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TRANSCRIPTOME ANALYSIS OF *PINELLIA TERNATA* DURING LEAF DEVELOPMENT

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Abstract

Pinellia ternata (Thunb.) Breit., a widely distributed medicinal plant in China, is highly valued for its alkaloids, guanosine, organic acids, and other bioactive compounds. While various transcription factor (TF) families have been implicated in *P. ternata* growth, development, and abiotic stress responses, the absence of a complete *P. ternata* genomic sequence has hindered deeper understanding of the precise molecular regulatory mechanisms governing *P. ternata* gene expression during leaf growth. In this study, we performed transcriptome and gene expression profiling of leaves across four developmental stages to unveil specific gene expression patterns for each stage. Sequencing of the leaf transcriptome encompassing these stages generated over 45.44 million raw reads, yielding approximately 42.04 million clean reads per sample. *De novo* assembly of high-quality reads generated 221,251 unigenes (UniGs) averaging 773 bp in length. Analysis of UniG sequences revealed dynamic transcriptomic changes occurring during *P. ternata* growth and development, including significant changes in expression of genes related to several TF families. Subsequent exploration of the annotated RNA-seq database uncovered numerous TFs exhibiting high-level, stable expression during specific leaf developmental stages, suggesting pivotal roles for some TFs in *P. ternata* leaf growth and development. This comprehensive analysis provides insights into the intricate regulatory networks underlying *P. ternata* leaf development, paving the way for future functional genomics studies and targeted improvement of this valuable medicinal plant.

Key words: Transcriptome; Sequencing; *Pinellia ternata*; Leaf; Development.

Introduction

The native plant *Pinellia ternata* (Thunb.) Breit., also known as Banxia in China, holds considerable medicinal value in China, Korea, Japan, and various other countries (Bai *et al.*, 2022), with extensive documentation supporting its efficacy in curing vomiting and cough, infected diseases, and inflammations (Peng *et al.*, 2019; Peng *et al.*, 2022). Studies majoring on modern pharmacological have uncovered an array of diverse *P. ternata* therapeutic properties, including cough suppression, anti-vomiting, expectorant effects, anti-bacterial, anti-tumor, hypnotic, etc. (Kong *et al.*, 2018). As a result, *P. ternata* is frequently incorporated in clinical traditional Chinese medicine (TCM) formulations and over 400 Chinese patented drugs, such as Banxia Cough Tablets, Banxia Tianma Pills, and Huoxiang Zhengqi Oral Liquid (Mao & He, 2020). However, previous studies pointed out that the current market demand each year for *P. ternata* exceeds 8,000 tons, greatly surpassing the combined production of these plants from cultivated and wild sources, highlighting the significant challenge in meeting market demand for *P. ternata*.

Next-generation sequencing (NGS) platforms, including Illumina High-Seq and Roche/454 systems, have revolutionized molecular research, due to their cost-effectiveness and high-throughput capabilities (Gase, 2012). Consequently, NGS platforms are widely utilized for applications related to *de novo* sequencing, comprehensive whole-genome and transcriptome analyses, and genome resequencing, and which can provide rich sequence-based information needed to enhance understanding of molecular mechanisms underlying gene functions (Morozova & Marra, 2008; Guo *et al.*, 2010). Notably, these efficient and cost-effective technologies are especially useful for characterizing gene functions of non-model organisms lacking reference genomes, such as *P. ternata* (Wang *et al.*, 2009; Chen *et al.*, 2023). In such cases, transcriptomic sequences or *de novo* genomic can serve as invaluable resources for gene expression and discovery, molecular marker development, gene localization, and comparative genomics, etc. (Wang *et al.*, 2014; Zhang *et al.*, 2016; Huang *et al.*, 2016; Xue *et al.*, 2019; He *et al.*, 2022; Guo *et al.*, 2022; Guo *et al.*, 2023; Xu *et al.*, 2023; Bo *et al.*, 2023; Duan *et al.*, 2023; Yin *et al.*, 2023).

In this study, we analyzed *P. ternata* leaves collected during four developmental stages using Illumina paired-end NGS sequencing technology in order to establish a comprehensive leaf transcriptome sequence database. Our analysis revealed gene sequences within this database that exhibited high homology with genes encoding transcription factors (TFs), including MYB, AP2-EREBP, bHLH, among others, providing new insights into *P. ternata* TF structures and functions.

Material and Methods

Plant materials: *P. ternata* seeds were provided by Weizhong Li (Guizhou University of Traditional Chinese Medicine). Plants of *P. ternata* were cultivated in plastic pots in 40-cm diameter and 25-cm depth containing peat moss and vermiculite in a 4:1 volume ratio in a lighted incubator (50% relative humidity, 25°C, 16-h light/8-h dark cycle). In this research, leaf developmental stages of *P. ternata* are characterized with below: defined as follows: leaf length = 2.8 cm represents Stage 1; leaf length = 3 cm represents Stage 2; leaf length = 4 cm represents Stage 3; and leaf length = 7.5 cm represents Stage 4 (Fig. 1). Leaf blades during each of the four growth stages were collected respectively and were frozen immediately in liquid nitrogen and then stored at -80°C for further experiments. Total RNA prepared from leaf samples was reverse transcribed to generate cDNA then cDNA was further processed for transcriptome analysis. DNA sequences were generated using an Illumina HiSeq 2000 system (Shenzhen BGI). Three biological replicates were harvested and processed for each growth stage.

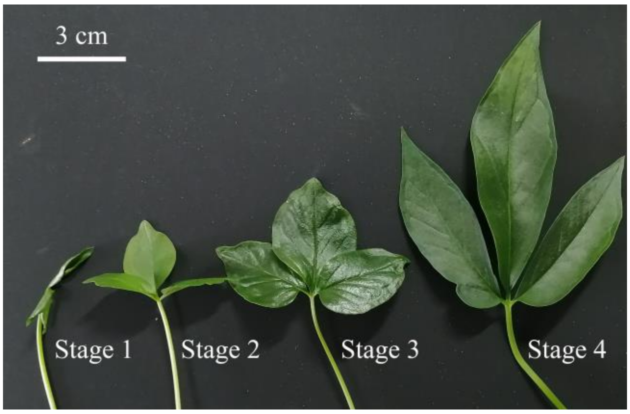

Fig. 1. *P. ternata* at four stages.

Transcriptome sequence processing and gene annotation: Leaf total RNA was extracted using CTAB then used to construct RNA-seq libraries. Libraries were sent to the Beijing Genomics Institution (BGI), where they were sequenced using the Illumina Genome Analyzer platform. Raw sequence data were filtered using SOAPnuke (v1.5.2) in three steps, as follows: 1) Deleting the reads including adapters (adapter contamination); 2) Deleting the reads with ratios of uncharted base ('N' base) to known bases of >10%; 3) Deleting the reads with > 50% low-quality bases (base quality scores of ≤15). The remaining high-quality clean reads were saved in FASTQ format (Chen *et al*., 2018). Thereafter, the clean reads were assembled by Trinity (v2.0.6) and afterwards, the assembly quality was evaluated using BUSCO (Grabherr *et al*., 2011). Subsequently, we mapped the clean sequence data to assembled unique gene sequences by Bowtie2 (v2.2.5) (Langmead & Salzberg, 2012), and afterwards the expression levels of genes were evaluated by RSEM (v1.2.8) (Li & Dewey, 2011). Candidate coding regions within unique gene sequence assemblies were identified using TransDecoder (v3.0.1) (Kim *et al*., 2015). Predicted protein sequences were aligned to SwissProt databases using BLAST and analyzed with Hmmscan to detect remote homologs, offering insights into the structures and functions of these proteins. Unigenes (UniGs) were identified using MISA (v1.0) (Thiel *et al*., 2003). Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) databases were employed to match the functions of the assembled UniG for further confirming the TF sequences.

Differential gene expression: DESeq was introduced to analyse the differential gene expression within group (Fold Change (FC) ≥ 2, the Adjusted P-value ≤ 0.001) (Wang *et al*., 2010). PossionDis was used to analyse the differential gene analysis between group (FC ≥ 2, the false-discovery rate (FDR) ≤ 0.001). A heatmap of differential gene clusters based on the differential gene set was generated using the pheatmap function. Identified differentially expressed genes (DEGs) underwent functional classification based on GO and KEGG annotation results. KEGG enrichment analysis (phyper function in R software) and GO enrichment analysis (TermFinder package) were performed to analyse the possible pathways and functions of the selected DEGs, respectively (https://metacpan.org/pod/GO::TermFinder). Q-value ≤0.05 means the candidate genes were significantly enriched.

Results and Discussion

Illumina paired-end sequencing analysis and *de novo* assembly of *P. ternata* sequences: The *P. ternata* transcriptome was analyzed using RNA-seq Analyzer II to elucidate leaf transcriptomic variations across different developmental stages. A comprehensive set of 12 cDNA libraries was generated from total RNA, resulting in the production of over 45.44 million raw reads yielding approximately 42.04 million clean reads per sample (Table 1). These high-quality reads produced from leaves collected during four developmental stages were integrated to generate the transcriptome profile of *P. ternata*. Trinity software was used to facilitate *de novo* package of these high-quality reads, culminating in the assembly of 221,251 UniGs with an average length of 773 bp. Notably, sequences spanning 200 bp to 3,000 bp constituted almost 96.81% of the total set of sequences, with 41,133 UniGs (22.26%) falling within the 200 bp to 300 bp range. The size distributions of these UniGs are depicted in Fig. 2.

Utilizing TransDecoder, ORFs of all UniGs were identified and extracted to obtain coding and protein sequences for use in gene cloning, explorations of phylogenetic relationships, and functional verification. Distinct stages exhibited clear dispersion while biologically converging with each other. Importantly, 3,133 identified UniGs were associated with 57 transcription factor families. Among these, the most highly represented families included MYB (311 UniGs), AP2-EREBP (301 UniGs), bHLH (269 UniGs), ABI3VP1 (232 UniGs), WRKY (212 UniGs), NAC (181 UniGs), C3H (146 UniGs), G2-like (132 UniGs), Trihelix (105 UniGs), Tify (104 UniGs), GRAS (102 UniGs), mTERF (85 UniGs), ARF (79 UniGs), C2C2-GATA (74 UniGs), and C2H2 (70 UniGs).

