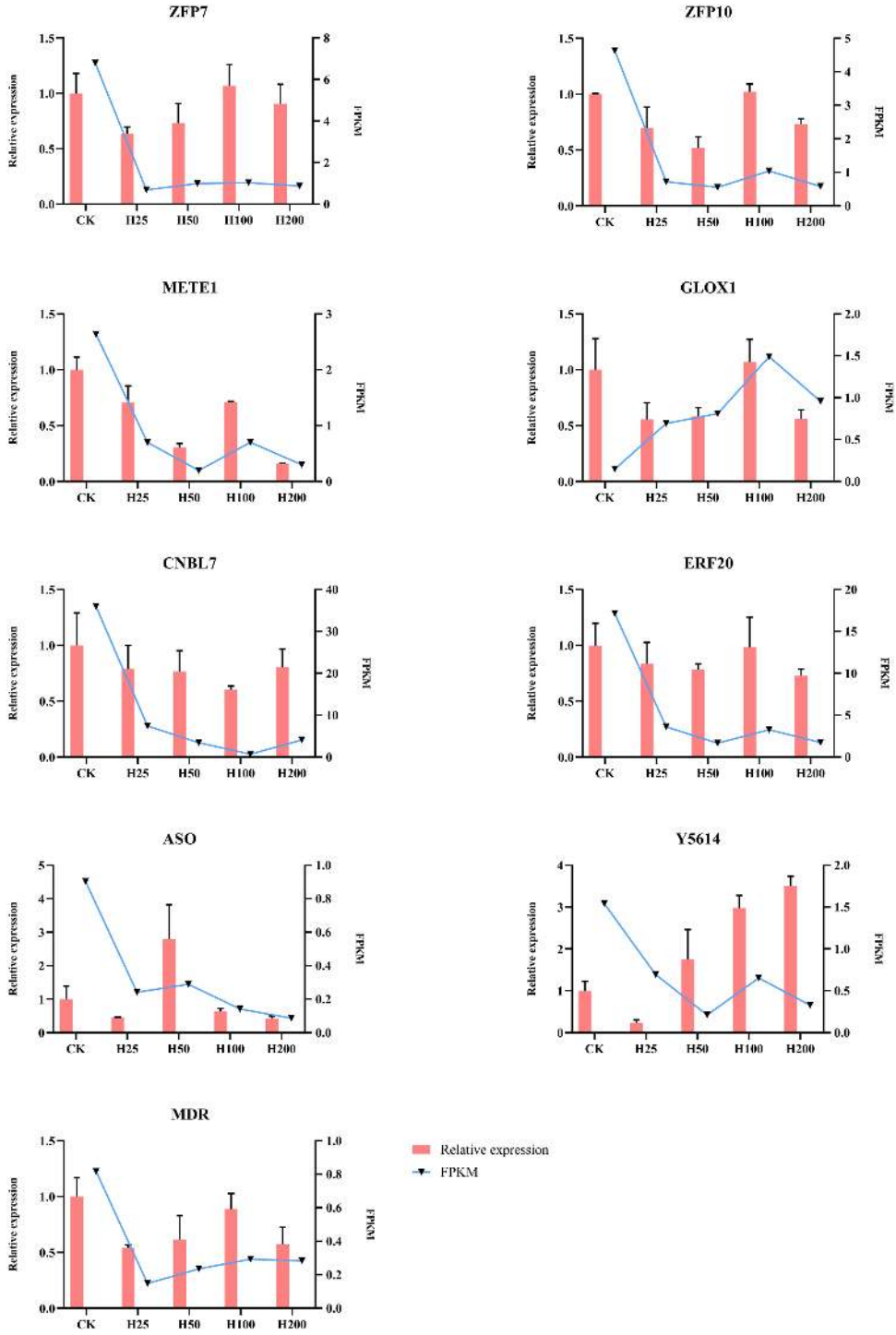

FIGURE 11
Expression of the selected differentially expressed genes (DEGs) by RNA sequencing (RNA-seq) and real-time quantitative PCR (RT-qPCR). The left and right y-axes are the RT-qPCR expression levels and RNA-seq data, respectively.

plasma membrane stability of the cell wall, ultimately enhancing plant Cd tolerance (Montesinos et al., 2012).

