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Combined Transcriptome and Metabolome Analysis of *Lupinus polyphyllus* Response to PEG Stress

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Abstract: Drought stress is a common abiotic stress, and *Lupinus polyphyllus* presents strong adaptability, but its drought resistance mechanism has not been explored. This study used PEG-6000 to simulate drought stress, and the H_2O_2 content, O_2^- generation rate and MDA content were determined. Transcriptome sequencing and untargeted metabolome analyses were also carried out on an Iceland germplasm and American B germplasm under different drought stress durations. The results showed that the gene regulation range in the American B germplasm was greater, whether genes were upregulated or downregulated. And the number of genes in the American B germplasm was higher than that in the Iceland germplasm. Additionally, the Iceland germplasm produced less peroxide under PEG stress than the Iceland germplasm. The Iceland germplasm was more stable than the American B germplasm under PEG stress, which can be shown in two aspects: peroxide content and gene regulation quantity. Joint transcriptomics and metabolomics analysis showed that genes and metabolites related to secondary and carbon metabolism were mainly involved in the response of *Lupinus polyphyllus* to PEG-simulated drought stress. The metabolites mainly included phenylalanine, tyrosine, trans-2-hydroxycinnamate, starch synthase, 1,4-alpha glucan branching enzyme and glycogen phosphorylase, and genes mainly included *COMT*, *F5H*, *REF1*, *CAD*, *UGT72E* and *TPS*. These results provided genetic resources and a theoretical basis for further molecular breeding of *Lupinus polyphyllus*.

Keywords: *Lupinus polyphyllus*; drought; metabolome; transcriptome; peroxide

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1. Introduction

Lupinus polyphyllus Lindl. is an annual herb with a height of up to 70 cm, which blooms from March to May and fruits from April to July. It has rich nutritional components, mainly including rich protein and high-quality fatty acids [1]. Plant legumes have been widely used as substitutes for animal proteins as dietary proteins due to their low cost [2], and have two types in nature: domesticated and non-domesticated. *Lupinus polyphyllus* originated in the Mediterranean region. The existence of domestication allows it to be transplanted into other areas [3], which also contributes to the significance of studying the drought tolerance of *Lupinus polyphyllus*.

Global climate change mainly causes drought stress, which has adverse effects on the production and development of crops in tropical and subtropical regions [4]. Drought tolerance has been studied in many plants, such as grape (*Vitis vinifera*) [4], southern yellow pine (*Pinus ponderosa*) [5], soybean (*Glycine max*) [6] and so on. *Lupinus polyphyllus* was shown to present preeminent tolerance to abiotic stresses [7], which demonstrated that the adaptive mechanism makes lupin the first choice for planting in acidic soil [8]. Genetic analysis can be used to predict the characteristics of lupin, such as anthracnose resistance [9], isoflavonoid release [10] and anther dehiscence [11]. The expression level of all genes can be controlled by transcriptome. Transcriptomics has also been applied to the study of drought tolerance in many plants such as alfalfa (*Medicago sativa*) [12], poplar

ma bamboo (*Dendrocalamus latiflorus*) [13], yellow lupine (*Lupinus luteus*) [14] and so on. Drought treatment destroyed the localization of auxin, changed the structure of the cell wall and led to the falling of lupin flowers when *Lupinus polyphyllus's* research was carried out [15,16]. Furthermore, drought conditions also caused changes in the level of amino acids [17] and carbon and nitrogen metabolism [18], but few studies focused on the relevant metabolic pathways and acting genes. *Lupinus polyphyllus* has important ornamental and nutritional values [19–21] and it was possible via artificial domestication to plant lupin across regions [22]. Therefore, it was of great value to study the causes of drought resistance of lupin.

The analysis of transcriptomics and metabolomics was carried out and qRT-PCR was performed to further confirm the differential gene by comparing drought-resistant germplasms (the Iceland germplasm) and non-drought-resistant germplasms (the American germplasm) and evaluating the plant physiological state and index of oxidative damage. A systematic study on the drought resistance mechanism of *Lupinus polyphyllus* was conducted, which indicated that Iceland may prove to have strong drought resistance after the synthesis of their data.

2. Materials and Methods

2.1. Sample Collection and the Setting of Drought Stress

Lupinus polyphyllus germplasms were collected in Yundingshan, Agan Town, Lanzhou City, Gansu Province, on 28 February 2019. Two germplasms of *Lupinus polyphyllus* including the 'Iceland' and the "American B" germplasm were applied for transcriptomes analysis. The seed of the two varieties of *Lupinus polyphyllus* was surface sterilized with 10% NaClO solution and soaked in water. The sand cultures method was used to grow the seedlings in the experiment. The seeds were placed equidistantly into 60 mm × 15 mm Petri dish seeds and germinated at about 20 °C in darkness for 4 days. After the lupine seeds had fully germinated, 10 uniformly growing seedlings were randomly selected from each culture dish and transplanted into a 100 mm × 80 mm nutrient bowl filled with 400 g of sand (with 50 uniform holes at the bottom). The bowl was then placed in a long box containing 200 mL of nutrient solution with ammonium and nitrate serving as inorganic nitrogen sources. Subsequently, the nutrient solution was replaced every two days. The seedlings were cultivated in an artificial climate chamber maintained at a temperature of 25/19 °C, with 16/8 h of light/darkness and a light exposure of 450 μmol m^{-2} s^{-1}, along with a relative humidity of 45–50%.

This study treated lupine seedlings with Hoagland nutrient solution as the control, and Hoagland nutrient solution containing 30% PEG-6000 as the PEG stress treatment after 7 weeks of culture based on the previous studies [23]. Solutions with −1.2 MPa osmotic potentials were prepared using PEG-6000, while the seedlings in the control pot were not affected by osmotic stress (0 MPa). Nutrient solutions were mixed in a water bath at 30 °C, stirred and transferred to pots. To prevent the concentration increase caused by the residual nutrient solution, each flowerpot should be cleaned thoroughly before changing the treatment solution. The osmotic pressure of the treatment solution was passed through a VAPRO® Vapor Pressure Defcit Osmometer (WESCOR Inc., Lewes, DE, USA). At 0, 1, 3, 5 and 7 d of the osmotic stress, the harvested leaf tissues of the "Iceland" and the "American B" germplasm were placed into liquid nitrogen immediately after weighing and packaging, and were immediately frozen for about 4 h. Subsequently, they were stored in an ultra-low temperature refrigerator (−80 °C) for subsequent determination of physiological and biochemical indicators. All treatments were randomly sampled with 4 replicates.

