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Phosphorus Alleviates Cadmium Damage by Reducing Cadmium Accumulation and Enhancing Antioxidant Enzymes at the Vegetative Phase in Soybean

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Abstract: Cadmium (Cd) is a toxic, non-essential heavy metal, with significant stress to plants such as soybean (*Glycine max*). High Cd concentration in the soil inhibits various stages of soybean growth, including seed germination, vegetative growth, and the reproduction stage. Phosphate, a vital macronutrient, has been shown to alleviate Cd-induced stress; however, the molecular mechanisms remain poorly understood. This study aimed to explore the interactive effects of Cd and phosphate on soybeans at the physiological, transcriptomic, and metabolic levels using a multi-omics approach. Experiments were conducted where soybean plants were treated with different concentrations of Cd and phosphate. The results indicated that Cd stress significantly reduced plant height, photosynthetic rate, and transpiration rate, while phosphorus application mitigated these effects, reducing Cd absorption in both roots and shoots. Furthermore, antioxidant enzyme activities (superoxide dismutase, catalase, and peroxidase) were significantly enhanced by phosphate under Cd stress, which scavenged reactive oxygen species (ROS) generated by cadmium, thereby protecting cells from oxidative stress damage. Transcriptome and metabolome analyses revealed substantial changes in gene expression and metabolite profiles in response to Cd and phosphate treatments. Notably, phosphorus treatment induced the up-regulation of genes involved in stress response, root development, and metal transport, while altering metabolic pathways related to phenolic acids, flavonoids, and lipids. This research provided new insights into the molecular mechanism by which phosphorus enhanced the activity of antioxidant enzymes, thereby improving the plant's antioxidant defense capacity and reducing the toxic effects of cadmium in soybeans, offering potential strategies for enhancing crop resilience against heavy metal contamination.

Keywords: soybean; cadmium stress; phosphate; antioxidant enzymes; multi-omics

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Academic Editor: Marzia Vergine

Received: 24 January 2025
Revised: 24 February 2025
Accepted: 27 February 2025
Published: 3 March 2025

Citation: Jia, S.; Zhao, X.; Huang, J.; Yao, X.; Xie, F. Phosphorus Alleviates Cadmium Damage by Reducing Cadmium Accumulation and Enhancing Antioxidant Enzymes at the Vegetative Phase in Soybean. *Agronomy* **2025**, *15*, 637. https://doi.org/10.3390/agronomy15030637

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1. Introduction

Cadmium (Cd) is a toxic, non-essential heavy metal, widely distributed in the environment as a result of human activities, such as mining, industrial emissions, and agricultural practices [1,2]. When present in high concentrations in soil, Cd accumulates in wheat, rice, and soybean, leading to detrimental effects on their growth, development, and productivity [3–5]. Soybean (*Glycine max*) is a globally important oilseed and protein crop that is particularly vulnerable to Cd-induced stress caused by the relatively efficient root uptake system, which hampers its yield and quality. When Cd-accumulated soybeans are

processed into food products, the heavy metal enters the human food chain. Prolonged consumption of food containing Cd can cause a wide range of health problems [6]. Phosphate, an essential nutrient for plants, plays a pivotal role in various physiological processes, including energy transfer, nucleic acid synthesis, and root development [7,8]. Previous studies have suggested that phosphate mitigates Cd toxicity by improving plant tolerance mechanisms [9]. However, the molecular mechanisms underlying this potential alleviation remain poorly understood.

Cd stress impacts multiple stages of soybean development. At the germination stage, elevated Cd levels reduce the germination rate and delay seedling emergence by interfering with enzymatic activity and hormonal balance [10]. At the vegetative growth stage, Cd inhibited root and shoot development, impaired root architecture by reducing elongation and lateral root formation, and decreased shoot height, leaf area, and biomass accumulation. These effects were partially attributed to Cd-induced disruption of chlorophyll synthesis, photosystem activity, and stomatal conductance, which in turn hinders carbon fixation and energy production [11,12]. At the reproductive stage, Cd stress led to abnormal flower development, poor pollination efficiency, and reduced seed set, ultimately lowering soybean yield and quality [13,14].

Furthermore, the toxic effects of Cd were passed on to subsequent generations through contaminated seeds, affecting their viability and growth potential [11].

At the physiological level, Cd stress activated the plant antioxidant defense system to counteract the accumulation of reactive oxygen species (ROS) generated by Cd exposure. Antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), along with non-enzymatic antioxidants like ascorbic acid and glutathione, played critical roles in scavenging ROS and protecting plant cells from oxidative damage [15]. Cd suppresses the expression of genes critical for antioxidant enzyme production. For example, Cd disrupts the Nrf2/ARE signaling pathway, a redox-sensitive transcriptional regulator responsible for activating genes like catalase CAT and SOD. Cd also disrupted ion homeostasis in soybeans, competing with essential ions such as calcium, magnesium, and iron, leading to nutrient imbalances that further impaired plant growth and function [16].

Phosphate is vital for plant growth, particularly under stress conditions. It enhanced root development, nutrient uptake, and water absorption, which were essential for mitigating the negative effects of Cd toxicity [17]. Phosphorus can react with Cd in the soil to form insoluble Cd-phosphate salts (e.g., $Cd_3(PO_4)_2$), thereby immobilizing Cd and reducing its bioavailability. For instance, in mangrove seedlings, exogenous phosphorus application decreased Cd uptake by promoting Cd-phosphate precipitation in the rhizosphere, which restricted Cd translocation to plant tissues [18]. Phosphate deficiency exacerbated Cd stress by impairing root structure and nutrient acquisition [19]. Calcium (Ca) application, often coupled with phosphorus, enhances Cd detoxification by competing with Cd for binding sites on root surfaces and in soil colloids. For example, $CaCl_2$ pre-treatment in faba bean seedlings reduced Cd accumulation and oxidative damage by stabilizing membrane integrity and promoting antioxidant defenses, which may involve Ca-P-mediated Cd immobilization [20]. Conversely, phosphate application has been shown to improve root biomass and enhance root penetration, enabling soybeans to access nutrients more efficiently, thus alleviating the impact of Cd stress [21]. Moreover, phosphate stimulates antioxidant enzyme activity, helping to detoxify ROS and reducing oxidative damage [22]. Phosphate also modulated the expression of genes involved in stress response pathways, including those related to root development, metal transport, and antioxidant defense, further enhancing Cd tolerance in plants [23–25].

Recent advances in the multi-omics approach encompassing transcriptomics and metabolomics have provided powerful tools to decipher the complex molecular responses

of plants to environmental stresses [26–28]. These integrative methods are particularly valuable in elucidating the genetic, biochemical, and physiological pathways involved in stress tolerance. Understanding how phosphate modulates the response of soybeans to Cd stress is essential for developing strategies to enhance crop resilience and ensure sustainable soybean production. The integration of transcriptomic and metabolomic data provides a comprehensive understanding of how phosphate affects soybean's response to Cd stress. Recent research has highlighted the potential of multi-omics approaches to uncover molecular insights into plant stress tolerance mechanisms [29]. This integrated analysis revealed novel pathways and biomarkers for enhancing soybean resilience to heavy metal stress.

The primary objective of this study was to investigate the impact of cadmium stress on soybeans and the potential ameliorative effects of phosphorus supplementation on this stress, focusing on the physiological, transcriptomic, and metabolic responses of the plants. Given that Cd is a non-essential heavy metal with toxic effects on plant growth, it is critical to understand how plants respond to Cd exposure and how nutrients like phosphorus mitigate these effects. The results from this study provided valuable insights into the molecular mechanisms of Cd toxicity and the role of phosphorus in enhancing soybean tolerance to this stress.

2. Materials and Methods

2.1. Plant Materials

This experiment was conducted in 2022 at the rain-shelter greenhouse of the experiment field at Shenyang Agricultural University (41°82′ N, 123°57′ E). The experiment used the soybean variety LiaoDou 10, which was selected in 2019 and 2020 for its sensitivity to both low phosphorus and cadmium stress, as the test material. The aim was to provide theoretical support for the breeding of high-quality, high-yield soybean varieties that are resistant to low phosphorus and cadmium, as well as for the high-yield, safe cultivation of cadmium-contaminated soils with phosphorus-sensitive soybean variety. The experimental materials were provided by the Soybean Research Institute of Shenyang Agricultural University. The experiment was conducted using sand culture, with black plastic pots used as the containers. Each pot has an inner diameter of 16 cm, a height of 25 cm, and three small drainage holes (0.3 cm in diameter) at the bottom. Each pot was filled with 5 kg of washed quartz sand.

2.2. Cadmium and Phosphorus Treatments

Two experiments were conducted in this study; in Experiment I, with the normal phosphorus concentration of 0.5 mmol/L, which was set as the control group, as previously reported by Yang et al. [30] and Zhao et al. [31]. Three cadmium stress treatments with concentrations of 0, 0.25, and 0.5 mg/L were set up, denoted as P2Cd0, P2Cd1, and P2Cd2, respectively. In Experiment II, a cadmium stress level of 0.25 mg/L was maintained, and three phosphorus treatments with concentrations of 0, 0.05, and 0.5 mmol/L were established, represented by P0Cd1, P1Cd1, and P2Cd1, respectively. The P2Cd0 treatment was conducted as the control group in this study. The growing soybean plants were maintained under a temperature regime of 28–25 °C (day:night) and a photo-period of 14:10 h (light:dark) cycle.