Differential expression, GO term enrichment, and KEGG analysis: To assess changes in *P. ternata* leaf gene expression profiles during plant growth, the transcriptional abundance of each gene was measured and expressed as fragments per kilobase per million mapped fragments (FPKM) values (Fig. 3). This analysis unveiled significant *P. ternata* leaf transcriptomic changes across the four developmental stages and numerous DEGs with a minimum 2-fold change in abundance between two compared stages (Table 2), revealing substantial variations in gene expression across the four developmental stages. Notably, an investigation into leaf suppressor gene expression patterns revealed pivotal roles for these genes in leaf signal transduction pathways. Furthermore, functional enrichment analysis of DEGs exhibiting conspicuous biological process-related changes across leaf developmental stages yielded GO and KEGG pathway-related terms that provided insights into the functions of these DEGs (Figs. 4 and 5).

Table 1. Summary of RNA-Seq datasets and mapped results for the twelve libraries.

Samples	Total raw reads (M)	Total clean reads (M)	Total clean bases (Gb)	Clean reads Q20 (%)	Clean reads Q30 (%)	Clean reads ratio (%)	Total clean reads (M)	Total mapping (%)	Uniquely mapping (%)
Stage 1-1	48.93	43.42	6.51	96.95	91.02	88.73	43.42	71.06	22.19
Stage 1-2	47.19	42.67	6.40	97.06	91.37	90.44	42.67	69.42	21.77
Stage 1-3	45.44	42.04	6.31	97.13	91.67	92.51	42.04	75.63	22.20
Stage 2-1	45.44	42.29	6.34	96.89	90.91	93.06	42.29	76.51	21.14
Stage 2-2	45.44	42.47	6.37	97.08	91.52	93.47	42.47	76.01	19.84
Stage 2-3	45.44	42.52	6.38	97.07	91.48	93.59	42.52	79.12	28.04
Stage 3-1	45.44	42.59	6.39	97.22	91.96	93.74	42.59	76.03	18.21
Stage 3-2	43.69	42.26	6.34	96.98	91.22	96.72	42.26	75.81	21.17
Stage 3-3	45.44	43.58	6.54	97.16	91.69	95.90	43.58	75.13	19.80
Stage 4-1	45.44	42.35	6.35	97.16	91.77	93.20	42.35	74.46	18.88
Stage 4-2	45.44	42.91	6.44	96.97	91.26	94.44	42.91	72.73	18.35
Stage 4-3	47.19	43.46	6.52	97.16	91.69	92.11	43.46	73.98	17.93

-1, -2, and -3: three biological replicates of each leaf sample

Table 2. DEGs among the different stages of *P. ternata* leaves.

Compare group	Up	Down	Total
Stage 1-vs-Stage 2	7,264	5,778	13,042
Stage 2-vs-Stage 3	405	590	995
Stage 3-vs-Stage 4	512	756	1,268
Stage 1-vs-Stage 4	9,673	7,844	17,517

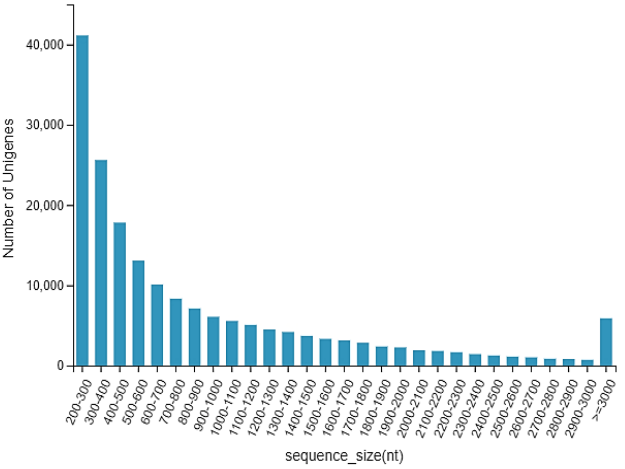

Fig. 2. Assembled transcript length distributions.

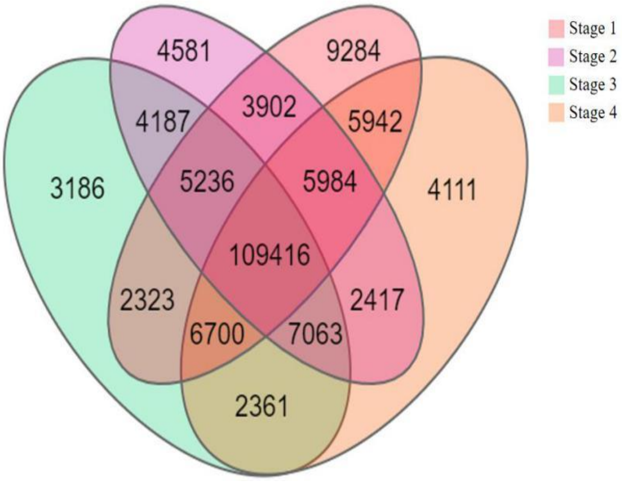

Fig. 3. Venn diagram of transcript abundance across development.

Transcriptome-wide identification of *P. ternata* TFs: Auxins are essential phytohormones that play key regulatory roles in plant growth and development. Binding of auxins to their receptors triggers signaling cascades that activate auxin response factors (ARFs). ARFs are DNA-binding TFs that bind to auxin response elements in promoters of auxin-responsive genes that synergistically orchestrate activities of auxin-regulated transduction signal pathways (Jiang *et al*., 2023). Due to the absence of an established *P. ternata* genomic database, we constructed an annotated leaf RNA-seq database for this species. Searching of this database for ARF sequences led to the identification of 79 TF UniGs encoding ARF TF family proteins. Among these, 3 DEGs were up-regulated and 25 DEGs were down-regulated in the Stage 1-vs-Stage 4 comparison (Table 3).

Given that gene expression levels are highly associated with gene functions, these results suggest crucial roles for ARFs in *P. ternata* growth and development. Specifically, relative expression levels of TRINITY_DN1467_c0_g1_i6-s2 and TRINITY_DN1983_c0_g1_i2-s1 were higher in Stage 1 leaves than in leaves collected during other stages, with no significant expression level differences in leaves observed for these ARFs among Stages 2, 3, and 4. Conversely, the relative expression level of TRINITY_DN150_c0_g1_i7-s4 was lower in Stage 1 leaves than in the leaves collected during other stages.

Various TF families, such as the GRAS family, have been reported to play essential roles in both plant development and stress resistance (Yang *et al*., 2023). In this work, the Stage 1-vs-Stage 4 comparison (Table 4) revealed differential expression of 102 *P. ternata* GRAS genes, of which 13 genes were mainly up-regulated, including TRINITY_DN4134 _c0_g1_i2-s10, TRINITY_ DN905_c3_g1_i2-s12, and TRINITY_DN787_c1_g1_i4-s11, while 18 genes were down-regulated, including TRINITY_DN568_c0_g1_i6-s3, TRINITY_DN1987 _c0_ g2_i1-s2, TRINITY_DN10168_c0 _g1_i2-s1, and other slightly down-regulated genes.

Scholars reported that the flowering process is associated with the MIKCc-type MADS-box family gene (Yang *et al*., 2022). We initially conducted a genome-wide analysis of MADS-box genes in *P. ternata*, to dig the possible regulating mechanism of the MADS-box gene in the leaf development of *P. ternata*, resulting in the identification of a total of 41 MADS-Box genes. For the Stage 1 vs. Stage 4 comparison, relative transcript levels of four MADS genes (TRINITY _DN16284_c0_g1_i1-s4, TRINITY_DN4339 _c0 _g1_i4-s10, TRINITY_DN4897_ c0_g2_i1-s8, and TRINITY _DN7059_c0_g1_i1-s7) gradually increased, while TRINITY_DN1302_c0_g1_i3-s1, TRINITY_DN1725 _c0 _g1_i1-s2, TRINITY_ DN20233_c0_g2_i1-s1, and TRINITY_DN5804_c0 _g1_ i1-s8 were initially down-regulated (Table 5).

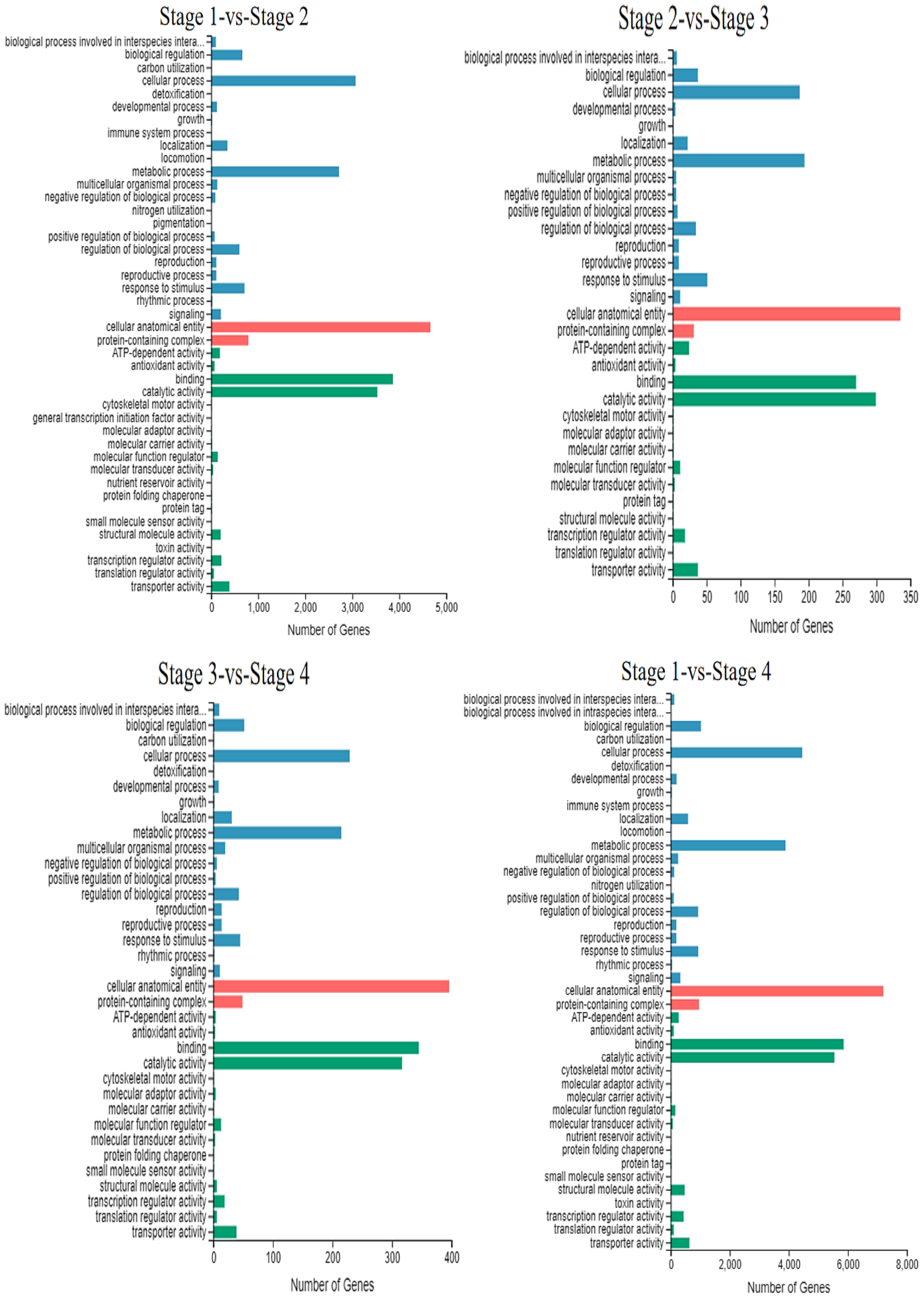

Fig. 4. Functional categories of the GO terms of all genes in different stages of *P. ternata* leaves. The red, green, and blue color represents cellular component, molecular function, and biological process, respectively.