Therefore, through a time course analysis of the stress responses, this study revealed that *C. chinensis* employs a two-phase adaptive process under Cd stress: rapid upregulation of the PME genes for immediate activation, followed by the downregulation of one pectate lyase gene and one pectinesterase inhibitor gene to maintain long-term cell wall homeostasis. Collectively, these responses enhance the Cd retention in the cell wall, modulate the cell wall structure and biomechanics, and thereby reduce the toxic effects of Cd on the plant. More importantly, the biosynthetic pathway of GPI anchor was significantly enriched in this study. We speculate that, under Cd stress, this pathway mediates the Cd stress response in *C. chinensis* roots by regulating the biomechanical properties of the cell wall and the synthesis of the cell wall components. Combined with changes

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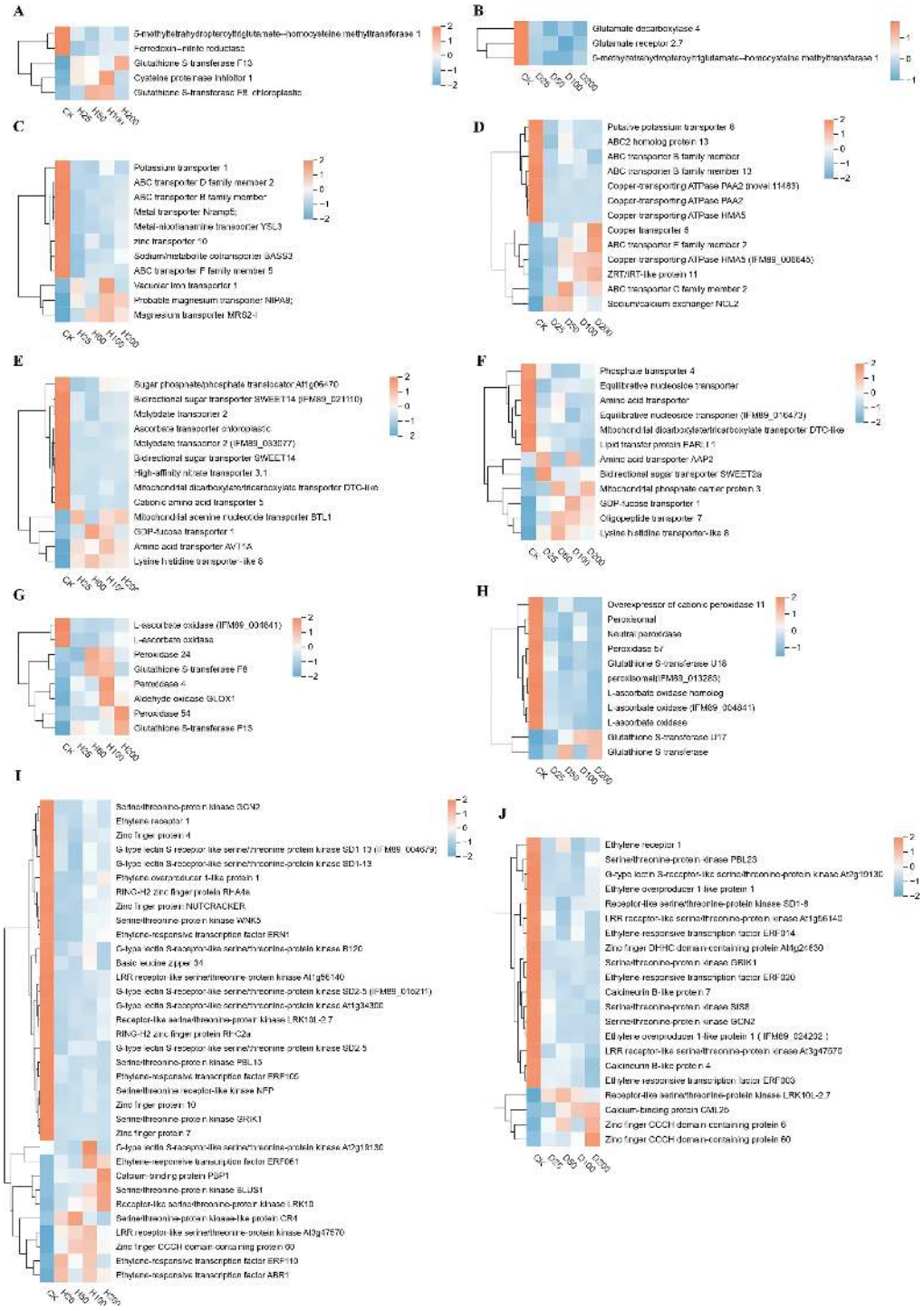

