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Root Physiological Changes and Transcription Analysis of *Iris domestica* in Response to Persistent Drought

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Abstract: *Iris domestica* is a popular gardening plant. Although the species is considered tolerant to drought, its growth and development are often affected by drought conditions. Therefore, revealing the regulatory mechanisms of drought tolerance in this species will aid in its cultivation and molecular breeding. In this study, morpho-physiological and transcriptome analyses of the roots of *I. domestica* plants were performed under persistent drought conditions. Peroxidase activity, proline content, and tectoridin content increased under sustained drought conditions. Transcriptome analysis showed that the roots of *I. domestica* seedlings respond to drought mainly by regulating the expression of drought-resistant genes and biosynthesis of secondary metabolites. This study provides basic data for identifying drought response mechanisms in *Iris domestica*.

Keywords: *Iris domestica*; drought; transcriptome; root; physiology

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1. Introduction

Drought is a global problem that also restricts the development of the planting industry, including the cultivation of Chinese medicinal materials [1,2]. Occasional drought threats have greatly affected the production and output of Chinese herbal medicines while reducing economic income [3]. The study of plants, particularly medicinal plants, and their physiological responses to water deficit is of great theoretical significance and has important practical implications. Root water uptake under drought conditions is insufficient to compensate for the loss of water owing to transpiration [4], which greatly restricts the growth and development of plants [5–8]. Plants have evolved various morphological, physiological, biochemical, and molecular strategies in response to drought stress [9,10]. Among these are the increased activity of protective enzymes (peroxidase, *POD*) and accumulation of osmolytes such as proline [11,12]. For example, Yang et al. found that the concentration of osmotic regulators and the activities of protective enzymes in *Bupleurum chinensis* were increased on the 12th and 20th days of drought [13]. These changes are associated with the expression of drought-induced functional and regulatory genes, which facilitates the adaptation of plants to drought [14,15]. In particular, the expression of *VP*, *POD*, and *LEA* genes improves the drought resistance of plants [16–18]. Moreover, recent studies have indicated that some transcription factors (TFs), such as *WRKY*, *NAC*, *MYB*, *bZIP*, *bHLH*, and *ERF*, can improve the drought tolerance of plants [19,20]. Transcriptome sequencing technology can be used to identify and analyze the functional regulatory genes in medicinal plants at the omics level, thus laying a foundation for the screening of superior varieties and molecular marker-assisted breeding [21].

Iris domestica (L.) Goldblatt & Mabb. (syn. *Belamcanda chinensis* [L.] DC.) is a perennial herb in the Iridaceae family that is widely distributed in China. The species is highly adaptable, drought-tolerant, and has a strong root system [22]. The elegant flower shape and high isoflavone content of this species contribute to its ornamental and medicinal value. In recent years, research on *I. domestica* has mainly focused on chemical identification,

medicinal value, efficacy in traditional Chinese medicine, and species origin. However, few studies have explored the physiological resistance of the species to environmental stress. Previous studies have shown that *I. domestica* has high drought tolerance [23]; however, the molecular mechanisms involved in the root drought response are unclear. In the present study, we aimed to examine the response of *I. domestica* roots to drought and reveal the mechanisms of drought resistance of this species. The results will facilitate the screening of high-quality drought-resistant germplasm resources.

2. Materials and Methods

This study was conducted at the Medicinal Plant Cultivation and Physiological and Ecological Practice Teaching Base (D08) of Jilin Agricultural University (Changchun, China). To maintain consistent experimental conditions (humidity, temperature, and light), all of the experiments were conducted in D08 with a natural photoperiod for approximately 12 h light, average humidity: 67.81%, average high temperature: 28 °C, and average low temperature: 21 °C.

Dried mature *I. domestica* seeds were collected at the Medicinal Botanical Garden (Jilin Agricultural University, Jilin, China) in October 2017 and planted in seeding pots in April 2018. One month later, approximately 5 cm tall, one-year-old plants from D08 were selected and transplanted into plant pots (14.5 cm high, inner diameter 20.5 cm; three plants per pot) also located in D08, and each was filled with approximately 4 kg of soil substrate. Each pot was placed in the ground, with the edge of the pot protruding from the ground. The soil physicochemical properties were as follows: 248 mg kg^{-1} available nitrogen, 17 mg kg^{-1} available phosphorus, 140 mg kg^{-1} available potassium, and a pH of 7.187. Normal field management (including weeding twice weekly and watering 150 mL daily) was performed throughout the experiment. Twelve pots, each containing three individual two-year-old plants, were used in this study. The plants were irrigated daily with 150 mL of water from 17:00 to 18:00 for four months. On day one of the fifth month, drought conditions were initiated and irrigation was stopped. Soil water content was recorded with a HH2 Soil Moisture Meter (Delta-T Devices Ltd., Cambridge, UK). The instrument probe was inserted half the depth of the pot and the soil moisture content was measured in three different parts of the pot. Root samples were collected on days 1 (control), 6, 13, and 27 of the drought experiment. Three pots were sampled each time and each pot contained three plants with similar growth patterns.

The roots were rinsed with tap water and surface moisture was removed using filter paper. The fresh weight (FW) of the roots was measured. To measure dry weight (DW), the roots were oven dried at 100 °C for 30 min initially and then at 60 °C until they reached a constant weight. The drying rate was calculated according to the formula: Drying rate = DW/FW × 100%. The dried roots powder (0.1000 g) were placed in a conical flask (50 mL) with 25 mL of 75% methanol solution. After ultrasonic treatment (frequency 40 kHz, temperature 25 °C) for 30 min, centrifugation at 3000× *g* rpm min^{-1} for 10 min, and filtration, the filtrate was fixed to 25 mL. The final volume was filtered by a 0.22 μm membrane and analyzed by high-performance liquid chromatography (HPLC). Five isoflavones were determined according to the methodology of Zhu et al. [24], with measurements taken from three pots, each containing three plants. The standard regression equations of tectorigenin, tectoridin, irigenin, iridin, and irisflorentin were $Y = -8 \times 10^{-6} + 0.0001X$ ($R^2 = 0.9999$), $Y = 0.0001 + 0.0002X$ ($R^2 = 0.9997$), $Y = -0.005 + 0.0002X$ ($R^2 = 0.9996$), $Y = -0.0004 + 0.0003X$ ($R^2 = 0.9999$), and $Y = -0.002 + 0.0002X$ ($R^2 = 0.9999$), respectively.