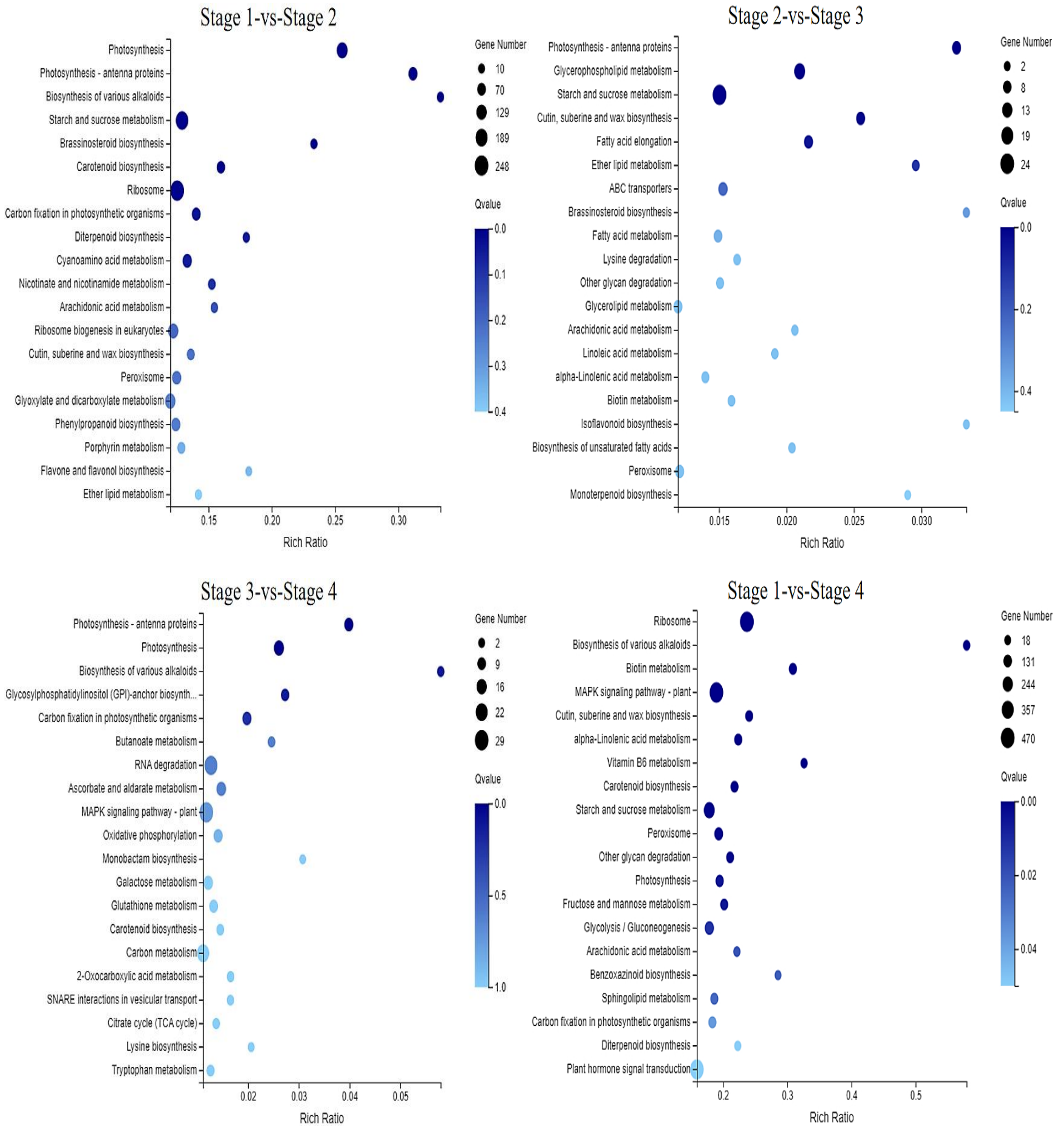

Fig. 5. The KEGG pathway terms of all genes in different stages of *P. ternata* leaves. The size represented gene number and the color represented Q-value.

Flores and his colleague revealed that transcription complexes (for example, MYB-bHLH-WD40 (MBW)) could act as master regulators of cellular processes via regulating multiple target genes' expressions in various plants (Flores *et al*., 2022). In this study, 311 MYB genes were identified in *P. ternata* (Table 6). Of these, expression levels of 61 genes (TRINITY_DN5289_c0_g1_i10-s4, TRINITY_DN1027_c0_g1_i4-s9, TRINITY_DN3379_c0_g1_i11-s9, and others) gradually increased in the Stage 1-vs-Stage 4 comparison, while 24 genes were initially down-regulated.

In Wang's study, the NAC (NAM-ATAF1/2-CUC) TF family has huge functions in plant cell growth and development, and plant cell adaption to adverse conditions (Wang *et al*., 2016). To understand NAC TF functions related to *P. ternata* leaf development, we identified 181 NAC TF transcripts in the *P. ternata* transcriptome then analyzed the expression profiles of these transcripts during the four developmental stages. Our results revealed that in the Stage 1 vs. Stage 2, there were 34 highly-expressed (H-Eed) and 5 lowly-expressed (L-Eed) NAF TF genes; in the Stage 2 vs. Stage, there were 322 H-Eed and 8 L-Eed genes; in the Stage 3 vs. Stage 4, there were 17 H-Eed and 3 L-Eed genes; and in the Stage 1 vs. Stage 4, there were 66 H-Eed and 14 L-Eed genes (Table 7).

Table 3. Identified genes expressed of ARF family in *P. ternata* leaves.

Gene ID	Description	log2 Fold change			
		Stage 1-vs-stage 2	Stage 2-vs-stage 3	Stage 3-vs-stage 4	Stage 1-vs-stage 4
TRINITY_DN10849_c0_g1_i2-s5	unnamed protein product [*Spirodela intermedia*]	-1.43	-1.17	0.16	-2.43
TRINITY_DN11731_c0_g1_i2-s3	hypothetical protein AMTR_s00057p00141830 [*Amborella trichopoda*]	-3.55	-1.62	1.44	-3.73
TRINITY_DN1359_c0_g1_i1-s1	PREDICTED: auxin response factor 6-like isoform X1 [*Nelumbo nucifera*]	-0.59	-1.55	-7.00	-9.14
TRINITY_DN1359_c0_g1_i2-s1	PREDICTED: auxin response factor 6-like isoform X1 [*Nelumbo nucifera*]	-2.68	0.07	-7.36	-9.95
TRINITY_DN1398_c0_g2_i2-s1	unnamed protein product, partial [*Vitis vinifera*]	-8.30		4.54	-3.84
TRINITY_DN1398_c0_g2_i6-s1	unnamed protein product [*Spirodela intermedia*]	-1.34	-0.04	-2.85	-4.22
TRINITY_DN14248_c0_g1_i8-s2	auxin response factor 15 isoform X2 [*Elaeis guineensis*]	-1.99	-2.00	-0.72	-4.70
TRINITY_DN1467_c0_g1_i6-s2	hypothetical protein AMTR_s00057p00141830 [*Amborella trichopoda*]	-6.44	-0.55	-0.86	-7.94
TRINITY_DN150_c0_g1_i7-s4	auxin response factor 23 [*Elaeis guineensis*]	2.03	-0.07	0.70	2.71
TRINITY_DN1686_c0_g1_i2-s7	PREDICTED: auxin response factor 6-like isoform X1 [*Nelumbo nucifera*]	-4.17	3.84	-2.05	-2.32
TRINITY_DN18280_c0_g1_i1-s11	unnamed protein product [*Spirodela intermedia*] > CAA7400308.1 unnamed protein product [*Spirodela intermedia*]	-1.44		4.17	2.62
TRINITY_DN193_c0_g1_i2-s2	unnamed protein product [*Spirodela intermedia*]	-1.99	-0.46	-1.23	-3.68
TRINITY_DN1983_c0_g1_i11-s1	auxin response factor 24 isoform X2 [*Elaeis guineensis*]	-1.63	-1.42	0.18	-2.84
TRINITY_DN1983_c0_g1_i1-s1	auxin response factor 23-like protein [*Cinnamomum micranthum* f. kanehirae]	-3.25	-1.27	0.69	-3.81
TRINITY_DN1983_c0_g1_i2-s1	hypothetical protein I3760_05G104100 [*Carya illinoinensis*]	-5.03			-5.10
TRINITY_DN254_c0_g2_i5-s2	unnamed protein product [*Spirodela intermedia*]	-1.92	0.49	-0.92	-2.34
TRINITY_DN2558_c0_g1_i1-s7	unnamed protein product [*Spirodela intermedia*]	0.13	-1.06	-3.40	-4.30
TRINITY_DN343_c0_g1_i2-s8	unnamed protein product [*Spirodela intermedia*]	-2.10	0.22	-1.01	-2.88
TRINITY_DN392_c1_g1_i1-s3	unnamed protein product [*Spirodela intermedia*]	-1.91	-0.05	-1.22	-3.17
TRINITY_DN392_c1_g1_i2-s3	unnamed protein product [*Spirodela intermedia*]	-2.19	-3.65	1.30	-4.55
TRINITY_DN416_c0_g1_i10-s7	auxin response factor 23 [*Cocos nucifera*]	0.60	0.49	1.12	2.23
TRINITY_DN416_c0_g1_i14-s7	auxin response factor 23 [*Cocos nucifera*]	-2.72	-0.64	-0.88	-4.19
TRINITY_DN510_c1_g1_i1-s2	TPA_asm: hypothetical protein HUJ06_019077 [*Nelumbo nucifera*]	-1.40	0.67	-1.88	-2.63
TRINITY_DN5644_c0_g1_i1-s1	auxin response factor 11-like protein [*Pinellia ternata*]	-2.43	-1.21	-0.47	-4.08
TRINITY_DN5888_c0_g1_i2-s4	unnamed protein product [*Spirodela intermedia*]	-1.07	-0.90	-1.09	-3.05
TRINITY_DN5888_c0_g2_i2-s4	auxin response factor 15 isoform X2 [*Elaeis guineensis*]	-1.47	-1.58	-1.13	-4.14
TRINITY_DN5888_c0_g2_i3-s4	auxin response factor 15-like isoform X2 [*Asparagus officinalis*]	-0.60	-1.79	-5.16	-7.53
TRINITY_DN996_c0_g1_i4-s8	auxin response factor 18 [*Elaeis guineensis*]	-0.02	-0.43	-2.56	-3.02

Table 4. Identified genes expressed of GRAS family in *P. ternata* leaves.

