

Analysis of the transcriptome revealed the molecular mechanisms underlying the growth of *Dendrobium nobile* under various epiphytic modes

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Abstract

Dendrobium nobile is a highly medicinal and ornamental orchid that is usually cultivated in various epiphytic modes. The gene expression and stem development of *D. nobile* can be influenced by its epiphytic modes. However, the relationship between genes and agronomic traits in this specie remains unclear. In this study, we collected two-year-old stems of *D. nobile* from different epiphytic modes for transcriptome sequencing. The aim of this study was to provide a theoretical basis for improving field cultivation and artificial breeding. We identified 239 common differential expression genes (cDEGs) among the different modes. These genes were mainly assigned to three pathways in KEGG analysis: MAPK signaling pathway-plant, flavonoid biosynthesis, and plant-pathogen interaction. Additionally, GO analysis revealed enrichment of some DEGs in response to chitin (13 cDEGs), salt stress (12 cDEGs), and water deprivation (10 cDEGs). Furthermore, through weighted gene co-expression network analysis, we detected 72 cDEGs that were correlated with stem length. Finally, we observed that the expression level of PR1 exist corrected with TGA2.2 and TGA1. Overall, this study identifies 72 candidate genes that may regulate the growth of *D. nobile* stems, providing basic evidence for designing a genetic breeding study.

1. Introduction

Dendrobium nobile, a perennial epiphytic herb of the genus *Dendrobium* in Orchidaceae, is one of the main species recorded in the Chinese Pharmacopoeia with both ornamental and medicinal value (Fan et al., 2023). However, over-exploitation of wild resources has led to the near depletion of *D. nobile*. In order to address the issue of low yield and high demand, various methods of artificial cultivation, such as greenhouse cultivation, stone epiphytic, and tree epiphytic techniques, have become popular for planting *D. nobile* (Zhong et al., 2022; Tan et al., 2020). Nonetheless, the molecular mechanism responsible for the differences in agronomic traits resulting from different modes of epiphytic growth remains unclear. Therefore, it is crucial to comprehend the molecular distinctions in *D. nobile* grown under different epiphytic modes for field planting and genetic enhancement purposes.

As a perennial epiphytic medicinal plant, the quality of *D. nobile* can be influenced by its epiphytic growth habits. Currently, the main cultivation substrates for *D. nobile* are pine bark, peat, sawdust, and coconut bran. Hu et al. discovered that the growth of *D. nobile* seedlings improved when different substrates, such as fungus residue, pine bark, and water moss, were mixed in specific proportions (Hu et al., 2019). Li et al. demonstrated that the growth state and chemical compositions of *D. nobile* could also be altered by changing the environmental conditions of cultivation (Li et al., 2018). Additionally, Guo et al. indicated that sawdust proved to be the best substrate for cultivation (Guo et al., 2016). Thus, these previous studies primarily focused on examining the effect of various epiphytic growth modes on the growth of *D. nobile*. Nonetheless, the molecular mechanism underlying the differences in agronomic traits of *D. nobile* at a molecular level has yet to be thoroughly investigated.

Plant growth is heavily influenced by environmental stresses such as drought, salt, and oxidative stress, which trigger changes in the expression of response genes, including transcription factors and defense-

related genes (Bashir et al., 2019). *MYB*, an important transcription factor, can regulate the expression of defense-related genes such as *PR1* (Wang et al., 2021). Pathogenesis-related protein (PR1) is a crucial defense protein that helps plants resist biotic and abiotic stresses. Over-expression of *GhMYB36* has been shown to enhance the expression of *PR1* under drought stress in *G. hirsutum* (Liu et al., 2022). Additionally, *PR1* has been found to be synergistically activated by *WRKY50* and *TGA* transcription factors in *Arabidopsis* (Hussain et al., 2018). In this study, we hypothesize that different epiphytic modes can induce different environmental stresses in *D. nobile*, which in turn influence its growth and development. We speculate that certain transcription factors and defense-related genes are involved in this response.

With the advance of the transcriptome sequencing, an increasing number of medicinal plants have been sequenced to uncover their molecular mechanism of growth and development in different conditions, such as *Bletilla striata* (Zou et al., 2021), *Dendrobium officinale* (Zhang et al., 2021), and *Lycium ruthenicum* (Qin et al., 2022). Building upon our previously constructed nucleotide database of *D. nobile* using PacBio SMRT (Li et al., 2022), this study outlines the transcriptomic profiling of 2-year-old stems of *D. nobile* cultivated in various epiphytic modes. Furthermore, we compared the agronomic characteristics of *D. nobile* under different epiphytic modes. Lastly, we employed weighted gene co-expression network analysis (WGCNA) to identify key genes related to stem length. Overall, our objective was to identify potential candidate genes for further investigation that are involved in the growth of *D. nobile*.

2. Materials and Methods

2.1 Plant materials

The stems of *D. nobile* were used as the research materials for this study. With a same altitude and environment, *D. nobile* plants were originally cultivated under four different epiphytic modes to provide a varied sampling pool. The epiphytic modes included Danxia stone (DS), Crushed stone (SS), Sawdust (JM), and Stump (SZ) (Figure S1). These plants were grown at the same altitude and in the same environment. Approximately 6 or 7 healthy stems, aged 2 years, were randomly selected from the Dendrobium Germplasm Garden of Chishui Xintian Company (105°740E, 28°560N) for each epiphytic pattern. Prior to sampling, the plant height and stem length were measured using a ruler. The diameter of the sampled stems was then measured using a vernier caliper, and the number of stem nodes was counted. Subsequently, the measured values for each agronomic trait within the same epiphytic pattern were averaged for further analysis. All stems were processed according to a previously described protocol. The samples were divided into four groups based on their planting modes, and each group consisted of three biological replicates for sequencing.

2.2 RNA extraction, cDNA library, and Illumina sequencing

Total RNA was extracted from each sampled stem using Trizol (Invitrogen, CA, USA) following the manufacturer's instructions. The extracted RNA, with an RIN number > 7.0, was further purified from the

total RNA (5 ug) using poly-T oligo-attached magnetic beads. Following purification, the mRNA was fragmented into small pieces using divalent cations at elevated temperatures. Subsequently, the cleaved RNA fragments were reverse-transcribed to generate the final cDNA library, according to the protocol for the mRNASeq sample preparation kit (Illumina, San Diego, USA). The average insert size for the paired-end libraries was 300 bp ($\pm$ 50 bp). Paired-end sequencing was performed on an Illumina Nova seq™ 6000.