2.2. Determination of Physiological Indexes of H_2O_2, O_2^- and MDA

To determine the physiological changes caused by drought, separated leaf tissues were collected to analyze the concentration of H_2O_2. According to the operating instructions of the hydrogen peroxide assay kit, the content of H_2O_2 was measured using KI iodine blue spectrophotometry. According to the operation instructions of the inhibition and generation

of the superoxide anions assay kit, the concentration of dissolved O_2^- was measured using the basic chemical analysis method (titration method). These reagent kits were purchased from the Nanjing Jiancheng Institute of Biotechnology. And the thiobarbituric acid method (TBA method) was used to determine the content of malondialdehyde (MDA) according to Chen et al.'s description [24].

2.3. Transcriptomics and Metabolomics Analysis of Iceland Germplasm and the American B Germplasm

2.3.1. RNA Extraction and RNA-Seq Sequence

The dried samples (0.2 g of leaf) were dehydrated for 0 h, 24 h and 72 h separately and were selected for the following examinations. Total RNA from the leaves collected from two lupines cultivated under drought stress and control treatments was extracted by using the Trizol reagent kit (Invitrogen, Carlsbad, CA, USA). The RNA quality and concentration were tested with the help of Agilent 2100 Bioanalyzer software (Agilent Technologies, Palo Alto, CA, USA) and an Ultra Micro UV Spectrophotometer (Beijing Dinghaoyuan Biotechnology Co., Ltd., Beijing, China), as well as the RNase free agarose gel electrophoresis technique. Then, Oligo (dT) beads were used to enrich mRNA. A library was created with an NEBNext Ultra RNA Library Prep Kit (New England Biolabs, Ipswich, MA, USA), which was used by Illumina. The cDNA library from leaf tissues of two lupines cultivated and treated with PEG-6000 at different time points was sequenced using Illumina Novaseq6000. These raw sequencing data are stored in the SRA database of the NCBI (https://www.ncbi.nlm.nih.gov/sra, accessed on 1 March 2024), with accession number PRJNA852358.

2.3.2. The Analysis of Differential Genes

The coverage adapter has been filtered for reads that either contained N greater than 10% or had been validated as low-quality readings, which referred to readings greater than 50% of the total, with a basic mass Q $\leq$ 20. Subsequently, a total of five databases including the NR, SwissProt, KEGG and COG (Clusters of Orthologous Groups) databases were mapped out to denote the function of the protein. Differential genes were screened by the standard of False Discovery Rate (FDR) < 0.05 and $|\log2FC| > 1$ and a volcanic map and heat map were made. And then, enrichment analysis of KEGG (https://www.genome.jp/kegg/pathway.html, accessed on 1 March 2024) and GO (http://geneontology.org/, accessed on 1 March 2024) was applied.

2.3.3. The Analysis of Metabolite

Samples (80 mg) were treated by drought stress mixed with 400 µL water, and 200 µL chloroform was added. The samples were then centrifuged for 10 min with a rotating speed of 12,000 rpm at 4 °C. A total of 80 µL methoxy amine pyridine hydrochloride was added to the supernatant (300 µL) of each sample. Then, the sample was incubated at 37 °C for 90 min. A total of 20 µL of n-hexane and 80 µL of BSTFA (N,O-Bis trifluoroacetamide, with 1% TMCS) was added to each sample. Afterward, all samples were incubated for 60 min at 70 °C. Then, all samples were kept for 30 min at room temperature, which was 25 °C. The derivative samples were analyzed by a gas chromatography system (Agilent Technologies Inc., Santa Clara, CA, USA) in specifications of 30 m × 0.25 mm × 0.25 µm, with separate derivatives on a DB-5MS fused silica capillary column (Agilent J&W Scientific, Folsom, CA, USA). We selected helium gas (>99.999%) as the carrier gas, with a constant flow rate of 1 mL·min^{-1} and 260 °C, and injected a volume of 1 µL in non-split mode, of which the temperature was 60 °C; then, 125 °C for 8 min; 210 °C for 4 min; 270 °C for 5 min; 305 °C for 10 min, and, finally, they were maintained at 305 °C for 3 min, with the MS quadrupole set at 150 °C, ion source (electron collision) set at 230 °C and collision energy set at 70 eV. In addition, the scanning range was set to 50 to 500 m/z, and data information collection was completed using ChemStation software (version E.02-02-1431, Agilent, Santa Clara,

CA, USA). The Fiehn or NIST database was applied to denote metabolites. Data analysis was accomplished by using the SIMCA 14.0 software package (Umetrics, Umea, Sweden).

2.3.4. Combined Analyses of the Data of Transcriptome and Metabolome Analyses

In order to better reveal drought stress, we chose to use different analytical methods to quantitatively map genes and metabolites directly to metabolic pathways. Therefore, we conducted KEGG pathway analysis on the transcriptome and metabolome, and the results showed that there were two identical enrichment pathways for DEGs and DAMs, namely flavonoid biosynthesis ($p < 0.05$) and isoflavone biosynthesis ($p < 0.01$). In addition, we also chose to downregulate differentially expressed genes (DEGs) and differentially accumulated metabolites (DAMs) and mapped them onto relevant KEGG pathway maps. A total of 9 key upregulated genes and 14 metabolites were simultaneously located in the isoflavonoid biosynthesis, whose KO ID is ko00943. Moreover, our additional results found that 14 DAMs and 2 key DEGs were simultaneously localized in flavonoid biosynthesis (ko00941), indicating that flavonoid biosynthesis plays an important role in drought stress in lupin beans.