The components of the nutrient solution were minor and modified according to Lin et al. [32]. The following was obtained: 3.6 mmol/L $CaSO_4 \cdot 2H_2O$, 2 mmol/L KNO_3, 18 μmol/L $FeSO_4 \cdot 7H_2O$, 18.9 μmol/L KCl, 9.3 μmol/L H_3BO_3, 0.9 μmol/L $MnSO_4 \cdot H_2O$, 0.9 μmol/L $ZnSO_4 \cdot 7H_2O$, 0.18 μmol/L $CuSO_4 \cdot 5H_2O$, 0.18 μmol/L $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 250 μmol/L $MgSO_4 \cdot 7H_2O$. The pH of the nutrient solution was adjusted to 6.0 with NaOH

or HCl. The phosphorus fertilizer added was potassium dihydrogen phosphate (KH_2PO_4). In the treatment, potassium ions (K^+) needed to be supplemented. For the treatment at a concentration of 0.05 mmol/L, 0.45 mmol/L of K^+ was added at 8:00 a.m., and distilled water was used for irrigation at 4:00 p.m. with 500 mL of each applied daily. The cadmium and phosphorus treatments were implemented from the vegetative cotyledons (VC) stage to the second trifoliolate stage (V2). Leaf samples were collected at the V2 stage.

2.3. Phenotyping and Physiological Measurement

For each experimental treatment, three replicates were set up for the measurement of plant height and stem diameter at the V2 stage, which was 14 days after the treatment. The determination of Cd content was carried out in accordance with the method described by Gill et al. [33]. Briefly, the leaf samples were dried at 80 °C for 6 h until a constant weight was achieved. Subsequently, they were ground into a fine powder and digested with a mixture of HNO_3 and $HClO_4$ (in a volume ratio of 3:1). The digested mixtures were then dissolved in deionized water and filtered through a 0.22 μm filter membrane. The Cd concentrations were measured by inductively coupled plasma—mass spectrometry (ICP-MS) [9].

On a sunny day, between 9:00 and 11:00 in the morning, physiological measurements were conducted at the trifoliolate (V2) stage of the soybean plants. The photosynthetic rate (Pn), and transpiration rate (Tr) were measured using a portable photosynthesis measuring instrument LI-6800 (LI-COR, Lincoln, NE, USA).

2.4. Determination of SOD, POD, and CAT Activity

Fourteen days after each treatment, three replicates were set up for the determination of SOD, POD, and CAT activity. Then, 0.2 g of leaf tissue was weighed and ground into homogenate in a pre-cooled mortar with 4 mL of pre-cooled phosphate buffer (PBS, pH 7.8). The homogenate was then transferred to a centrifuge tube and centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant was collected as the crude enzyme solution. Then, the activity of superoxide dismutase (SOD) was analyzed by applying the nitrogen blue tetrazolium method [34]. The peroxidase (POD) activity was measured using the guaiacol method, while the catalase (CAT) activity was detected through potassium permanganate titration [35,36].

2.5. Metabolomics Analysis

A total of five treatments, namely P0Cd1, P1Cd1, P2Cd1, P2Cd0, and P2Cd2, with three replicates for each treatment, were collected for metabolite analysis. Fresh leaf tissues were ground into powder using a grinder MM 400 (Retsch, Haan, Germany). For each sample, 50 milligrams were taken and mixed with 1200 μL of a pre-chilled (at −20 °C) 70% methanolic aqueous internal standard extract. The samples were then vortexed for 30 s at 30-min intervals, for a total of six vortexing cycles. Following this, the samples were centrifuged at 12,000× *g* for 3 min. After the first centrifugation, the supernatant was carefully transferred and subjected to a second centrifugation step at 12,000× *g* for 3 min at 4 °C. Subsequently, the supernatant was filtered through an MF-Millipore membrane with a pore size of 0.22 μm. The filtered sample was then transferred into an injection vial and stored for subsequent UPLC-MS/MS analysis.

The sample extracts were analyzed using a combined UPLC–ESI–MS/MS system. The UPLC component was an ExionLC™ AD (https://sciex.com.cn/, accessed on 2 December 2022), and the tandem mass spectrometry system was also sourced from the same provider (https://sciex.com.cn/, accessed on 2 December 2022). An Agilent SB—C18 column (particle size: 1.8 μm; dimensions: 2.1 mm × 100 mm) was employed for separation. The mobile phase consisted of two solvents. Solvent A was pure water containing 0.1% formic

acid, and solvent B was acetonitrile with 0.1% formic acid. The gradient elution program was as follows: the program started with a composition of 95% solvent A and 5% solvent B, which was maintained for 9 min. Over the next period, a linear gradient was applied to change the composition to 5% solvent A and 95% solvent B. This 5% A and 95% B composition was held constant for 1 min. Subsequently, the composition was adjusted back to 95% A and 5% B within 1.1 min and then maintained at this ratio for 2.9 min. The flow rate of the mobile phase was set at 0.35 mL/min. The column oven was maintained at a temperature of 40 °C. A sample injection volume of 2 μL was used. The effluent from the UPLC column was then alternately connected to an ESI-triple quadrupole-linear ion trap (QTRAP)-MS for mass spectrometric analysis.

The ESI source was operated at a temperature of 550 °C. In positive ion mode, the ion spray voltage was set at 5500 V, while in negative ion mode, it was set at −4500 V. The pressures of ion source gas I (GSI), gas II (GSII), and curtain gas (CUR) were set at 50 psi, 60 psi, and 25 psi, respectively. The collision-activated dissociation (CAD) was set to the high setting. QQQ scans were acquired as MRM experiments with the collision gas (nitrogen) set to the medium setting for the multiple reaction monitoring (MRM) experiments. The declustering potential (DP) and collision energy (CE) for each individual MRM transition were further optimized. A specific set of MRM transitions was monitored for each time period, corresponding to the metabolites eluted during that particular time frame.

2.6. RNA Isolation and Transcriptomics Sequencing

Total RNA was extracted from 100 mg leaf samples of five treatments, including P0Cd1, P1Cd1, P2Cd1, P2Cd0, and P2Cd2, with three replicates for each treatment, using TRIzol™ reagent (Invitrogen, Carlsbad, CA, USA). Isolated RNA samples were identified and accurately quantified using two specialized instruments. A Qubit fluorescence quantifier was employed to precisely measure the amount of RNA present. Additionally, a Qsep400 (Bioptic, Taipei, Taiwan) high-throughput bio-fragment analyzer was used to further characterize the RNA, likely in terms of fragment size and integrity. Sequencing libraries were prepared using the NEBNext UltraTM RNA Library Prep Kit for Illumina [New England Biolabs (NEB), Ipswich, MA, USA]. The entire library construction process was carried out strictly in accordance with the detailed instructions provided by the kit's manufacturer. During this procedure, index codes were incorporated into the libraries. These index codes serve a crucial role, as they enable the attribution of specific sequences to each individual sample during subsequent analysis. The sequencing of the constructed libraries was conducted on an Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA). This sequencing service was provided by Metware Biotechnology Inc. (Wuhan, China).

2.7. Preprocessing and De Novo Assembly

Data quality control was performed using fastp to remove reads containing adapters. Paired-end reads were discarded under the following two conditions: 1. When the number of 'N' nucleotides in any sequencing read exceeded 10% of the read length. 2. When any sequencing read contained low-quality bases (with a quality score, $Q \leq 20$) accounting for more than 50% of the read length. All subsequent analyses were based on the remaining clean reads. We used Bowtie2 version 2.2.3 to build the genome index. Then, we aligned the clean data to the reference genome using HISAT2 version 2.1.0. HTSeq version 0.6.0 was used to determine the read count for each gene in every sample. To estimate the gene expression levels in each sample, we calculated the fragments per kilobase million mapped reads (FPKM). DESeq2 was employed for differential gene expression analysis. Genes were identified as differentially expressed genes (DEGs) if they met the following criteria: a

q-value ≤ 0.05 and an absolute value of log2_ratio ≥ 1. We used the TBtools (Toolbox for Biologists) v1.098765 [37] to conduct Venn diagram and heat map analyses based on the RNA sequencing data from this study.

2.8. Quantification of Gene Expression Levels and Differential Expression Analysis

The reference genome of soybean and its corresponding annotation files were downloaded from the website, http://ftp.ensemblgenomes.org, accessed on 15 February 2023 [38]. We used HISAT to create an index for the reference genome. Subsequently, the clean reads were aligned to this indexed reference genome. To quantify gene expression levels, we employed feature counts to calculate gene alignment statistics. Based on the gene length, we calculated the Fragments Per Kilobase Million (FPKM) values for each gene. To identify differentially expressed genes between two groups, we utilized DESeq2. The Benjamini and Hochberg correction was applied to the *p*-values obtained from the analysis. The corrected *p*-values and the log2 fold change were set as the thresholds for determining significant differential expression.

2.9. Quantitative Real-Time PCR (qRT-PCR)

cDNA synthesis was carried out using the PrimeScript™ RT Reagent Kit (TaKaRa, Beijing, China) following the manufacturer's detailed instructions. Primer 3.0 was utilized for primer designing of quantitative real-time polymerase chain reaction (qRT-PCR). The specific information regarding these primers is provided in Supplementary Table S1. The qRT-PCR was performed using TB Green® Premix Ex Taq™ II FAST qPCR (TaKaRa, Dalian, China) on the CFX96™(Bio-Rad, Berkeley, CA, USA). All qRT-PCR experiments were replicated three times to ensure the reliability of the results. The relative expression analysis was conducted using the $2^{-\Delta\Delta}$CT method [39]. The reference gene used in this calculation was GmEF1a. The samples treated with distilled water at each time point served as the control group.

2.10. Statistical Analysis

Data analyses were conducted using the SPSS 22.0 statistical package (SPSS, Chicago, IL, USA). An Analysis of Variance (ANOVA) was performed to evaluate the effects of different treatments. If a significant treatment effect was detected ($p < 0.05$), Duncan's Multiple Range Test was applied to identify significant differences among the treatments at a significance level of $p < 0.05$.

3. Results

3.1. Physiological Measurement

In this study, the results demonstrated that the plant height, net photosynthetic rate, and transpiration rate were significantly suppressed in both the cadmium-treated groups (0.25 mg/L and 0.5 mg/L) compared to the cadmium-free group (Figure 1). Phosphorus treatment was found to significantly reduce the absorption of cadmium by the aboveground parts and roots (Figure 1).

