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Transcriptomic Insights into Seed Germination Mechanisms of the Bamboo *Qiongzhuea tumidinoda*

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Abstract: Seed germination is a complex developmental process and a critical stage in plant development. The mechanism of seed germination in *Qiongzhuea tumidinoda* remains unclear. In this study, the transcriptomic analysis of four germination stages was conducted to reveal the regulatory mechanism. Totals of 2352, 5523, and 4533 differentially expressed genes (DEGs) were identified in S2 vs. S1, S3 vs. S1, and S4 vs. S1, respectively. A total of 998 DEGs were identified during seed germination. Enrichment studies indicated that the DEGs were mainly involved in plant hormone signal transduction and phenylpropanoid biosynthesis pathways. In addition, 131 transcription factors were differentially expressed, of which ERFs and MYBs may play pivotal roles in seed germination. To sum up, *TGA4*, *IAA24*, *SAUR32*, *AHK4*, and *HCT4* may regulate seed germination.

Keywords: *Qiongzhuea tumidinoda*; seed germination; transcriptome; expression pattern

1. Introduction

Seed germination is the first step for plant growth and development and is a crucial stage in a plant's life cycle, starting from imbibition and ending up with the protrusion of the radicle from the seed coat [1]. The reserve mobilization, metabolic activation, and vacuolization of endosperm cells become the earliest signs of germination, followed by embryo cell growth, endosperm rupture, testa rupture, and ultimately, radicle emergence [2]. Seed germination is a complex physiological, biochemical, and metabolic program, regulated by various genetic and environmental factors. The metabolism of carbohydrates, proteins, and fats provides energy for seedling growth during this period [3]. As a major source of cellular energy, carbohydrates are closely related to the seed germination process [4]. In plant seeds, starch is the main carbohydrate reserve and accumulates in the endosperm [5]. Starch and sucrose are metabolized through a complex and coordinated pathway by multiple enzymes, such as alpha-amylase (α-amylase), beta-amylase (β-amylase), and sucrose synthase [6]. Starch can be resolved into soluble glucose by α-amylase, which is induced by a lack of sugar and gibberellic acid (GA) [7]. GAMYB activates α-amylase expression by binding to GA-responsive elements in its promoter in rice [8].

The regulation of seed germination is also mediated by different phytohormones, such as GA, abscisic acid (ABA), and ethylene [9]. Seed germination is promoted by GA and inhibited by ABA and is determined by the relative ratio of GA/ABA [9]. Ethylene influences seed germination by reducing the effect of ABA [10]. The ABA-responsive central genes *ABA-INSENSITIVE 3* (*ABI3*), *ABI4*, and *ABI5* play important roles in regulating seed germination. *Arabidopsis* loss-of-function single mutants of *ABI3* resulted in losing viability during seed germination [11]. *ABI4* decreases seed germination by interacting with vitamin

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C defective 2 (VTC2) and respiratory burst oxidase homolog D (RbohD) and promoting ROS accumulation in *Arabidopsis* under salinity stress [12]. *ABI5* can inhibit seed germination by interacting with BRI1-EMS-suppressor 1 (BES1) [13]. *NTM1-like 8* (*NTL8*) acts in the regulation of GA-induced germination [14].

The mechanisms of seed germination in plants may be similar, such as plant hormone and radicle protrusion. Different plants have some of their own mechanisms, especially for gene expression regulation during germination. With the rapid development of high-throughput sequencing, RNA-seq has become an effective research tool in investigating the regulatory genes and mechanisms of seed germination, such as *Arabidopsis*, maize, soybean, wheat, and rice [15–19]. However, changes in the gene expression of seed germination in bamboo, especially in *Qiongzhuea tumidinoda*, are still obscure. As an important landscape plant, *Q. tumidinoda* is a specific bamboo species in southwest China, with high economic, ecological, and ornamental values [20]. Sexual propagation is limited in bamboo for the long flowering cycle and short seed viability, so asexual propagation is its main reproductive way. However, asexual propagation significantly reduces genetic diversity and adaptability [21]. Thus, studying seed germination is also important for selecting and breeding bamboo seedlings. In this study, we profiled progressively developing seeds from imbibition to radicle protrusion (10 mm) by a transcriptomic approach. GO analysis indicated the differential involvement of DEGs in biological processes in the four stages during seed germination. KEGG analysis suggested that the DEGs were mainly involved in plant hormone signal transduction and phenylpropanoid biosynthesis pathways. The expression patterns of the selected DEGs were validated using qPCR. These findings provide a valuable reference to investigate the molecular mechanism underlying seed germination in other bamboo plants.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

The seeds of *Q. tumidinoda*, used in this experiment, were harvested in April from Daguan County, Zhaotong City, Yunnan Province, China (104°00 N, 28°11 E). The seeds without lemma were soaked with 75% ethanol for 1 min and 1% sodium hypochlorite for 30 min and then flushed with distilled water three times. The seeds were planted in sterile Petri dishes with moistened filter paper and cultured in a 24 °C chamber under a 12 h light/12 h dark photoperiod. The seeds were collected with radicle lengths of 0 mm, 1 mm, 5 mm, and 10 mm (S1, S2, S3, and S4), frozen immediately in liquid nitrogen, and stored at −80 °C. Each sample was pooled with three seeds. Three biological replications were performed.