2.4. Quantitative Real-Time PCR (qRT-PCR) Validation

We randomly selected 20 DEGs for RNA seq data validation and measured their relative expression levels using qRT-PCR technology. The extraction method and reagent kit used for total RNA are the same as RNA-seq sequence. Then, the total RNA was reverse-transcribed into cDNA using a Prime Script RT kit (TaKaRa, Shiga, Japan) with TaKaRa and a gDNA eraser. Then dilute the cDNA 20 times as the final template concentration for qRT PCR reaction. According to the manufacturer's instructions, the Light Cycle ® 96 Real-time PCR system (Roche Life Sciences, Shanghai, China) and SYBR® The Premium Ex TaqTM II kit (Takara Biomedical Technology, Dalian, China) were used for qRT PCR, with each biological repetition being the mean of 3 technical repetitions. The PCR amplification reaction conditions were as follows: first, 94 °C for 5 min; second, 40 cycles at 95 °C for 15 s and 60 °C for 1 min and finally, the assumed *actin* gene of *Lupinus polyphyllus* was used as an endogenous control using the $2^{-\Delta\Delta CT}$ method, which calculates the relative level of gene expression [25]. In addition, a specific primer design was employed using Primer 6.24 software (Quebec City, QC, Canada).

3. Results

3.1. The Differences of Plant Phenotype under Different Degrees of PEG Stress

All specimens were divided into three groups in this study, including the control group, 24 h for the PEG stress group and 72 h for the PEG stress group. All of the leaves were stretched, and the color was normal in the control group. The PEG (polyethylene glycol) solution was used to simulate the PEG stress. The edge of the leaf began to curl slightly when under PEG stress for 24 h. With the longer exposure to PEG stress, its curling degree grew more seriously, and it eventually withered (Figure 1). It can be observed that the condition of the Iceland germplasm was better than the American B germplasm.

3.2. Responses of Physiological Indexes

Physiological index results demonstrated that levels of H_2O_2 (hydrogen peroxide), O_2^- (superoxide anion) and MDA (malondialdehyde) in the American B germplasm were higher than that of the Iceland germplasm, which showed that the American B germplasm was much more sensitive to PEG stress. Similarly, the Iceland germplasm also showed the potential for better drought resistance to lower levels of H_2O_2, O_2^- and MDA compared to that of the American B germplasm. Therefore, it was possible for the Iceland germplasm to be drought-resistant due to the biological structure and cell specialty (Figure 2).

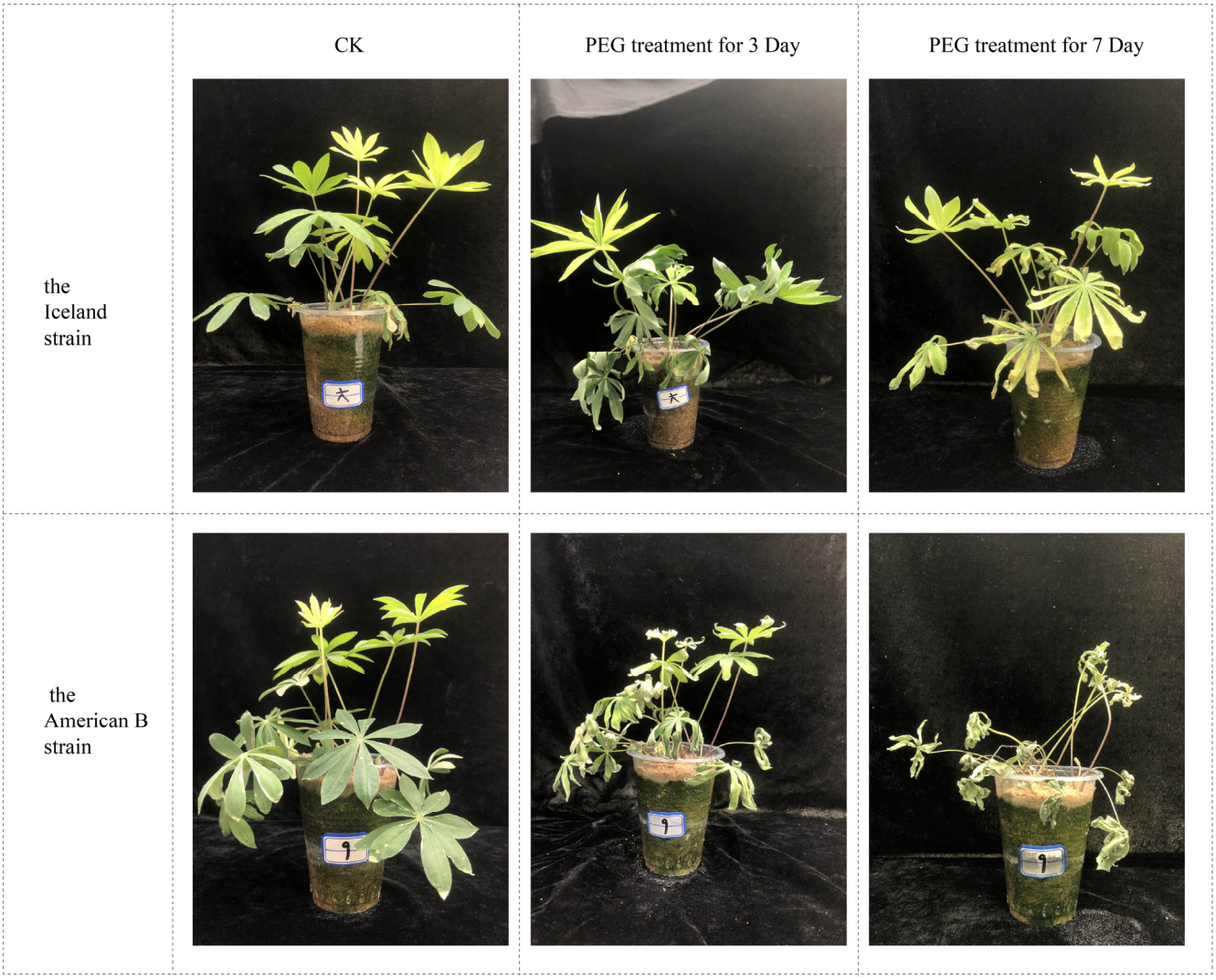

Figure 1. *Lupinus polyphyllus* under PEG stress. The figure shows the results of PEG drought treatment 0D, 3D, and 7D in the American B germplasm and the Iceland germplasm, respectively.