The activities of antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in the first trifoliate leaf at the V2 stage were measured under different cadmium (Cd) and phosphorus (P) treatments. Compared to the control (P2Cd0), the SOD, POD, and CAT activities significantly decreased in the Cd treatment groups (0.25 mg/L and 0.5 mg/L) (Figure 2). The phosphorus supplementation treatment was able to enhance the activities of SOD, POD, and CAT under cadmium treatment at a concentration of 0.25 mg/L.

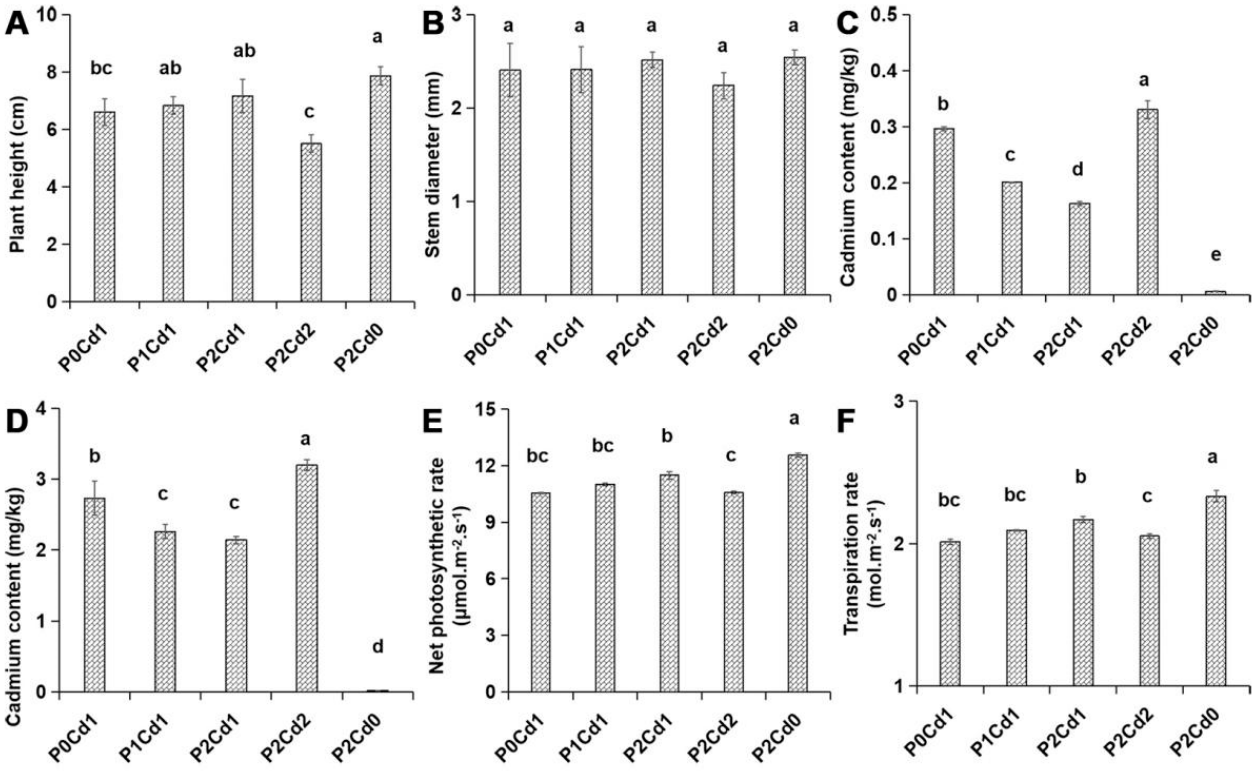

Figure 1. Plant height, stem diameter, cadmium content, net photosynthetic rate, and transpiration rate measurements at the second trifoliolate stage. P0Cd1 (0 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment), P1Cd1 (0.05 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment), P2Cd1 (0.5 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment), P2Cd2 (0.5 mmol/L phosphorus treatment and 0.5 mg/L cadmium treatment), and P2Cd0 (0.5 mmol/L phosphorus treatment and 0 mg/L cadmium treatment). (**A**) Plant height; (**B**) stem diameter; (**C**) cadmium content of above ground; (**D**) cadmium content of root; (**E**) net photosynthetic rate; (**F**) transpiration rate. Different small letters on vertical bars indicate significant differences between means at the $p < 0.05$ level.

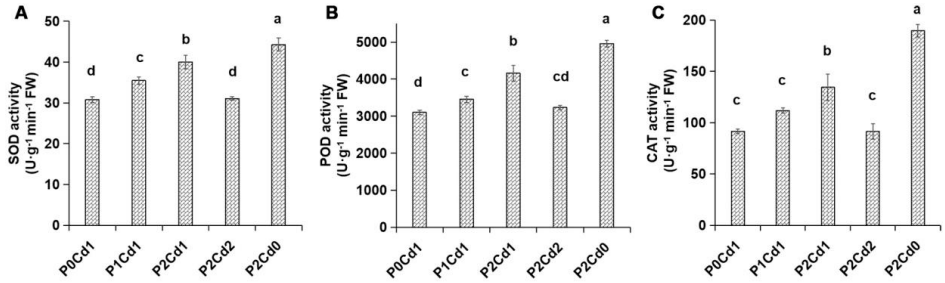

Figure 2. Effect of phosphorus treatments on the activities of antioxidant enzymes under cadmium stress at the second trifoliolate stage. P0Cd1 (0 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment), P1Cd1 (0.05 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment), P2Cd1 (0.5 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment), P2Cd2 (0.5 mmol/L phosphorus treatment and 0.5 mg/L cadmium treatment), and P2Cd0 (0.5 mmol/L phosphorus treatment and 0 mg/L cadmium treatment). (**A**) Superoxide dismutase (SOD) activity; (**B**) peroxidase (POD) activity; (**C**) catalase (CAT) activity. Different small letters on vertical bars indicate significant differences between means at the $p < 0.05$ level.

3.2. Transcriptome and Metabolic Analyses

Principal component analysis (PCA) was carried out on the transcriptome and metabolome independently to visually illustrate whether disparities existed among the sample groups in both omics domains (Figure 3A,B). The PCA analysis revealed that

the three replicates within each treatment group exhibited a relatively robust correlation, whereas conspicuous differences were observable among the distinct treatment groups. Notably, in the transcriptome, the discrepancies between the cadmium-treated group and the cadmium-free control group were highly pronounced. More than 13 metabolomes were detected in this study, with the majority classified as flavonoids (22.55%), phenolic acids (13.94%), lipids (11.83%), and amino acids and derivatives (10.22%) (Figure 3C).

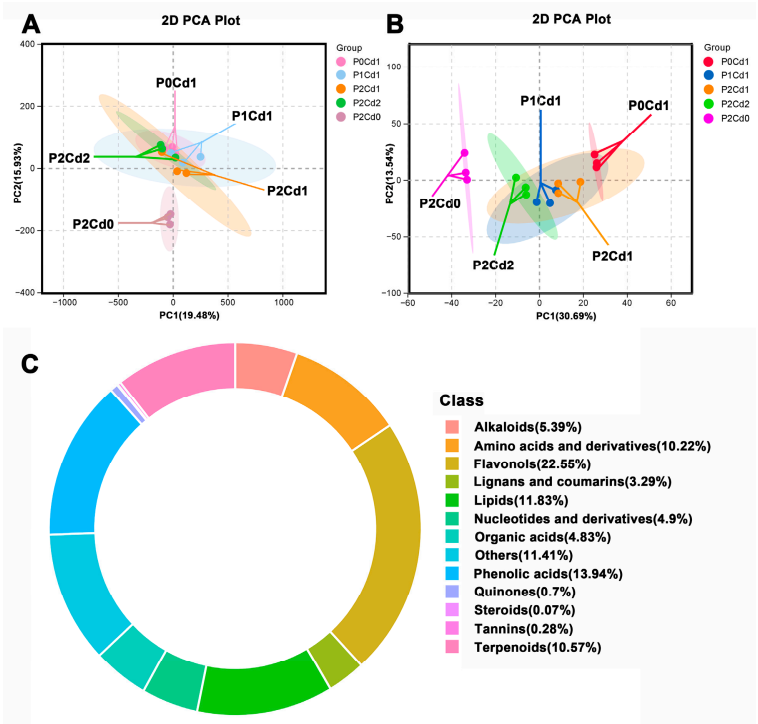

Figure 3. Principal component analysis (PCA) of the transcriptome and metabolome and classification of the metabolome in this study. (**A**) Principal component analysis of transcriptome; (**B**) principal component analysis of metabolome; (**C**) metabolome classification.

3.3. Differently Expressed Genes (DEGs) and Differential Metabolomes Analysis

In this study, average clean reads of 49,435,860, 48,825,590, 46,156,759, 44,731,373, and 54,768,364 were generated from P0Cd1, P1Cd1, P2Cd1, P2Cd2, and P2Cd0, respectively. A total of 931, 830, 380, 2465, 3375, and 134 DEGs were identified in the comparisons of P1Cd1_vs_P0Cd1, P2Cd1_vs_P0Cd1, P2Cd1_vs_P1Cd1, P2Cd0_vs_P2Cd1, P2Cd0_vs_P2Cd2, and P2Cd2_vs_P2Cd1 in response to different cadmium and phosphorus treatments. The highest number of 3375 DEGs was detected in the comparison between the cadmium-treated group (0.5 mg/L) and the cadmium-free control group, while the lowest number of 134 DEGs was observed in the comparison of different cadmium treatments (0.25 mg/L and 0.5 mg/L) under the same phosphorus treatment with a concentration of 0.5 mmol/L.