2.3 De novo assembly, unigene annotation and functional classification

De novo assembly, unigene annotation, and functional classification were performed as described in our previous study (Li et al., 2022). In brief, De novo assembly of the transcriptome was performed with Trinity 2.4.0 (Grabherr et al., 2011). Trinity groups transcripts into clusters based on shared sequence content. Such a transcript cluster was very loosely referred to as a gene. The longest transcript in the cluster was chosen as the gene sequence (aka Unigene). Then, all assembled Unigenes were aligned against the non-redundant (Nr) protein database (<http://www.ncbi.nlm.nih.gov/>), Gene Ontology (GO) (<http://www.geneontology.org>), SwissProt(<http://www.expasy.ch/sprot/>), Kyoto Encyclopedia of Genes and Genomes (KEGG)(<http://www.genome.jp/kegg/>) and eggNOG (<http://eggnogdb.embl.de/>) databases using DIAMOND with a threshold of E value < 0.00001 (Buchfink et al., 2015).

2.4 Differential expression genes analysis

To analyze the differential expression of genes, we used the Salmon tool to count the expression levels of each assembled unigene (Patro et al., 2017). The expression levels were calculated using the TPM method (Mortazavi et al., 2008). The differentially expressed genes (DEGs) were selected based on the following criteria: $\log_2$ (fold change) > 1 or $\log_2$ (fold change) < -1, and statistical significance with a p-value < 0.05. We performed this analysis using the R package edgeR (Robinson et al., 2010).

Subsequently, we screened for overlap between the DEGs in the DS vs JM and DS vs SZ comparisons for further analysis.

2.5 Weighted gene co-expression network analysis

We constructed gene co-expression networks using the WGCNA package in the R software (version 4.0.5) (Langfelder et al., 2008). The common DEGs obtained from the previous analysis were further divided into three modules using WGCNA. We also calculated the correlation among different modules. To examine the associations between the modules and stem length, we estimated module-trait associations through correlation analysis.

2.6 Quantitative real-time PCR (qRT-PCR) analysis

To validate the accuracy of the transcriptome data, we selected five differential genes (DEGs) for qRT-PCR verification. We used 2-year-old stems of *D. nobile* from the DS, JM, and SZ as materials. Total RNA extraction was performed using a BIO FIT polysaccharide polyphenol biological kit, and cDNA was synthesized using a Tiangen reverse transcription kit. For each sample, we performed three technical

replicates of the qRT-PCR assay with three biological replicates. Gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The primers used for qRT-PCR can be found in Table S1.

2.7 Statistical analysis

We analyzed and visualized the stem data using R language (version 4.0.5). For picture assembly, we used Adobe Illustrator CS6 software (version 2021). To determine significant differences, we conducted one-way ANOVA followed by Dun-can's test. Statistical significance was identified at a p-value < 0.05.

3. Results

3.1 Epiphytic mode affects the agronomic traits

To study the differences in the agronomic traits of *D. nobile*, four different agronomic traits were measured for each of the four different epiphytic modes (Figure S1). The plant height in the SS mode was the highest, with an average of 33 cm, and there was a significant difference between Crushed stone (SS) and Danxia stone (DS), Sawdust (JM) and Stump (SZ) modes (Fig. 1A). The stem length in the DS mode was the shortest, with an average of 16.3 cm, and there was a significant difference between DS and SS, JM, and SZ modes (Fig. 1B). The stem diameter in the JM mode was the highest, with an average of 10.34 mm, and there was a significant difference between JM and SS modes (Fig. 1C). The number of stem nodes in the SS mode was the highest, with an average of 9, and there was a significant difference between DS and SS, JM, and also between SS and SZ modes (Fig. 1D). Interestingly, the average values of plant height, stem length, stem diameter, and number of stem nodes in the DS mode were the lowest.

3.2 Transcriptome sequencing quality statistics

To analyze the molecular mechanism underlying *D. nobile's* response to the four different epiphytic modes, the stems of 2-year-old plants were collected with three biological repeats for RNA-seq analysis, resulting 5.42–7.84 Gb of clean data for each sample (Table S1). A total of 574,679,536 reads were obtained through RNA-seq of all samples, with an average of 48,039,961 reads detected per sample. After removing low-quality data (including sequences with connectors and low quality), we obtained 561,314,055 valid sequences, which accounted for 97.67% of the total number of sequences. The average GC content of the samples was 46.34%. The length distribution of splicing genes is shown in Fig. 3. The figure shows that 42.68% of genes had a length distribution between 200-300bp, and only 0.03% of genes had a length lower than 200bp (Fig. 2A). Additionally, 42,389 genes were annotated in the NR database, with 37,521 genes annotated from *D. catenatum*, 1,220 from *Phalaenopsis equestris*, 618 from *Quercus. suber*, and the remaining genes annotated from other species (Fig. 2B). Finally, a sample correlation analysis showed that the samples collected from DS, JM, and SZ remained highly similar, while those collected from SS were distinct (Figure S2). This result indicates that the data related to SS samples will be excluded from further analysis.

3.3 Screening of common DEGs

Differential expression genes (DEGs) were screened based on the principle of $FDR < 0.05$ and $|\log_2FC| > 1$. The number of DEGs (Fig. 3A) between DS and SZ, DS and JM, JM and SZ was 2244 (662 up-regulated and 1582 down-regulated), 1401 (573 up-regulated and 828 down-regulated), and 849 (510 up-regulated and 339 down-regulated) respectively. Figure 1B illustrates that the average stem length in DS was lower compared to the other groups. Hence, the common DEGs (cDEGs) between DS vs JM and DS vs SZ were selected for further analysis. The Venn diagram depicted a total of 698 cDEGs (Fig. 3B). The expression levels of these 698 cDEGs were displayed in a cluster heat map (Fig. 3C). Out of the 698 cDEGs, 239 were annotated for further GO and KEGG analysis. It is worth noting that among these cDEGs, DS had 175 up-regulated and 64 down-regulated genes, and the expression trend of each cDEG was consistent between DS vs SZ and DS vs JM.