Gene ID	Description	log2 Fold change			
		Stage 1-vs-Stage 2	Stage 2-vs-Stage 3	Stage 3-vs-Stage 4	Stage 1-vs-Stage 4
TRINITY_DN10168_c0_g1_i2-s1	unnamed protein product [*Spirodela intermedia*]	-3.25	-4.68	0.69	-7.26
TRINITY_DN1055_c2_g1_i1-s1	unnamed protein product [*Spirodela intermedia*]	-2.00	-0.55	-1.15	-3.69
TRINITY_DN14001_c0_g1_i2-s2	unnamed protein product [*Spirodela intermedia*]	-1.62	-1.75	-1.46	-4.82
TRINITY_DN17089_c0_g2_i1-s11	PREDICTED: scarecrow-like protein 8 [*Solanum tuberosum*]			1.86	2.13
TRINITY_DN1747_c0_g1_i1-s6	unnamed protein product [*Spirodela intermedia*]	2.11	-0.34	0.64	2.44
TRINITY_DN1905_c0_g1_i2-s2	unnamed protein product [*Spirodela intermedia*] > CAA7389169.1 unnamed protein product [*Spirodela intermedia*]	-2.12	-2.33	1.31	-3.11
TRINITY_DN1987_c0_g2_i1-s2	unnamed protein product [*Spirodela intermedia*]	-3.31	-4.52	0.00	-7.77
TRINITY_DN2196_c0_g1_i5-s8	unnamed protein product [*Spirodela intermedia*]	-1.80	2.39	-3.40	-2.83
TRINITY_DN23480_c0_g1_i1-s9	scarecrow-like protein 32 [*Phoenix dactylifera*]	3.35	0.59	-0.83	3.13
TRINITY_DN2506_c0_g1_i8-s12	scarecrow-like protein 32 [*Phoenix dactylifera*]	2.78	0.72	-0.02	3.51
TRINITY_DN28987_c0_g1_i1-s3	PREDICTED: protein SHORT-ROOT-like [*Nelumbo nucifera*] > DAD25208.1 TPA_asm: hypothetical protein HUJ06_026672 [*Nelumbo nucifera*]	-4.17	1.77	-1.82	-4.21
TRINITY_DN3257_c0_g1_i1-s3	protein SCARECROW 2 [*Elaeis guineensis*]	-2.10	-1.98	1.44	-2.60
TRINITY_DN3259_c0_g1_i14-s6	hypothetical protein GW17_00029411 [*Ensete ventricosum*] > RWW39582.1 hypothetical protein BHE74_00055080 [*Ensete ventricosum*]	4.22	0.32	-1.96	2.60
TRINITY_DN36877_c0_g1_i2-s2	PREDICTED: DELLA protein SLR1-like [*Nelumbo nucifera*]	-1.45	-2.07	-0.44	-3.93
TRINITY_DN4134_c0_g1_i2-s10	scarecrow-like protein 32 [*Phoenix dactylifera*]	4.53	-0.50	0.72	4.80
TRINITY_DN4317_c0_g1_i10-s4	protein SCARECROW 2 [*Elaeis guineensis*]	-0.40	-3.64	0.88	-3.12
TRINITY_DN4348_c0_g1_i6-s2	unnamed protein product [*Spirodela intermedia*]	-1.27	-0.81	-1.31	-3.37
TRINITY_DN4850_c0_g1_i3-s11	scarecrow-like protein 32 [*Phoenix dactylifera*]	3.16	-0.81	1.28	3.67
TRINITY_DN4906_c0_g1_i2-s7	scarecrow-like protein 9 [*Asparagus officinalis*] > ONK76916.1 uncharacterized protein A4U43_C02F1220 [*Asparagus officinalis*]	-2.58	4.09	0.63	2.17
TRINITY_DN568_c0_g1_i11-s3	unnamed protein product [*Spirodela intermedia*]	-2.82	-0.01	-0.22	-3.01
TRINITY_DN568_c0_g1_i6-s3	unnamed protein product [*Spirodela intermedia*]	-1.69	-2.70	-4.41	-8.79
TRINITY_DN6750_c0_g1_i2-s7	unnamed protein product [*Spirodela intermedia*]	-5.56	6.05	-6.13	-5.63
TRINITY_DN729_c0_g1_i10-s1	unnamed protein product [*Spirodela intermedia*] > CAA7389169.1 unnamed protein product [*Spirodela intermedia*]	-1.95	-1.56	0.14	-3.35
TRINITY_DN787_c1_g1_i10-s11	scarecrow-like protein 9 [*Elaeis guineensis*] > XP_010937597.1 scarecrow-like protein 9 [*Elaeis guineensis*] >XP_029123935.1 scarecrow-like protein 9 [*Elaeis guineensis*]	0.25	0.13	-6.59	-6.23
TRINITY_DN787_c1_g1_i4-s11	unnamed protein product [*Spirodela intermedia*]	1.61	-2.94	5.06	3.76
TRINITY_DN8075_c0_g1_i4-s3	unnamed protein product [*Spirodela intermedia*]	0.67	-4.70	6.48	2.46
TRINITY_DN8075_c0_g1_i5-s3	unnamed protein product [*Spirodela intermedia*]	0.12	1.41	0.95	2.51
TRINITY_DN8075_c0_g1_i7-s3	unnamed protein product [*Spirodela intermedia*]	-3.87	-0.10	0.03	-3.91
TRINITY_DN905_c3_g1_i2-s12	unnamed protein product [*Spirodela intermedia*]	2.92	-0.09	1.63	4.48
TRINITY_DN905_c3_g1_i5-s12	unnamed protein product [*Spirodela intermedia*]	0.89	1.60	-0.31	2.15
TRINITY_DN9776_c0_g3_i1-s4	GRAS family protein RAM1-like [*Dioscorea cayenensis* subsp. *rotundata*]	2.68	-5.67	0.03	-2.91

Table 5. Identified genes expressed of MADS family in *P. ternata* leaves.

Gene ID	Description	log2 Fold change			
		Stage 1-vs-Stage 2	Stage 2-vs-Stage 3	Stage 3-vs-Stage 4	Stage 1-vs-Stage 4
TRINITY_DN1302_c0_g1_i3-s1	B-type MADS-box protein PI-1 [*Anthurium andraeanum*]	-4.23			-4.30
TRINITY_DN16284_c0_g1_i1-s4	A-type MADS-box protein FUL1 [*Anthurium andraeanum*]	3.28	-0.83	0.63	3.10
TRINITY_DN1725_c0_g1_i1-s2	hypothetical protein GW17_00053183 [*Ensete ventricosum*] > RZR88446.1 hypothetical protein BHM03_00016027 [*Ensete ventricosum*]	-0.59	-2.52	-1.26	-4.33
TRINITY_DN20233_c0_g2_i1-s1	unnamed protein product [*Spirodela intermedia*]	-3.23	2.08	-4.27	-5.45
TRINITY_DN4339_c0_g1_i4-s10	A-type MADS-box protein FUL1 [*Anthurium andraeanum*]	3.67	-4.02	4.09	3.79
TRINITY_DN4897_c0_g2_i1-s8	MADS-box transcription factor 23 isoform X2 [*Cinnamomum micranthum* f. *kanehirae*]	-21.32	22.29	0.18	2.23
TRINITY_DN5804_c0_g1_i1-s8	PREDICTED: floral homeotic protein AGAMOUS-like [*Nelumbo nucifera*]	-4.01	3.05	-1.44	-2.37
TRINITY_DN7059_c0_g1_i1-s7	unnamed protein product [*Spirodela intermedia*]	3.60	4.74	-6.22	2.13

Trihelix family TFs are closely related to the plant development, as well as response to biotic and abiotic stresses (Mo *et al*., 2019). A total of 105 trihelix TF genes were identified in *P. ternata*. To explore the potential functions of trihelix family TFs in *P. ternata* leaf development, we analyzed leaf transcript levels of trihelix TFs across the four developmental stages. The results of this analysis discovered 7 H-Eed trihelix TF genes and 6 H-Eed genes in the Stage 1 vs.Stage 2; 3 H-Eed trihelix TF genes and 2 L-Eed genes in the Stage 2 vs.Stage 3; 2 H-Eed trihelix TF genes and 5 L-Eed trihelix TF genes in the Stage 3 vs. Stage 4; 11 H-Eed trihelix TF genes and 14 L-Eed trihelix TF genes in the Stage 1 vs. Stage 4 (Table 8).

Tian *et al*., revealed that the pivotal functions of the WRKY TF family in biological processes, for instance, plant growth and development, biotic and abiotic stress responses, and secondary metabolite synthesis, (Tian *et al*., 2023). In this study, 212 WRKY TF genes were identified in *P. ternata*. Among which, 95 were H-Eed genes and 11 were L-Eed genes in the Stage 1 vs. Stage 4 (Table 9). Collectively, the abovementioned results revealed TFs with distinct expression profiles during *P. ternata* leaf development, while also highlighting TFs specific to different developmental stages. However, additional research studies are needed to verify the functions of TF genes identified in this study.