3.3. The Analysis of KEGG Enrichment

We conducted principal component analysis (PCA) based on the transcriptional expression profiles of two *Lupinus polyphyllus* treated with PEG-6000 at different times. The first two principal components explained the variance of 66.8% (PC1) and 17.9% (PC2) between samples of two lupine germplasm under different osmotic stresses (Figure 3A). Compared with CK, as the PEG-6000 treatment time continued, the number of DEGs increased in two *Lupinus polyphyllus* (Figure 3B). Compared with CK, the Icelandic germplasm had the highest number of DEGs mapped to metabolic pathways at 72 h of stress (Figure 3C). Secondly, there was a significant enrichment in secondary metabolic biosynthesis, amino acid biosynthesis and carbon metabolism pathways. In the comparison group of A-CK vs. A-72 h, we identified that DEGs were mainly enriched in metabolic pathways, secondary metabolic biosynthesis and carbon metabolism pathways (Figure 3D). On the whole, the KEGG enrichment analyses indicated that biosynthesis of secondary metabolites and metabolic pathways played a critical role in the Iceland germplasm and the American B germplasm.

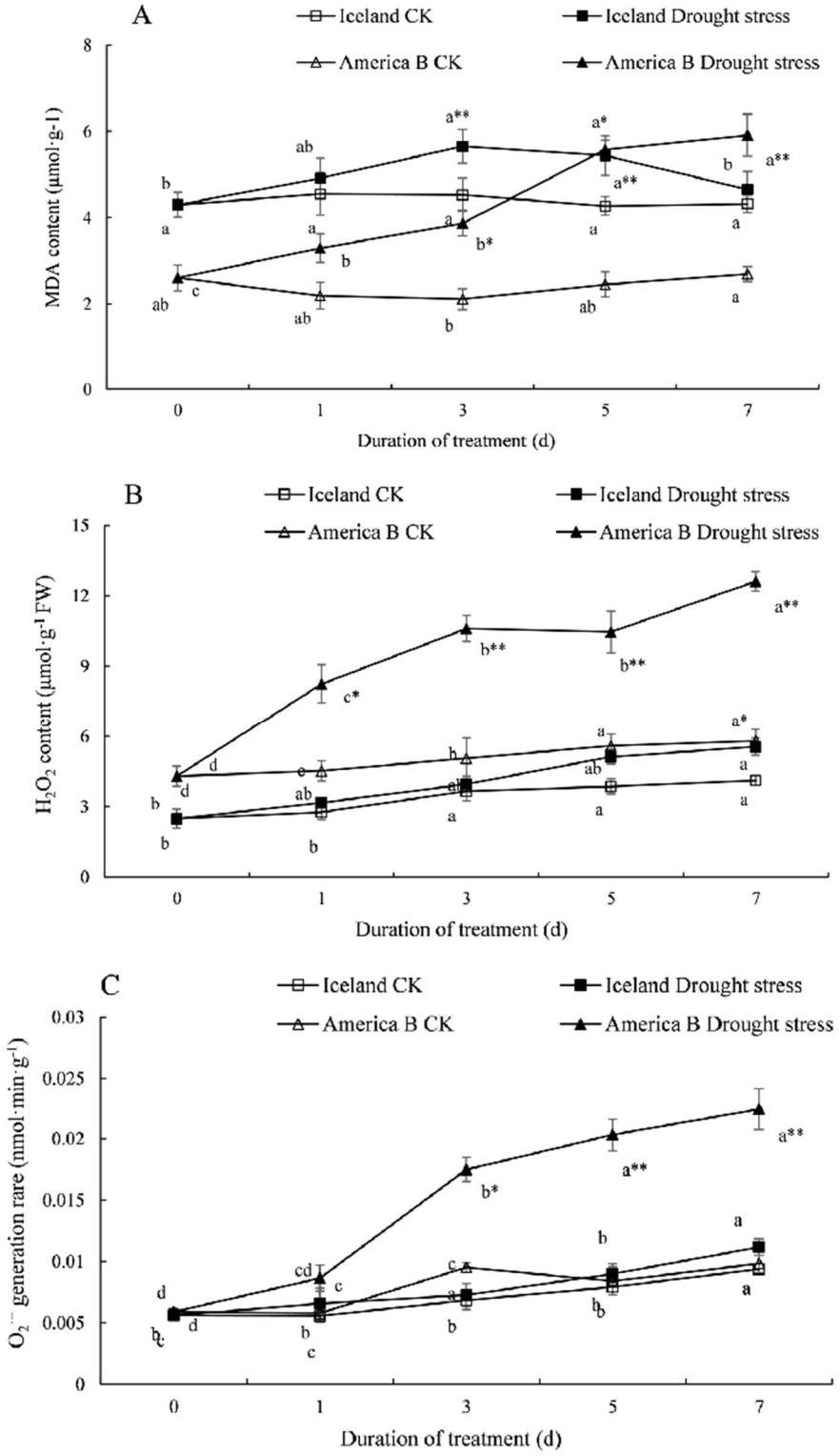

Figure 2. Determination of peroxide content. (**A**): MDA content. (**B**): H_2O_2 content. (**C**): O_2^- generation rate. ** indicates extremely significant differences at the $p < 0.01$ level between two materials at same stage, and * indicates significant differences at the $p < 0.05$ level.