FIGURE 12
Heatmap of the differentially expressed genes (DEGs) encoding proteins. The coloring of the heatmap denotes the gene content and distinguishes strongly expressed genes (colored in *red*) compared with weakly expressed genes (in *green*). **(A, B)** Heatmap of the DEGs encoding Cd chelation. **(C, D)** Heatmap of the DEGs encoding heavy metal transporters. **(E, F)** Heatmap of the DEGs encoding proteins related to Cd transport. **(G, H)** Heatmap of the DEGs encoding proteins involved in the antioxidant pathway. **(I, J)** Heatmap of the DEGs encoding proteins related to the signal transduction pathway. Figures 2–8 are completed in the Metware Cloud Platform and in Adobe Illustrator 2021.

in the PME gene activity, whether this pathway primarily modulates the cell wall biomechanical properties in *C. chinensis* roots by regulating pectin synthesis and metabolism, and whether the proteins within this pathway directly regulate pectin synthesis or metabolism, will be the core focus of future research.

4.4 *C. chinensis* genes related to Cd transport

HMAs, ABC transporters, NRAMPs, ZIPs, CDFs, YSL transporters, and CAX proteins are involved in the absorption,

transport, separation, and detoxification of HMs in plants (He, 2021). In this study, 11 and 13 DEGs associated with metal ion transport were identified in the 24-h and 10-day Cd stress groups, respectively (Supplementary Table 10). Cluster analysis performed on the variations in their expression levels. P-type ATPases are a class of transporters that function as ion pumps using energy generated by ATP hydrolysis (Axelsen and Palmgren, 2001). Among them, P1B-ATPases are involved in the uptake, transport, and detoxification of HMs in plants. OsHMA2 in rice is involved in the translocation of Zn and Cd from the roots to the aboveground parts. At the same time, OsHMA3 specifically transports Cd and sequesters it in root cell vacuoles, thereby exerting a detoxification effect. In the model plant *Arabidopsis thaliana*, AtHMA3 is localized to the vacuolar membrane and can sequester Zn and Cd in the vacuoles for detoxification (Takahashi et al., 2012). The ZIP transporter family not only mediates the transport of Zn^{2+} and Fe^{2+} but also regulates Cd^{2+} uptake by plant roots. For example, the zinc transporter ZNT1 can mediate the influx of Cd^{2+}, and the iron-regulated transporter BcIRT1 is the primary transporter of Cd in Chinese cabbage (Pence et al., 2000; Wu et al., 2021). As a non-essential element for plants, Cd can enter root cells in ionic form through the ion channels and transporters of essential elements such as Zn, Ca, and Fe (Dong, 2020). In this study, the magnesium transporter and vacuolar iron transporter 1 genes were significantly upregulated in the 24-h stress groups, while the potassium transporter 1 and zinc transporter 10 genes were significantly downregulated.

The NRAMP family can not only bind to and transport some essential transition divalent metals but also transport toxic metals such as Cd, Pb, and Hg (Bozzi et al., 2016). Nramp5 has been identified as a transporter protein located on the rice cell membrane, which is involved in transporting Cd from external solutions into rice roots (Sasaki et al., 2012). YSL proteins can participate in the absorption of complexed Cd and facilitate the translocation of metal–nicotianamine complexes across plant cell membranes. SnYSL3 in *Solanum nigrum* transports nicotianamine complexes containing Fe, Cu, Zn, and Cd, and studies have confirmed that its Cd transport rate increases under Cd stress (Dong, 2020; Feng et al., 2017). In our study, the *Nramp5* and *YSL3* genes were significantly downregulated in the 24-h stress groups. ABC transporters are also proteins that can transport free or chelated HMs through biofilms and squeeze them into apoplasts or vacuoles to achieve detoxification (Jasinski et al., 2008). Previous studies have shown that ABC transporters play an important role in the process of Cd sequestration in plant vacuoles. The ABCC subfamily has been widely studied; for example, the AtABCC1 and AtABCC2 proteins can transport HMs such as As, Cd, and Hg into vacuoles (Park et al., 2012).