2.2. RNA Sequencing and Transcriptomic Analysis

The total RNA was isolated from the frozen seeds using a Plant Total RNA Isolation Kit (Vazyme Biotechnology, Nanjing, China), following the manufacturer's protocol. The RNA quality and concentration were verified using a Nanodrop 2000 spectrophotometer (Thermo Scientific, New York, NY, USA), and one microgram of RNA per sample was sent to Novogene Co., Ltd. (Tianjin, China) for RNA sequencing using an Illumina HiSeq platform. An average of 9.5 Gb of raw data for each sample was generated. The quality of the raw data was evaluated with FastQC (Babraham Institute, Cambridge, UK). Reads containing adapters or poly N or with low quality were cut to obtain clean data. The sequence duplication level, GC content, Q20, and Q30 were calculated for the clean data. The de novo assembly of the transcriptome was generated using Trinity software (version 2.1.1) [22]. All the assembled unigenes were annotated based on Nt, Nr, Pfam, KOG/COG, SwissProt, KEGG, and GO databases [23].

2.3. DEG Analysis

Gene expression levels were quantified based on fragments per kilobase per million mapped reads (FPKM) [23]. The differential expression analysis was conducted using DESeq2 software (version 1.4.5) [24]. Genes with a $|\log2(\text{FoldChange})| > 1$ and an adjusted p-value < 0.05 were regarded as DEGs [25]. The principal component analysis of the expression levels was used to estimate the differences among the samples. Heatmaps were generated using TBtools v2.210, according to our previous report [26]. The functional classification of the DEGs was analyzed using GO and KEGG enrichment analyses. The significantly enriched terms and pathways were selected with a corrected p-value < 0.05. The transcription factors were analyzed using Plant TFDB [27].

2.4. Weighted Gene Co-Expression Network Analysis (WGCNA)

The relationships between the identified genes and the germination stage were analyzed using WGCNA in R language according to previously reported methods [28]. The soft threshold was set at 12 ($R^2 > 0.9$) to ensure the network applicable to a scale-free topology. Co-expression modules were analyzed using the function 'blockwiseModules', with the default settings of the minModuleSize at 50 and the cutheight at 0.25.

2.5. The qPCR Analysis

Fifteen candidate genes were selected to detect the reliability of the RNA-seq by qPCR. The qPCR reactions were conducted according to our protocol [29]. The reactions were performed as follows: starting at 95 °C for 3 min, followed by 40 cycles at 95 °C for 10 s and at 60 °C for 30 s. The melting curve was implemented for confirming the specificity of the reactions, and *18S* was used as an internal reference gene. The primers were designed using Primer Premier software (version 5.0) and listed in Table S1. The relative expression levels of the genes were determined using the $2^{-\Delta\Delta CT}$ method [30]. Three biological replicates were performed for each sample. The mean transcript values were normalized using log2 fold to generate heatmaps with TBtools [26].

3. Results

3.1. Transcriptome Assembly and Annotation

To explore the molecular mechanism of *Q. tumidinoda* seed germination, the germinating seeds at S1, S2, S3, and S4 were collected (Figure 1A). Twelve libraries were constructed and subjected to RNA sequencing analysis. The Pearson's heatmap showed that there was a high correlation degree within each group of samples, indicating that all the samples had good biological reproducibility (Figure 1B). PCA clustered samples into four groups, consistent with the four stages of the germinating seeds (Figure 1C). These results indicated that the data had high quality.

The sequencing and assembly information was summarized in Table S2. More than 27 million raw reads were gained for each library, the number of which ranged from 27,430,370 to 35,700,035. After removing the low-quality data, 109.46 Gb of clean reads was generated, with the average reads for each sample being 9.12 Gb, and the Q20 and Q30 values were all higher than 97% and 92%, respectively. The average GC content was 55.24%, and the average mapped reads accounted for 71.22%. The clean reads from the 12 libraries were de novo assembled and merged into 95,230 unigenes using the Trinity approach. The N50 length and average gene length were 1091 and 1953 bp, respectively (Table S3). There were 33,103 unigenes with length < 500 bp, 29,389 unigenes with length ranging from 500 to 1000 bp, 18,786 unigenes with length ranging from 1000 to 2000 bp, and 13,952 unigenes with length > 2000 bp. These unigenes were matched with seven databases (NR, NT, KO, SwissProt, PFAM, GO, and KOG) to obtain annotation information

(Table S4). Of all the annotated unigenes, 48,092 (50.5%) unigenes annotated to the NR database, 54,334 (57.05%) unigenes annotated to the NT database, 15,931 (16.72%) unigenes annotated to the KO database, 32,722 (34.36%) unigenes annotated to the SwissProt database, 34,228 (35.94%) unigenes annotated to the PFAM database, 34,224 (35.93%) unigenes annotated to the GO database, and 8888 (9.33%) unigenes annotated to the KOG database. In total, 66,644 (69.98%) unigenes were assigned to at least one database.