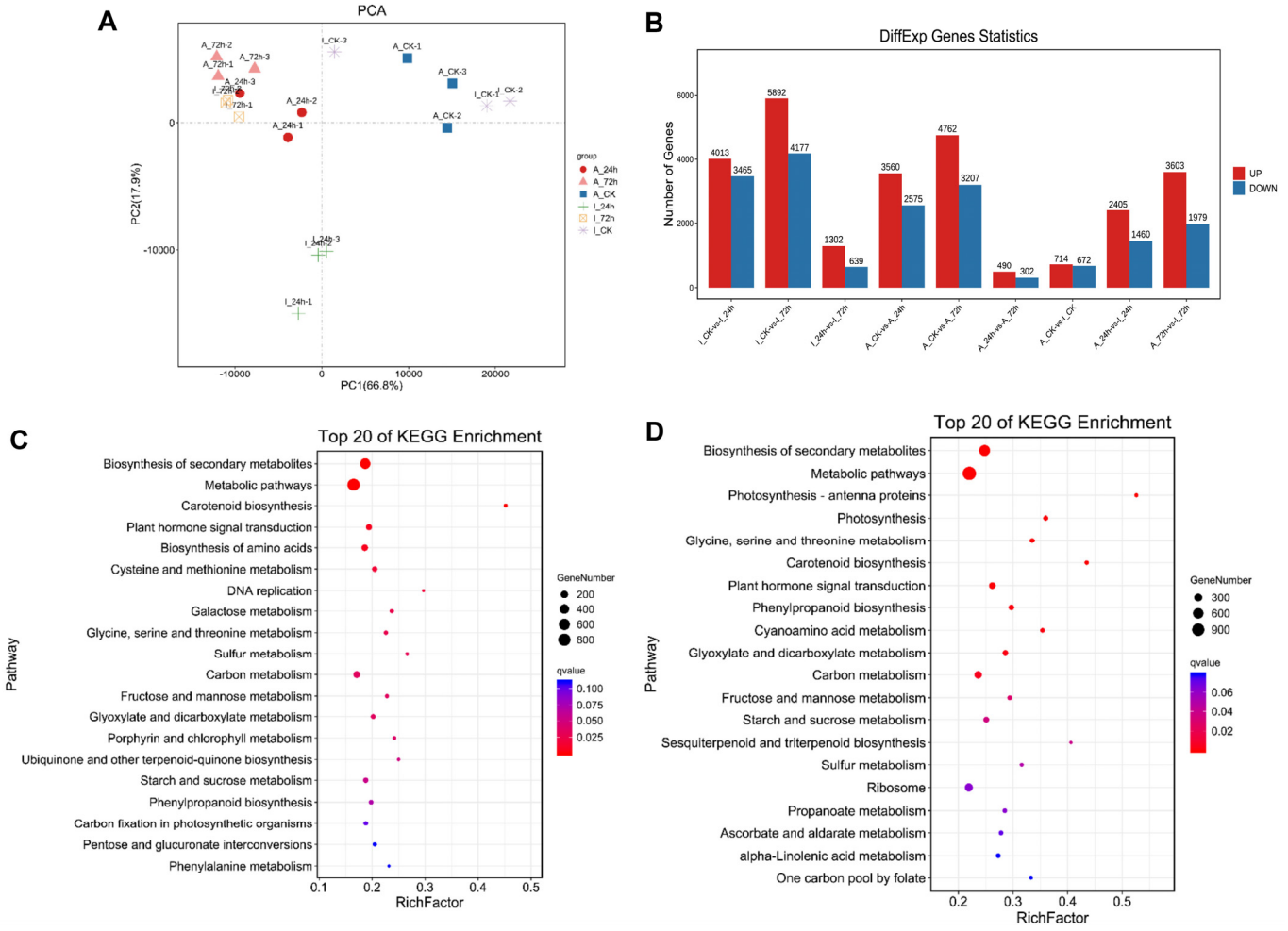

Figure 3. Summary and analysis of transcriptome from two *Lupinus polyphyllus* treated with PEG-6000 at different times. (**A**): PCA of transcriptome of samples from the Iceland and American B germplasm during plant development. (**B**): DEGs of the Iceland and American B germplasm during plant development. (**C**): KEGG pathway analysis of Iceland under PEG stress for 72 h. (**D**): KEGG pathway analysis of the American B germplasm under PEG stress for 72 h.

3.4. The Enrichment Analysis of Differential Genes Function

The analysis of the cellular component, molecular function and biological process was completed by mapping GO database. The specific structure and metabolic pathway between the Iceland germplasm and American B germplasm were demonstrated, such as glycoprotein binding, iron coordination entity transport and the homeostatic process (Figure 4). Oxidoreductase activity played an important role in Iceland germplasm and American B germplasm under PEG stress. Significant apoptosis also occurred in the American B germplasm instead of the Iceland germplasm. Moreover, the acyl−CoA metabolic process played a significant role in PEG stress of the Iceland germplasm and American B germplasm. The results show that the sulfur compound metabolic process exhibits significant differences under PEG stress, which indicates that the genes related to light stimulation and external biological stimulation are significantly different.