3.4 Analysis of KEGG and GO of 239 cDEGs

The function of the 239 cDEGs was analyzed using GO and KEGG analysis, and the results are presented in Fig. 5. The GO analysis showed enrichment mainly in three functional categories: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) (Fig. 4A). The KEGG analysis classified the results into five categories: Metabolism, Genetic Information Processing, Environmental Information Processing, Cellular Processes, and Organismal Systems (Fig. 4C).

The GO functional annotation results revealed that the 239 cDEGs were primarily annotated in 622 aspects, of which 166 aspects were statistically significant with a p -value < 0.05 . The top 50 GO terms (Fig. 4A) showed that the main biological processes were the regulation of transcription, DNA-templated (29 genes), DNA-binding transcription factor activity (27 genes), and oxidation-reduction process (15 genes). The top cellular components were the nucleus (72 genes), cytoplasm (49 genes), and cytosol (30 genes). As for molecular function classification, the prominent terms were protein binding (40 genes), DNA-binding transcription factor activity (27 genes), and ATP binding (23 genes). Notably, the top three significant GO terms were response to chitin (13 genes), secondary metabolite biosynthetic process (12 genes), and DNA-binding transcription factor activity (29 genes) (Fig. 4B).

A total of 239 DEGs that overlapped were annotated in 66 pathways through KEGG analysis. Among these pathways, 11 pathways were found to be statistically significant with a p -value of less than 0.05. Figure 4D displays the top 20 pathways, which include MAPK signaling pathway-plant (dct04016, zma04016, han04016, nsy04016, thj04016, smo04016), flavonoid biosynthesis (dct00941, nnu00941), plant-pathogen interaction (csat04626, dct04626, han04626, jcu04626, aly04626), Circadian rhythm (dct04712, dct04712, zma04712, bdi04712), Phenylpropanoid biosynthesis (cic00940, dct00940, sita00940, rcu00940), alpha-Linolenic acid metabolism (dct00592, han00592), Ubiquinone and other terpenoid-quinone biosynthesis (dct00130), Linoleic acid metabolism (dct00591, cann00591), Carotenoid biosynthesis (dct00906, egr00906, zma00906), Phosphatidylinositol signaling system (zma04070, dct04070, smo04070), and other pathways. Among them, MAPK signaling pathway-plant, plant-pathogen interaction, and flavonoid biosynthesis were the top three significant pathways, which are crucial for the growth of a plant under environmental stresses.

3.5 A weighted gene co-expression network analysis (WGCNA) of 239 cDEGs

To further understand the function of co-expression genes, 239 overlapped DEGs were analyzed by a weighted gene co-expression network (Fig. 5). These overlapped DEGs were divided into three different modules including Meblue (66 genes), Mebrown (167 genes), Megrey (6 genes) (Fig. 5A). Moreover, the co-expression levels of some genes in the same module were extremely high (Fig. 5B). In addition, as an important index of growth, the stem length was selected for correlation analysis with these cDEGs in three modules. The result displayed that these genes in Megrey ($r = -0.67$, $p\text{-value} = 0.049$) and Meblue ($r = 0.72$, $p\text{-value} = 0.030$) were significantly correlated with the stem length (Fig. 5C).

Subsequently, these genes in Meblue and Megrey were analyzed using GO analysis and KEGG analysis. The results of the GO analysis showed that genes in Meblue were mainly annotated in response to chitin, calcium ion binding and regulation of striated muscle contraction (Figure S3A), while genes in Megrey were mainly annotated in chalcone biosynthetic process, naringenin-chalcone synthase activity and cannabinoid biosynthetic process (Figure S3B). The results of the KEGG analysis showed that genes in Meblue were mainly annotated in Plant-pathogen interaction, Phosphatidylinositol signaling system and MAPK signaling pathway-plant (Figure S3C), while genes in Megrey were annotated in Flavonoid biosynthesis, Circadian rhythm-plant, MAPK signaling pathway-plant (Figure S3D). Consequently, the plant-pathogen interaction pathway was selected for further analysis.

3.6 Analysis of plant-pathogen interaction pathway related genes and *PR1* gene

KEGG analysis of Meblue genes (66 genes) revealed that the plant-pathogen interaction pathway was significantly enriched. Consequently, seven genes associated with this pathway were selected for further analysis, namely *CML30*, *CML42*, *CML19*, *WRKY6*, *WRKY53*, *WRKY11* and *PR1*. In comparison to JM and SZ samples, the expression levels of *CML30*, *CML42* and *CML19* were found to be up-regulated in DS (Fig. 6A), indicating their potential role in regulating stomatal closure in *D. nobile* cell walls. Additionally, String analysis (<https://cn.string-db.org>) demonstrated co-expression and protein homology among *WRKY6*, *WRKY11* and *WRKY53* (Fig. 6B). Previous studies have shown that certain *WRKY* transcription factors can influence the expression of *PR1* (Hussain et al., 2018). However, the inconsistent expression trend between *PR1* and *WRKY6*/*WRKY11*/*WRKY53* suggests that other regulatory mechanisms may be involved.

To investigate additional transcription factors that regulate the expression of *PR1*, three pathways associated with *PR1* expression were selected for further analysis in Kyoto Encyclopedia of Genes and Genomes (KEGG, <https://www.kegg.jp/entry/K13449>), including MAPK signaling pathway (map04016), Plant hormone signal transduction (map04075), and Plant-pathogen interaction (map04626). Among these pathways, eight transcription factors (*VIP1*, *TGA1*, *TGA2.2*, *WRKY22*, *NPR1*, *TGA21*, *TGA3*, *TGA2.3*) potentially involved in regulating *PR1* expression were chosen for correlation analysis. The results

revealed a strong association between the expression of *PR1* and *TGA2.2* ($r = 0.83$, $p < 0.01$), followed by *TGA1* ($r = 0.81$, $p < 0.05$) as shown in Fig. 6C, and both of which are annotated within the Plant hormone signal transduction pathway. Therefore, these findings suggest that the Plant hormone signal transduction pathway may play a pivotal role in regulating the expression of *PR1* in JM and SZ groups.

3.7 qRT-PCR validation

qRT-PCR validation was conducted to confirm the reliability of transcriptome data, where 6 cDEGs were selected and their expression levels were verified. The *actin* gene of *D. nobile* served as a reference control. Results showed that the relative expression levels of 5 cDEGs were consistent with transcriptome sequencing data (Fig. 7).