In this study, under short-term stress (24 h), the Mg transporter genes and vacuolar iron transporter 1 (*VIT1*) were significantly upregulated. In contrast, the potassium transporter genes and the zinc transporter 10 gene were downregulated (Figure 12C). These results suggest that, during early Cd stress, *C. chinensis* may enhance the activity of Mg- and Fe-related transporters to maintain ion homeostasis and cope with Cd stress while simultaneously reducing the Cd influx by inhibiting specific competitive absorption channels for Cd, such as those mediated by Zn/Fe pathways. Notably, the upregulation of *VIT1* suggests its potential role in the sequestration of Cd into vacuoles, a mechanism consistent with previous findings in other plant species (Pence et al., 2000; Wu et al., 2021). *Nramp5* and *YSL3*, genes known to mediate Cd transport, were downregulated (Sasaki et al., 2012; Dong, 2020; Feng et al., 2017). This suggests that *C. chinensis* may inhibit Cd transport by suppressing the expression of these two genes, which differs from the commonly observed upregulation of their orthologs in other plants, indicating a distinct regulatory mechanism for Cd transport in this species. Under prolonged stress (10 days), the upregulation of the genes encoding a copper transporter (*COPT6*), a sodium/calcium exchanger (*NCL2*), *ZIP11*, and members of the ABC-C family was observed (Figure 12D). This implies the activation of a different set of response mechanisms during long-term acclimation. Specifically, the induced expression of the ABC-C family genes is consistent with the enhanced vacuolar sequestration for detoxification, a functional analogy to the ABCC proteins reported in rice (Jasinski et al., 2008). Nevertheless, it exhibits a more pronounced temporal correlation in *C. chinensis*.

Furthermore, we identified 13 and 12 DEGs associated with amino acids, sugars, nucleosides, and lipids under 24-h and 10-day Cd stress, respectively (Supplementary Table 11 and Figures 12E, F). Amino acids are among the most abundant biosynthetic precursors in plants involved in plant osmoregulation and protein synthesis. Some intermediate metabolites also participate in plant signal transduction and HM compartmentalization processes (Wei et al., 2023; Zhao, 2021). Phosphate significantly inhibits the Cd uptake by rice roots and its translocation to the aboveground parts (Zhao, 2021).

In this study, lysine histidine transporter-like 8 (LHT8) and GDP-fucose transporter 1 were upregulated in response to stress of varying durations. The amino acid transporter *AVT1A* gene was significantly upregulated under 24-h stress, while the sugar phosphate translocator, high-affinity nitrate transporter 3.1, molybdate transporter, and bidirectional sugar transporter *SWEET14* were significantly downregulated. Under 10-day stress, the bidirectional sugar transporter *SWEET2a* and the oligopeptide transporter 7 genes were significantly upregulated, whereas the amino acid transporter and phosphate transporter 4 were considerably downregulated (Figures 12E, F). The upregulation of LHT8, the significant enrichment of the lysine biosynthesis pathway in the KEGG analysis, and its association with the pivotal role of the lysine degradation pathway, as revealed by metabolomic data, strongly suggest that lysine metabolism may play a central role in Cd detoxification in *C. chinensis*. In contrast, the observed downregulation of the phosphate transporters is inconsistent with previous findings of phosphate supplementation inhibiting Cd uptake in rice (Zhao, 2021). This discrepancy indicates a unique mechanism in *C. chinensis*, where the modulation of phosphorus metabolism may alter the chemical speciation of Cd or influence the cellular signaling pathways involved in Cd stress. The underlying mechanism merits further investigation.

4.5 *C. chinensis* genes involved in the antioxidant pathway

ROS refers to a group of partially reduced or activated oxygen molecules. Although Cd lacks redox activity, it can induce ROS

production in plants and trigger oxidative stress (Zheng et al., 2022). H_2O_2 is a key signaling molecule in plants: excessive accumulation can cause severe oxidative damage and impair cellular functions, including inhibition of photosynthesis and ATP production, as well as induction of lipid peroxidation and DNA damage (Lin and Aarts, 2012; Singh et al., 2010). Plants have evolved an antioxidant system to resist oxidative stress, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and guaiacol peroxidase (GPX), to scavenge ROS. In addition, GST, peroxidase (POD), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and some non-enzymatic substances including ascorbic acid (AsA), reduced GSH, and proline, are also capable of scavenging excess ROS (Leng, 2021; Zhang, 2020).