To explore the interactive effects of Cd and phosphate on soybeans through transcriptomic and metabolic analyses, we concentrated on two key aspects. First, under a specific cadmium-stress condition, we analyzed how treatments with different phosphorus concentrations (0.05 mmol/L and 0.5 mmol/L) alleviated cadmium stress. Specifically, we compared the gene expression and metabolite profiles between the following pairs: P1Cd1_vs_P0Cd1 and P2Cd1_vs_P0Cd1. In these comparisons, a total of 93 differentially expressed genes (DEGs) and 38 differential metabolites were identified (Figure 4). This allowed us to understand how different levels of phosphorus supplementation affected the soybean's response to a fixed cadmium concentration.

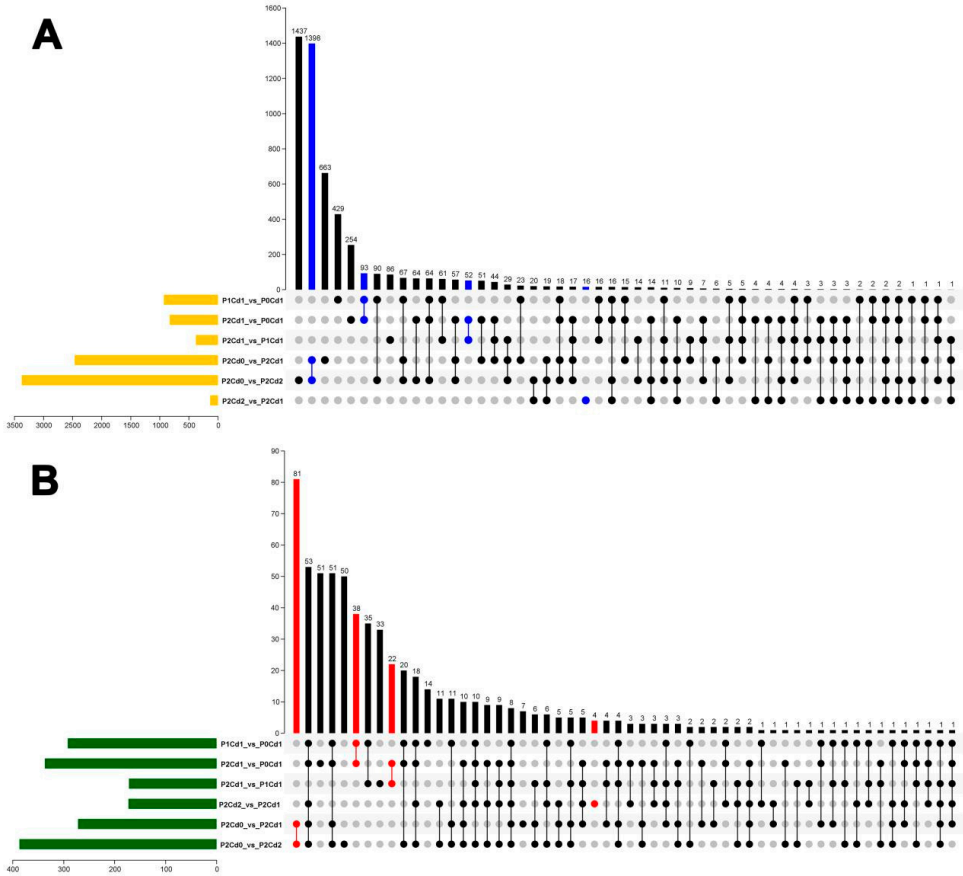

Figure 4. Venn diagram of differentially expressed gene number and differential metabolite number in the comparison pairs of P1Cd1 vs. P0Cd1, P2Cd1 vs. P0Cd1, P2Cd1 vs. P1Cd1, P2Cd0 vs. P2Cd1, P2Cd0 vs. P2Cd2, and P2Cd2 vs. P2Cd1. P0Cd1 (0 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment), P1Cd1 (0.05 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment), P2Cd1 (0.5 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment), P2Cd2 (0.5 mmol/L phosphorus treatment and 0.5 mg/L cadmium treatment), and P2Cd0 (0.5 mmol/L phosphorus treatment and 0 mg/L cadmium treatment). (**A**) Transcriptome analysis; (**B**) metabolome analysis.

Second, under a normal phosphorus concentration of 0.5 mmol/L, we investigated the impacts of different cadmium treatments (0.25 mg/L and 0.5 mg/L) on soybeans. We compared the gene expression and metabolite profiles in the pairs: P2Cd0_vs_P2Cd1 and P2Cd0_vs_P2Cd2. In these cases, 1398 DEGs and 81 differential metabolites were shared (Figure 4). This analysis helped us to determine how varying cadmium levels influenced the soybean's physiological state when phosphorus was at a constant level. The number of differential metabolomes detected from the phosphorus and cadmium treatments ranged from 172 to 473, providing a comprehensive view of the metabolic changes occurring in soybeans under different Cd–P treatment combinations.

Under cadmium stress with a concentration of 0.25 mg/L, phosphorus treatment led to the induction of 31 up-regulated genes and 54 down-regulated genes (Figure 5A). The up-regulated genes encompassed Calvin cycle protein CP12-1 and CP12-2, MLP-like protein, BTB/POZ and TAZ domain-containing protein, 3-hexulose-6-phosphate isomerase, as well as DNA-binding domain-containing protein belonging to the AP2/ERF-ERF transcript factor family, among others. Regarding the down-regulated genes, they were involved in the *bHLH*, *MYB*, *HB-HD-ZIP*, and *B3* transcript factor families and included stem-specific protein TSJT1, filament-like plant protein, receptor-like protein EIX2, and putative lipid-transfer protein DIR1, among others. The metabolome data indicated

that 11 and 27 metabolites were up-regulated and down-regulated, respectively, in both P1Cd1_vs_P0Cd1 and P2Cd1_vs_P0Cd1 comparisons (Figure 5B). A total of 38 differential metabolites were categorized into lipids (14 compounds), phenolic acids (9 compounds), flavonoids (5 compounds), and others (Figure 6A). All but one of the top 10 up-regulated metabolites were identified as phenolic acids, with MWS2066 being identified as triterpene saponin (Figure 6B). Meanwhile, the top 10 down-regulated metabolites were identified as coumarins, free fatty acids, amino acids and their derivatives, flavonols, isoflavones, and other flavonols.

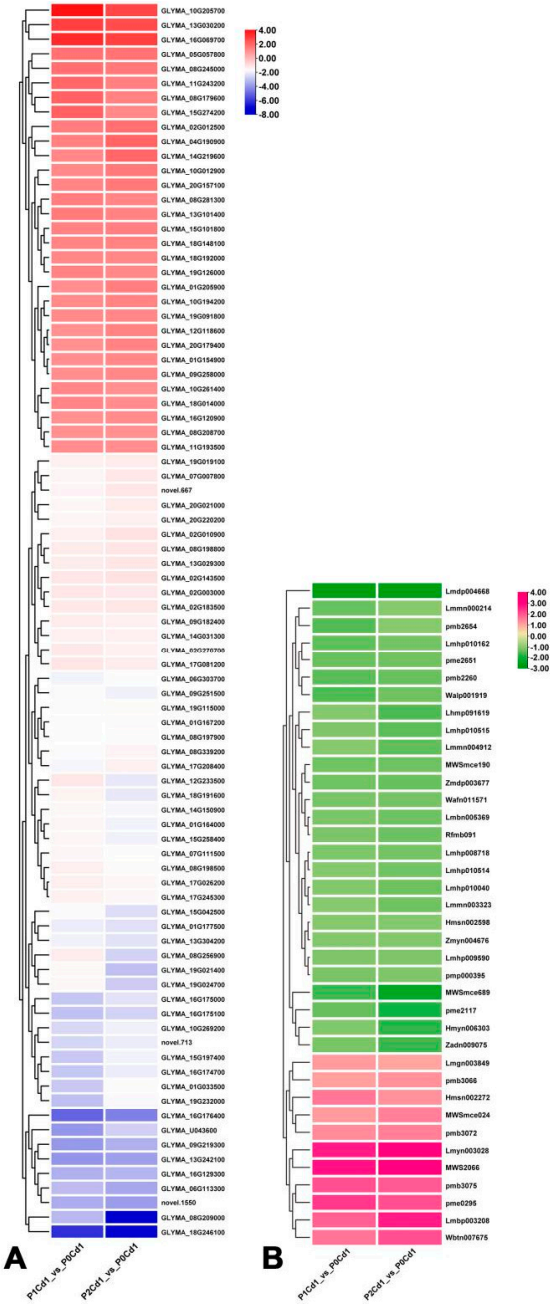

Figure 5. Heatmap of differentially expressed genes and differential metabolites shared between P1Cd1_vs_P0Cd1 and P2Cd1_vs_P0Cd1. P0Cd1 (0 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment), P1Cd1 (0.05 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment), and P2Cd1 (0.5 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment). (**A**) Transcriptome analysis; (**B**) metabolome analysis.