4. Discussion

Previous studies have demonstrated that the growth of *D. catenatum* can be influenced by cultivation conditions. Specifically, leaves of *D. catenatum* cultivated on rock and pear substrates were observed to be sparse and small, while stems appeared short and purple, with a well-developed root system (Lin et al., 2017). In our study, we discovered significant differences in stem length among different epiphytic modes for *D. nobile*. Notably, specimens cultivated on Danxia stone exhibited the shortest stem length, suggesting that JM, SS, and SZ may offer more favorable modes for the growth of *D. nobile*. This finding aligns with Zhong et al.'s observations where stone cultivation resulted in shorter stem lengths but higher dendrobine content (Zhong et al., 2022).

To date, GO analysis and KEGG analysis have been extensively employed to elucidate the functional roles of genes in various medicinal plants, including *Salvia miltiorrhiza* (Chang et al., 2019), *Panax notoginseng* (Yang et al., 2022), and *Polygonum cuspidatum* (Wang et al., 2021). In this study, GO analysis confirmed that 239 overlapping DEGs were significantly enriched in response to chitin, water deprivation, wounding, and other processes. Interestingly, compared to JM and SZ samples, certain overlapped DEGs annotated for chitin response, water deprivation response, salt stress response, and defense exhibited up-regulation specifically in DS sample. This suggests that *D. nobile* cultivated on DS is more susceptible to environmental stresses such as drought and salinity. Furthermore, these 239 overlapping DEGs were predominantly associated with MAPK signaling pathway regulation, plant-pathogen interaction modulation, and flavonoid biosynthesis. Previous studies have demonstrated the crucial role of these pathways in plant defense mechanisms which can ultimately influence plant growth (Gai et al., 2020).

WGCNA is commonly employed to identify key genes in various studies (Wang et al., 2022; Wang et al., 2022). Zeng et al. discovered 12 highly correlated genes associated with cold stress or recovery treatment in rice through co-expression network analysis (Zeng et al., 2022). Additionally, Xu et al. utilized co-expression network analysis to select 12 unigenes encoding crucial enzymes and 42 unigenes encoding transcription factors that potentially regulate chlorogenic acid metabolism (Xu et al., 2022). In our study, we identified a set of 72 significant genes strongly correlated with stem length in *D. nobile*. Subsequently, eight chitin-responsive genes were annotated using GO analysis, while nine genes were annotated within

the plant-pathogen interaction pathway via KEGG analysis, indicating that pathogens may be the primary biotic stressors impeding stem growth on DS.

Notably, the expression level of *PR1* in JM and SZ was higher than that in DS, while the expression levels of CML30, CML42 and CML19 in JM and SZ were lower than that in DS. This indicates that *D. nobile* grows under different modes and responds to environmental stresses in different ways. The *D. nobile* plants grown on DS overcame environmental stresses by regulating stomatal closure, while those grown on JM and SZ overcame environmental stresses by regulating the expression of *PR1*. *PR1*, an important defense-related gene, can be regulated by various transcription factors such as *MYB*, *WRKY*, and *TGA*. In our study, we found that the expression of *PR1* may be regulated by two different transcription factors, *TGA2.2* and *TGA1*.

In this study, we investigated the agronomic traits and genome transcription profiling of *D. nobile* under four different epiphytic conditions. Our results showed that 72 genes were significantly correlated with stem length, and these genes were primarily involved in responding to chitin and were annotated in the plant-pathogen interaction pathway. Additionally, we identified two transcription factors that may regulate the expression of *PR1*. Overall, the genes predicted in this study can serve as valuable genetic resources for enhancing genetic improvement and furthering our understanding of the molecular mechanism underlying stem growth in *D. nobile*, providing potential candidate genes.

Declarations

Author Contributions

DX and YH conceived, supervised and writing-reviewed the manuscript. CL, LQ, DX and YH originally wrote the draft and writing-reviewed the draft. CL, LQ, YZ, HQ, LL and QL performed the experiments. CL, LQ, DT carried out the analysis. DX and YH designed the experiments. YH and DX co-founded and co-administrated the project.

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Conflicts of Interest

All authors have approved the final version of the manuscript and declare that no conflicts of interest exist that could be perceived as prejudicial to the impartiality of the reported research.

Data Availability Statement

These polished sequences have been uploaded to NCBI with the SRA accession number PRJNA807080.

Ethical Approval:

Not applicable. This is not a study of human or animal.