Based on the enrichment analysis of the DEGs and DEMs, purine–pyrimidine metabolism, photosynthesis, amino acid anabolism, and DNA/RNA anabolic processes were either upregulated or downregulated. Consequently, we speculated that Cd stress induces an oxidative stress response in *C. chinensis*, which in turn triggers ROS production. In this study, 8 and 11 DEGs associated with this response were identified in the 24-h and 10-day stress groups, respectively (Supplementary Table 12; Figures 12G, H).

Enrichment analysis of the DEGs and DEMs indicated significant upregulation or downregulation of fundamental metabolic pathways, including purine and pyrimidine metabolism, photosynthesis, amino acid anabolism, and DNA/RNA biosynthesis. This provides direct omics evidence for the classic path of "Cd → metabolic disorder → ROS generation." Furthermore, an increase in GST activity was detected, which is consistent with previously reported findings that GST plays a key role in detoxification and antioxidation by catalyzing the conjugation of GSH with peroxides and electrophilic toxins, including the oxidative by-products induced by Cd (Leng, 2021; Zhang, 2020). This confirms the conserved function of this enzyme in the response of *C. chinensis* to Cd stress. In this study, 8 and 11 DEGs associated with this process were identified in the 24-h and 10-day stress groups, respectively (Supplementary Table 12; Figures 12G, H).

In addition, POD activity was activated under 24 h of Cd stress, but was inhibited after 10 days of exposure. This suggests that *C. chinensis* may initially employ POD to rapidly scavenge peroxides (e.g., H_2O_2) in the early stages of stress, while its antioxidant strategy likely shifts as stress persists. More importantly, GST U18 was significantly downregulated under long-term stress, but exhibited no significant change under short-term treatment. This indicates that different GST isoforms may exert stage-specific functions during Cd stress, and the downregulation of GST U18 may represent a component of metabolic reprogramming during prolonged adaptation—rather than merely a weakened defense response—potentially highlighting a novel regulatory node. Furthermore, L-ascorbate oxidase activity was significantly inhibited, suggesting that *C. chinensis* may reduce ascorbate oxidation by suppressing L-ascorbate oxidase activity, thereby increasing the ascorbate levels and, consequently, lowering the ROS levels in the plant. Combined with the upregulation of GST, this strongly suggests that *C. chinensis* might rely particularly on the AsA–GSH cycle to cope with Cd-induced oxidative stress. In contrast, the activities of SOD and CAT did not change significantly in this study, which stands in potential contrast to reports in some other plants (e.g., *Sedum alfredii*) where the SOD and CAT activities are often markedly induced (Jin et al., 2008). This differential response of the antioxidant system may reflect the unique redox regulation strategy of *C. chinensis*, a perennial medicinal plant.

4.6 *C. chinensis* genes related to the signal transduction pathway

Under HM stress, metal ions stimulate the pathways involved in plant signal perception and transduction, regulate relevant TFs, and ultimately activate or repress a range of genes to confer defense and detoxification against HM stress (Wang et al., 2013). The main signaling pathways in response to HM stress include calcium, hormone, and MAPK signaling (Leng et al., 2020).

Ca^{2+} is a common second messenger ion in eukaryotes, and fluctuations in the intracellular free Ca^{2+} concentration are among the earliest events in the perception of environmental changes (Köster et al., 2022). Studies have shown that Cd treatment promotes the positive response of Ca^{2+} signaling channels in plant cells. An elevated intracellular Ca^{2+} concentration is sensed by highly sensitive Ca^{2+} sensors, which convert it into biological signals and transmit it to Ca^{2+} binding proteins and calcium-sensitive proteins to enhance stress tolerance (Leng et al., 2020; Ren et al., 2022). In addition, calcium-dependent, calmodulin-dependent, and calcineurin B-like protein kinases were involved in Cd accumulation in *Ulva compressa* (González et al., 2021). We speculated that *C. chinensis* may also enhance Cd tolerance by activating the expression of the Ca^{2+}-binding proteins and reducing the plant Cd accumulation by inhibiting the calcineurin B-like protein expression. In this study, 34 and 21 DEGs associated with signal transduction pathways were identified in the 24-h and 10-day stress groups, respectively (Supplementary Table 13; Figures 12I, J). The calcium-binding protein PBP1 was significantly upregulated in the 24-h stress group; in the 10-day stress group, the calcineurin B-like protein was significantly downregulated, while the calcium-binding protein CML25 was significantly upregulated. This aligns well with the aforementioned mechanism in which Cd stress triggers fluctuations in intracellular calcium signaling, which, in turn, stimulates the calcium-binding proteins to transmit signals and initiate defense responses. Furthermore, the downregulation of the calcineurin B-like protein under short-term stress suggests that, similar to that in *U. compressa*, the reduced activity of calcineurin B-like proteins may contribute to the decreased Cd accumulation in *C. chinensis* cells (González et al., 2021). These findings not only support the hypothesis that *C. chinensis* enhances its Cd tolerance by activating calcium-binding protein expression but also corroborate the conserved role of calcium signaling as an early response mechanism to Cd stress.