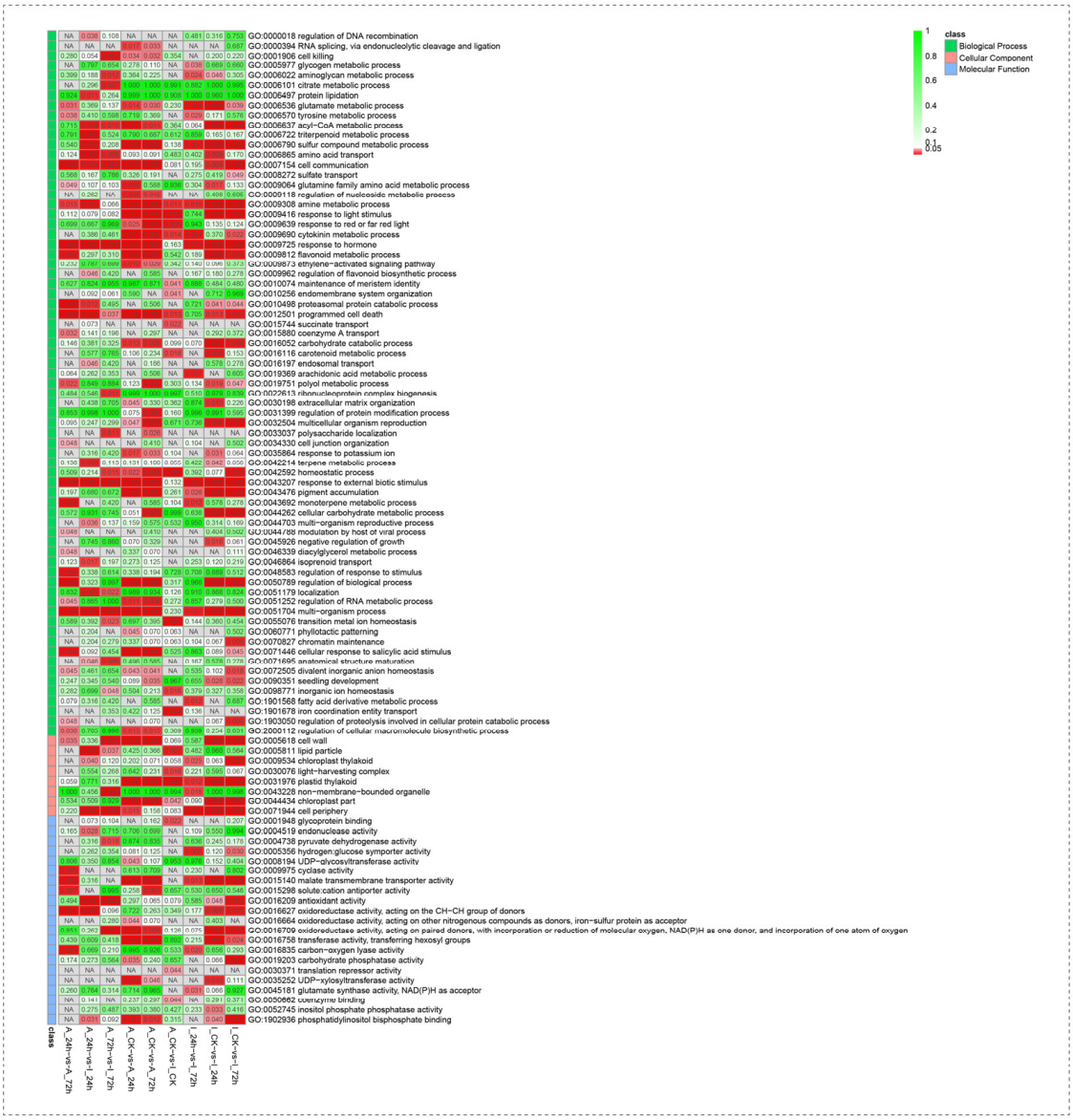

Figure 4. The result of GO analysis.

3.5. The Analysis of Differential Metabolic Pathways

The enrichment analysis of KEGG has shown that pathways were related to differential genes. There were significant differences in the biosynthesis of benzene-C between the Iceland germplasm and American germplasm B under PEG stress. There were significant differences between pentose and gluconate under PEG stress (Figure 5). What is consistent with GO is that the materials related to photosynthesis have also changed under PEG stress, such as carbon metabolism, photosynthesis and photosynthesis-antenna proteins. In addition, cyanoamino acid metabolism, carotenoid biosynthesis, DNA replication and plant hormone signal transduction are also involved in the resistance of *Lupinus polyphyllus* to PEG stress.

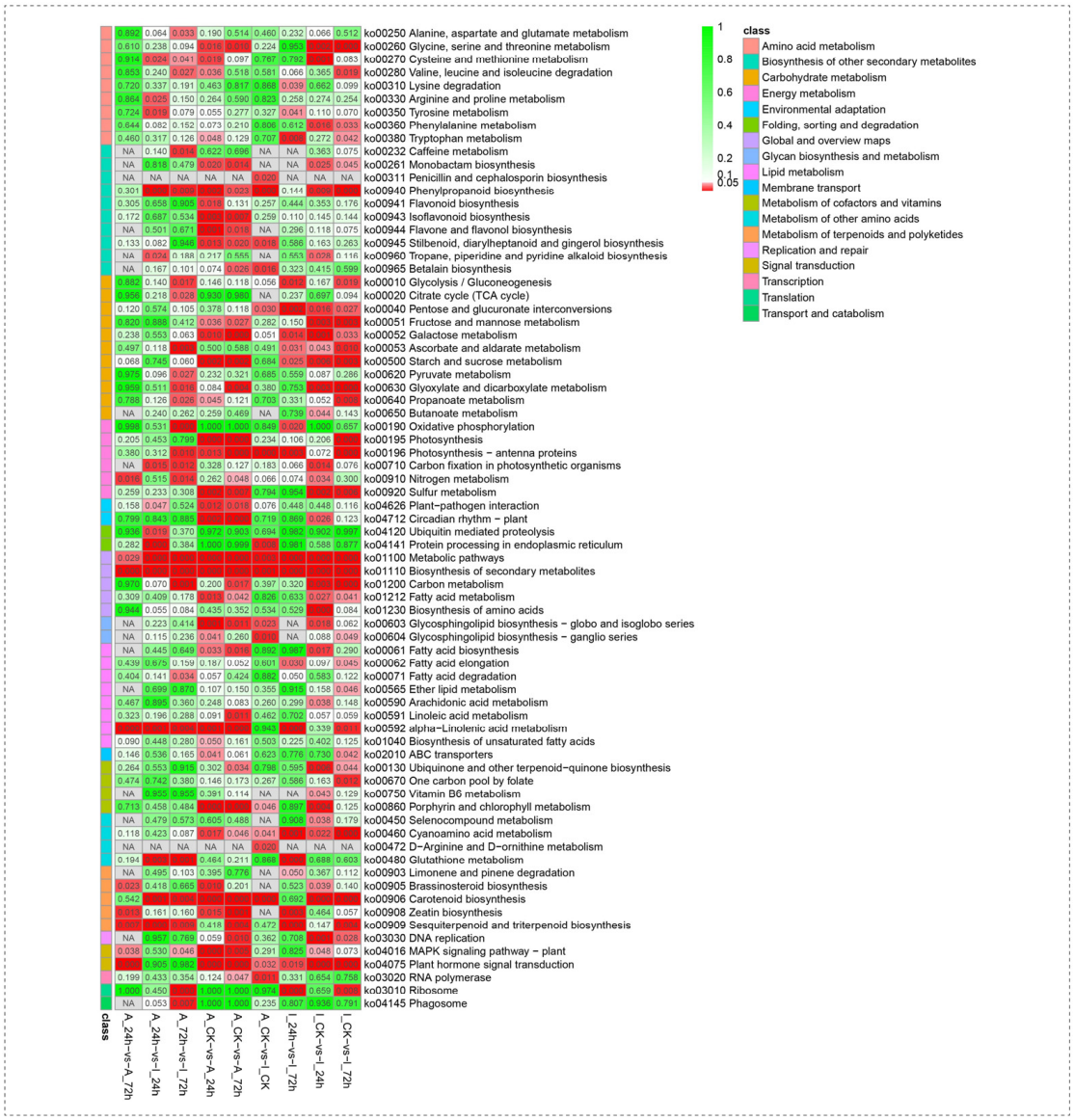

Figure 5. The result of KEGG analysis.

3.6. Correlation between Differential Genes and Pathways

Metabolomics mainly analyzes endogenous small molecules with relative analysis numbers less than 1000, which are mainly involved in organism metabolism and maintain normal growth and development. The first 25 genes and metabolites with the most significant differences were selected for cluster analysis. Different genes and metabolites demonstrated a good clustering trend (Figure 6). Genes including oleosin 5, brassinosteroid insensitive 1-associated receptor kinase 1-like isoform X1 and Xaa-Pro aminopeptidase pep showed a close cluster relationship. The larger clustering was proteasome subunit alpha type-7-like, which could regulate genetic information processing and folding, sorting and degradation of retinoblastoma-related protein and retrotransposon-related protein. In addition, these metabolites with significant differences included L-Saccharopine, N6-Trimethyl-L-lysine, 3-hydroxy-3-methylpentanedioic acid, 4-Methoxybenzaldehyde and N-Acetyl-DL-glutamic acid.