The crude extract from 0.50 g fresh samples was extracted with solvent (phosphate buffer solution for *POD*, and sulfosalicylic acid for Pro test). A microplate reader (Spectra Max 190, Molecular Devices, San Jose, CA, USA) was used to determine absorbance. *POD* activities and Pro content were analysed with Peroxidase assay kit, Proline assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), respectively.

Based on the obtained physiological parameters, samples collected at days 1, 13, and 27 were used for transcriptome analysis. Samples from day 6 were not included

because they exhibited little change in soil moisture. Total RNA was extracted from root samples using an RNAprep Pure Plant Plus Kit (TianGen Biotech, Beijing, China) following the manufacturer's instructions. Total RNA was qualified and quantified using a Bioanalyzer 2100 and RNA 1000 Nano LabChip Kit (Agilent Technologies, Santa Clara, CA, USA) with RNA integrity number >7.0. Poly(A) RNA was subjected to two rounds of purification from total RNA (5 μg) using poly-T oligo-attached magnetic beads. Following purification, the mRNA was fragmented into small pieces using divalent cations under elevated temperatures. The cleaved RNA fragments were reverse-transcribed to create the final cDNA library in accordance with the protocol for the mRNA-Seq sample preparation kit (Illumina, San Diego, CA, USA). The average insert size for the paired-end libraries was 300 bp (±50 bp). Paired-end sequencing was conducted on an Illumina NovaSeq 6000 platform (LC Sciences, Houston, TX, USA) following the vendor's recommended protocol.

Cutadapt and Perl in-house scripts were used to trim adapter sequences and low-quality and undetermined bases. Sequence quality (Q20, Q30, and GC content) of the clean data was verified using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) accessed on 14 May 2021. All downstream analyses were based on high-quality clean data. De novo assembly of the transcriptome was performed using Trinity 2.4.0 [25]. Trinity groups transcripts into clusters based on the shared sequence content. Such a transcript cluster is very loosely referred to as "gene". The longest transcript in the cluster was selected as the "gene" sequence (i.e., unigene).

All assembled unigenes were aligned against the non-redundant (Nr) protein (http://www.ncbi.nlm.nih.gov/) accessed on 14 May 2021, Gene Ontology (GO) (http://www.geneontology.org) accessed on 14 May 2021, SwissProt (http://www.expasy.ch/sprot/) accessed on 14 May 2021, Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/) accessed on 14 May 2021, and eggNOG (http://eggnogdb.embl.de/) accessed on 14 May 2021 databases using DIAMOND with an E-value threshold of <0.00001 [26]. The Salmon method was used to determine the expression levels of unigenes by calculating the transcripts per million [27]. Gene expression levels were calculated and normalized using the trimmed mean of M values. Differentially expressed unigenes were selected by statistical significance ($p < 0.05$) and $\log_2$ (fold change) >1 or <−1 using the R package edgeR [28].

Total RNA was extracted according to the method described above. cDNA was synthesized by quantitative reverse transcription PCR (qRT-PCR), as previously described [10]. PCR was then performed in an Mx 3000 P instrument (Agilent Technologies) using SYBR Premix Ex TaqTM II (Takara Biomedical Technology Co., Ltd., Beijing, China). The 20 μL qRT-PCR reaction volumes contained 1 μL template, 1 μL of upstream and downstream primers (10 μM each), 7 μL RNase-Free d_2H_2O, and 10 μL of SYBR Premix Ex TaqII. The cycling protocol consisted of the initial denaturation step at 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s, 55 °C for 30 s, and 72 °C for 30 s. Relative transcript expression was assessed using the $2^{-\Delta\Delta CT}$ method and the *EF1-β* gene as the reference [29–31]. Three independent replicates were prepared for each treatment. The primers used for qRT-PCR are presented in File S1 (Supplementary Materials).

Statistical analysis of the physiological data was conducted using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Significant differences among treatments were determined using one-way ANOVA and Duncan's multiple comparison analysis ($p < 0.05$). Simple graphs and enriched circle plots were drawn by Origin 2018 software and OmicShare tools, a free online platform for data analysis (https://www.omicshare.com/tools) accessed on 7 November 2021.

3. Results

3.1. Soil Water Content and Root Physiological Characteristics

To understand the responses to drought stress in *I. domestica*, plants were initially irrigated with 150 mL water daily until the drought simulation, after which point irrigation was interrupted for 27 days. The soil water content during the experiment decreased from 22.83% to 5.7%; the decrease was initially sharp and then became gradual (Figure 1).

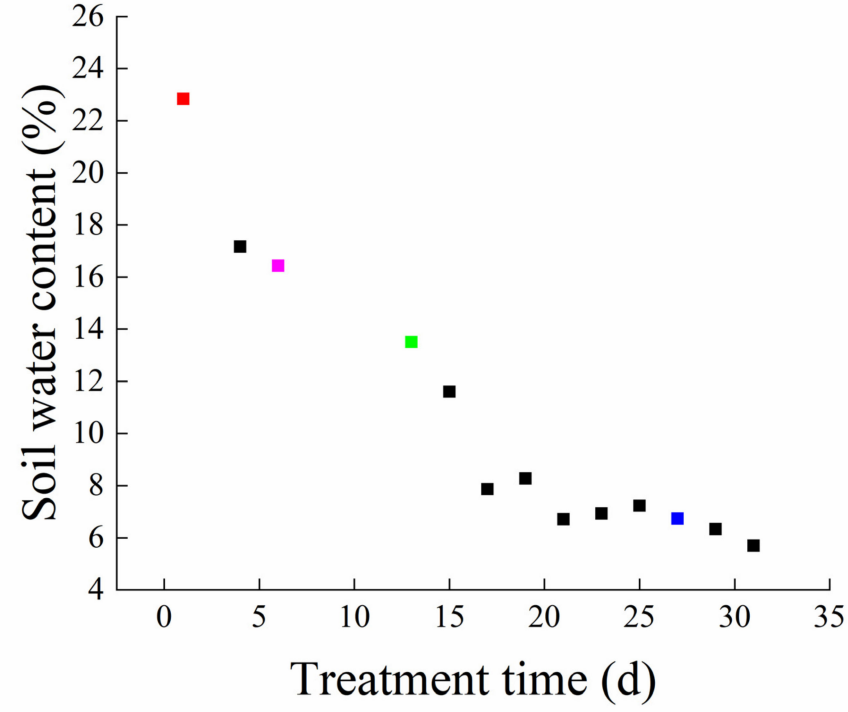

Figure 1. Changes in soil moisture under persistent drought. Red, pink, green, and blue squares represent 0 d, 6 d, 13 d, and 27 d samples, respectively.