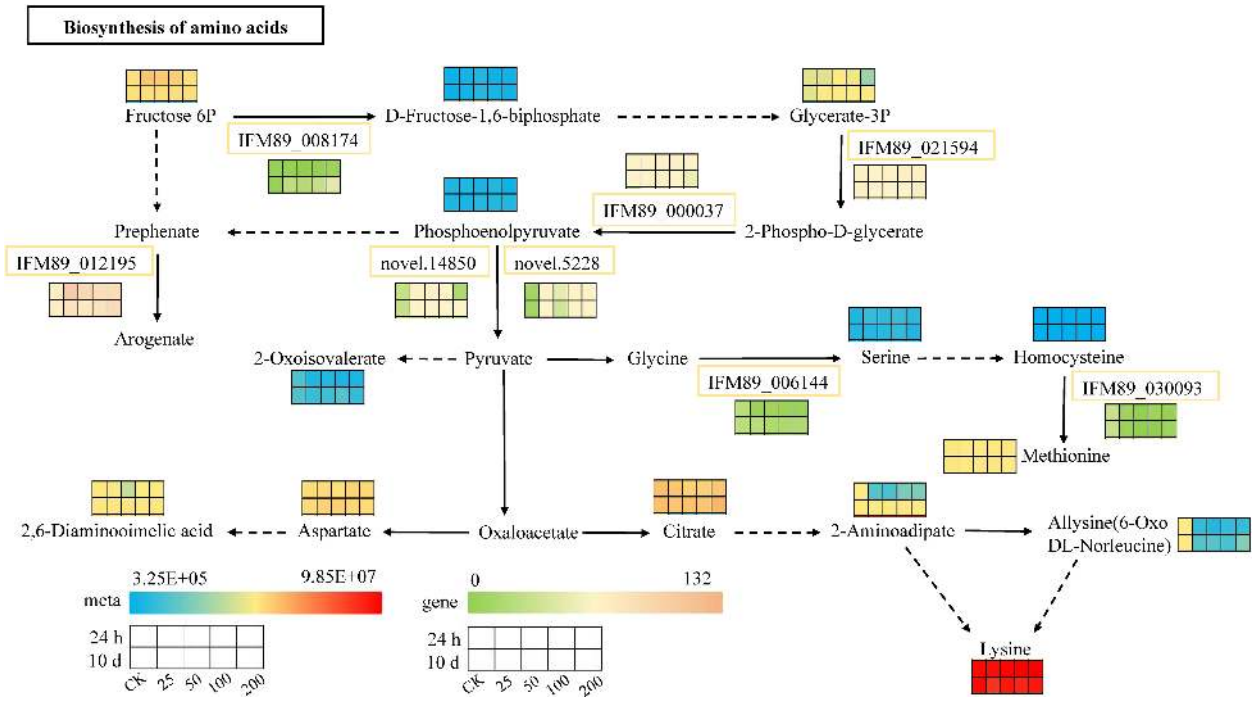

FIGURE 13
Alterations in the biosynthesis of amino acid pathways induced by Cd stress. The schematic representation shows metabolites (circles) and the corresponding genes (squares).