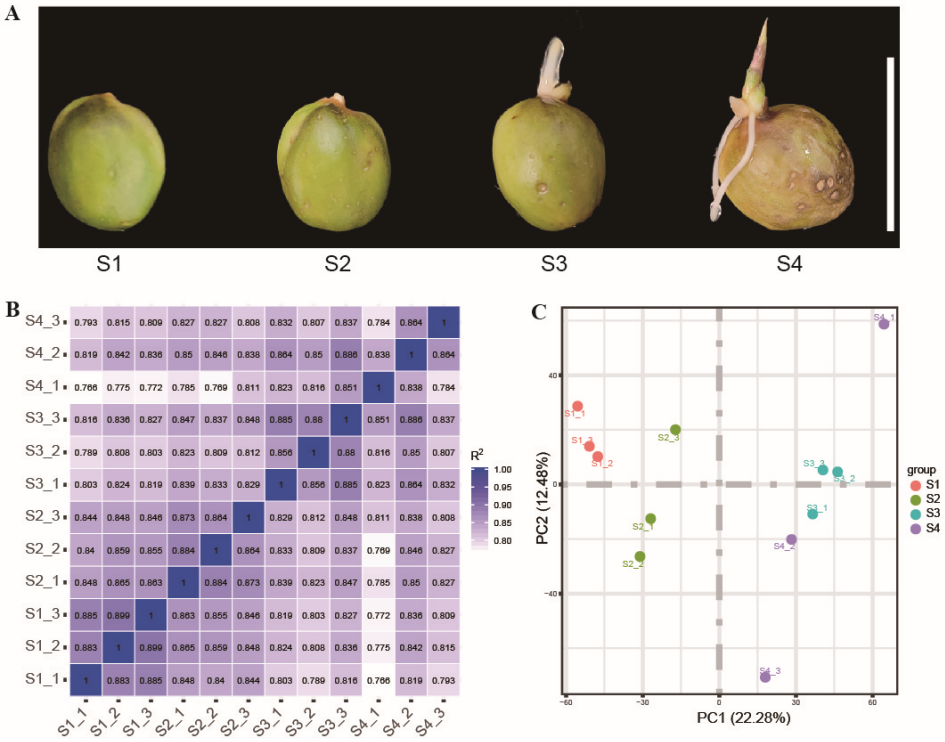


Figure 1. (**A**) Dynamic changes during the seed germination. Bar = 2 cm. (**B**) Correlation analysis heatmap of *Q. tumidinoda* seeds in four stages. Colors show the relevance level of each sample, ranging from low (white) to high (blue). (**C**) PCA of genes identified from *Q. tumidinoda* seeds in four stages.

3.2. DEGs During Seed Germination

DEGs were identified as follows: 2352 between S2 and S1 (1448 up and 904 down), 5523 between S3 and S1 (3322 up and 2201 down), and 4533 between S4 and S1 (2310 up and 2223 down) (Figure 2A). There were more DEGs in the S3 vs. S1 and S4 vs. S1 comparisons (the rapid extension period of hypocotyl in seed germination) than those in the S2 vs. S1 comparison. The number of upregulated DEGs was higher than that of the downregulated DEGs in S2 vs. S1, S3 vs. S1, and S4 vs. S1.

Phenylpropanoid biosynthesis (ko00940) was the most enriched pathway between S2 and S1, followed by fatty acid elongation (ko00062) and plant hormone signal transduction (ko04075). For S3 and S1, phenylpropanoid biosynthesis (ko00940) and plant hormone signal transduction (ko04075) were the top two pathways. Ribosome (ko03010) was the top pathway that the DEGs enriched, followed by the phenylpropanoid biosynthesis (ko00940), starch and sucrose metabolism (ko00500), and plant hormone signal transduction (ko04075) pathways in S4 and S1 (Figure 2B). These results showed that the phenylpropanoid biosynthesis and plant hormone signal transduction pathways may act in the seed germination of *Q. tumidinoda.*