The drying rate of the roots showed an upward trend and was significantly higher ($p < 0.01$) at day 13 (21.66%) than at day 1 (17.03%) (Figure 2a). The content of five isoflavones was measured in the roots. Drought conditions were not conducive to the accumulation of iridin, tectorigenin, irigenin, and irisflorentin, but rather contributed to the accumulation of tectoridin, which was significantly higher at day 13 than at day 1 (Figure 2b). The *POD* activity and proline content in the roots increased from day 6 in response to drought, and were both significantly higher than those at day 1 ($p < 0.01$; Figure 2c,d).

3.2. Quality Control and Annotation

In total, 133.28 GB valid reads were obtained from the transcriptome data. The proportion of Q30 was greater than 94% and the GC content ranged from 47.26% to 53.42%, indicating good data sequencing quality. After assembly, 116,612 unigenes were obtained with an average length of 368 bp. The highest functional annotation rate was obtained using eggNOG (43.80%), followed by the NR database (40.56%) and KEGG (25.52%). The unigenes matched the sequences from the genomes of *Asparagus officinalis*, *Quercus suber*, *Elaeis guineensis*, *Phoenix dactylifera*, *Musa acuminata*, *Ananas comosus*, and other species (Figure 3; Supplementary Materials, File S2).

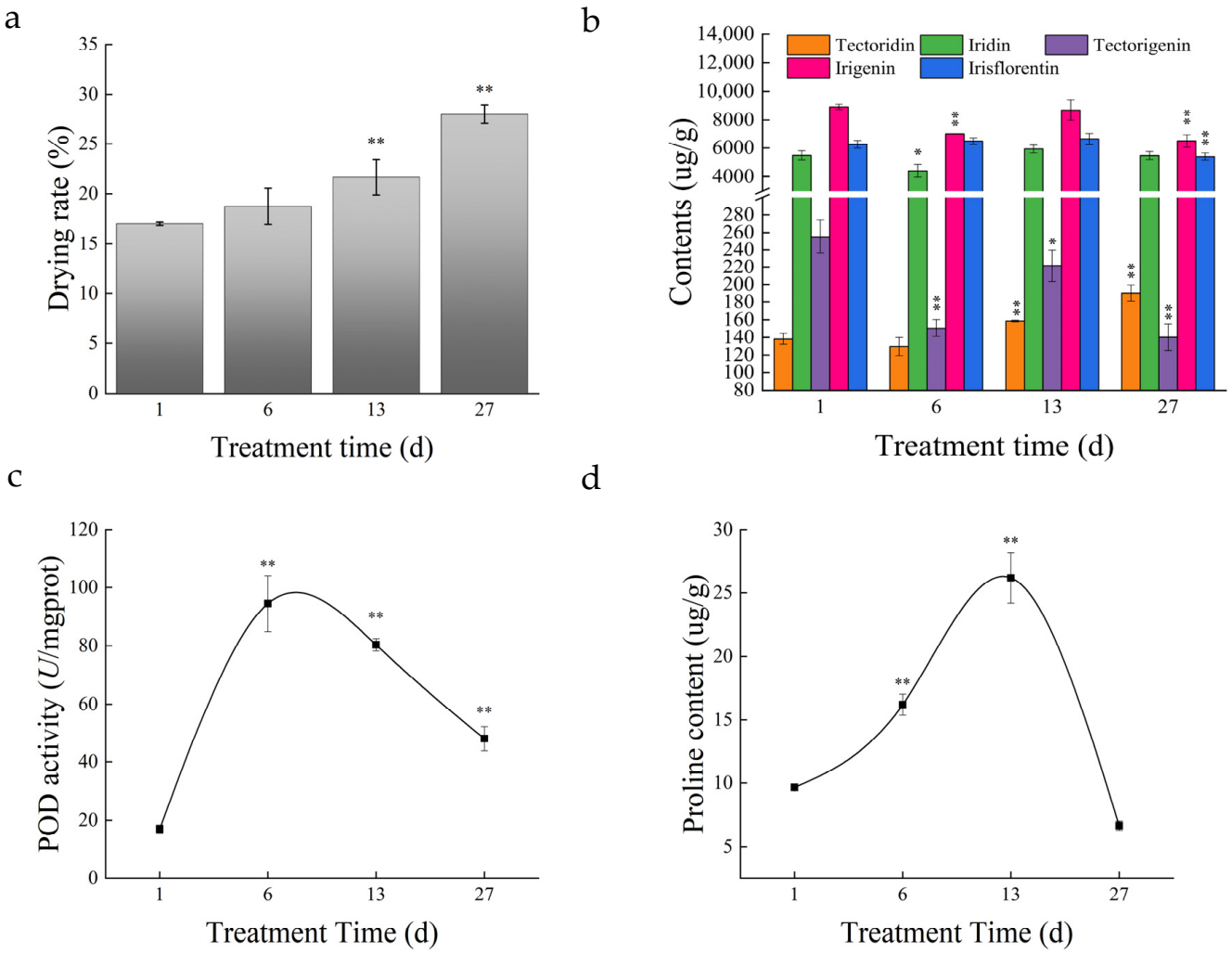

Figure 2. Drying rate (the ratio of dry weight to fresh weight) (**a**), isoflavone contents (**b**), *POD* activities (**c**), and Proline (**d**) of roots in *I. domestica* (* indicates a significant correlation at the 0.05 level, ** indicates a significant correlation at the 0.01 level).

3.3. Gene Expression Analysis

Twenty genes were randomly selected to verify the transcriptome sequencing results by qRT-PCR; a strong positive correlation was determined between the results of RNA sequencing and qRT-PCR. The correlation coefficient for day 13 versus day 1 (R^2 = 0.77) and day 27 versus day 1 (R^2 = 0.63) indicates the reliability of the transcriptome sequencing data (Figure 4).

In total, 252,522 transcripts were obtained from sequencing libraries. We found 1230 unigenes that were differentially expressed in the root samples during the drought period. The number of differentially expressed genes (DEGs) gradually increased over time; 308 and 1059 DEGs were detected at 13 and 27 d of drought treatment, respectively. Of those, 63 and 265 DEGs were upregulated and 245 and 794 DEGs were downregulated, respectively. The differential expression of the genes varied at different time points (Figure 5a) under the drought conditions.