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Figures

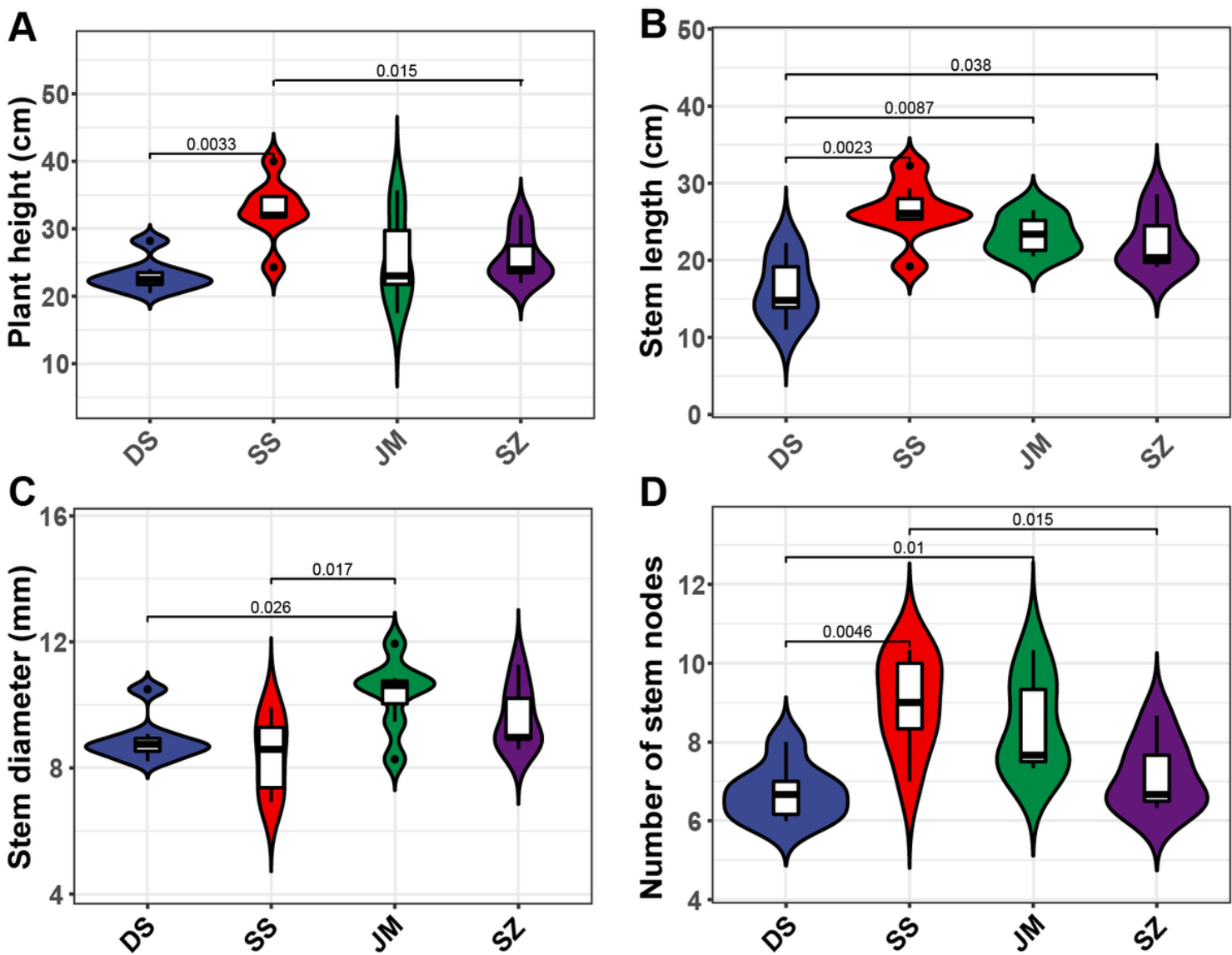


Figure 1

Comparison of agronomic traits of *D. nobile* under different epiphytic modes. A. Plant height, B. Stem length, C. Stem diameter, D. Stem nodes.

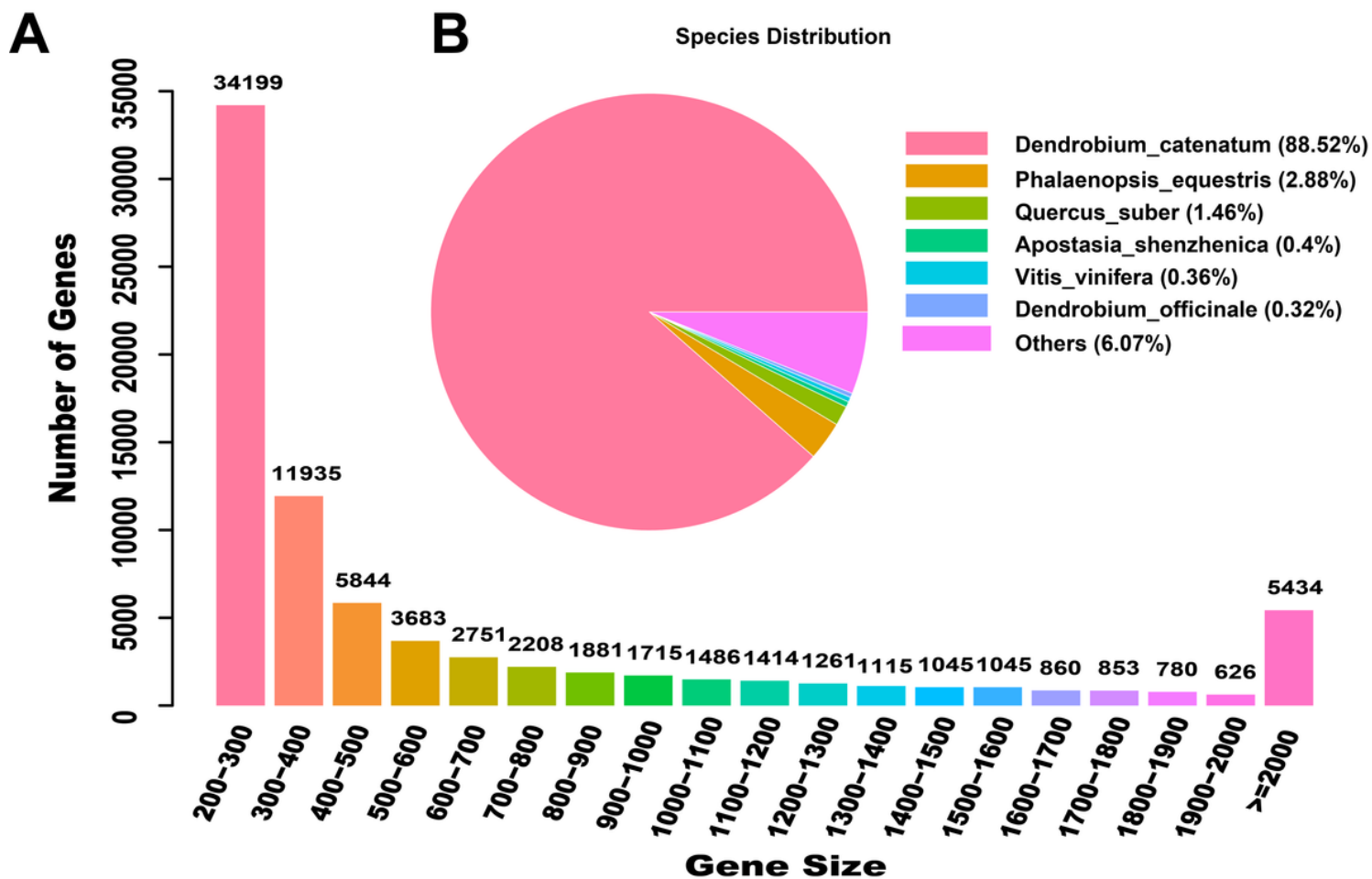


Figure 2

Gene size and annotated species. A. Length distribution of gene, B. Species distribution.