Conclusions

In this study *P. ternata* leaf transcriptome and gene expression profiles across four developmental stages were analyzed, resulting in the identification of specific gene expression patterns for each developmental stage. Over 45.44 million raw reads were generated yielding approximately 42.04 million clean reads per sample. High-quality reads were *de novo* assembled into 221,251 UniGs with an average length of 773 bp. Analysis of these UniGs revealed that dynamic transcriptomic changes occurred during *P. ternata* growth and development. Searches of the annotated *P. ternata* leaf RNA-seq database revealed the identities of 3,133 TF-encoding UniGs belonging to 57 TF families. Some of these genes exhibited high-level, stable expression during specific stages of *P. ternata* leaf development. These results shed light on the intricate regulatory networks underlying *P. ternata* leaf development, thereby supporting future research efforts to enhance understanding of the functional genomics of this species toward achieving targeted improvement of this important medicinal plant.

Acknowledgments

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Table 6. Identified genes expressed of MYB family in *P. ternata* leaves.

Gene ID	Description	log2 Fold change			
		Stage 1-vs-Stage 2	Stage 2-vs-Stage 3	Stage 3-vs-Stage 4	Stage 1-vs-Stage 4
TRINITY_DN1027_c0_g1_i4-s9	unnamed protein product [*Spirodela intermedia*]	23.24	-0.11	-1.54	8.33
TRINITY_DN1034_c0_g1_i5-s1	transcription factor AS1 [*Elaeis guineensis*] > XP_010922323.1 transcription factor AS1 [*Elaeis guineensis*] > XP_010922324.1 transcription factor AS1 [*Elaeis guineensis*] > XP_010922325.1 transcription factor AS1 [*Elaeis guineensis*]	-1.27	-0.28	-0.67	-2.21
TRINITY_DN10450_c0_g1_i2-s12	unnamed protein product [*Spirodela intermedia*]	-0.59	2.73	0.01	2.17
TRINITY_DN10726_c0_g2_i2-s8	transcription factor AS1 [*Elaeis guineensis*] > XP_010922323.1 transcription factor AS1 [*Elaeis guineensis*] > XP_010922324.1 transcription factor AS1 [*Elaeis guineensis*] & gt; XP_010922325.1 transcription factor AS1 [*Elaeis guineensis*]	-1.35	-0.36	-2.46	-4.15
TRINITY_DN11079_c0_g1_i1-s9	hypothetical protein GOBAR_DD18503 [*Gossypium barbadense*]	3.10	1.56	-1.09	3.53
TRINITY_DN11088_c0_g1_i1-s7	hypothetical protein CUMW_287410 [*Citrus unshiu*]	3.71	0.48	-0.85	3.37
TRINITY_DN1206_c1_g3_i1-s8	R2R3 MYB transcription factor 29, partial [*Salvia miltiorrhiza*]	1.34	3.51	-2.62	2.26
TRINITY_DN1206_c2_g1_i5-s8	myb-related protein MYBAS1-like isoform X2 [*Phoenix dactylifera*]	-0.25	2.88	0.33	2.94
TRINITY_DN1206_c5_g1_i1-s8	myb-related protein Hv33-like [*Cocos nucifera*]	-5.57	7.60	-5.43	-3.42
TRINITY_DN1327_c0_g1_i4-s6	unnamed protein product [*Spirodela intermedia*]	4.73	-3.11	0.91	2.57
TRINITY_DN14102_c0_g2_i2-s5	unnamed protein product [*Spirodela intermedia*]	3.35	1.03	-1.43	2.96
TRINITY_DN14102_c0_g2_i3-s5	hypothetical protein CUMW_287410 [*Citrus unshiu*]	3.09	1.99	-0.97	4.20
TRINITY_DN14151_c0_g1_i3-s1	unnamed protein product [*Spirodela intermedia*]	-1.90	1.58	-3.39	-3.68
TRINITY_DN1445_c0_g1_i12-s5	unnamed protein product [*Spirodela intermedia*] > CAA7391552.1 unnamed protein product [*Spirodela intermedia*]	2.04	0.05	0.71	2.79
TRINITY_DN14452_c0_g1_i1-s1	ScMYB21 protein [Saccharum hybrid cultivar Co 86032]	-2.99	2.35	-2.42	-3.06
TRINITY_DN1458_c0_g1_i10-s8	unnamed protein product [*Brassica napus*]	4.73	1.68	-2.06	4.36
TRINITY_DN151_c1_g1_i5-s4	unnamed protein product [*Spirodela intermedia*]	5.40	-1.17	-1.18	3.01
TRINITY_DN151_c1_g1_i8-s4	unnamed protein product [*Spirodela intermedia*]	2.62	-1.33	0.90	2.25
TRINITY_DN15451_c0_g2_i1-s10	unnamed protein product [*Spirodela intermedia*]	1.34	-1.06	3.54	3.85
TRINITY_DN1606_c0_g1_i5-s2	hypothetical protein TEA_022903 [*Camellia sinensis* var. *sinensis*]	-4.42	0.79	1.30	-2.28
TRINITY_DN16270_c0_g2_i2-s5	MYB-like transcription factor ETC3 [*Dioscorea cayenensis* subsp. *rotundata*]	4.28	-0.87	-0.84	2.59
TRINITY_DN1731_c0_g1_i3-s2	transcription factor AS1 [*Elaeis guineensis*] > XP_010922323.1 transcription factor AS1 [*Elaeis guineensis*] & gt; XP_010922324.1 transcription factor AS1 [*Elaeis guineensis*] & gt; XP_010922325.1 transcription factor AS1 [*Elaeis guineensis*]	-0.20	-1.56	-0.42	-2.15
TRINITY_DN1760_c0_g1_i7-s10	transcription factor MYB44 [*Elaeis guineensis*]	0.84	1.24	0.16	2.24
TRINITY_DN1760_c0_g1_i8-s10	unnamed protein product [*Spirodela intermedia*]	2.55	0.74	0.43	3.74
TRINITY_DN1826_c0_g2_i1-s9	unnamed protein product [*Spirodela intermedia*]	3.15	0.17	-0.94	2.36
TRINITY_DN1848_c0_g1_i2-s5	unnamed protein product, partial [*Vitis vinifera*]	3.14	-0.99	0.10	2.26
TRINITY_DN1848_c0_g2_i1-s5	unnamed protein product [*Spirodela intermedia*]	3.54	-0.51	0.32	3.35
TRINITY_DN18555_c0_g1_i1-s11	transcription factor MYB4 isoform X2 [*Elaeis guineensis*]		0.87	2.89	4.13
TRINITY_DN18780_c0_g1_i1-s11	transcription factor MYB30-like [*Phoenix dactylifera*]	3.59	-0.23	1.87	5.17
TRINITY_DN18975_c0_g1_i1-s5	myb protein [*Oryza sativa*]	1.68	-2.18	3.34	2.78

Table 6. (Cont'd.).

Gene ID	Description	log2 Fold change			
		Stage 1-vs-Stage 2	Stage 2-vs-Stage 3	Stage 3-vs-Stage 4	Stage 1-vs-Stage 4
TRINITY_DN2001_c0_g1_i11-s10	unnamed protein product [*Spirodela intermedia*] > CAA7391552.1 unnamed protein product [*Spirodela intermedia*]	2.67	0.16	-0.10	2.74
TRINITY_DN20519_c0_g1_i1-s12	transcription factor MYB14 [*Cinnamomum micranthum* f. *kanehirae*]	-2.05	4.06	2.08	4.09
TRINITY_DN20665_c0_g1_i1-s1	myb-related protein MYBAS1-like isoform X2 [*Phoenix dactylifera*]	-0.38	2.88	0.12	2.60
TRINITY_DN2095_c1_g1_i4-s2	PREDICTED: transcription factor RAX2-like isoform X2 [*Nelumbo nucifera*] > DAD31193.1 TPA_asm: hypothetical protein HUJ06_010044 [*Nelumbo nucifera*]	-1.78	-3.99	0.18	-5.57
TRINITY_DN2121_c0_g1_i4-s9	hypothetical protein BHE74_00036003 [*Ensete ventricosum*] > RZS23446.1 hypothetical protein BHM03_00056390 [*Ensete ventricosum*]	2.81	2.37	0.19	5.32
TRINITY_DN2164_c0_g1_i8-s10	MYB170 [*Populus tomentosa*]	1.82	-0.57	1.14	2.43
TRINITY_DN2190_c0_g1_i10-s10	unnamed protein product [*Spirodela intermedia*]	1.58	-0.15	1.50	2.98
TRINITY_DN2198_c0_g1_i4-s8	R2R3-MYB transcription factor MYB2 [*Epimedium sagittatum*]		6.81	-4.36	2.85
TRINITY_DN2198_c0_g1_i9-s8	transcription factor MYB61 [*Elaeis guineensis*]	1.50	6.03	-10.07	-2.53
TRINITY_DN22084_c0_g1_i1-s4	unnamed protein product [*Ananas comosus* var. *bracteatus*]	4.46	-2.29	0.04	2.23
TRINITY_DN2248_c0_g1_i10-s5	transcription factor MYB44 [*Elaeis guineensis*]	0.46	1.55	0.19	2.18
TRINITY_DN2248_c0_g1_i9-s5	transcription factor MYB44-like [*Phoenix dactylifera*]	7.56	1.51	-2.82	6.24
TRINITY_DN22788_c0_g1_i1-s8	protein ODORANT1-like [*Durio zibethinus*]		3.77	-1.90	2.26
TRINITY_DN23107_c0_g2_i1-s8	unnamed protein product [*Spirodela intermedia*]	-2.51	3.91	-3.88	-2.46
TRINITY_DN2353_c0_g1_i3-s7	unnamed protein product [*Spirodela intermedia*]	4.19	0.34	-2.06	2.48
TRINITY_DN23548_c0_g1_i1-s12	LHY homologue1 [*Lemna gibba*]	1.34	1.36	-0.53	2.20
TRINITY_DN2651_c0_g1_i4-s11	unnamed protein product [*Spirodela intermedia*] > CAA7391552.1 unnamed protein product [*Spirodela intermedia*]	-0.53	1.05	2.22	2.73
TRINITY_DN2730_c0_g2_i1-s5	transcription factor MYB124-like isoform X1 [*Phoenix dactylifera*]	-1.18	-1.14	-0.07	-2.39
TRINITY_DN2894_c1_g1_i1-s11	R2R3-MYB transcription factor MYB16, partial [*Pinus taeda*]	-1.94	2.97	1.52	2.57
TRINITY_DN29393_c0_g1_i1-s12	unnamed protein product [*Spirodela intermedia*]			2.43	2.72
TRINITY_DN29778_c0_g1_i2-s1	hypothetical protein TEA_022903 [*Camellia sinensis* var. *sinensis*]	-3.03	-0.69	-3.63	-7.36
TRINITY_DN29778_c0_g1_i3-s1	unnamed protein product [*Spirodela intermedia*]	-3.09	-2.97	-0.94	-6.98
TRINITY_DN3020_c0_g1_i3-s3	transcription factor AS1 [*Elaeis guineensis*] > XP_010922323.1 transcription factor AS1 [*Elaeis guineensis*] & gt; XP_010922324.1 transcription factor AS1 [*Elaeis guineensis*] & gt; XP_010922325.1 transcription factor AS1 [*Elaeis guineensis*]	-0.74	-1.34	-1.85	-3.91
TRINITY_DN3020_c0_g1_i5-s3	transcription factor AS1 [*Elaeis guineensis*] & gt; XP_010922323.1 transcription factor AS1 [*Elaeis guineensis*] & gt; XP_010922324.1 transcription factor AS1 [*Elaeis guineensis*] > XP_010922325.1 transcription factor AS1 [*Elaeis guineensis*]	-1.02	-1.56	-1.15	-3.70
TRINITY_DN31244_c0_g1_i1-s1	myb-related protein Hv33-like [*Cocos nucifera*]	-1.99	2.83	-4.77	-3.94
TRINITY_DN32159_c0_g1_i1-s2	Transcription factor DIVARICATA [*Apostasia shenzhenica*]	0.60	-1.16	-3.67	-4.20
TRINITY_DN3291_c0_g1_i5-s9	unnamed protein product [*Spirodela intermedia*]	-0.78	0.06	-8.51	-9.22
TRINITY_DN331_c5_g1_i1-s6	myb-related protein MYBAS2 [*Setaria italica*] & gt; RCV18783.1 hypothetical protein SETIT_3G331100v2 [*Setaria italica*]	6.62	-1.47	1.52	6.66