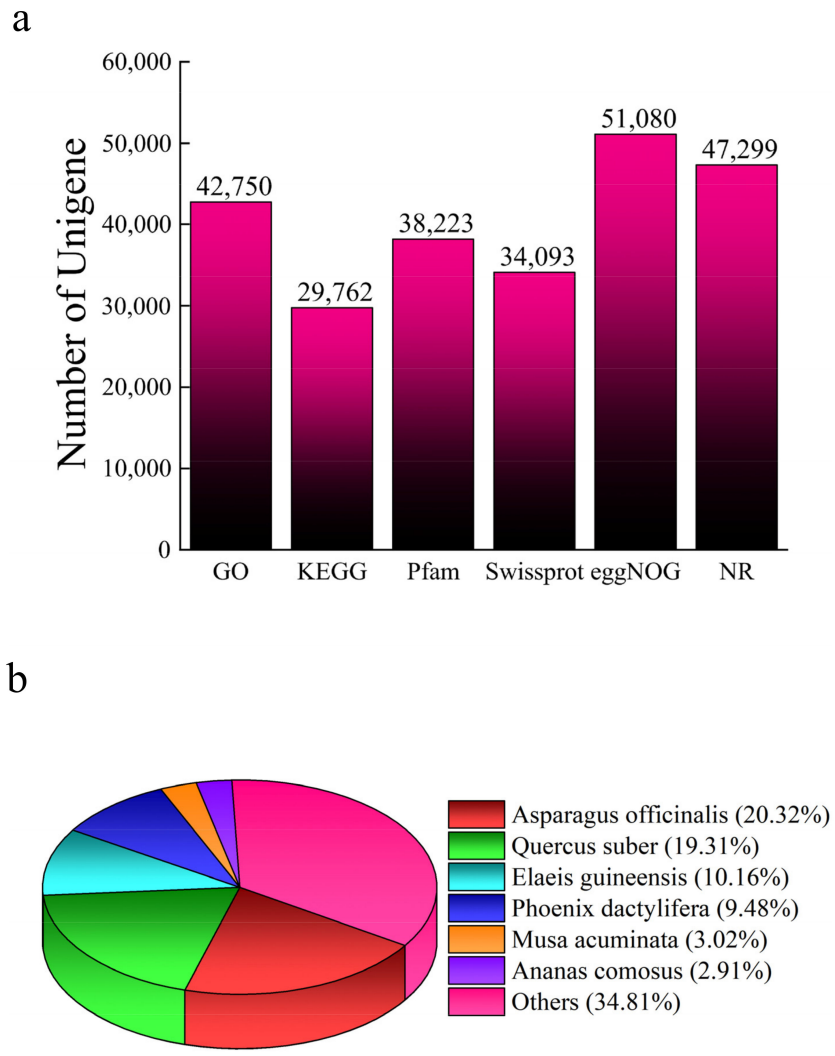

Figure 3. Unigenes annotated in different databases (**a**) and distributed into different species (**b**).

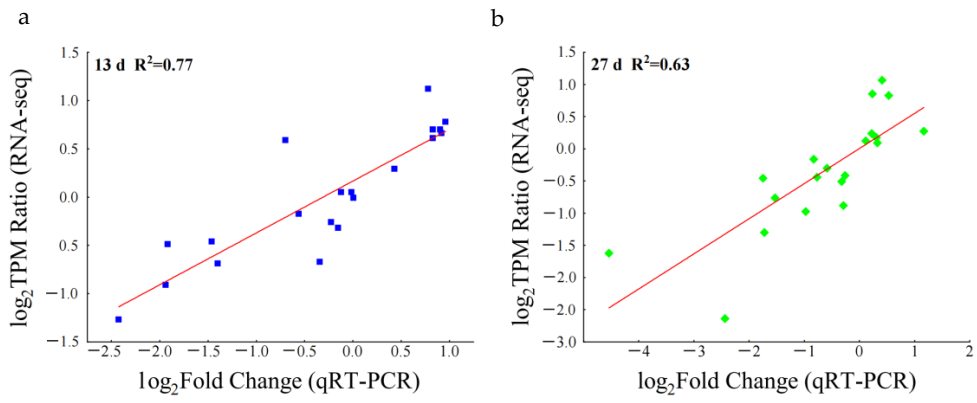

Figure 4. Correlation between RNA-seq and qRT-PCR results of samples collected at days 13 (**a**) and 27 (**b**). X-axis denoted Log_2 (the ratio of qRT-PCR) using the method $2^{-\Delta\Delta Ct}$ to calculate the relative expression level. Y-axis represented the TPM values in RNA-Seq data. Each square in the figure (**a**,**b**) represents the amount of gene expression.

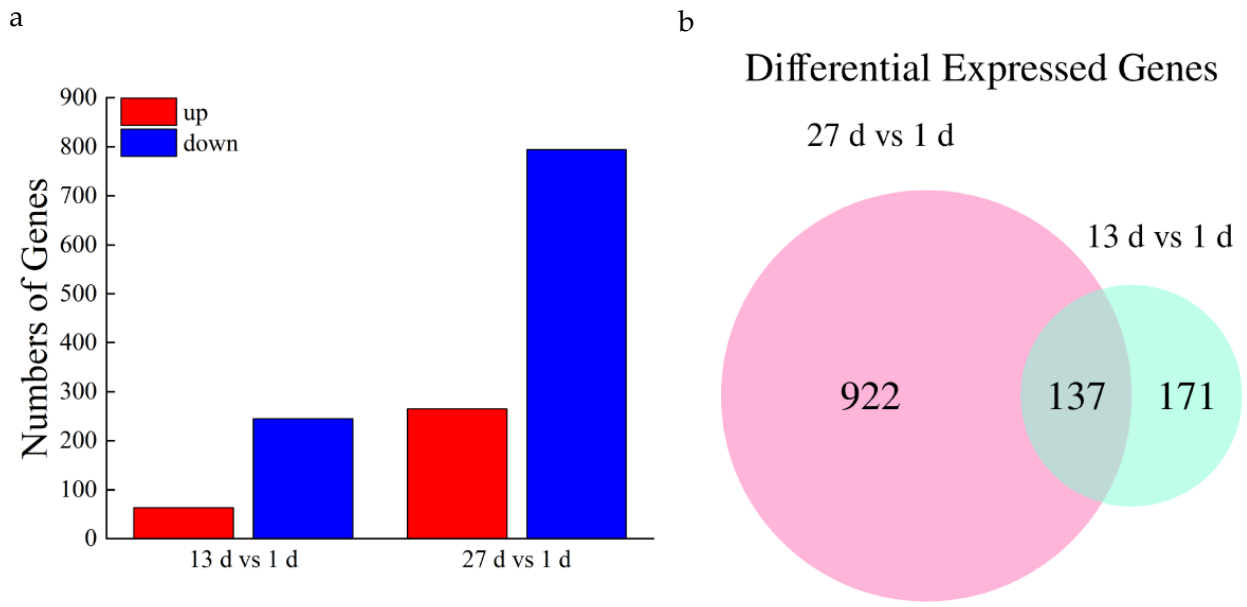

Figure 5. Histogram (**a**) and Venn diagram (**b**) of differentially expressed genes.