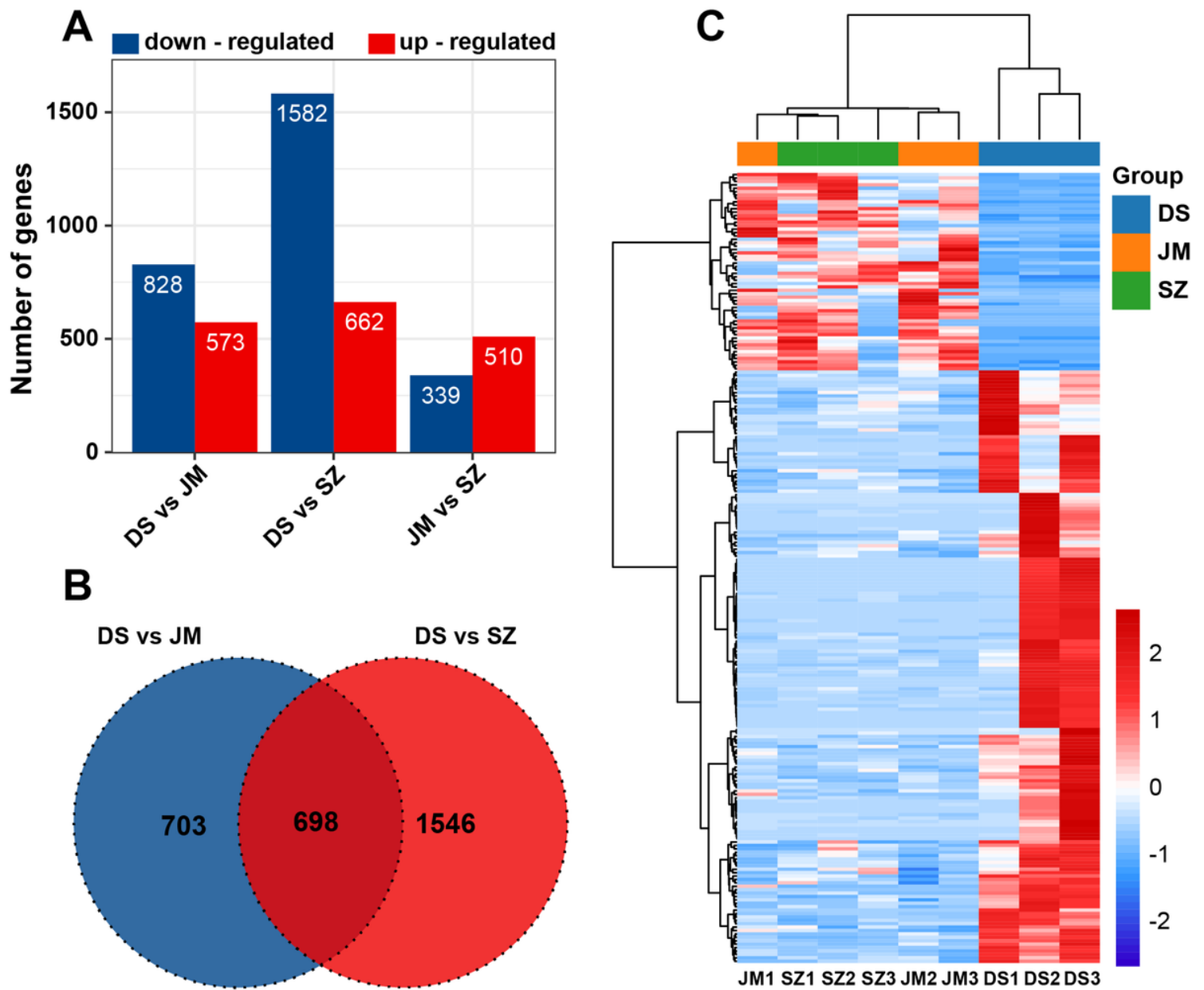


Figure 3

Statistics of DEGs. A. Number statistics of DEGs, B.Venn plot of different comparison groups. C.Expression levels of the 698 cDEGs.