Table 6. (Cont'd.).

Gene ID	Description	log2 Fold change			
		Stage 1-vs-Stage 2	Stage 2-vs-Stage 3	Stage 3-vs-Stage 4	Stage 1-vs-Stage 4
TRINITY_DN3312_c0_g3_i1-s7	unnamed protein product [*Microthlaspi erraticum*]	2.70	-0.57	0.04	2.20
TRINITY_DN3379_c0_g1_i11-s9	unnamed protein product [*Spirodela intermedia*] >CAA7391552.1 unnamed protein product [*Spirodela intermedia*]	6.22	1.53	0.29	8.00
TRINITY_DN3379_c0_g1_i20-s9	unnamed protein product [*Spirodela intermedia*] & gt; CAA7391552.1 unnamed protein product [*Spirodela intermedia*]	3.02	-0.46	-0.20	2.38
TRINITY_DN3379_c0_g1_i4-s9	unnamed protein product [*Spirodela intermedia*] & gt; CAA7391552.1 unnamed protein product [*Spirodela intermedia*]	1.74	1.98	-0.26	3.50
TRINITY_DN3573_c0_g1_i9-s3	unnamed protein product [*Spirodela intermedia*] & gt; CAA7401945.1 unnamed protein product [*Spirodela intermedia*]	-1.61	-0.46	-0.53	-2.60
TRINITY_DN3626_c0_g1_i1-s7	unnamed protein product [*Spirodela intermedia*]	2.91	1.07	-0.94	3.06
TRINITY_DN3660_c0_g1_i10-s5	unnamed protein product [*Spirodela intermedia*]	0.23	-4.82		-4.56
TRINITY_DN37709_c0_g1_i1-s2	transcription factor CPC-like isoform X2 [*Quercus suber*] & gt; XP_030931413.1 transcription factor CPC-like [*Quercus lobata*]	-2.46	1.31	-3.16	-4.31
TRINITY_DN4198_c0_g1_i4-s7	unnamed protein product [*Spirodela intermedia*] & gt; CAA7391552.1 unnamed protein product [*Spirodela intermedia*]	2.52	0.21	0.13	2.86
TRINITY_DN4329_c0_g1_i12-s5	unnamed protein product [*Spirodela intermedia*]	3.61	-1.18	0.30	2.74
TRINITY_DN4329_c0_g1_i6-s5	protein REVEILLE 1 [*Cinnamomum micranthum* f. *kanehirae*]	3.66	-0.97	1.05	3.74
TRINITY_DN4881_c0_g1_i1-s4	myb-related protein P-like protein [*Cinnamomum micranthum* f. *kanehirae*]	0.72	-2.64	-2.64	-4.52
TRINITY_DN4986_c0_g1_i1-s11	unnamed protein product [*Spirodela intermedia*]	-2.51	3.68	1.00	2.17
TRINITY_DN5095_c0_g1_i3-s2	unnamed protein product [*Spirodela intermedia*] & gt; CAA7401945.1 unnamed protein product [*Spirodela intermedia*]	2.15	-1.83	2.43	2.72
TRINITY_DN5289_c0_g1_i10-s4	unnamed protein product [*Spirodela intermedia*]	21.73	-22.26	21.39	21.69
TRINITY_DN5357_c0_g1_i3-s4	MYB-like transcription factor ETC3 [*Dioscorea cayenensis* subsp. *rotundata*]	3.33	-1.13	0.26	2.47
TRINITY_DN5557_c0_g1_i7-s9	protein REVEILLE 1 [*Cinnamomum micranthum* f. *kanehirae*]	5.45	0.17	-1.83	3.79
TRINITY_DN584_c0_g1_i6-s10	unnamed protein product [*Spirodela intermedia*]	2.24	-0.61	1.30	2.95
TRINITY_DN61_c0_g1_i6-s8	unnamed protein product [*Spirodela intermedia*]	1.79	0.59	-0.35	2.04
TRINITY_DN61_c2_g1_i2-s8	MYB-related transcription factor [*Salvia miltiorrhiza*] & gt; AGN52158.1 MYB-related transcription factor [*Salvia miltiorrhiza*]	-0.17	2.23	-5.08	-3.02
TRINITY_DN6130_c0_g1_i3-s4	unnamed protein product [*Spirodela intermedia*]	1.20	-2.99	-1.79	-3.57
TRINITY_DN7525_c0_g1_i6-s6	unnamed protein product [*Spirodela intermedia*]	6.31	-1.12	-1.17	4.05
TRINITY_DN7612_c0_g1_i1-s12	transcription factor MYB44-like [*Rhodamnia argentea*]	-0.62	1.80	1.91	3.12
TRINITY_DN7733_c0_g2_i1-s7	unnamed protein product [*Spirodela intermedia*]	1.34	-1.06	1.95	2.26
TRINITY_DN775_c0_g1_i1-s10	unnamed protein product [*Spirodela intermedia*]	2.33	1.68	0.53	4.60
TRINITY_DN775_c0_g1_i3-s10	myb-related protein MYBAS1-like isoform X2 [*Phoenix dactylifera*]	3.35	1.10	0.20	4.66
TRINITY_DN958_c1_g1_i1-s9	unnamed protein product [*Spirodela intermedia*]	4.49	1.67	-2.67	3.48

Table 7. Identified genes expressed of NAC family in *P. ternata* leaves.

Gene ID	Description	log2 Fold change			
		Stage 1-vs-Stage 2	Stage 2-vs-Stage 3	Stage 3-vs-Stage 4	Stage 1-vs-Stage 4
TRINITY_DN10201_c0_g1_i4-s5	hypothetical protein HPP92_019243 [*Vanilla planifolia*]	5.12	1.10	0.15	6.31
TRINITY_DN10416_c0_g1_i1-s11	hypothetical protein OsJ_33216 [*Oryza sativa* Japonica Group]	-0.59	1.76	1.57	2.75
TRINITY_DN10459_c0_g1_i1-s12	transcription factor JUNGBRUNNEN 1 isoform X1 [*Citrus clementina*] & gt; XP_006484909.1 transcription factor JUNGBRUNNEN 1 isoform X1 [*Citrus sinensis*] & gt; ESR50366.1 hypothetical protein CICLE_v10032203mg [*Citrus clementina*] & gt; KDO51868.1 hypothetical protein CISIN_1g021785mg [*Citrus sinensis*] & gt; GAY68152.1 hypothetical protein CUMW_261950 [*Citrus unshiu*]	4.46	-2.29	3.69	5.83
TRINITY_DN10543_c0_g1_i3-s11	unnamed protein product [*Spirodela intermedia*] & gt; CAA7404572.1 unnamed protein product [*Spirodela intermedia*]	1.58	0.34	0.13	2.08
TRINITY_DN11891_c0_g1_i15-s11	NAC domain-containing protein 68-like [*Phalaenopsis equestris*]	3.37	-0.10	0.92	4.21
TRINITY_DN12042_c0_g1_i1-s10	NAC domain-containing protein 83-like [*Abrus precatorius*]			2.47	2.76
TRINITY_DN12284_c0_g1_i1-s4	NAC domain-containing protein 87-like [*Tripterygium wilfordii*] & gt; KAF5735256.1 NAC domain-containing protein [*Tripterygium wilfordii*]	3.74	-1.99	1.00	2.75
TRINITY_DN12333_c0_g2_i1-s2	NAC domain-containing protein 53-like isoform X2 [*Asparagus officinalis*]	0.12	-1.01	-1.76	-2.65
TRINITY_DN12741_c0_g1_i1-s8	NAC domain-containing protein 90-like [*Phoenix dactylifera*]	7.41	0.54	-2.51	5.40
TRINITY_DN1335_c0_g1_i4-s12	PREDICTED: NAC domain-containing protein 68-like [*Nelumbo nucifera*] & gt; DAD42013.1 TPA_asm: hypothetical protein HUJ06_000243 [*Nelumbo nucifera*]	-0.33	1.94	2.00	3.61
TRINITY_DN13553_c0_g1_i8-s9	unnamed protein product [*Spirodela intermedia*] & gt; CAB1184518.1 unnamed protein product [*Spirodela intermedia*]	4.25	0.45	-1.82	2.85
TRINITY_DN1487_c1_g1_i6-s5	unnamed protein product [*Spirodela intermedia*] & gt; CAB1184518.1 unnamed protein product [*Spirodela intermedia*]	5.77	0.18	-1.39	4.58
TRINITY_DN15699_c0_g1_i1-s3	protein CUP-SHAPED COTYLEDON 1 [*Zea mays*] & gt; AIB04825.1 NAC transcription factor, partial [synthetic construct] & gt; ONM22681.1 NAC domain containing protein 38 [*Zea mays*]	-2.17			-2.20
TRINITY_DN15759_c0_g1_i1-s11	transcription factor JUNGBRUNNEN 1 [*Abrus precatorius*]	3.46	-1.32	4.01	6.13
TRINITY_DN1593_c0_g1_i17-s7	unnamed protein product [*Spirodela intermedia*]	1.99	1.81	0.50	4.28
TRINITY_DN1593_c0_g1_i4-s7	NAC domain-containing protein 48-like [*Phalaenopsis equestris*]	4.52	2.01	-1.04	5.50
TRINITY_DN16074_c0_g1_i2-s3	NAC domain-containing protein 71 [*Triticum urartu*]	-1.69	-0.44	-0.19	-2.26
TRINITY_DN16074_c0_g1_i3-s3	NAC domain-containing protein 90-like [*Pyrus ussuriensis* x *Pyrus communis*]	-2.11	-0.25	-0.20	-2.54
TRINITY_DN167_c0_g1_i5-s10	unnamed protein product [*Spirodela intermedia*]	2.67	1.75	-0.76	3.66
TRINITY_DN171_c0_g1_i11-s5	unnamed protein product [*Spirodela intermedia*]	3.07	2.15	0.05	5.24
TRINITY_DN171_c0_g1_i2-s5	NAC domain-containing protein 68-like [*Phalaenopsis equestris*]	2.78	-0.17	0.02	2.60
TRINITY_DN171_c0_g1_i7-s5	unnamed protein product [*Spirodela intermedia*]		6.17	0.51	7.06
TRINITY_DN180_c0_g1_i4-s11	unnamed protein product [*Spirodela intermedia*]	0.10	2.28	0.99	3.34
TRINITY_DN180_c0_g3_i2-s11	unnamed protein product [*Spirodela intermedia*]	1.97	3.98	2.03	7.95
TRINITY_DN180_c0_g3_i3-s11	unnamed protein product [*Spirodela intermedia*]	0.29	3.16	0.53	3.96