The common unigenes at different physiological stages were presented using a Venn diagram (Figure 5b). Compared with day 1, a total of 137 genes were significantly differentially co-expressed at days 13 and 27 of root water stress. These co-expressed genes may be related to the moisture deprivation response. Additionally, a large number of genes that respond to drought stress were induced at day 27 (Figure 5b).

3.4. GO and KEGG Enrichment Analysis

To identify the major biological processes that are expressed under drought conditions, we performed GO enrichment analysis of DEGs at three physiological stages using a false discovery rate < 0.05 as the threshold value. Based on the GO enrichment results, the 20 most significant accessions were selected to explain the physiological changes. In roots, the terms "nucleosome", "nucleosome assembly", and "nucleosomal DNA binding" were enriched at day 13 and further enriched at day 27 compared with day 1, suggesting root adaptability in response to drought. At day 13, the abundantly enriched GO terms were "protein autophosphorylation", "abscisic acid-activated signaling pathway", "ethylene-activated signaling pathway", "cell periphery", and "pectinesterase inhibitor activity", whereas the special terms "extracellular region", "cell wall", "response to water deprivation", "apoplast", "lignin biosynthetic process", and "peroxidase activity" were enriched in the roots at day 27 (Figure 6; Supplementary Materials, File S3).

According to the KEGG pathway enrichment analysis, 191 DEGs were involved in 18 different pathways at day 1. In addition, 46 DEGs were annotated in seven different pathways at day 13 and 145 DEGs were annotated in 15 different pathways at day 27. In these root pathways, phenylpropanoid biosynthesis, alanine, aspartate, and glutamate metabolism, as well as protein processing in the endoplasmic reticulum, were enriched in the upregulated DEGs throughout all treatment periods; the enriched pathways of downregulated DEGs were pentose and glucuronate interconversions, plant hormone signal transduction, phenylpropanoid biosynthesis, linoleic acid metabolism, and caffeine metabolism. Additionally, in the roots, plant hormone signal transduction and arginine and proline metabolism were enriched in all treatments; the number of DEGs gradually increased with the increasing treatment time. Additionally, we found that plant hormone signal transduction was the most enriched pathway in root genes, revealing their important role in water deficiency (Figure 7; Supplementary Materials, File S4).

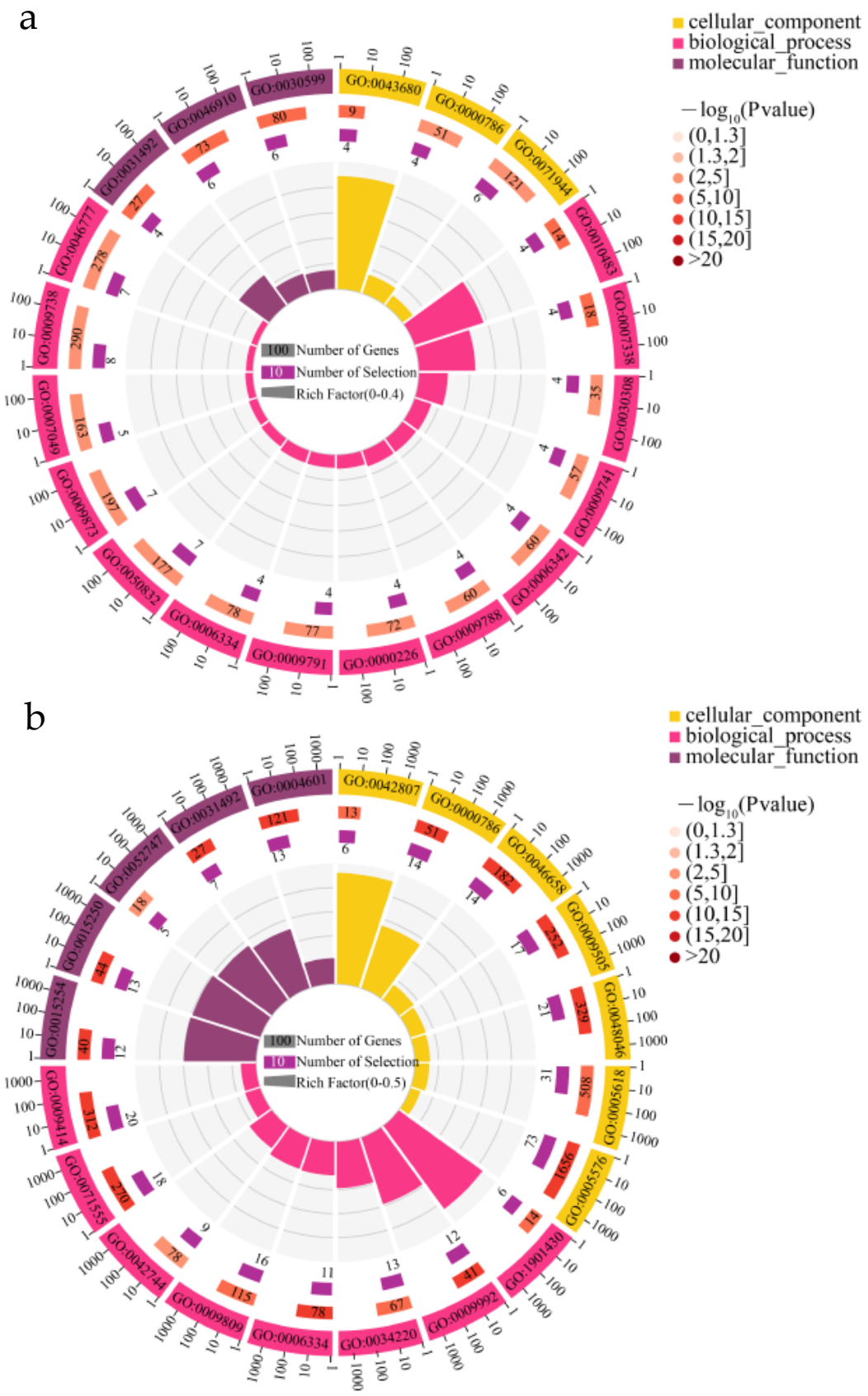

Figure 6. GO analysis of differential gene: 13 d vs. 1 d (**a**) and 27 d vs. 1 d (**b**).