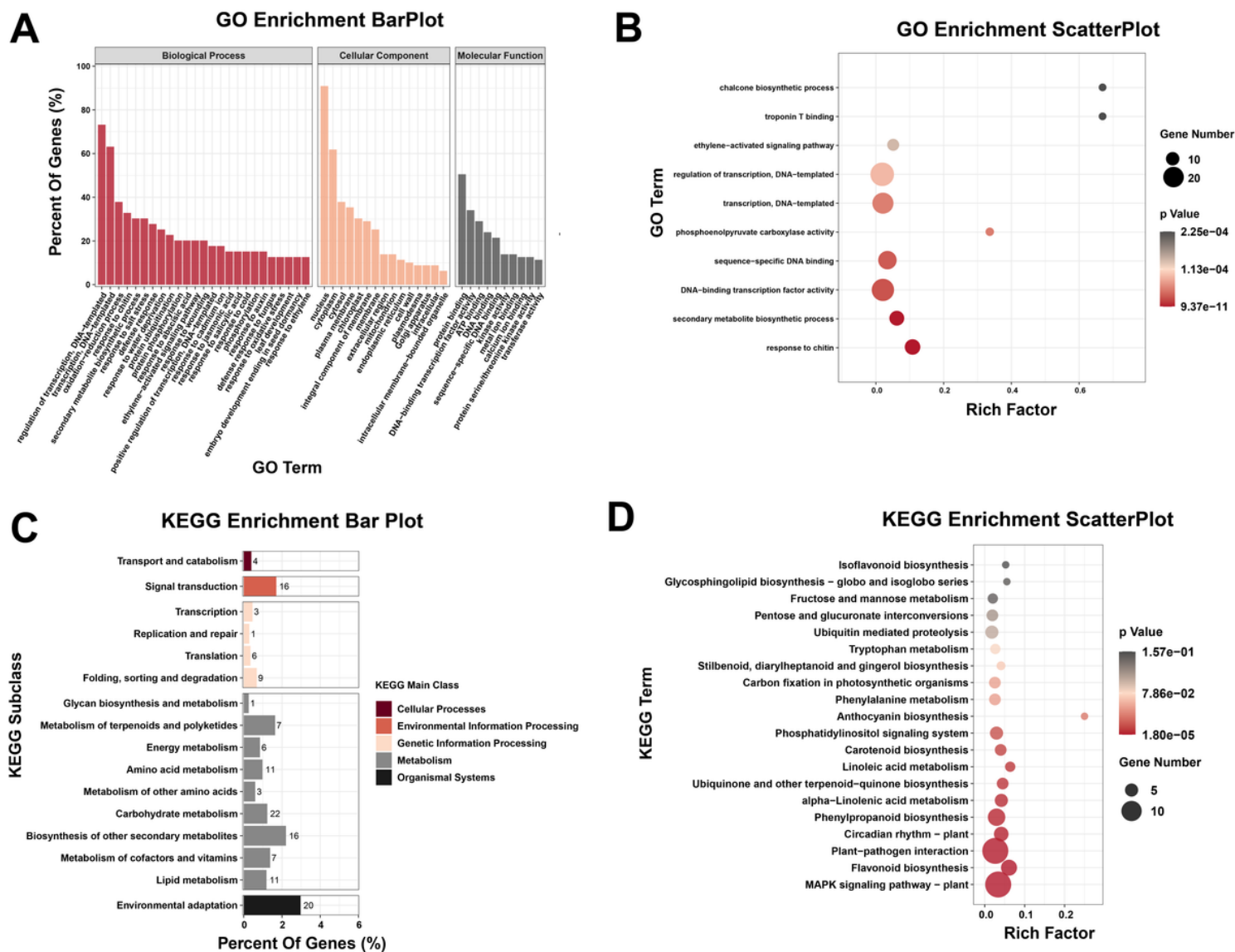


Figure 4

GO analysis and KEGG analysis of 239 cDEGs. .A. GO enrichment bar plot of 239 cDEGs, .B. GO enrichment scatter plot of 239 cDEGs, .C. KEGG enrichment bar plot of 239 cDEGs, D. KEGG enrichment scatter plot of 239 cDEGs.

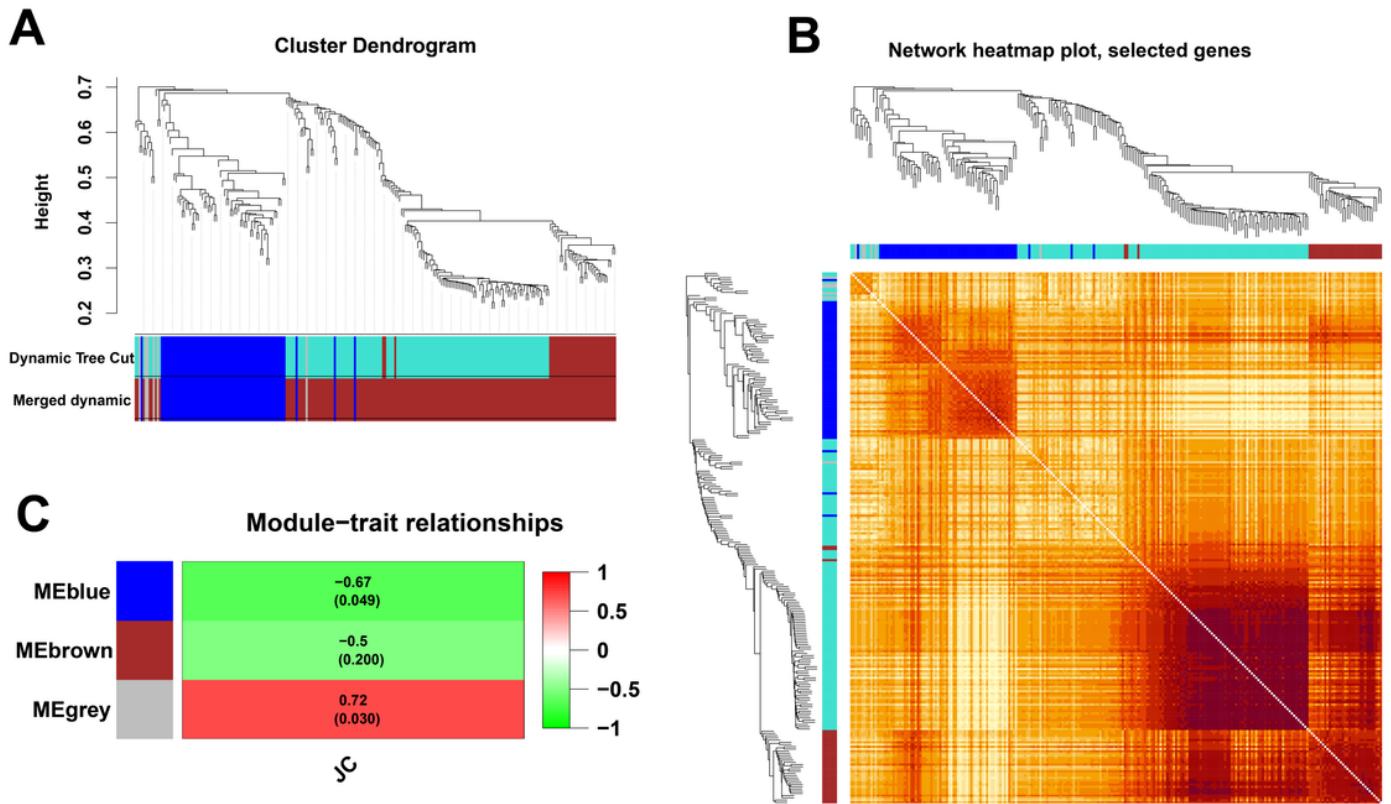


Figure 5

WGCNA of 239 overlapped DEGs. A. Hierarchical cluster analysis to detect co-expression clusters with corresponding color assignments. Each color represents a module in the constructed gene co-expression network by WGCNA. B. Heatmap depicting the Topological Overlap Matrix (TOM) of selected genes for weighted co-expression network analysis, while light color represents lower overlap and red represents higher overlap. C. Module-trait relationships. Each cell contains the corresponding correlation and p-value; red indicates the positive correlation and green indicates the negative correlation.