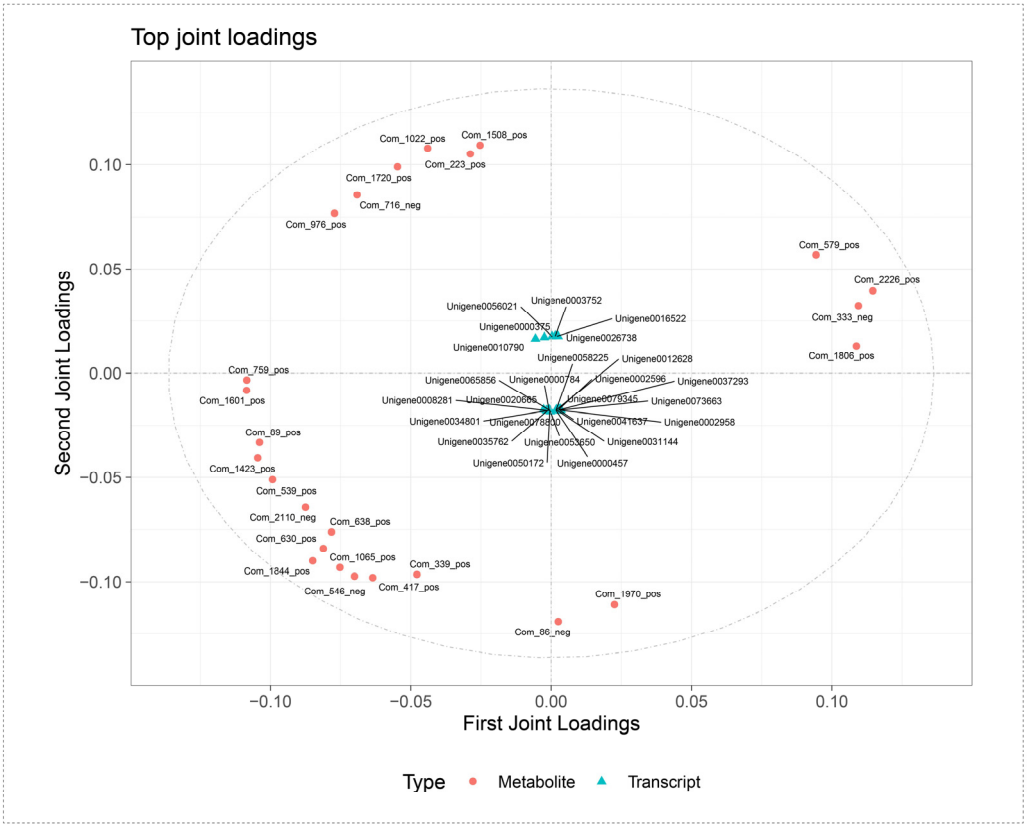

Figure 6. Inter-group element correlation analysis displayed through 02 model analysis, unigene and differential metabolites. The cluster is displayed as a single gene and differential metabolites.

3.7. Verification of DEGs Using qRT-PCR

To verify the accuracy of RNA-seq data of *Lupinus polyphyllus* under PEG-6000 stress, a total of 20 DEGs participating in *Lupinus polyphyllus* under PEG stress were randomly selected for qRT PCR in this study. The results showed that the qRT PCR results were generally consistent with the RNA seq data, with a high correlation coefficient (R2 = 0.8711), which confirmed the RNA-seq results (Figure 7).

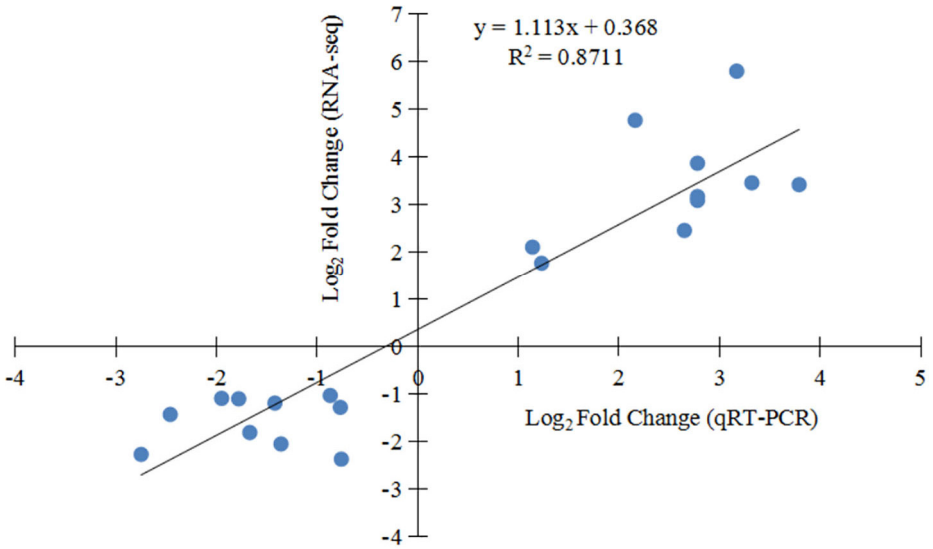

Figure 7. qRT-PCR validations of DEGs characterized by RNA-seq.