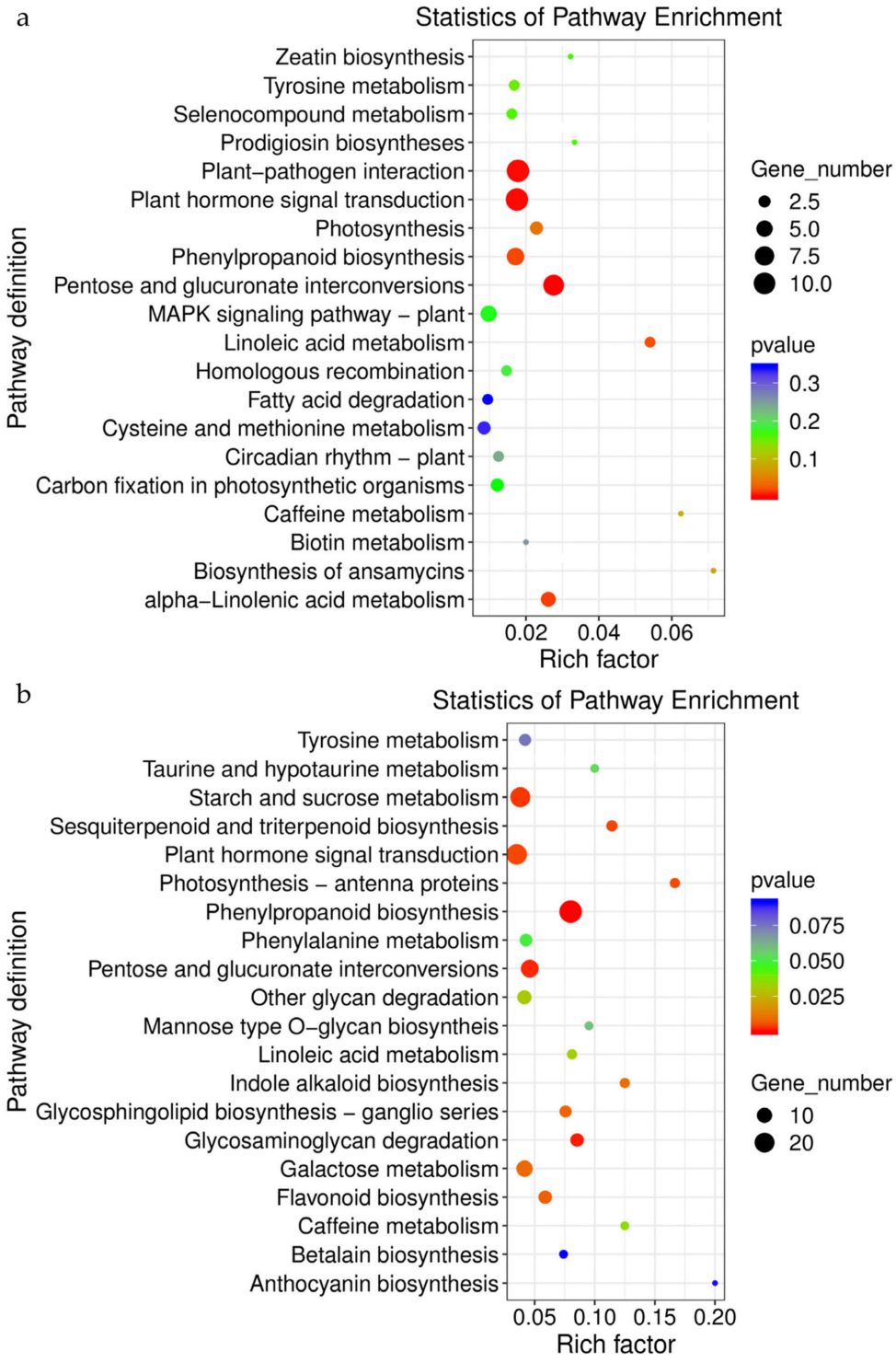

Figure 7. KEGG enrichment of differential gene: 13 d vs. 1 d (**a**) and 27 d vs. 1 d (**b**).

3.5. DEGs Related to Water Deprivation and Oxidative Stress

Seventeen genes identified by GO analysis responded to water deprivation, including pyrophosphate-energized vacuolar membrane proton pump (*VP*), late embryogenesis abundant protein (*LEA*), and alcohol dehydrogenase 1 (*ADH*). With the prolongation of drought time, the expression of the *VP* gene (TRINITY_DN60175_c1_g5) increased gradually (3.89-fold at day 13 and 4.99-fold at day 27 compared with that on day 1), while the expression of *ADH* (TRINITY_DN50206_c1_g1) decreased slowly (−3.95-fold

at day 13 and −6.45-fold at day 27 compared with that on day 1). Interestingly, *LEA* (TRINITY_DN56480_c0_g6) was upregulated (3.37-fold at day 27 compared with day 1), while its expression at day 13 was not significantly upregulated ($p > 0.05$).

Several antioxidant genes, such as those encoding *thioredoxin* (3 genes) and *POD* (8 genes), were also identified. Compared with day 1, one *thioredoxin* gene (TRINITY_DN55997_c1_g2) was upregulated 2.78-fold and the others were downregulated. Similar to the *thioredoxin* gene, all POD genes were predicted to be downregulated; this was the case, with one notable exception, POD gene (TRINITY_DN51139_c0_g5), which was upregulated 4.85-fold at day 13 (Figure 8a). These results suggest that different genes encoding for the same protein may play different roles in plants.