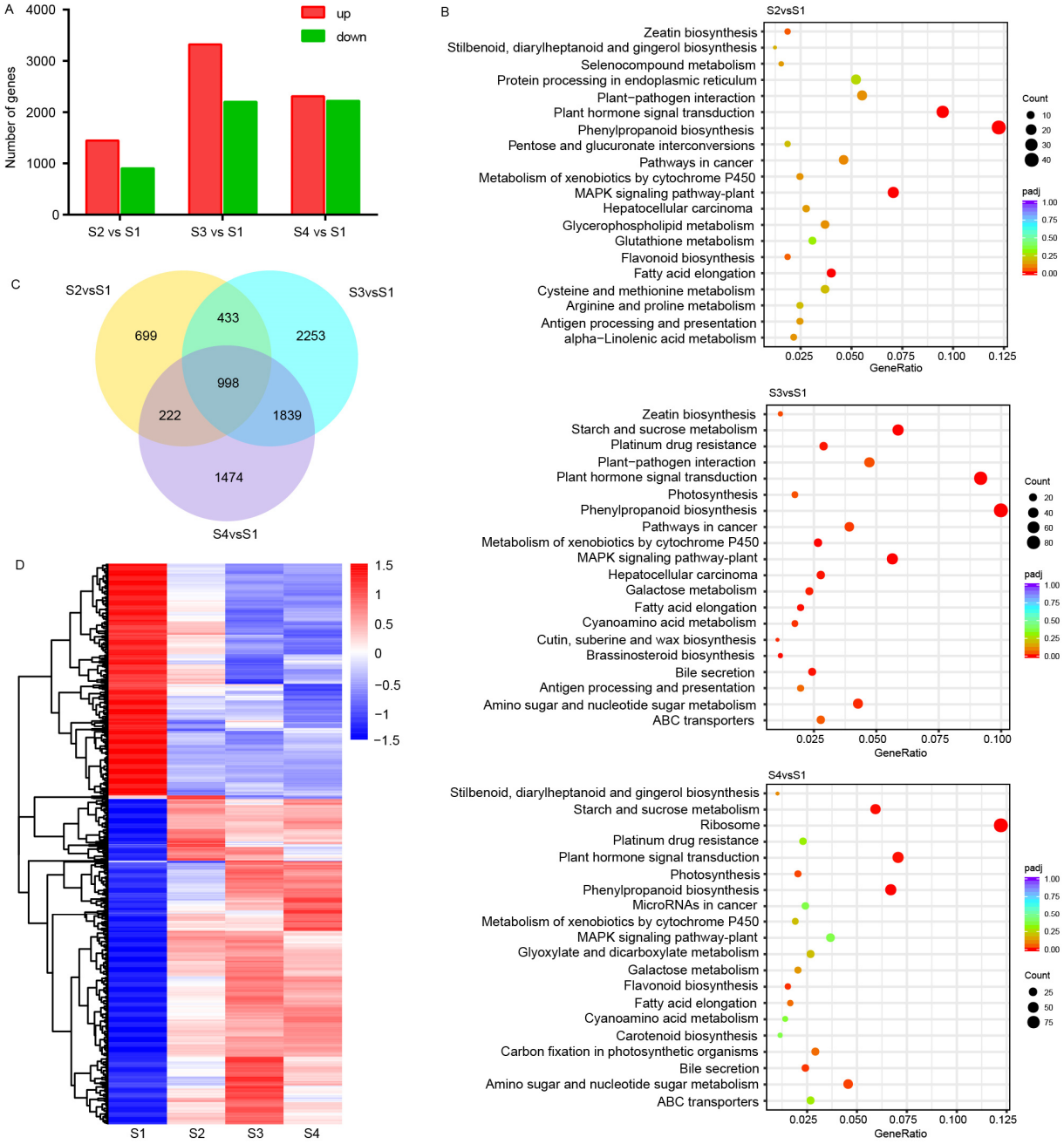


Figure 2. Preliminary analysis of the transcriptome of *Q. tumidinoda* seeds. (**A**) The number of DEGs identified in the developing seeds compared to those in S1. (**B**) Bubble diagrams of the enrichment of the KEGG pathways of S2 vs. S1, S3 vs. S1, and S4 vs. S1. (**C**) Venn diagrams showing overlapping of DEGs in four stages. (**D**) Heatmap DEGs in *Q. tumidinoda* seeds. The color scale at the right represented relative expression levels.

To further confirm the DEGs during the germination process, a Venn diagram was constructed among S2 vs. S1, S3 vs. S1, and S4 vs. S1 (Figure 2C). The number of DEGs shared between S4 vs. S1 and S3 vs. S1 was 2837, while that shared between S3 vs. S1 and S2 vs. S1 was 1431, and that shared between S4 vs. S1 and S2 vs. S1 was 1220, suggesting that the large numbers of DEGs continuously expressed in the rapid extension stage of hypocotylmay be related to seed germination. A total of 998 DEGs were found in all three comparisons. The heatmap of the 998 DEGs showed that 581 DEGs and 417 DEGs were upregulated and downregulated, respectively (Figure 2D).

3.3. GO and KEGG Enrichment Analyses

GO analysis was performed, and the top 30 enriched GO terms were selected for further analysis (Figure 3A and Table S5). According to the GO annotation, the DEGs were divided into three main categories: cellular component, biological process, and molecular function. The enriched DEGs were primarily observed in the GO terms of the transcription factor activity, sequence-specific DNA binding (GO:0003700), and nucleic-acid-binding transcription factor activity (GO:0001071). The GO terms related to oxidation were also significantly enriched, such as oxidoreductase activity (GO:0016705), peroxidase activity (GO:0004601), and antioxidant activity (GO:0016209). The DEGs related to these terms should be paid close attention to in further research.