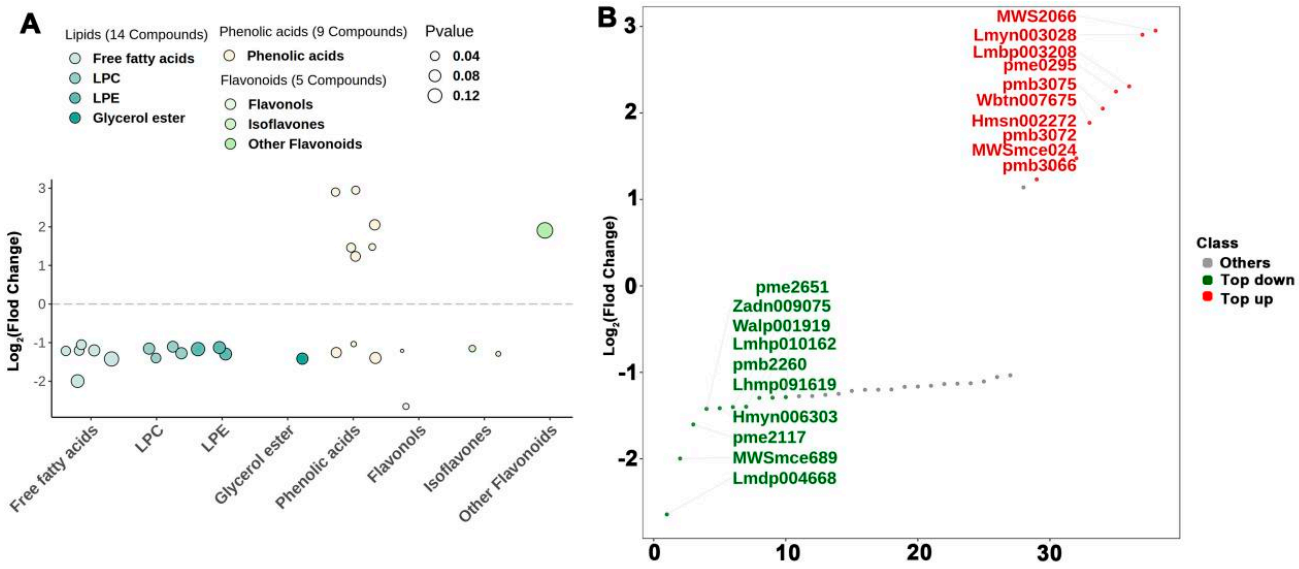

Figure 6. Classification and dynamic distribution of differential metabolites shared between P1Cd1_vs_P0Cd1 and P2Cd1_vs_P0Cd1. P0Cd1 (0 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment), P1Cd1 (0.05 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment), and P2Cd1 (0.5 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment). (**A**) Classification of differential metabolites; (**B**) dynamic distribution of differential metabolites.

Under the same phosphorus treatment, with a concentration of 0.5 mmol/L, 1398 differentially expressed genes (DEGs) were detected between the different cadmium-treated groups (0.25 mg/L and 0.5 mg/L) and the cadmium-free control group. The heat map of 63 DEGs with a Log_2 fold change (Log_2FC) greater than 4.0 or less than −4.0, as well as 81 differential metabolites shared by P2Cd0_vs_P2Cd1 and P2Cd0_vs_P2Cd2, was plotted (Figure 7). The up-regulated genes comprised ruBisCO-associated protein, protein SODIUM POTASSIUM ROOT DEFECTIVE, chlorophyll a-b binding protein, organ-specific protein, aldehyde oxidase GLOX1, isoleucine N-monooxygenase, long-chain-alcohol oxidase *FAO4A*, ethylene-responsive transcription factor *ERF027*, and transcription factor *MYB30* isoform X1, among others. The down-regulated genes were involved in isoflavone 2′-hydroxylase, flavonoid 6-hydroxylase, chalcone reductase, short-chain dehydrogenase, putative branched-chain-amino-acid aminotransferase, zinc finger protein, *bZIP* transcription factor *bZIP72*, zinc transporter 1-like precursor, Kunitz type trypsin inhibitor, ethylene-response factor, ethylene-responsive transcription factor *ERF098*, and hypothetical protein.

The differential metabolites data demonstrated that 9 and 72 compounds were up-regulated and down-regulated, respectively, in both the P2Cd0_vs_P2Cd1 and P2Cd0_vs_P2Cd2 comparisons. Of the 81 differential metabolites, 31 compounds were classified as flavonoids, 10 compounds as phenolic acids, and 8 compounds as alkaloids (Figure 8). The predominant segment of the top up-regulated metabolites was identified as phenolic acids, while the predominant down-regulated metabolites were identified as flavonoids.

A total of 173 differential metabolites were identified through the comparison of P2Cd2 and P2Cd1 treatments (Figure 9A). Sixty-seven (38.7%) of the compounds were classified as flavonoids and 27 (15.6%) as terpenoids. The top 10 up-regulated metabolites were identified as flavonoids, terpenoids, alkaloids, and organic acids (Figure 9B). However, the top 10 down-regulated metabolites were identified as terpenoids, phenolic acids, lignans and coumarins, amino acids and their derivatives, and alkaloids.

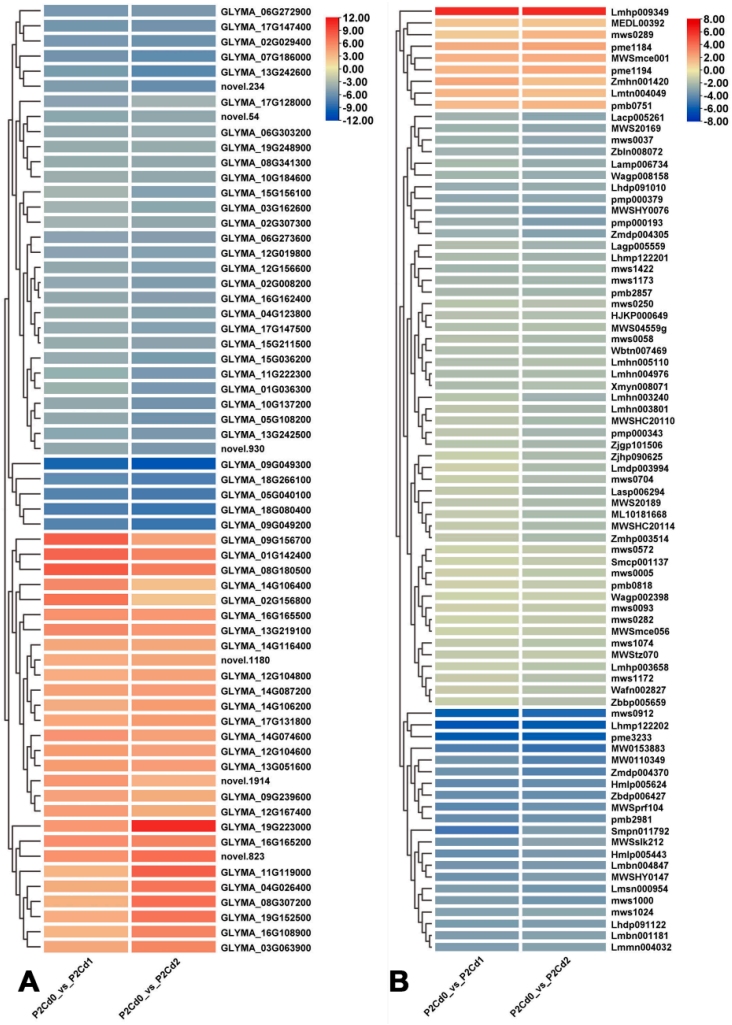

Figure 7. Heat map of differentially expressed genes and differential metabolites shared between P2Cd0_vs_P2Cd1 and P2Cd0_vs_P2Cd2. P2Cd0 (0.5 mmol/L phosphorus treatment and 0 mg/L cadmium treatment), P2Cd1 (0.5 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment), and P2Cd2 (0.5 mmol/L phosphorus treatment and 0.5 mg/L cadmium treatment). (**A**) Transcriptome; (**B**) metabolome.

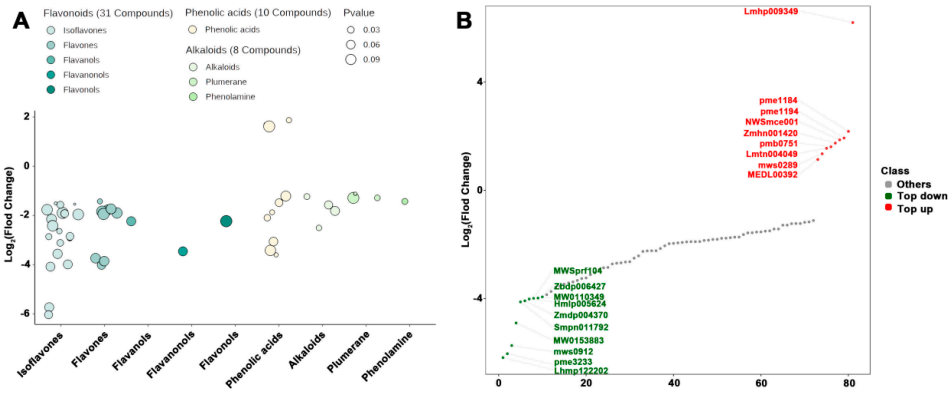

Figure 8. Classification and dynamic distribution of differential metabolites shared between P2Cd0_vs_P2Cd1 and P2Cd0_vs_P2Cd2. P2Cd0 (0.5 mmol/L phosphorus treatment and 0 mg/L cadmium treatment), P2Cd1 (0.5 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment), and P2Cd2 (0.5 mmol/L phosphorus treatment and 0.5 mg/L cadmium treatment). (**A**) Classification of differential metabolites; (**B**) dynamic distribution of differential metabolites.

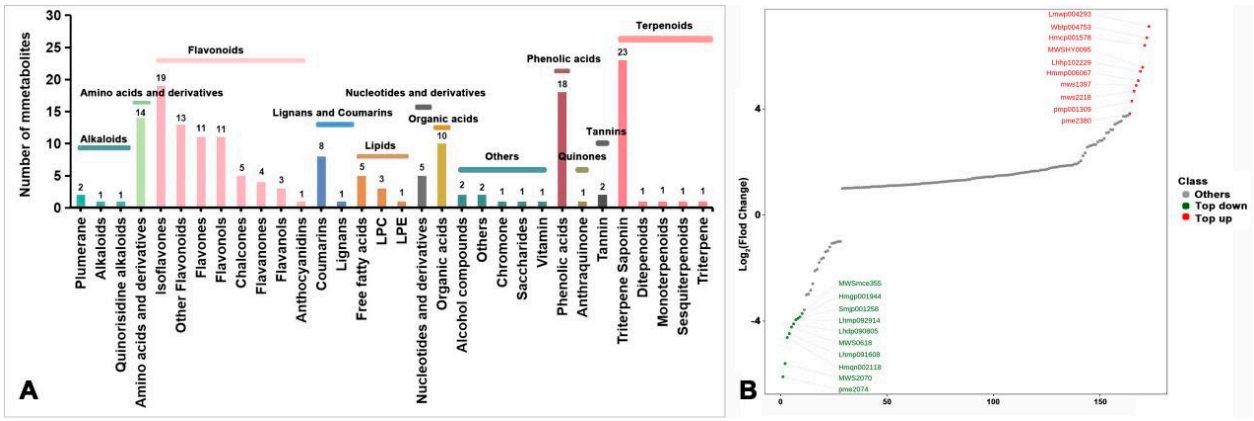

Figure 9. Classification and dynamic distribution of differential metabolites for P2Cd2 compared to P2Cd1. P2Cd1 (0.5 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment) and P2Cd2 (0.5 mmol/L phosphorus treatment and 0.5 mg/L cadmium treatment). (**A**) Classification of differential metabolites; (**B**) dynamic distribution of differential metabolites.