Table 7. (Cont'd.).

Gene ID	Description	log2 Fold change			
		Stage 1-vs-Stage 2	Stage 2-vs-Stage 3	Stage 3-vs-Stage 4	Stage 1-vs-Stage 4
TRINITY_DN180_c0_g3_i4-s11	unnamed protein product [*Spirodela intermedia*]	0.94	2.80	2.30	6.00
TRINITY_DN18661_c0_g1_i1-s11	NAC domain-containing protein 48 [*Oryza brachyantha*]	1.77	1.78	1.52	5.05
TRINITY_DN18840_c0_g1_i1-s7	unnamed protein product [*Spirodela intermedia*]	2.33	3.53	1.93	7.77
TRINITY_DN19139_c0_g1_i1-s3	unnamed protein product [*Spirodela intermedia*]	-1.16	1.84	2.32	3.02
TRINITY_DN21024_c0_g1_i2-s2	hypothetical protein COCNU_04G009560 [*Cocos nucifera*]	-1.19	-1.58	-0.27	-3.02
TRINITY_DN21081_c0_g1_i4-s2	NAC domain-containing protein 68 [*Elaeis guineensis*]	1.22	1.43	0.29	2.90
TRINITY_DN2403_c1_g1_i3-s5	PREDICTED: NAC domain-containing protein 35 [*Nelumbo nucifera*]	1.84	-2.52	-2.72	-3.40
TRINITY_DN24074_c0_g2_i1-s4	NAC domain-containing protein 68 [*Elaeis guineensis*]	2.95	0.05	-0.54	2.44
TRINITY_DN25623_c0_g1_i2-s5	NAC domain-containing protein 68 [*Cocos nucifera*]	1.68	0.60	1.67	3.94
TRINITY_DN2575_c0_g1_i1-s7	NAC domain-containing protein 100 [*Elaeis guineensis*]	1.92	3.07	-0.49	4.52
TRINITY_DN2575_c0_g2_i5-s7	unnamed protein product [*Spirodela intermedia*]	3.97	1.51	0.29	5.79
TRINITY_DN2603_c0_g1_i10-s8	NAC domain-containing protein 2 [*Glycine soja*]	5.52	1.50	-2.24	4.79
TRINITY_DN2612_c0_g1_i9-s9	NAC domain-containing protein 68 [*Cocos nucifera*]	2.50	1.38	1.01	4.87
TRINITY_DN2682_c0_g1_i5-s4	NAC domain-containing protein 35 [*Elaeis guineensis*]	-0.15	-1.45	-1.81	-3.42
TRINITY_DN2798_c1_g1_i1-s4	protein NTM1-like 9 [*Phoenix dactylifera*]	21.18	-21.55	6.89	7.20
TRINITY_DN2843_c0_g1_i7-s6	unnamed protein product [*Spirodela intermedia*] > CAB1184518.1 unnamed protein product [*Spirodela intermedia]*	0.59	1.76	0.09	2.44
TRINITY_DN3072_c0_g1_i3-s3	PREDICTED: NAC domain-containing protein 35 [*Nelumbo nucifera*]	-0.20	-1.55	-1.33	-3.07
TRINITY_DN30870_c0_g1_i1-s1	NAC protein 1 [*Elaeis guineensis*]	-1.46	4.33	0.15	3.01
TRINITY_DN3262_c0_g1_i7-s12	hypothetical protein HPP92_019243 [*Vanilla planifolia*]		3.06	1.29	4.75
TRINITY_DN3409_c0_g1_i6-s1	NAC domain-containing protein 71 [*Triticum urartu*]	-0.77	-1.31	0.04	-2.03
TRINITY_DN3409_c0_g1_i9-s1	PREDICTED: NAC domain-containing protein 40 isoform X2 [*Tarenaya hassleriana*]	-2.34	-3.80		-6.10
TRINITY_DN3652_c0_g1_i1-s6	unnamed protein product [*Spirodela intermedia*] > CAB1184518.1 unnamed protein product [*Spirodela intermedia*]	4.83	-1.51	-1.07	2.26
TRINITY_DN4030_c0_g1_i1-s1	hypothetical protein HPP92_019243 [*Vanilla planifolia*]	1.71	1.59	-0.84	2.43
TRINITY_DN44_c0_g1_i12-s8	unnamed protein product [*Spirodela intermedia*]	1.45	3.05	0.50	4.99
TRINITY_DN44_c0_g1_i2-s8	NAC domain-containing protein 68-like protein [*Cinnamomum micranthum* f. *kanehirae*]	1.36	2.14	0.51	3.98
TRINITY_DN484_c0_g1_i10-s10	unnamed protein product [*Spirodela intermedia*]	3.50	1.47	0.67	5.66
TRINITY_DN484_c0_g1_i9-s10	unnamed protein product [*Spirodela intermedia*]	2.52	0.07	0.69	3.25
TRINITY_DN4882_c0_g1_i1-s12	unnamed protein product [*Spirodela intermedia*]		2.37	5.55	8.30
TRINITY_DN4882_c0_g1_i4-s12	unnamed protein product [*Spirodela intermedia*]	1.34	1.78	5.61	8.73

Table 7. (Cont'd.).

Gene ID	**Description**	**log2 Fold change**			
		Stage 1-vs-Stage 2	**Stage 2-vs-Stage 3**	**Stage 3-vs-Stage 4**	**Stage 1-vs-Stage 4**
TRINITY_DN4888_c0_g1_i1-s11	unnamed protein product [*Spirodela intermedia*]	3.24	3.00	2.82	9.04
TRINITY_DN4888_c0_g1_i6-s11	unnamed protein product [*Spirodela intermedia*]	2.50	2.50	4.49	9.46
TRINITY_DN503_c0_g1_i6-s9	unnamed protein product [*Spirodela intermedia*] > CAB1184518.1 unnamed protein product [*Spirodela intermedia*]	1.90	0.77	0.30	2.94
TRINITY_DN5466_c0_g2_i4-s12	unnamed protein product [*Spirodela intermedia*]	1.13	3.40	2.30	6.84
TRINITY_DN5788_c0_g1_i9-s12	NAC domain-containing protein 22 [*Dendrobium catenatum*]	2.73	-0.82	0.88	2.74
TRINITY_DN5873_c1_g1_i1-s3	NAC domain-containing protein 67-like [*Lactuca sativa*] > PLY96701.1 hypothetical protein LSAT_6X56480 [*Lactuca sativa*]	-3.38	1.89	-0.63	-2.15
TRINITY_DN601_c0_g1_i1-s11	unnamed protein product [*Spirodela intermedia*]	5.38	2.63	0.79	8.77
TRINITY_DN601_c0_g1_i4-s11	unnamed protein product [*Spirodela intermedia*]	2.59	1.37	3.79	7.71
TRINITY_DN6037_c0_g1_i3-s12	NAC protein [*Lilium pumilum*]	-0.08	2.08	1.10	3.09
TRINITY_DN6683_c0_g1_i6-s8	NAC domain-containing protein 68-like [*Phoenix dactylifera*]	0.35	1.71	0.27	2.31
TRINITY_DN726_c0_g1_i9-s12	LOW QUALITY PROTEIN: NAC domain-containing protein 13-like [*Dioscorea cayenensis* subsp. *rotundata*]	1.14	-0.32	1.33	2.17
TRINITY_DN7413_c0_g1_i11-s12	NAC protein 1 [*Cocos nucifera*]	2.22	-0.08	0.54	2.68
TRINITY_DN7777_c0_g1_i12-s2	hypothetical protein CRG98_025956 [*Punica granatum*]	-1.79	-0.26	-0.15	-2.19
TRINITY_DN813_c0_g2_i2-s9	NAC domain-containing protein 68-like [*Phalaenopsis equestris*]	2.33	-2.04	1.85	2.13
TRINITY_DN8157_c0_g1_i1-s4	unnamed protein product [*Spirodela intermedia*]	7.40	-6.20	3.45	4.73
TRINITY_DN8590_c0_g1_i1-s4	unnamed protein product [*Spirodela intermedia*] > CAA7391186.1 unnamed protein product [*Spirodela intermedia*]	0.49	-6.90		-6.32
TRINITY_DN8697_c0_g1_i2-s1	unnamed protein product [*Spirodela intermedia*]	-3.13	-3.82	-0.94	-7.87
TRINITY_DN8922_c0_g1_i1-s7	NAC domain-containing protein 1 [*Phyllostachys edulis*]	4.60	2.53	-0.33	6.80
TRINITY_DN9273_c0_g1_i1-s11	uncharacterized protein A4U43_C07F7860 [*Asparagus officinalis*]	1.34	-0.10	2.21	3.45
TRINITY_DN9273_c0_g1_i5-s11	hypothetical protein C4D60_Mb03t19160 [*Musa balbisiana*]		2.33	2.73	5.45
TRINITY_DN9456_c0_g1_i6-s7	unnamed protein product [*Spirodela intermedia*] > CAA7404572.1 unnamed protein product [*Spirodela intermedia*]	3.55	0.83	-1.24	3.16
TRINITY_DN965_c0_g2_i10-s12	unnamed protein product [*Spirodela intermedia*]	0.72	1.74	1.36	3.81
TRINITY_DN965_c0_g2_i7-s12	unnamed protein product [*Spirodela intermedia*] > CAB1184518.1 unnamed protein product [*Spirodela intermedia*]	-0.34	2.31	0.16	2.11
TRINITY_DN965_c1_g2_i1-s12	unnamed protein product [*Spirodela intermedia*]	3.95	1.12	1.92	6.99
TRINITY_DN9768_c0_g1_i1-s5	NAC domain-containing protein 90-like [*Elaeis guineensis*]	6.04	-1.18	0.18	5.02
TRINITY_DN9768_c0_g1_i2-s5	NAC domain-containing protein 90-like [*Phoenix dactylifera*]	21.17	-1.35	-1.85	4.59

Table 8. Identified genes expressed of trihelix family in *P. ternata* leaves.