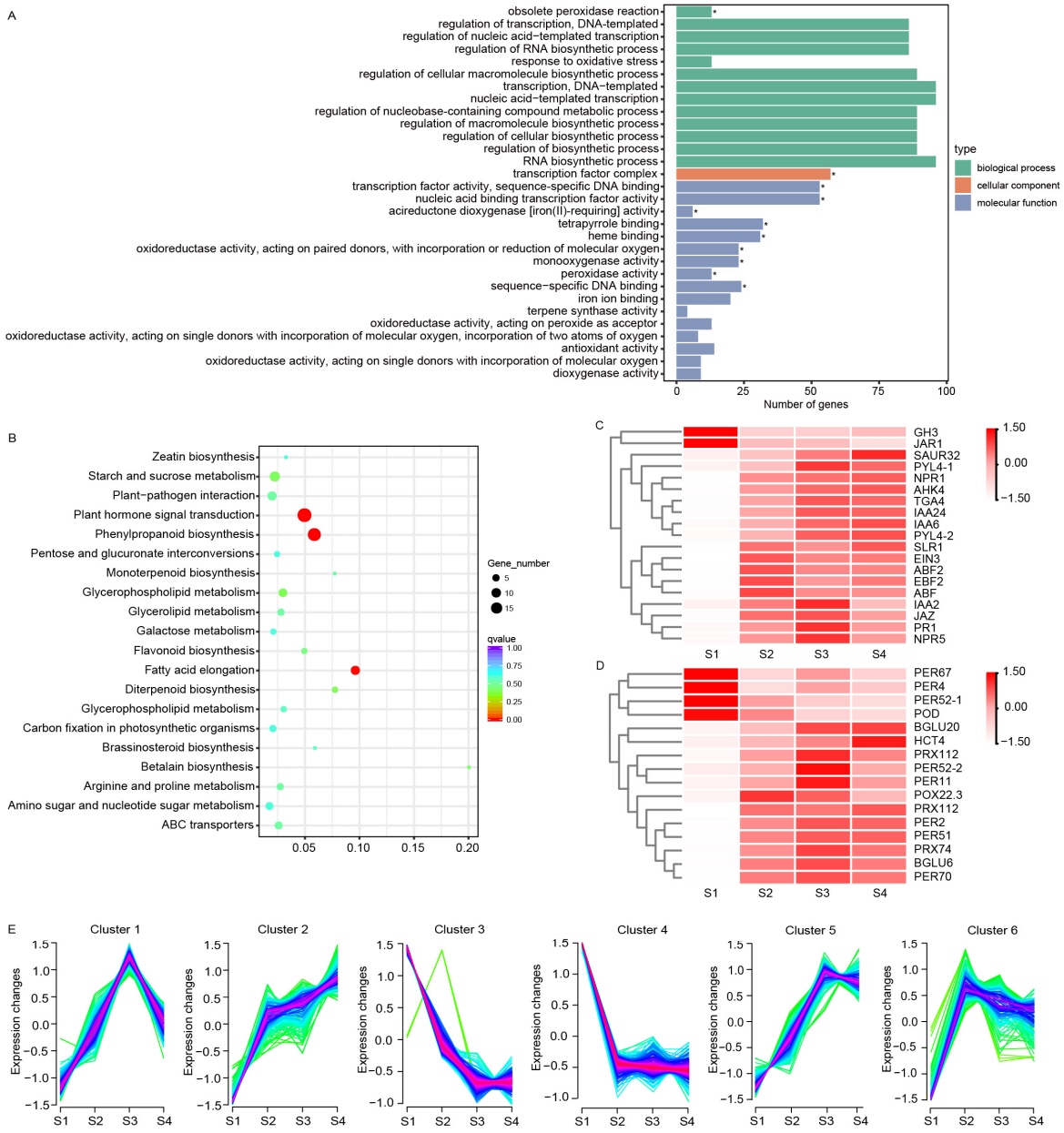


Figure 3. GO (**A**) and KEGG (**B**) enrichment analyses of DEGs in *Q. tumidinoda* seeds. Heatmaps of genes related to phenylpropanoid biosynthesis (**C**) and plant hormone signal transduction (**D**) in *Q. tumidinoda* seeds in four stages. (**E**) Cluster of DEGs in *Q. tumidinoda* seeds. * indicated the significantly enriched terms with p-value < 0.05.

KEGG enrichment analysis was also performed to investigate the potential roles of DEGs in seed germination (Figure 3B). Among these enriched pathways, plant hormone signal transduction (ko04075), phenylpropanoid biosynthesis (ko00940), and fatty acid elongation (ko00062) were the top three enriched categories which may be involved in the regulation of seed germination. Starch and sucrose metabolism (ko00500) was also in the top 20 enriched KEGG pathways. A total of nineteen DEGs (seventeen up and two down) and sixteen DEGs (twelve up and four down) were enriched in plant hormone signal transduction and phenylpropanoid biosynthesis (Figure 3C,D). These DEGs (like *SAUR32*, *TGA4*, *IAA24*, *AHK4*, and *HCT4*) may be involved in seed germination.

3.4. Co-Expression Network Analysis with WGCNA

Cluster analysis was used to classify the 998 DEGs into six clusters (Figure 3E). Gene expression levels rapidly peaked at S3 in cluster 1 and at S2 in cluster 6 and then quickly declined. The gene expressions in clusters 2 and 5 were elevated during germination. The expression trend of clusters 3 and 4 showed a consistently decreasing trend. Clusters 2, 3, 4, and 5 may be involved in seed germination. To further investigate the genes related to seed germination, the data were further analyzed using WGCNA. A total of 95,230 genes were assigned to eight co-expression modules with different colors (brown, red, blue, yellow, pink, turquoise, and green) (Figure 4A). The detailed information was listed in Table S6. Four gene modules exhibited the strongest correlation with seed germination (Figure 4B). Genes were closely linked to S1 in the red module (cor = 0.95), S2 in the brown module (cor = 0.98), and S4 in the pink and turquoise modules (cor = 0.94 and 0.9). KEGG enrichment analysis showed that the plant hormone signal transduction (ko04075), phenylpropanoid biosynthesis (ko00940), and MAPK signaling (ko04016) pathways were the top three enriched categories in the red module. Oxidative phosphorylation (ko00190) was the top enriched category in the brown module. Beta-alanine metabolism (ko00410) and endocytosis (ko04144) were the top two enriched categories in the pink module. Ribosome (ko03010) and ribosome biogenesis in eukaryotes (ko03008) were the top two enriched categories in the turquoise module (Figure S1). There were 109 common genes in the four clusters and four gene modules (Figure S2), of which *SAUR32* and *HCT4* acting in plant hormone signal transduction and phenylpropanoid biosynthesis pathways may play the key roles.

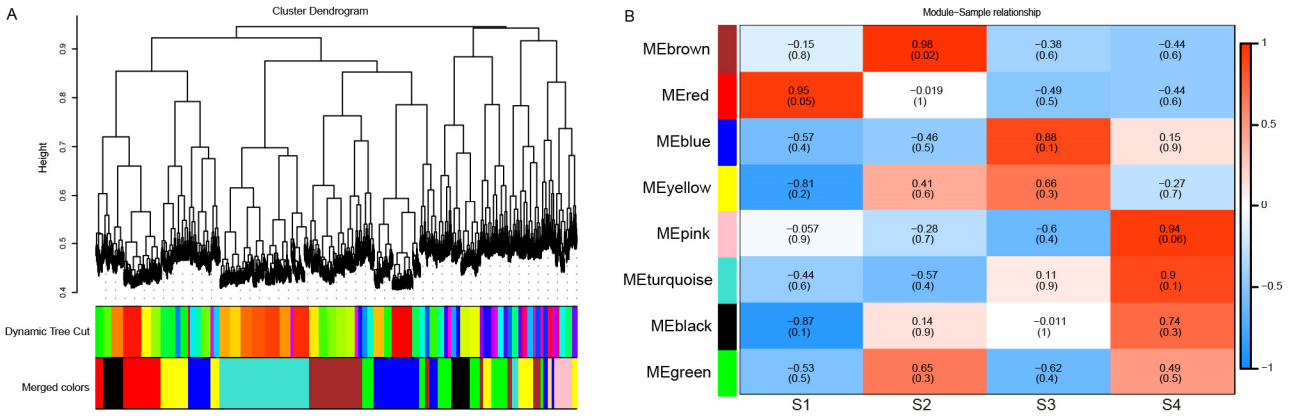


Figure 4. WGCNA-based identification of the key genes associated with seed germination. (**A**) Hierarchical clustering tree indicated the co-expression modules identified using WGCNA at different stages. Different modules were identified by different colors. A gene was represented by each leaf of the cluster tree. (**B**) The heatmap chart showing module–trait relationships. Red and blue indicated the positive and negative correlations between the module and stages.