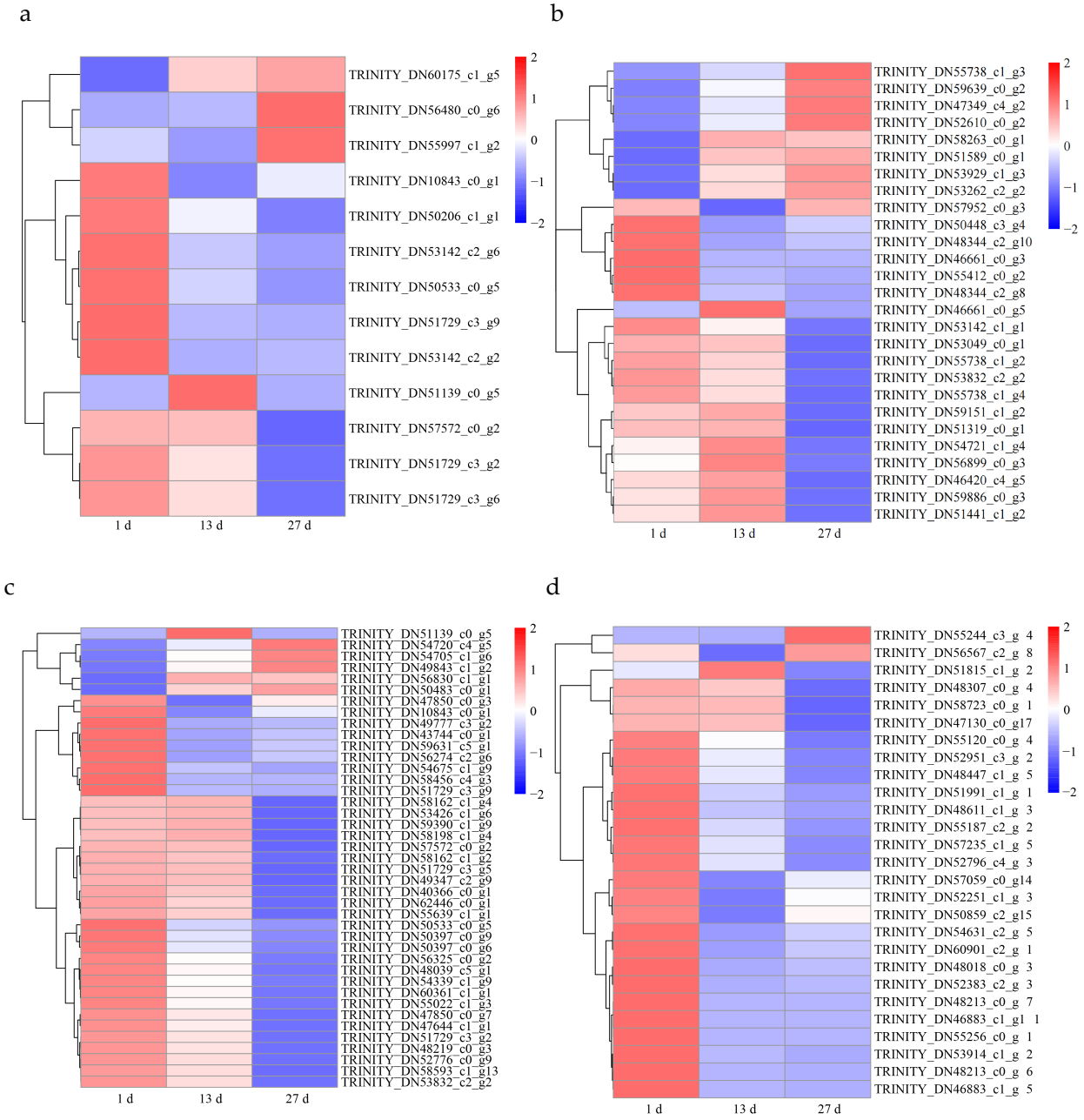

Figure 8. Heatmap diagram of differentially expressed genes (DEGs): *peroxide* and *thioredoxin* (**a**); glucose metabolism (**b**); secondary metabolites (**c**); transcription factors (**d**).

3.6. Expression of Genes Involved in Metabolism and Biosynthesis

Several metabolic and biosynthetic processes were activated under drought conditions, such as the "starch and sucrose metabolism" pathway (map00500) and "secondary metabolite biosynthetic pathway" (GO:0044550). Many genes involved in the starch synthase process were upregulated; ISA3 (TRINITY_DN47349_c4_g2) was upregulated 4.23-fold at 27 d. More genes at 13 d were upregulated than at 27 d, but the fold change of DEGs was greater at 27 d. In addition, genes encoding endoglucanase, glucan endo-1,3-beta-glucosidase, and plasmodesmata callose-binding protein were downregulated at 13 and 27 d (Figure 8b).

Many DEGs were associated with secondary metabolism. GO and KEGG enrichment identified 74 DEGs involved in secondary metabolism (Supplementary Materials, File S4). Among these, the transcription levels of 41 genes related to phenylpropanoid biosynthesis pathways were affected by drought stress (Figure 8c).

3.7. Transcription Factors Responding to Drought Stress

We identified 27 TFs, comprising *WRKY* (8), *NAC* (5), *MYB* (5), *bZIP* (1), *bHLH* (4), and *ERF* (4). On day 13 versus day 1 and day 27 versus day 1, 7 and 24 TFs were identified, respectively. Interestingly, the number of downregulated genes was greater than that of upregulated genes; only one gene was upregulated (TRINITY_DN51815_c1_g2) at 13 d and two genes were upregulated (TRINITY_DN55244_c3_g4 and TRINITY_DN56567_c2_g8) at 27 d compared with day 1 of the experiment. In addition, the four TFs of the *WRKY* family (TRINITY_DN48213_c0_g7, TRINITY_DN48213_c0_g6, TRINITY_DN57059_c0_g14, and TRINITY_DN52383_c2_g3) were differentially expressed in both 13 d versus 1 d and 27 d versus 1 d; these transcripts were possibly related to drought tolerance (Figure 8d; Supplementary Materials, File S5).

4. Discussion

Plant responses to drought are complex. The plant root system absorbs water from soil and is the first organ to perceive a decrease in soil water potential, triggering complex water stress response mechanisms to alleviate water stress. To cope with drought, plants increase the activity of protective enzymes such as *POD*, accumulate solutes to adjust the osmotic potential in cells, and elevate secondary metabolites; however, the understanding of drought resistance of *I. domestica* is incomplete. In this study, we analyzed the characteristic *I. domestica* response to drought at the physiological level and used transcriptome sequencing to analyze the molecular basis of the response.

The drying rate, as the ratio of DW to FW, is directly proportional to the economic value of the *I. domestica* crop. The drying rate of roots increases under drought conditions. In our study, the drying rate of the roots increased as drought conditions progressed, which has been attributed to the capacity of the roots with larger biomass to absorb soil moisture [32]. Plants produce a large quantity of reactive oxygen species (ROS) under adverse conditions (such as drought), which leads to oxidative stress [33]. Plants possess antioxidant enzymes and systems, such as *POD* enzymes, that eliminate ROS [34]. The relatively high *POD* activity throughout the drought experiment and the significantly higher levels at day 6 than at day 1 ($p < 0.01$) corroborated the role of *POD* in ROS scavenging and cell membrane structure protection in *I. domestica*. Consistent with *POD* enzyme activities, the genes encoding *POD* were induced by drought. These results were in accordance with those reported by Yang et al. [35]. In addition, secondary polyphenol metabolites in plants can also scavenge ROS [36–39]. Drought stress increases the content of isoflavones such as tectoridin, which may be related to the removal of ROS in *I. domestica* [40,41]. Drought is not conducive to the accumulation of other isoflavones, which may be because of isoflavone transformation or their transport across tissues [42]. We identified genes related to the biosynthesis of phenylpropanoid, which often contains polyphenolic structures [43].