3.4. Verification of DEGs Involved in Different Cadmium and Phosphorus Treatments

Nine DEGs were randomly selected from the 1398 DEGs obtained from the comparisons of P2Cd0_vs_P2Cd1 and P2Cd0_vs_P2Cd2. These included the coding genes for Calvin cycle protein CP12-1 (*GLYMA_02G012500*) and CP12-2 (*GLYMA_10G012900*), which were subjected to quantitative real-time PCR analysis. The primers utilized for qRT-PCR were detailed in Supplementary Table S1. The qRT-PCR results exhibited a similar expression pattern when compared to the RNA-Seq data (Figure 10).

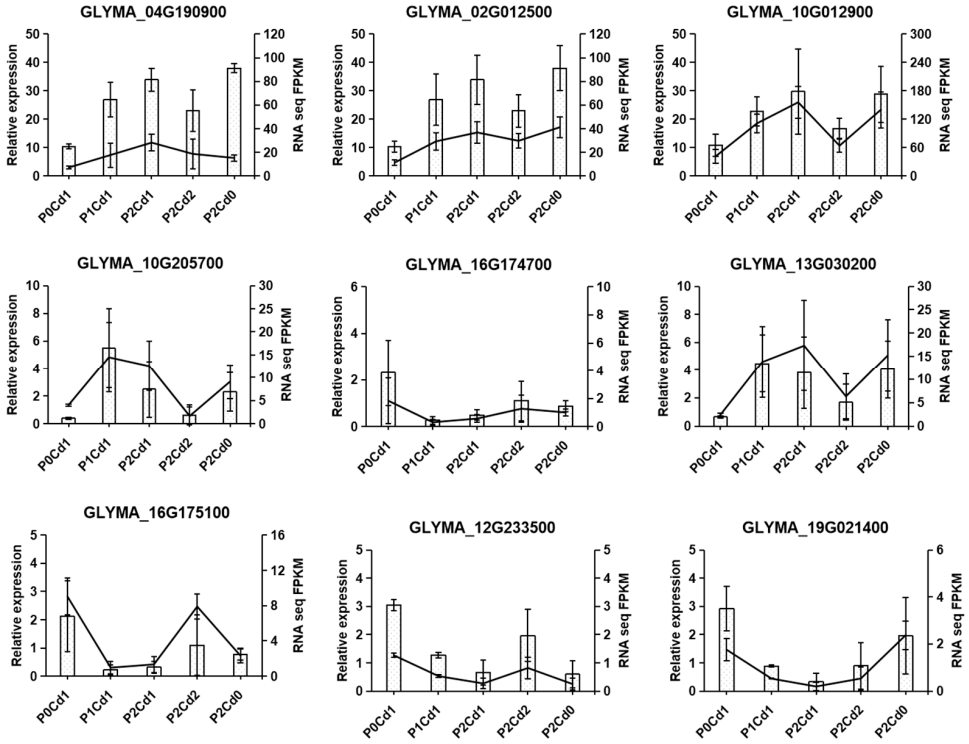

Figure 10. Relative expression of differentially expressed genes selected from P2Cd0_vs_P2Cd1 and P2Cd0_vs_P2Cd2. P2Cd0 (0.5 mmol/L phosphorus treatment and 0 mg/L cadmium treatment), P2Cd1 (0.5 mmol/L phosphorus treatment and 0.25 mg/L cadmium treatment), and P2Cd2 (0.5 mmol/L phosphorus treatment and 0.5 mg/L cadmium treatment). The bar chart represents the FPKM values from RNA sequencing data, while the line chart represents gene expression from qRT-PCR.

4. Discussion

4.1. Impact of Cadmium on Soybean Growth and Physiology

The results confirmed that Cd exposure significantly inhibited soybean growth, including reductions in plant height, photosynthetic rate, and transpiration rate, as previously reported in other studies [11,13]. The reduction in these physiological parameters was indicative of Cd-induced oxidative stress and the disruption of various physiological processes, such as photosynthesis and water uptake. Cadmium-induced inhibition of photosynthesis was likely due to the oxidative damage caused by the generation of reactive oxygen species (ROS) under stress conditions. This observation aligned with findings by Andresen et al. [40] and Shamsi et al. [41], who reported that cadmium exposure adversely affected photosynthetic efficiency and transpiration in soybeans.

Antioxidative enzymes, including SOD, POD, and CAT, played crucial roles in detoxifying ROS produced under metal stress. These results showed that the activities of these enzymes were significantly reduced in the cadmium-treated groups compared to the cadmium-free control group (Figure 2). This suggested that cadmium exposure disrupted the antioxidant defense system, leading to an accumulation of ROS that damaged cellular components. This result was consistent with previous reports showing that Cd stress elevated ROS production in plants [42,43], leading to cellular damage if not adequately managed by antioxidant defense systems.

Interestingly, this study observed that the application of phosphorus ameliorated the negative effects of Cd on soybean plants. Phosphorus treatment reduced Cd accumulation in both the roots and above-ground parts of the plants. This result aligned with earlier studies suggesting that phosphorus reduced Cd uptake by competitive inhibition at root surfaces [44,45]. Furthermore, phosphorus supplementation enhanced the activities of SOD, POD, and CAT under cadmium stress, particularly at the lower cadmium concentration (0.25 mg/L). This finding suggested that phosphorus acted as an enhancer of the antioxidative defense system, helping the plant to better cope with cadmium-induced oxidative stress. Similar findings have been reported by Maqbool et al. [46], who observed that phosphorus modulated antioxidative enzyme activities under heavy metal stress, thereby promoting plant tolerance. These findings underscored the importance of phosphorus not only as a critical nutrient but also as a potential tool to mitigate the toxic effects of Cd in plants.

4.2. Transcriptomic and Metabolic Responses to Cadmium and Phosphorus Treatment

The transcriptome and metabolome data provided deeper insights into the molecular mechanisms driving the plant response to cadmium and phosphorus treatments. Principal component analysis (PCA) of both transcriptomic and metabolomic data revealed a clear separation between the different treatment groups (Figure 3). Notably, cadmium stress resulted in substantial changes in gene expression profiles, with a marked difference between the cadmium-treated and cadmium-free control groups. This highlighted the significant impact of cadmium on soybean metabolism and gene expression.

Through RNA sequencing, a total of 931–3375 differentially expressed genes (DEGs) were identified across the different comparisons, with the largest number of DEGs detected between the cadmium-treated and cadmium-free groups (Figure 4). Genes involved in stress responses, including transcription factors (e.g., *MYB*, *AP2/ERF-ERF*), signal transduction pathways, and biosynthetic processes, were among the most differentially expressed. The upregulation of genes related to photosynthesis (e.g., Calvin cycle proteins) and oxidative stress response (e.g., SOD, POD) suggested that cadmium induced both a protective and adaptive response in soybean plants.

Metabolomic analysis revealed significant shifts in metabolite profiles under different cadmium and phosphorus treatments. A total of 172–473 differential metabolites were detected, with flavonoids, phenolic acids, and terpenoids being the most abundant categories (Figures 6 and 8). These metabolites were known to play roles in plant defense against abiotic stress, including heavy metal stress. For example, phenolic acids were potent antioxidants that scavenged ROS, while flavonoids were involved in stabilizing cell membranes and chelating heavy metals.

In the comparison between phosphorus treatments under cadmium stress (P2Cd1 vs. P0Cd1), upregulation of phenolic acids and flavonoids was observed, which aligned with previous findings indicating that these compounds were often increased in response to metal stress. Similarly, terpenoids and alkaloids were upregulated in the high cadmium (0.5 mg/L) treatment, indicating that these metabolites contributed to the plant detoxification processes.

Phenolic acids were often involved in the plant defense response, likely playing a part in the neutralization of ROS generated during Cd exposure [47]. On the other hand, flavonoids, which were generally considered protective against oxidative stress, were down-regulated in response to higher Cd concentrations, potentially reflecting a shift in the plant's metabolic priorities to focus on other protective pathways.

The combination of transcriptomic and metabolomic data also highlighted the differential regulation of key stress-related pathways under varying levels of phosphorus and cadmium treatments. For example, genes associated with the ethylene signaling pathway (e.g., *ERF027* and *MYB30*) were differentially expressed under Cd stress, consistent with the known role of ethylene in mediating plant stress responses [48]. Additionally, the up-regulation of RuBisCO-associated protein suggested that phosphorus supplementation enhanced photosynthetic efficiency under Cd stress, which was crucial for maintaining plant growth.

4.3. Gene-Environment Interactions and Phosphorus's Role in Stress Tolerance

This study underscored the complex interplay between Cd stress and phosphorus availability in soybeans. Phosphorus, an essential macronutrient for energy metabolism and signaling, played a multi-faceted role in mitigating Cd toxicity. The up-regulation of genes involved in root development and nutrient transport, alongside the enhanced antioxidant response, suggested that phosphorus helps the plant to better tolerate Cd stress by facilitating nutrient uptake and improving cellular protection mechanisms. This was particularly important since Cd competed with essential ions such as calcium and magnesium, leading to a nutrient imbalance that further exacerbated stress [49].