In the short- and long-term treatment comparison groups, 18 and 9 DEGs were associated with serine/threonine protein kinases, respectively. Among these, 13 and 8 DEGs were significantly downregulated, accounting for 72.22% and 88.89% of the total, respectively. MAPK is a highly conserved signaling molecule, and the MAPK signaling cascade consists of MAPKKKs, MAPKKs, and MAPKs (Jalmi and Sinha, 2015). MAPK signal transduction plays a crucial role in cell proliferation, plant development, hormone physiology, and abiotic stress signal transmission. As serine/threonine protein kinases, MAPKs can be activated by phosphorylation-related response factors when plants are exposed to stress (Zhang, 2020). However, in contrast to numerous reports indicating the activation of the MAPK signaling pathway under Cd stress, the present study observed a significant downregulation of the majority of the serine/threonine protein kinases (Supplementary Table S13; Figures 12I, J). This discrepancy may be attributed to the timing of sample collection: the transcriptomes analyzed herein represent steady-state responses at 24 h and 10 days post-stress onset. As MAPK activation is typically rapid and transient, it is plausible that, by these later time points, the initial signaling surge had already returned to basal levels or entered a phase of feedback inhibition (Zhang and Zhang, 2022).

Regulation of the plant hormone levels helps plants alleviate Cd-induced stress (Labidi et al., 2021). Abscisic acid (ABA) is involved in Cd signal transduction in *Solanum tuberosum* roots. It can also promote the synthesis of the metal chelators GSH and PCs by regulating cysteine synthesis. ABA signaling induces the expression of the genes containing a G-box in their promoters, and basic leucine zipper (b-ZIP) proteins are TFs that specifically bind to G-boxes, serving as key components of the ABA signaling pathway (Stroiński et al., 2013). In addition, ZFP3 and its family of C2H2-type zinc finger proteins (ZFPs) have been identified as negative regulators of ABA signaling (Joseph et al., 2014). Cd induces a rapid increase in ethylene content in plants over a short period; however, ethylene itself cannot induce cell death and only acts as an essential component in chemically induced cell death (Iakimova et al., 2008). Furthermore, ethylene can regulate GSH synthesis to mitigate the impact of Cd stress on photosynthesis and enhance plant tolerance to Cd (Zhang, 2020). In this study, we observed downregulation of the key components of the ethylene biosynthesis and signaling pathways, as well as ZFP3, a negative regulator of ABA signaling. This aligns with the established view that plants adapt to stress by rebalancing hormone homeostasis (Labidi et al., 2021). The predominant suppression of ZFP activity suggests that *C. chinensis* may activate broader phytohormone signaling by inhibiting the expression of this ABA negative regulator. The ethylene response exhibited a distinct time-dependent pattern: three ethylene-responsive TFs were significantly upregulated after 24 h of stress, whereas all ethylene-related genes were significantly downregulated in the 10-day stress group (Supplementary Table S13; Figures 12I, J). This dynamic shift likely reflects a physiological trade-off between immediate stress alarm and long-term adaptation, potentially preventing excessive ethylene production and the consequent induction of programmed cell death (Iakimova et al., 2008).

4.7 Response of the biosynthesis amino acid pathway to Cd stress in *C. chinensis*

We analyzed the genes and metabolites involved in the biosynthesis of amino acids. The results indicated that all relevant genes exhibited altered expression (Supplementary Tables S14, S15;

Figure 13). Carbohydrates can directly or indirectly facilitate metabolite flux during amino acid biosynthesis (Nunes-Nesi et al., 2010). In the glycolytic step, fructose 6P→D-fructose-1,6-bisphosphate, the *IFM89_008174* gene exhibited a clear time-dependent pattern: it showed no significant change in the 24-h Cd-treated group, but was significantly upregulated in the 10-day group, whereas the levels of the two metabolites in this pathway remained unchanged. Therefore, under Cd stress, *C. chinensis* may stabilize the levels of fructose 6P and D-fructose-1,6-bisphosphate by regulating the expression of *IFM89_008174*, thereby maintaining the supply of the carbon skeletons required for downstream amino acid synthesis. Arogenate dehydratase (ADT) plays a crucial role in the phenylalanine biosynthesis pathway by catalyzing the conversion of arogenate into the aromatic amino acid L-phenylalanine, thereby enhancing the metabolic flux toward phenylalanine synthesis. Consequently, the metabolic flux of its substrate, arogenate, is particularly critical in the phenylalanine biosynthetic pathway (Yoo et al., 2021). No genes associated with ADT expression were identified in this study; however, genes involved in the synthesis of its substrate, arogenate, were detected. In the upstream segment of this pathway, the expression of the genes *IFM89_021594* and *IFM89_000037* was downregulated. In the downstream segment, the expression of the gene *IFM89_012195* was upregulated (Supplementary Tables S14, S15; Figure 13). While the abundance of arogenate was not quantified in this study, the differential expression of the genes in this pathway underscores the significant roles of arogenate and phenylalanine biosynthesis in the response of *C. chinensis* to Cd stress. Pyruvate acts as a central hub in cellular metabolism and is a key metabolic node linking multiple amino acid pathways (Multhoff et al., 2024). This study revealed that two upregulated genes, *novel.5228* and *novel.14850*, directly regulate pyruvate metabolism. Pyruvate is involved in multiple branches of amino acid biosynthesis. In the glycine-to-methionine pathway, the downregulation of *IFM89_006144* and *IFM89_030093* did not cause significant changes in the abundance of serine and methionine (Supplementary Tables S14, S15; Figure 13), suggesting the existence of posttranscriptional compensation or alternative metabolic pathways maintaining serine and methionine homeostasis against Cd stress. In the lysine biosynthetic branch, no related DEGs were identified. However, the abundance of some metabolites in this branch changed significantly, while the final lysine biosynthesis level remained unchanged (Supplementary Tables S14, S15; Figure 13). This implies the presence of metabolic bypass mechanisms that regulate the lysine biosynthetic pathway to stabilize the synthesis of lysine, which possesses antioxidant properties (Okla et al., 2023), thereby alleviating Cd-induced oxidative stress. Collectively, the associations between these DEGs and metabolites indicate that *C. chinensis* maintains amino acid homeostasis under Cd stress through the regulation of key hub nodes and compensatory mechanisms.