3.5. Analysis of Differentially Expressed Transcription Factors

The 998 DEGs encode 131 transcription factors (93 upregulated and 38 downregulated) from 27 families (Figure 5A). The largest families were MYB and ERF (13, 9.92%), followed by WRKY (10, 7.63%), which may have great effects on regulating seed germination (Figure 5B). The heatmap of 13 MYBs and 13 ERFs showed that most MYBs and ERFs were upregulated during seed germination (Figure 5C,D). The KEGG analysis showed that 131 differentially expressed transcription factors were significantly enriched in the term of the plant hormone signal transduction (Figure 5E). Cluster analysis was used to classify one hundred thirty-one transcription factors into six clusters (Figure 5F). The expression levels of the transcription factors rapidly peaked at S3 and then quickly declined in cluster 1 and slightly in declined in cluster 2. The gene expressions in clusters 4 and 5 were elevated during germination. The expression trends of clusters 3 and 6 showed a consistently decreasing trend. The genes in clusters 3, 4, 5, and 6 were considered as the key transcription factor genes, like *ERF1*, *ERF12*, *MYB30*, and *MYB44*.

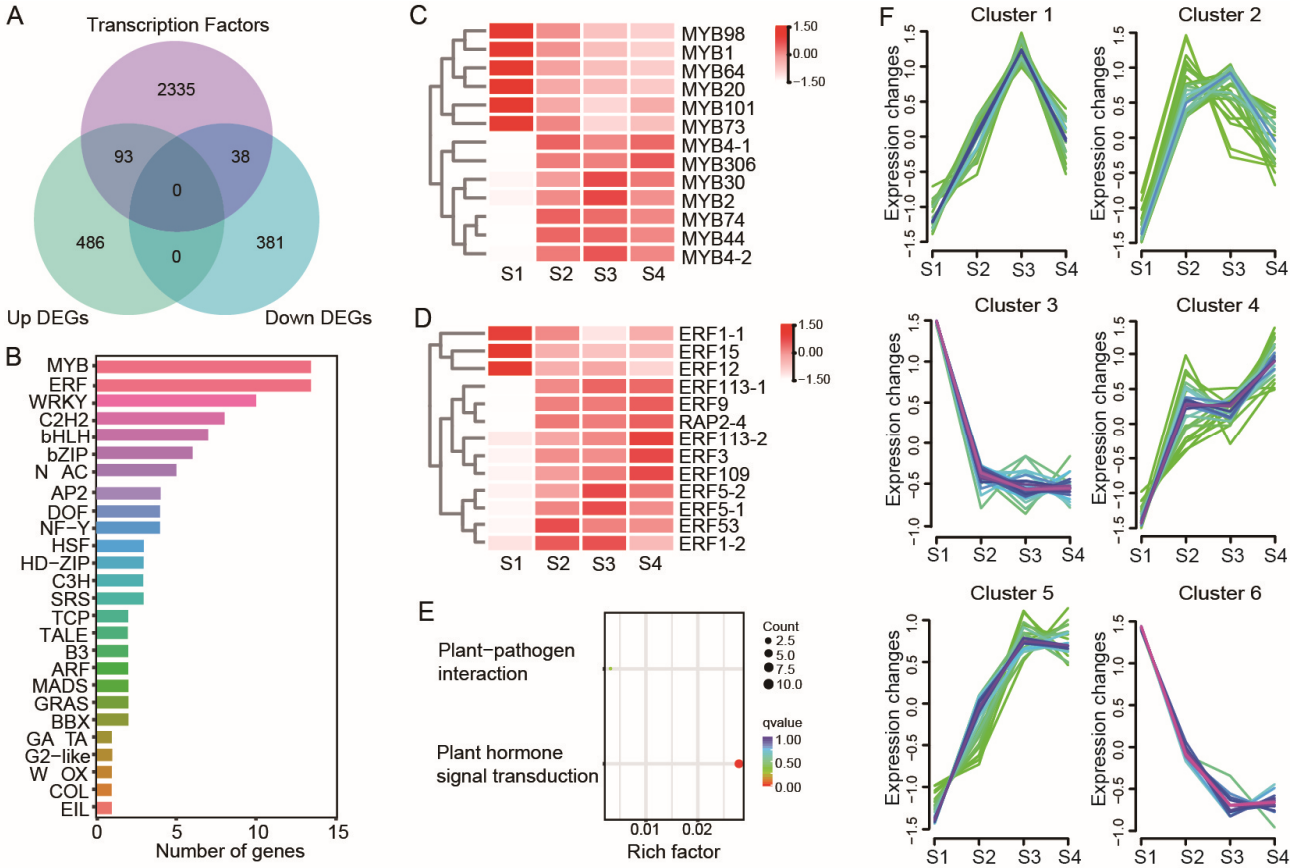


Figure 5. (**A**) Venn diagrams showing overlapping of DEGs and transcription factors. (**B**) The main families of differentially expressed transcription factors. The heatmaps of differentially expressed MYBs (**C**) and ERFs (**D**) in *Q. tumidinoda* seeds. (**E**) KEGG enrichment analysis of differentially expressed transcription factors in *Q. tumidinoda* seeds. (**F**) The expression changes of the DEGs in six clusters in different stages.

3.6. The qPCR Validation

Fifteen candidate genes were randomly selected to detect the reliability of RNA-seq using qPCR (Figure 6). These candidate genes were mainly selected from the DEGs related to plant hormone signal transduction (*SAUR32*, *TGA4*, *IAA24*, *AHK4*, *EIN3*, *PYL4-1*, *NPR5*, and *PR1*) and phenylpropanoid biosynthesis (*HCT4*, *PER70*, and *PRX112*). Four transcription factors (ERF1, ERF12, MYB30, and MYB44) belonging to the ERF and MYB gene families

were also selected. The qPCR results were consistent with the RNA-seq results, suggesting the accuracy and reliability of RNA-seq.