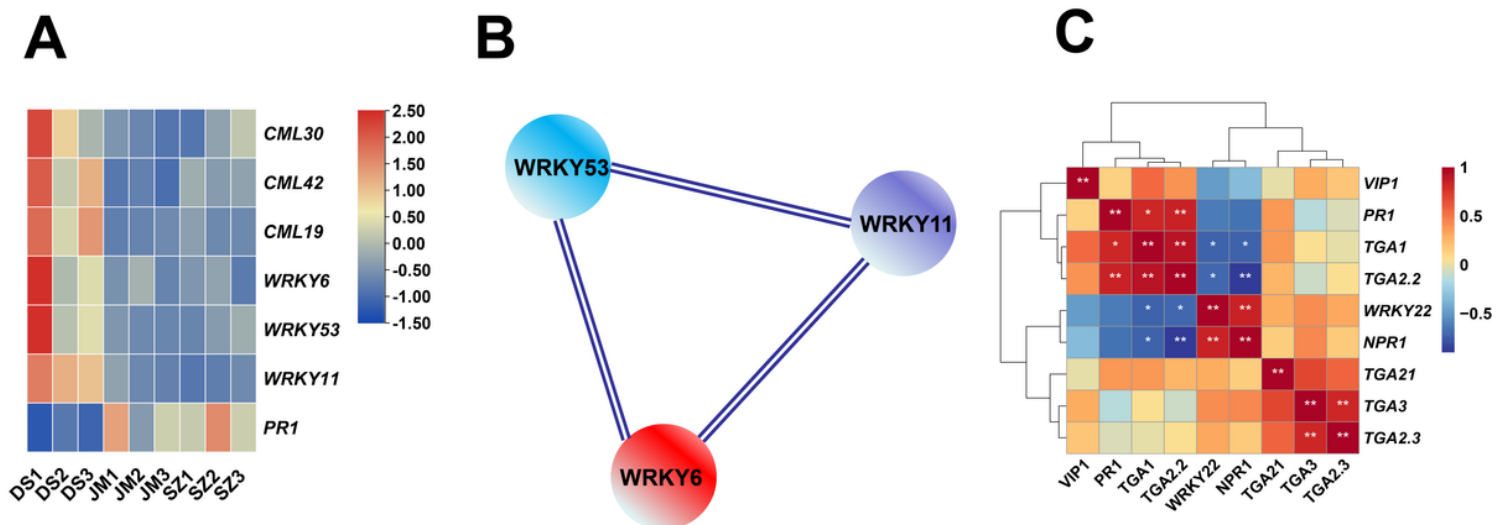


Figure 6

Analysis of plant-pathogen interaction pathway related genes and *PR1* gene. A. Expression levels of genes related to the plant-pathogen interaction pathway, B. Co-expression genes of *D. nobile*, C. Heat map of correlation analysis.

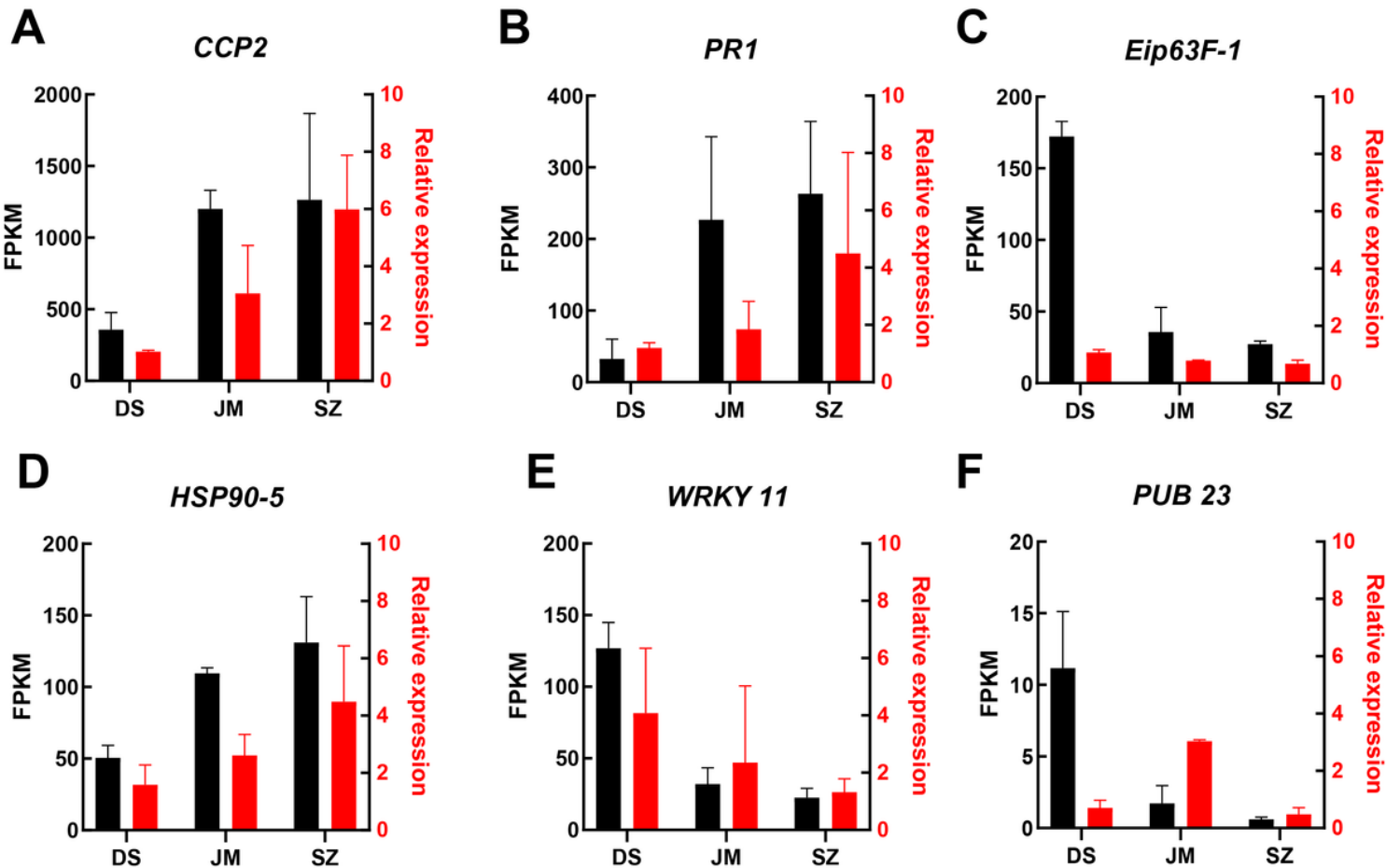


Figure 7

qRT-PCR analyses of overlapped DEGs.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [FigureS1ThegrowingenvironmentofD.nobile.tif](#)
- [FigureS2Thecorrelationanalysisofsamples..tif](#)
- [FigureS3GOanalysisandKEGGanalysisof72keyDEGs.tif](#)
- [TableS1Transcriptomesequencingresults.xlsx](#)
- [TableS2TheinformationofqPCRprimers.xlsx](#)