Phosphorus's ability to regulate stress-responsive genes further supported its role in enhancing stress tolerance. Our analysis identified several genes involved in antioxidant defense, metal transport, and ion homeostasis that were up-regulated in response to phosphorus treatment. This included genes encoding *bHLH* and *MYB* transcription factors, which were known to regulate responses to abiotic stress [50,51]. By modulating the expression of these genes, phosphorus supplementation likely helps the plant to fine-tune its response to the oxidative and ionic stress induced by Cd. The interplay between Cd stress and P availability in soybeans highlights a critical gene-environment interaction where P acts as a modulator of plant resilience. The up-regulation of antioxidant-related genes (e.g., flavonoid biosynthesis enzymes) in soybean under P supplementation parallels findings in plants like horse gram (*Macrotyloma uniflorum*) and Bengal gram (*Cicer arietinum*), where heavy metals induce oxidative stress but are countered by enhanced antioxidant enzyme activity (e.g., superoxide dismutase, catalase) [49]. Notably, MYB and bHLH transcription

factors, critical for abiotic stress responses in soybean [52], are similarly implicated in arsenic (As) tolerance in rice (*Oryza sativa*) and lead (Pb) detoxification in *Arabidopsis* [53].

4.4. Differential Metabolites and Gene Expression Validation

The top up-regulated metabolites in the metabolomic analysis included phenolic acids, flavonoids, and terpenoids, which were consistent with the differential expression of key genes related to secondary metabolite biosynthesis, such as flavonoid 6-hydroxylase and isoflavone 2′-hydroxylase (Figure 7). The overlap between the differential metabolites and genes underscored the intricate relationship between gene expression and metabolic shifts in response to cadmium and phosphorus treatments. The accumulation of phenolic acids and flavonoids in Cd-stressed soybeans aligns with metabolomic responses observed in tea plants (*Camellia sinensis*) exposed to HMs, where secondary metabolites act as metal chelators and free radical scavengers [54]. For example, in *Hordeum vulgare*, phytochelatins, and flavonoids are induced under copper (Cu) and zinc (Zn) stress, reflecting a shared metabolic strategy to mitigate oxidative damage [54]. The overlap between gene expression (e.g., flavonoid 6-hydroxylase) and metabolite profiles in soybeans underscores the integration of genetic and biochemical pathways in stress adaptation.

To verify the findings from transcriptomic analysis, quantitative real-time PCR (qRT-PCR) was performed for nine DEGs selected from the high-confidence DEGs, including those involved in photosynthesis and stress responses. The qRT-PCR results confirmed the patterns observed in the RNA-Seq data, providing further validation of the observed gene expression changes (Figure 10).

5. Conclusions and Implications for Agricultural Practices

This study provides a detailed, multi-dimensional analysis of the effects of Cd stress and phosphorus supplementation on soybeans. Our findings not only confirmed that Cd exposure significantly affected soybean growth and physiology but also demonstrated that phosphorus supplementation alleviated many of the negative impacts associated with Cd toxicity. By integrating transcriptomic and metabolomic data, the study preliminarily revealed that phosphorus alleviated Cd-induced stress through mechanisms such as protecting photosynthetic pigments, reducing Cd absorption, and enhancing antioxidant enzyme activity.

These results have important implications for agricultural practices, particularly in regions where soil contamination with heavy metals like Cd is a concern. Phosphorus fertilization, beyond its traditional role in promoting plant growth, may offer a viable strategy for mitigating the adverse effects of heavy metal contamination in crops like soybeans. Future research should focus on further elucidating the molecular pathways regulated by phosphorus under Cd stress, as well as exploring other potential nutrient interventions to support crop resilience in contaminated environments.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15030637/s1, Table S1: Details information of the qRT-PCR primers used in this study.

Author Contributions: Conceptualization, X.Y.; Formal analysis, S.J., X.Z. and X.Y.; Investigation, S.J., X.Z. and J.H.; Resources, F.X.; Data curation, S.J. and X.Z.; Writing—original draft, S.J.; Writing—review and editing, J.H., X.Y. and F.X.; Visualization, J.H.; Supervision, F.X.; Project administration, F.X. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Liaoning Agriculture Major Project (2022JH1/10200002) and the grants [2021YFD1201102] from the National Key Research and Development Plan of the Ministry of Science and Technology.

Data Availability Statement: The datasets generated and analyzed during this study are available upon reasonable request from the corresponding authors. The RNA-Seq data of this study have been deposited at NCBI under BioProject ID PRJNA1212477.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Bojorquez, M.G.; Frias-Espericueta, D.; Voltolina, D. Removal of cadmium and lead by adapted strains of *Pseudomonas aeruginosa* and *Enterobacter cloacae*. *Rev. Int. Contam. Ambient.* **2016**, *32*, 407–412. [CrossRef]
2. Clemens, S.; Ma, J.F. Toxic heavy metal and metalloid accumulation in crop plants and foods. *Annu. Rev. Plant Biol.* **2016**, *67*, 489–512. [CrossRef]
3. Bashir, A.; Rizwan, M.; Ali, S.; Adrees, M.; Rehman, M.Z.U.; Qayyum, M.F. Effect of composted organic amendments and zinc oxide nanoparticles on growth and cadmium accumulation by wheat: A life cycle study. *Environ. Sci. Pollut. Res.* **2020**, *27*, 23926–23936. [CrossRef]
4. Rizwan, M.; Ali, S.; Adrees, M.; Rizvi, H.; Zia-ur-Rehman, M.; Hannan, F.; Qayyum, M.F.; Hafeez, F.; Ok, Y.S. Cadmium stress in rice: Toxic effects, tolerance mechanisms, and management: A critical review. *Environ. Sci. Pollut. Res.* **2016**, *23*, 17859–17879. [CrossRef]
5. Liu, L.; Shang, Y.K.; Li, L.; Chen, Y.H.; Qin, Z.Z.; Zhou, L.J.; Yuan, M.; Ding, C.B.; Liu, J.; Huang, Y.; et al. Cadmium stress in Dongying wild soybean seedlings: Growth, Cd accumulation, and photosynthesis. *Photosynthetica* **2018**, *56*, 1346–1352. [CrossRef]
6. Wang, P.; Chen, H.; Kopittke, P.M.; Zhao, F.-J. Cadmium contamination in agricultural soils of China and the impact on food safety. *Environ. Pollut.* **2019**, *249*, 1038–1048. [CrossRef]
7. Satheesh, V.; Tahir, A.; Li, J.; Lei, M. Plant phosphate nutrition: Sensing the stress. *Stress Biol.* **2022**, *2*, 16. [CrossRef]
8. Yoshitake, Y.; Yoshimoto, K. Intracellular phosphate recycling systems for survival during phosphate starvation in plants. *Front. Plant Sci.* **2023**, *13*, 1088211. [CrossRef]
9. Jia, H.; Hou, D.; O'Connor, D.; Pan, S.; Zhu, J.; Bolan, N.S.; Mulder, J. Exogenous phosphorus treatment facilitates chelation-mediated cadmium detoxification in perennial ryegrass (*Lolium perenne* L.). *J. Hazard. Mater.* **2020**, *389*, 121849. [CrossRef]
10. Lee, Y.; Jang, J.; Jeon, Y.; Kim, H.; Jang, G.; Yoon, Y. Assessing the effects of accumulated Cd(II) on seed germination and root development of Arabidopsis thaliana. *Appl. Biol. Chem.* **2021**, *64*, 87. [CrossRef]
11. Haider, F.U.; Liqun, C.; Coulter, J.A.; Cheema, S.A.; Wu, J.; Zhang, R.; Wenjun, M.; Farooq, M. Cadmium toxicity in plants: Impacts and remediation strategies. *Ecotoxicol. Environ. Saf.* **2021**, *211*, 111887. [CrossRef]
12. Parmar, P.; Kumari, N.; Sharma, V. Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress. *Bot. Stud.* **2013**, *54*, 45. [CrossRef]
13. Andresen, E.; Lyubenova, L.; Hubáček, T.; Bokhari, S.N.H.; Matoušková, Š.; Mijovilovich, A.; Rohovec, J.; Küpper, H. Chronic exposure of soybean plants to nanomolar cadmium reveals specific additional high-affinity targets of cadmium toxicity. *J. Exp. Bot.* **2020**, *71*, 1628–1644. [CrossRef]
14. Ali, M.; Muazu, L. A Review on the Effects of Cadmium Stress on Growth and Development of Plants. *South Asian Res. J. Biol. Appl. Biosci.* **2020**, *2*, 33–37. [CrossRef]
15. Lai, X.; Yang, X.; Rao, S.; Zhu, Z.; Cong, X.; Ye, J.; Zhang, W.; Liao, Y.; Cheng, S.; Xu, F. Advances in physiological mechanisms of selenium to improve heavy metal stress tolerance in plants. *Plant Biol.* **2022**, *24*, 913–919. [CrossRef]
16. Niu, L.; Li, C.; Wang, W.; Zhang, J.; Scali, M.; Li, W.; Liu, H.; Tai, F.; Hu, X.; Wu, X. Cadmium tolerance and hyperaccumulation in plants—A proteomic perspective of phytoremediation. *Ecotoxicol. Environ. Saf.* **2023**, *256*, 114882. [CrossRef]
17. Lynch, J.P. Root phenes for enhanced soil exploration and phosphorus acquisition: Tools for future crops. *Plant Physiol.* **2011**, *180*, 529–540. [CrossRef]
18. Zhang, M.; Jin, Z.; Zhang, X.; Wang, G.; Li, R.; Qu, J.; Jin, Y. Alleviation of Cd phytotoxicity and enhancement of rape seedling growth by plant growth–promoting bacterium *Enterobacter* sp. Zm-123. *Environ. Sci. Pollut. Res.* **2020**, *27*, 33192–33203. [CrossRef]
19. Khan, F.; Siddique, A.B.; Shabala, S.; Zhou, M.; Zhao, C. Phosphorus plays key roles in regulating plants' physiological responses to abiotic stresses. *Plants* **2023**, *12*, 2861. [CrossRef]
20. Nouairi, I.; Jalali, K.; Essid, S.; Zribi, K.; Mhadhbi, H. Alleviation of cadmium-induced genotoxicity and cytotoxicity by calcium chloride in faba bean (*Vicia faba* L. var. minor) roots. *Physiol. Mol. Biol. Plants* **2019**, *25*, 921–931. [CrossRef]
21. Grant, C.A. Influence of phosphate fertilizer on cadmium in agriculture soils and crops. *Pedologist* **2011**, *54*, 143–155.
22. Lennicke, C.; Cochemé, H.M. Redox metabolism: ROS as specific molecular regulators of cell signaling and function. *Mol. Cell* **2021**, *81*, 3691–3707. [CrossRef] [PubMed]
23. Cui, G.; Ai, S.; Chen, K.; Wang, X. Arbuscular mycorrhiza augments cadmium tolerance in soybean by altering accumulation and partitioning of nutrient elements, and related gene expression. *Ecotoxicol. Environ. Saf.* **2019**, *171*, 231–239. [CrossRef] [PubMed]