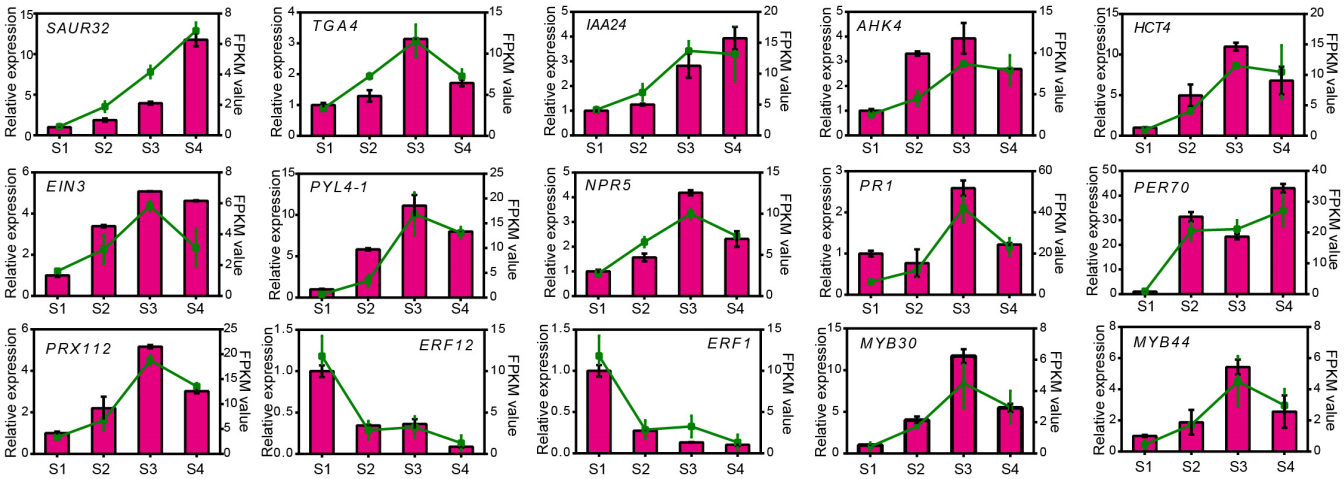


Figure 6. The expression levels from qPCR (bars) and FPKM from RNA sequencing (lines) of the selected genes. The qPCR results were analyzed based on three biological and three technical replicates.

4. Discussion

Seed germination begins with water uptake and ends with radicle emergence. The radicle growing out of the seed is a key stage during germination. Seed germination is a complex physiological and biochemical process and controlled by the interactions of many external and internal factors [1]. Gene expressions related to phytohormones, carbohydrates, cell-wall metabolism, transcription, and translation changed in this process [31]. In this study, twelve libraries for the transcriptome sequencing of *Q. tumidinoda* seeds at four stages were constructed. Altogether, 109.46 Gb of clean data was obtained, the average reads were 9.12 Gb, the base of the Q30 percentage was 92%, and 95,230 unigenes were annotated (Table S2). There were more upregulated genes than downregulated genes in S2 vs. S1, S3 vs. S1, and S4 vs. S1. A total of 998 DEGs were obtained during seed germination. The enrichment analysis of the KEGG pathways showed that the DEGs were significantly enriched in plant hormone signal transduction and phenylpropanoid biosynthesis pathways. Thus, it was proposed that these two pathways were significantly related to *Q. tumidinoda* seed germination.

Plant hormones play important roles in seed germination, and nearly all the plant hormones were involved in regulating seed germination, including abscisic acid (ABA), auxin indole-3-acetic acid (AUX), ethylene (ET), cytokinin (CTK), and salicylic acid (SA) [32]. ABA is the key hormone that represses seed germination, whereas auxin, JA, and ET retain and induce seed germination by controlling ABA signaling [9]. The ABA signal transduction pathway contains three major components, SNF1-related protein kinase 2 (SnRK2), type 2C protein phosphates (PP2Cs), and PYR/PYL/RCAR (ABA receptors). ABA binds to PYR/PYL/RCAR and forms ternary complexes with PP2Cs, thereby abrogating the inhibitory effects on SnRK2, resulting in the activation of the ABA signaling pathway [33]. In the present study, genes encoding PYL4 were upregulated during the seed germination (Figure 3C), suggesting that ABA may regulate the seed germination. AUX is a vital signaling molecule acting in plant development, seed dormancy, and germination [34]. *IAA2/6/24* and *SAUR32* were highly upregulated, similar to previous results obtained for *Fritillaria taipaiensis* P.Y.Li [35], suggesting that auxin may positively regulate the seed germination of *Q. tumidinoda.* Small auxin-up RNA (SAUR) is an important gene family involved in the auxin signaling pathway [36]. The seed germination of *saur32* is less sensitive