4. Discussion

Plants initiated multiple mechanisms to coordinate with each other, which was in response to drought stress. Suzuki et al.'s research has shown that plant molecular responses to drought and high-temperature stress are unique [26]. The plants responded to different types of stress in various ways, which means it has been supposed that the responses to various types of stress can be combined [27]. We found that the curling degrees of plant leaves also increased with the aggravation of water loss by comparing the apparent responses under drought stress in this study. Curly leaves can reduce water evaporation by reducing blade area, which is an apparent physiological response of plants. Actually, the response mechanism of plants to various types of stress is extremely complicated [28]. Peroxides are secreted by plants under various types of stress, and peroxides can cause damage to plant tissues and cells. Excessive accumulation of H_2O_2 can cause autophagy and programmed cell death of chloroplasts and peroxisomes in plant leaves [29,30]. MDA and O_2^- are the most commonly used physiological indicators for detecting plant oxidative stress [31]. Both the Iceland germplasm and the American B germplasm produced peroxides under drought stress. However, the content of peroxide produced by both was distinct. This study found that compared with American germplasm B, the Icelandic germplasm had lower levels of H_2O_2, O_2^- and MDA content under PEG-simulated drought stress, indicating better drought resistance. It was speculated that different growth environments bestow *Lupinus polyphyllus* with different characteristics. Metabolome data also demonstrated differences in plant characteristics between the two germplasms. Plant characteristics varied with the geographical environment as similar examples occurred in Rhodophyta [32]. In total, the ability of the American B germplasm to respond to PEG-simulated drought stress was weaker than that of the Iceland germplasm, which may be attributed to different geographical conditions.

Phenylpropanoids are precursors of many key secondary metabolite pathways, such as flavonoids and anthocyanins, which mainly enhance plant drought resistance by regulating cell osmotic balance [33,34]. Wang et al. [35] reported significant accumulation of aromatic amino acid phenylalanine in wild soybeans (drought-tolerant type). Jia et al. [36] identified a significant accumulation of phenylalanine in drought-induced poplar trees (*Populus*). Generally speaking, under drought stress, the content of free amino acids in plants increases, and some studies have also confirmed that higher levels of phenylalanine in plants were related to their drought resistance [37]. Phenylalanine ammonia lyase (*PAL*) is a key enzyme involved in the biosynthesis of isoterpenoid antioxidant compounds. Previous studies have investigated the antioxidant response of two maize inbred lines during the nutritional period. The results showed that the total antioxidant capacity of the leaves of the two maize inbred lines increased rapidly during the drought period, and the activity of *PAL* was also significantly increased during the stress period. [38]. Many compounds with protective effects, such as cork, lignin, flavonoids and phenylpropene, come from this pathway [39]. Based on the metabolomics and transcriptomics data of this study, we found that after 72 h of drought stress, metabolites involved in the phenylpropanoid biosynthesis pathway, such as phenylalanine, tyrosine, trans-2-hydroxycinnamate and coumarin, showed an upregulated expression trend. Genes such as *COMT*, *F5H*, *REF1*, *CAD* and *UGT72E* were all upregulated in expression. These results indicate that *Lupinus polyphyllus* Guss. can upregulate the expression of metabolites and genes related to the phenylalanine biosynthesis pathway under drought stress, increase phenylalanine content and enhance plant antioxidant function to resist damage caused by drought stress.

Carbon assimilation is mainly responsible for the synthesis of osmotic regulating substances, and starch participates in plant osmotic regulation mainly in the form of its degradation into glucose, enhancing plant resistance to damage caused by drought environments [40]. Starch and sucrose metabolism play a key regulatory role in enhancing the tolerance of *Verbena officinalis* to drought stress [41]. Jacobsen et al. [42] also found that as the leaf water potential decreased and the ABA content increased, barley (*Hordeum vulgare*) seedling leaves' alpha-amylase activity increased, but drought affecting beta-amylase did

not cause any impact. Trehalose-6-phosphate synthase (*TPS*) plays an important role in the trehalose synthesis pathway and is a key enzyme. Compared with wild-type maize (*Zea may*), transgenic maize containing *TPS1* significantly increased the total root length and diameter, the number of primary and secondary lateral roots, the active root absorption area, total root area and root vitality [43]. Trehalose is related to the tolerance of plants to abiotic stress. Lyu et al. [44] found that the fusion gene of *TPSP* was introduced into tomato (*Solanum lycopersicum*) from Escherichia coli, and the trehalose level increased in seeds overexpressing the *TPSP* gene. And it was also found that overexpression of fucose and its related metabolites in tomato seeds can serve as stress signals, actively respond to heat stress responses and enhance stress tolerance. Li et al. [45] found significant differences in the expression levels of genes and metabolites related to starch and sucrose metabolism pathways under drought stress in two maize inbred lines with different drought resistance after 7 days of drought stress. This may be involved in the response of maize to drought stress. In addition, sugars participate in many metabolic and signaling pathways in plants, playing an important role in defense responses against biotic and abiotic stress [46]. This study found that sugar showed a downward trend under drought stress in *Lupinus polyphyllus*, with genes such as maltase glycosylase, sucrose synthase, granular bound starch synthase, glycogen phosphorylase, glucose-6-phosphate isomerase, alpha-trehalose, alpha-amylase, maltase, 4-alpha-glucan transferase, isoamylase, 1,4-alpha glucan branching enzyme (GBE) and glycogen phosphorylase showing an upregulated expression trend under drought stress, indicating that metabolites in the sucrose amylase metabolic pathway and soluble sugar enzyme genes coordinate to participate in plant response to drought stress.

5. Conclusions

This study performed a comprehensive analysis of the physiological, transcriptomic, and metabolomic responses of two distinct germplasms with varying drought resistance to PEG-induced drought stress. The findings revealed that genes and metabolites associated with secondary and carbon metabolism were primarily involved in the adaptation of *Lupinus polyphyllus* to PEG-simulated drought stress. The metabolites primarily included phenylalanine, tyrosine, trans-2-hydroxycinnamate, starch synthase, GBE and glycogen phosphorylase. The genes primarily included *COMT*, *F5H*, *REF1*, *CAD*, *UGT72E* and *TPS*. These genes and metabolites were upregulated in the drought-resistant Iceland germplasm under PEG-induced drought stress. The result suggested that these metabolites played a pivotal role in the molecular mechanism of *Lupinus polyphyllus* response to PEG-induced drought stress. Further research is required to elucidate the molecular functions of the upstream and downstream genes that regulate these metabolites in *Lupinus polyphyllus* under drought stress, aiming to provide genetic resources and theoretical grounding for molecular breeding of *Lupinus polyphyllus*.

Author Contributions: S.C., W.D. and H.M. contributed to the study conception and design. Material preparation, data collection and analysis were performed by S.C. and W.D. The writing—original draft was written by S.C. Methodology was partially carried out by W.D. H.M. contributed to editing and revising the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in this study are included in the article.

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Conflicts of Interest: The authors have no relevant financial or non-financial interests to disclose.

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