24. Wei, X.; Fu, Y.; Yu, R.; Wu, L.; Wu, Z.; Tian, P.; Li, S.; Yang, X.; Yang, M. Comprehensive sequence and expression profile analysis of the phosphate transporter gene family in soybean. *Sci. Rep.* **2022**, *12*, 20883. [CrossRef] [PubMed]
25. Wang, Q.; Wang, J.; Yang, Y.; Du, W.; Zhang, D.; Yu, D.; Cheng, H. A genome-wide expression profile analysis reveals active genes and pathways coping with phosphate starvation in soybean. *BMC Genom.* **2016**, *17*, 192. [CrossRef]
26. Hasin, Y.; Seldin, M.; Lusis, A. Multi-omics approaches to disease. *Genome Biol.* **2017**, *18*, 83. [CrossRef]
27. Maan, K.; Baghel, R.; Dhariwal, S.; Sharma, A.; Bakhshi, R.; Rana, P. Metabolomics and transcriptomics based multi-omics integration reveals radiation-induced altered pathway networking and underlying mechanism. *NPJ Syst. Biol. Appl.* **2023**, *9*, 42. [CrossRef]
28. Mersha, T.B. From Mendel to multi-omics: Shifting paradigms. *Eur. J. Hum. Genet.* **2024**, *32*, 139–142. [CrossRef]
29. Yan, Q.; Zhang, G.; Zhang, X.; Huang, L. A review of transcriptomics and metabolomics in plant quality and environmental response: From bibliometric analysis to science mapping and future trends. *Metabolites* **2024**, *14*, 272. [CrossRef]
30. Yang, A.; Kong, L.; Wang, H.; Yao, X.; Xie, F.; Wang, H.; Ao, X. Response of Soybean Root to Phosphorus Deficiency under Sucrose Feeding: Insight from Morphological and Metabolome Characterizations. *BioMed Res. Int.* **2020**, *2020*, 2148032. [CrossRef]
31. Zhao, H.; Yang, A.; Kong, L.; Xie, F.; Wang, H.; Ao, X. Proteome characterization of two contrasting soybean genotypes in response to different phosphorus treatments. *AoB Plants* **2021**, *13*, plab019. [CrossRef] [PubMed]
32. Lin, K.; Williams, D.V.; Zeng, M.; Ahmed, I.M.; Dai, H.; Cao, F.; Wu, F. Identification of low grain cadmium accumulation genotypes and its physiological mechanism in maize (*Zea mays* L.). *Environ. Sci. Pollut. Res.* **2022**, *29*, 20721–20730. [CrossRef]
33. Gill, S.S.; Khan, N.A.; Tuteja, N. Cadmium at high dose perturbs growth, photosynthesis, and nitrogen metabolism while at low dose it upregulates sulfur assimilation and antioxidant machinery in garden cress (*Lepidium sativum* L.). *Plant Sci.* **2012**, *182*, 112–120. [CrossRef] [PubMed]
34. Beyer Jr, W.F.; Fridovich, I. Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. *Anal. Biochem.* **1987**, *161*, 559–566. [CrossRef] [PubMed]
35. Aebi, H. Catalase in vitro. *Methods Enzymol.* **1984**, *105*, 121–126.
36. Beffa, R.; Martin, H.V.; Pilet, P.E. In vitro oxidation of indoleacetic acid by soluble auxin-oxidases and peroxidases from maize roots. *Plant Physiol.* **1990**, *94*, 485–491. [CrossRef]
37. Chen, C.; Chen, H.; Zhang, Y.; Thomas, H.R.; Frank, M.H.; He, Y.; Xia, R. TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. *Mol. Plant.* **2020**, *13*, 1194–1202. [CrossRef] [PubMed]
38. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **2001**, *25*, 402–408. [CrossRef]
39. Song, J.; Sun, Z.; Saud, S.; Fahad, S.; Nawaz, T. Exploring the deleterious effects of heavy metal cadmium on antioxidant defense and photosynthetic pathways in higher plants. *Plant Stress* **2024**, *15*, 100716. [CrossRef]
40. Shamsi, I.H.; Wei, K.; Jilani, G.; Zhang, G.P. Interactions of cadmium and aluminum toxicity in their effect on growth and physiological parameters in soybean. *J. Zhejiang Univ. Sci. B* **2007**, *8*, 181–188. [CrossRef]
41. Muradoglu, F.; Gundogdu, M.; Ercisli, S.; Encu, T.; Balta, F.; Jaafar, H.Z.; Zia-Ul-Haq, M. Cadmium toxicity affects chlorophyll a and b content, antioxidant enzyme activities and mineral nutrient accumulation in strawberry. *Biol. Res.* **2015**, *48*, 11. [CrossRef] [PubMed]
42. Jung, H.I.; Lee, B.R.; Chae, M.J.; Lee, E.J.; Lee, T.G.; Jung, G.B.; Kim, M.S.; Lee, J. Ascorbate-mediated modulation of cadmium stress responses: Reactive oxygen species and redox status in Brassica napus. *Front. Plant Sci.* **2020**, *11*, 586547. [CrossRef] [PubMed]
43. Yang, S.; Xie, J.; Li, Q. Oxidative response and antioxidative mechanism in germinating soybean seeds exposed to cadmium. *Int. J. Environ. Res. Public Health* **2012**, *9*, 2827–2838. [CrossRef]
44. Chen, Q.; Hu, Y.; Yang, L.; Zhu, B.; Luo, F. Phosphorus regulates the level of signaling molecules in rice to reduce cadmium toxicity. *Curr. Issues Mol. Biol.* **2022**, *44*, 4070–4086. [CrossRef] [PubMed]
45. Maqbool, A.; Rizwan, M.; Yasmeen, T.; Arif, M.S.; Hussain, A.; Mansha, A.; Ali, S.; Alshaya, H.; Okla, M.K. Phosphorus fertilizers enhance the phytoextraction of cadmium through *Solanum nigrum* L. *Plants* **2022**, *11*, 236. [CrossRef]
46. Chen, C.; Wu, Y.; Li, J.; Wang, X.; Zeng, Z.; Xu, J.; Liu, Y.; Feng, J.; Chen, H.; He, Y.; et al. TBtools-II: A "one for all, all for one" bioinformatics platform for biological big-data mining. *Mol. Plant* **2023**, *16*, 1733–1742. [CrossRef]
47. Anwar, A.; Wang, Y.; Chen, M.; Zhang, S.; Wang, J.; Feng, Y.; Xue, Y.; Zhao, M.; Su, W.; Chen, R.; et al. Zero-valent iron (nZVI) nanoparticles mediate SlERF1 expression to enhance cadmium stress tolerance in tomato. *J. Hazard. Mater.* **2024**, *468*, 133829. [CrossRef]
48. Qin, S.; Liu, H.; Nie, Z.; Rengel, Z.; Gao, W.; Li, C.; Zhao, P. Toxicity of cadmium and its competition with mineral nutrients for uptake by plants: A review. *Pedosphere* **2020**, *30*, 168–180. [CrossRef]
49. Li, J.; Han, G.; Sun, C.; Sui, N. Research advances of MYB transcription factors in plant stress resistance and breeding. *Plant Signal. Behav.* **2019**, *14*, 1613131. [CrossRef]

50. Guo, J.; Sun, B.; He, H.; Zhang, Y.; Tian, H.; Wang, B. Current understanding of bHLH transcription factors in plant abiotic stress tolerance. *Int. J. Mol. Sci.* **2021**, *22*, 4921. [CrossRef]
51. Dhalaria, R.; Kumar, D.; Kumar, H.; Nepovimova, E.; Kuča, K.; Torequl Islam, M.; Verma, R. Arbuscular Mycorrhizal Fungi as Potential Agents in Ameliorating Heavy Metal Stress in Plants. *Agronomy* **2020**, *10*, 815. [CrossRef]
52. Banerjee, S.; Agarwal, P.; Choudhury, S.R.; Roy, S. MYB4, a member of R2R3-subfamily of MYB transcription factor functions as a repressor of key genes involved in flavonoid biosynthesis and repair of UV-B induced DNA double strand breaks in Arabidopsis. *Plant Physiol. Biochem. PPB* **2024**, *211*, 108698. [CrossRef] [PubMed]
53. Ma, D.; Guo, Y.; Ali, I.; Lin, J.; Xu, Y.; Yang, M. Accumulation characteristics of plant flavonoids and effects of cultivation measures on their biosynthesis: A review. *Plant Physiol. Biochem. PPB* **2024**, *215*, 108960. [CrossRef]
54. Cuypers, A.; Vanbuel, I.; Iven, V.; Kunnen, K.; Vandionant, S.; Huybrechts, M.; Hendrix, S. Cadmium-induced oxidative stress responses and acclimation in plants require fine-tuning of redox biology at subcellular level. *Free. Radic. Biol. Med.* **2023**, *199*, 81–96. [CrossRef] [PubMed]

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