to ABA compared with that of the wild type [37]. *SAUR32* and *SAUR76* are involved in cell division [37,38]. Ethylene can interfere with ABA signaling to counteract ABA inhibition and promote seed germination under stress conditions [39]. Overexpressing *EIN3* enhances seed germination under stress [40]. In this work, one *EIN3* gene was upregulated during seed germination. Genes acting in ethylene signal transduction mostly were increased, suggesting that ethylene may positively participate in *Q. tumidinoda* seed germination (Figure 3C). Genes involved in cytokines and SA were also differentially expressed during seed germination, which may be pivotal for seed germination in *Q. tumidinoda* CTK positively regulates seed germination and functions by interacting with ABA signaling in dicot species. A DEG annotated as *AHK4* was found ewith the increasing trend, which is a cytokinin-binding receptor and transduces cytokinin signals across the membrane, was found and showed an increasing trend [41]. This implied that CTK may play a positive role in the seed emergence process. SA is involved in plant defense system and elevates plant tolerance to biotic and abiotic stresses [42]. However, information on the role of SA in seed germination is still limited. Herein, four DEGs (*NPR1*, *NPR5*, *TGA4*, and *PR1*), annotated as SA signaling genes, were found with upregulation trends, which was consistent with the results obtained for *Polygonatum cyrtonema* Hua [32]. *NRT1.1* and *TGA4* induce root hair development by suppressing the longitudinal elongation of trichoblast cells under nitrate treatment [43]. Therefore, SA may act in seed germination in *Q. tumidinoda*, and the functions of related genes need further investigation. Overall, these findings indicated that these genes involved in plant hormone signal transduction play the key roles in seed germination in *Q. tumidinoda*.

The process of seed germination contains the formation of a series of secondary metabolites, generated from phenylpropanoid biosynthesis. Phenylpropanoids are the most important component of plant secondary metabolites in seed development [44]. Seed germination in foxtail millet is closely connected with phenylpropanoid-related pathways under drought stress [45]. Several studies have reported that phenolic substances regulate seed germination, including yellow-seeded rapeseed (*Brassica napus*) and *Arabidopsis* Heynh. transparent testa mutants [46,47]. Flavonoids are involved in plant growth and development, like pathogen resistance and seed coat development [48]. In this study, a shikimate O-hydroxycinnamoyltransferase gene (*HCT4*) with an upward trend was found (Figure 3D). The promotional role of shikimate in seed germination has been reported in rice [49]. *HCT*s play dual roles downstream as well as upstream of the 3-hydroxylation step in the phenylpropanoid pathway [50]. HCTs control the turnover and biosynthesis of major phenolic compounds in plants, for example, lignin and chlorogenic acid [51]. The upregulations of HCTs areare associated with the accumulation of C and H lignins in *Paphiopedilum armeniacum* seed, which enhances the stability and hydrophobicity of the plant tissue [52]. The vast majority of the DEGs involved in the phenylpropanoid biosynthesis pathway belonged to the peroxidase gene family and were upregulated during seed germination (Figure 3D), similar to findings in maize seeds [53]. The GO terms related to oxidation were also significantly enriched, like peroxidase activity (Figure 3A). The *peroxidase* genes function in cellular homeostasis and oxidative stress tolerance [54]. These results indicated that *HCT4* and peroxidase genes may play important roles in seed germination.

Starch metabolism also plays important roles in seed germination. During germination, starch is degraded by α-amylase to provide energy sources for germination and precursors for growth [7]. The starch and sucrose metabolism (ko00500) pathway was significantly enriched in S4 vs. S1 according to the KEGG analysis (Figure 2B). Genes related to starch degradation have been identified. The transcriptomic results showed that the level of the gene encoding α-amylase (AMY) was increased during seed germination, suggesting

that starch in *Q. tumidinoda* seed was also degraded to contribute to the energy needed for germination.

A total of 131 differentially expressed transcription factors were identified during the seed germination process. Consistent with a previous study [9], the MYB and ERF families were the largest families (Figure 5B), suggesting that these transcription factor families may play essential roles in seed germination. ERF family genes are thought to act in water absorption and abscisic acid signaling in rice seed germination [55]. MYBs promote seed dormancy by regulating genes related to ABA synthesis [56]. In this study, 13 MYBs and 13 ERFs were significantly changed (Figure 5C,D), implying that they may mediate seed germination in *Q. tumidinoda*.

Compared with the known germination genes in model plants, such as rice and *Arabidopsis* [57,58], *ERF12*, *MYB30*, *PYL*s, *ABF*s, *JAZ*, and *AMY* were also detected in *Q. tumidinoda*, with the similar expression trends, suggesting that the regulatory mechanism of seed germination may be conservative in plants. However, some germination genes in rice and moso bamboo [59] were not differentially expressed in *Q. tumidinoda*, like *GA3ox2* and *GA2ox*s, which may because of the specificity of the species. The discovery of the potential roles of candidate genes will open up new avenues to investigate the mechanisms of *Q. tumidinoda* seed germination. The transcriptome can only represent gene changes at the transcriptional level. Combined with the proteome, the transcriptome will better reveal gene changes at the protein level in the future. Future studies may also analyze the functions of the identified candidate genes through CRISPR or overexpression assays.

5. Conclusions

In this work, the molecular regulation of the seed germination in *Q. tumidinoda* was investigated using transcriptomic analysis. Transcriptomic analysis identified 998 DEGs during *Q. tumidinoda* seed germination. KEGG analysis showed that the plant hormone signal transduction and phenylpropanoid biosynthesis pathways were significantly enriched and may have important roles in seed germination. In addition, 131 transcription factors were differentially expressed, of which ERFs and MYBs may play the key roles in seed germination. To sum up, *TGA4*, *IAA24*, *SAUR32*, *AHK4*, and *HCT4* may regulate seed germination. These results strengthen our understanding of the regulation mechanism of seed germination and provide a theoretical basis for the breeding of *Q. tumidinoda* in the future.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae11040430/s1: Figure S1: Bubble diagram of the enrichment of the KEGG pathway of four gene modules. Figure S2: Venn diagrams showing overlapping of metabolites in WGCNA and mfuzz analysis. Table S1: Primers used in this study. Table S2: Summary of the transcriptome sequencing data of the 12 libraries. Table S3: Results of transcriptome unigenes. Table S4: The annotation information of the unigenes. Table S5: The top 30 enriched GO terms of the DEGs. Table S6: The genes in different modules.

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