To gain a deeper understanding of the drought resistance of *I. domestica*, we performed mRNA sequencing. First, we validated our transcriptome results using qRT-PCR technol-

ogy, with the correlation coefficient for day 13 versus day 1 (R^2 = 0.77) and day 27 versus day 1 (R^2 = 0.63). The correlation was not very high, which may be due to differences in experimental principles and techniques. We found similarities with other studies, in which the correlation coefficients were 0.80 and 0.83 for *Epichloë gansuensis* and *Arachis hypogaea* L., respectively, suggesting that our sequencing results were relatively reliable for further information mining. Additionally, we identified several genes related to water deprivation and oxidative stress. Vacuolar proton pyrophosphatases play important roles in drought resistance. The *VP* gene from *Ammopiptanthus nanus* was strongly induced by dehydration [44] and its ectopic expression significantly enhanced the drought tolerance of transgenic maize [16]. Transformation of the Arabidopsis *VP* gene conferred strong drought tolerance in alfalfa [45]. Taken together, these results indicate that *VP* is involved in plant resistance to drought. Another gene, *LEA*, was significantly upregulated in *I. domestica*, and similar observations were reported for *Phaseolus vulgaris* [46] and *Lepidium apetalum* [47]. Transcriptional regulation of plant stress resistance genes is an important approach by which plants cope with unfavorable external environments [48,49].

Transcription factors can specifically combine with the *cis*-acting elements of the target genes to regulate downstream stress resistance genes. Currently, TF families, including *bHLH*, *WRKY*, *NAC*, *MYB*, *ERF*, and *bZIP* families, are well known for their role in plant stress tolerance [50]. Following the GO and KEGG enrichment analyses, protein–protein interaction analysis of DEGs and TFs was performed to identify the key genes involved in the drought tolerance of *I. domestica*. These structural genes may be regulated by *WRKY51* TF (TRINITY_DN48213_c0_g7). This TF family is reportedly involved in the plant response to abiotic stress; Wang et al. (2013) confirmed that the expression of *WRKY51* was upregulated by drought stress [51]. Therefore, *WRKY51* may play an important role in the *I. domestica* drought response. In addition, glucan endo-1,3-beta-*glucosidase* (TRINITY_DN46661_c0_g3) and *CYP81B57* (TRINITY_DN58456_c4_g3) may contribute to drought tolerance in this species (Figure 9).

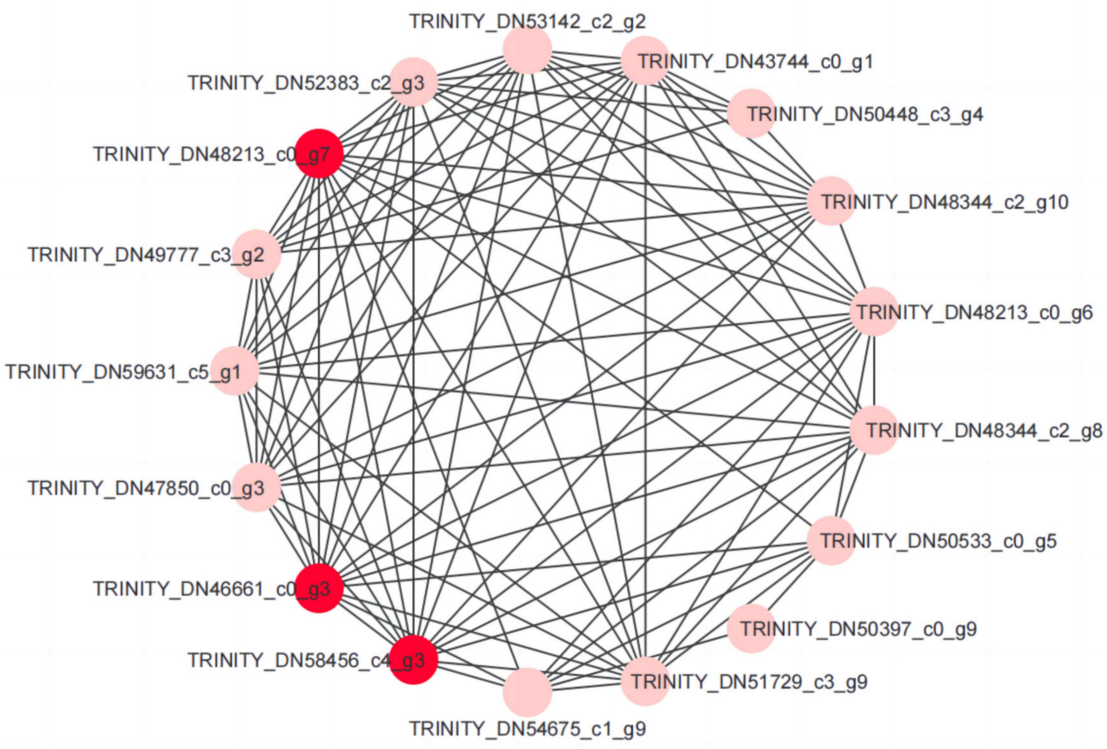

Figure 9. Construction of protein–protein interaction network model of drought-tolerant candidate genes in *I. domestica* by Cytoscape software. The red circles are hub genes.

5. Conclusions

In this study, we investigated the physiological changes and performed a transcription analysis of *I. domestica* roots in response to persistent drought by naturally reducing the soil water content. A total of 1230 DEGs were established in the root samples during the drought period. GO and KEGG enrichment analysis results showed that these DEGs mainly involve water deprivation, oxidative stress, metabolism, biosynthesis, and TFs responding to drought stress. Further analyses of the DEGs and TFs revealed that *WRKY51* (TRINITY_DN48213_c0_g7), glucan endo-1,3-beta-*glucosidase* (TRINITY_DN46661_c0_g3), and *CYP81B57* (TRINITY_DN58456_c4_g3) were hub genes involved in the drought stress response of *I. domestica*. Although these functional genes were successfully screened in this study, their gene function was not verified. Therefore, future studies should focus on the function of these genes under drought conditions. Overall, this study provides basic data for identifying drought response mechanisms in *I. domestica*.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae8121162/s1, File S1: Primer information for qRT-PCR. File S2: Clean data and assembly stats for *Iris domestica*. File S3: GO enrichment of DEGs under different stages of drought stress. File S4: KEGG enrichment of DEGs at different stages of drought stress. File S5: Transcription factor families.

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