5 Conclusion

This study employed ICP-MS and found that the roots comprise the main site for Cd accumulation in *C. chinensis*. Accordingly, taking the roots of *C. chinensis* as the research material, this study combined metabolomic and transcriptomic techniques to systematically explore the response mechanism of *C. chinensis* to Cd stress. Metabolomic analysis showed that Cd stress significantly decreased the contents of various metabolites in the roots of *C. chinensis*. Flavonoid biosynthesis, lysine biosynthesis and degradation, oxidative phosphorylation, DNA-related metabolism, and cofactor biosynthesis pathways were significantly enriched under Cd stress. The DEGs identified by transcriptome sequencing are mainly involved in metal ion uptake and transport, metal chelate formation, cell wall metabolism, antioxidant pathways, and signal transduction processes. Comprehensive analysis of the metabolomic and transcriptomic results indicated that the amino acid biosynthesis pathway is a key pathway for *C. chinensis* in the response to Cd stress.

However, this study remains preliminary in its exploration of the molecular mechanisms of Cd uptake and stress response in *C. chinensis*, warranting further investigation into the mechanisms, gene functions, and protein activities. For example, *C. chinensis* roots may enhance the cell wall Cd-binding capacity via PME-catalyzed pectin demethylation under Cd stress, but H_2O_2 regulates the PME activity (Wang et al., 2022). Thus, future work should examine whether modulating the H_2O_2 levels to increase PME activity can enhance the root cell wall Cd adsorption. Arogenate and phenylalanine biosynthesis may contribute to Cd stress response, but arogenate was not quantified in metabolomics, requiring further functional characterization. *IFM89_006144* and *IFM89_030093* directly regulate serine and methionine, but the levels of these two amino acids showed no significant differences. Therefore, the mechanisms by which these two genes exert their unique roles in the Cd stress response of *C. chinensis* require further investigation. The response mechanism of GST U18 under prolonged Cd stress also requires in-depth investigation.

Data availability statement

The data reported in this paper have been deposited in the OMIX, China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (https://ngdc.cncb.ac.cn/omix: accession no. OMIX015621 and OMIX015623).

Author contributions

YN: Conceptualization, Writing – original draft, Data curation. ZY: Writing – review & editing. ZW: Methodology, Writing – review & editing. SH: Writing – review & editing, Methodology. LX: Writing – review & editing, Methodology. CD: Formal analysis, Writing – review & editing. YJ: Formal analysis, Writing – review & editing. HZ: Writing – review & editing, Formal analysis. XS: Writing – review & editing, Resources. WW: Project administration, Writing – review & editing. SW: Writing – review & editing, Resources. MD: Writing – review & editing, Supervision. WH: Project administration, Methodology, Writing – review & editing, Funding acquisition.

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Conflict of interest

Authors SW and MD were employed by the company Xi'an Ande Pharmaceutical Co, Ltd.

The remaining author(s) declared that this work 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/fpls.2026.1733160/full#supplementary-material

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