Gene ID	Description	log2 Fold change Stage 1-vs-Stage 2	Stage 2-vs-Stage 3	Stage 3-vs-Stage 4	Stage 1-vs-Stage 4
TRINITY_DN1089_c0_g3_i4-s5	trihelix transcription factor GTL1 isoform X2 [*Phoenix dactylifera*]	-0.67	-1.61	-0.89	-3.16
TRINITY_DN11317_c0_g1_i3-s6	Myb_DNA-bind_4 domain-containing protein [*Cephalotus follicularis*]	4.24	-1.06	0.23	3.39
TRINITY_DN1155_c1_g1_i1-s5	unnamed protein product [*Spirodela intermedia*]	5.20	-0.14	-0.69	4.37
TRINITY_DN120_c0_g1_i1-s1	unnamed protein product [*Spirodela intermedia*]	-1.49	-1.27	-1.72	-4.45
TRINITY_DN120_c0_g2_i1-s1	trihelix transcription factor GTL1 isoform X2 [*Phoenix dactylifera*]	-0.58	-1.17	-0.93	-2.67
TRINITY_DN120_c0_g2_i3-s1	unnamed protein product [*Spirodela intermedia*]	-1.54	-0.45	-1.01	-2.97
TRINITY_DN120_c0_g2_i4-s1	PREDICTED: trihelix transcription factor GTL1 isoform X2 [*Nelumbo nucifera*] > DAD36061.1 TPA_asm: hypothetical protein HUJ06_006701 [*Nelumbo nucifera*]	-0.69	-0.56	-1.09	-2.30
TRINITY_DN14949_c0_g1_i1-s7	PREDICTED: trihelix transcription factor ASIL2-like [*Musa acuminata* subsp. *malaccensis*] > CAG1834182.1 unnamed protein product [*Musa acuminata* subsp. *malaccensis*]	0.49	1.18	0.80	2.47
TRINITY_DN15399_c0_g1_i2-s10	hypothetical protein SADUNF_Sadunf16G0297900 [*Salix dunnii*]	3.15	-3.83	4.13	3.47
TRINITY_DN15821_c0_g1_i1-s3	PREDICTED: trihelix transcription factor GTL1-like isoform X2 [*Musa acuminata* subsp. *malaccensis*]	-2.87			-2.92
TRINITY_DN1769_c0_g1_i6-s8	hypothetical protein C4D60_Mb08t11000 [*Musa balbisiana*]	2.16	-2.99	-1.26	-2.04
TRINITY_DN1845_c0_g1_i3-s10	unnamed protein product [*Spirodela intermedia*]	6.24	-1.00	1.27	6.54
TRINITY_DN24034_c0_g1_i3-s2	unnamed protein product [*Spirodela intermedia*]	-3.87	1.24	-4.51	-7.12
TRINITY_DN3235_c0_g1_i3-s5	unnamed protein product [*Spirodela intermedia*]	-0.12	1.66	-7.00	-5.47
TRINITY_DN3271_c1_g1_i6-s7	LOW QUALITY PROTEIN: trihelix transcription factor ASR3-like [*Elaeis guineensis*]	3.39	1.50	-0.32	4.54
TRINITY_DN3467_c0_g1_i5-s1	trihelix transcription factor GTL1 isoform X2 [*Elaeis guineensis*]	0.16	-1.54	-1.08	-2.43
TRINITY_DN3950_c0_g1_i7-s11	unnamed protein product [*Spirodela intermedia*]	-2.17		5.42	3.22
TRINITY_DN5323_c0_g2_i1-s10	trihelix transcription factor GTL1 isoform X2 [*Phoenix dactylifera*]	1.68	0.66	0.71	3.04
TRINITY_DN55_c1_g1_i1-s3	unnamed protein product [*Spirodela intermedia*]	-1.66	-1.14	-2.80	-5.57
TRINITY_DN616_c0_g3_i1-s2	trihelix transcription factor GTL1 isoform X2 [*Phoenix dactylifera*]	-2.98	0.62	0.18	-2.18
TRINITY_DN7105_c0_g1_i4-s8	trihelix transcription factor GTL1 isoform X2 [*Ricinus communis*]	1.19	2.26	-1.19	2.28
TRINITY_DN8536_c0_g1_i3-s1	Trihelix transcription factor GT-2 [*Zea mays*]	-3.94	2.15	-2.70	-4.50
TRINITY_DN8536_c0_g3_i1-s1	hypothetical protein HHK36_019293 [*Tetracentron sinense*]	-5.00	2.56	-3.02	-5.48
TRINITY_DN8588_c0_g2_i1-s6	hypothetical protein C4D60_Mb04t32090 [*Musa balbisiana*]	5.32	-1.68	1.04	4.69
TRINITY_DN9677_c1_g1_i1-s10	unnamed protein product [*Spirodela intermedia*]	1.15	0.00	1.90	3.09

Table 9. Identified genes expressed of WRKY family in *P. ternata* leaves.

Gene ID	Description	log2 Fold change			
		Stage 1-vs-Stage 2	Stage 2-vs-Stage 3	Stage 3-vs-Stage 4	Stage 1-vs-Stage 4
TRINITY_DN10125_c0_g1_i4-s1	B-box zinc finger protein 23-like [*Phoenix dactylifera*]	0.19	2.05	0.76	2.98
TRINITY_DN1126_c0_g1_i10-s10	unnamed protein product [*Spirodela intermedia*]	3.19	-0.86	0.11	2.43
TRINITY_DN1126_c0_g1_i13-s10	PREDICTED: probable WRKY transcription factor 70 [*Musa acuminata* subsp. *malaccensis*] > CAG1835452.1 unnamed protein product [*Musa acuminata* subsp. *malaccensis*]	3.30	-1.14	0.29	2.46
TRINITY_DN11458_c0_g1_i4-s11	putative WRKY transcription factor 70 [*Cinnamomum micranthum* f. *kanehirae*]	2.50	-2.22	3.66	3.94
TRINITY_DN11892_c0_g1_i6-s11	unnamed protein product [*Spirodela intermedia*]	2.97	1.45	-0.01	4.35
TRINITY_DN1256_c0_g1_i5-s5	probable WRKY transcription factor 31 [*Phoenix dactylifera*]	2.14	0.79	1.66	4.54
TRINITY_DN1256_c0_g1_i7-s5	WRKY transcription factor 42 [*Cocos nucifera*]	3.94	0.33	-0.16	4.07
TRINITY_DN13_c0_g1_i10-s12	WRKY transcription factor WRKY24 [*Cocos nucifera*]	-0.96	2.42	1.88	3.31
TRINITY_DN13_c0_g1_i11-s12	WRKY transcription factor WRKY24 isoform X2 [*Elaeis guineensis*]	2.24	1.82	0.46	4.49
TRINITY_DN13_c0_g1_i3-s12	transcription factor WRKY33 [*Lindera glauca*]	3.46	2.00	-0.40	5.03
TRINITY_DN13_c0_g1_i8-s12	transcription factor WRKY33 [*Lindera glauca*]	0.40	0.79	1.25	2.42
TRINITY_DN13545_c0_g1_i3-s5	PREDICTED: WRKY transcription factor 22 [Nelumbo nucifera] > DAD47084.1 TPA_asm: hypothetical protein HUJ06_017021 [*Nelumbo nucifera*]	1.36	0.82	-0.09	2.10
TRINITY_DN137_c0_g1_i10-s5	probable WRKY transcription factor 51 [*Populus alba*] > TKR78686.1 putative WRKY transcription factor 51 [*Populus alba*]	9.21	-1.41	-1.13	6.64
TRINITY_DN137_c0_g1_i13-s5	probable WRKY transcription factor 51 [*Populus alba*] > TKR78686.1 putative WRKY transcription factor 51 [*Populus alba*]	9.06	-2.59	0.01	6.47
TRINITY_DN146_c0_g1_i17-s5	unnamed protein product [*Spirodela intermedia*] > CAB1184584.1 unnamed protein product [*Spirodela intermedia*]	-0.76	0.62	-3.89	-3.99
TRINITY_DN146_c0_g1_i2-s5	unnamed protein product [*Spirodela intermedia*] > CAB1184584.1 unnamed protein product [*Spirodela intermedia*]	2.15	0.07	0.19	2.41
TRINITY_DN146_c0_g1_i9-s5	unnamed protein product [*Spirodela intermedia*] > CAB1184584.1 unnamed protein product [*Spirodela intermedia*]	1.26	-0.55	1.37	2.08
TRINITY_DN1796_c0_g1_i6-s5	PREDICTED: probable WRKY transcription factor 38 [*Musa acuminata* subsp. *malaccensis*] > CAG1837781.1 unnamed protein product [*Musa acuminata* subsp. *malaccensis*]	7.48	-2.26	-1.69	3.53
TRINITY_DN181_c0_g1_i21-s11	unnamed protein product [*Spirodela intermedia*]	5.53	-6.23	6.17	5.51
TRINITY_DN181_c0_g1_i7-s11	PREDICTED: probable WRKY transcription factor 38 [*Musa acuminata* subsp. *malaccensis*] > CAG1837781.1 unnamed protein product [*Musa acuminat*a subsp. *malaccensis*]	5.55	-6.24	8.15	7.47
TRINITY_DN181_c0_g1_i8-s11	unnamed protein product [*Spirodela intermedia*]	-4.05			-4.08
TRINITY_DN181_c0_g1_i9-s11	unnamed protein product [*Spirodela intermedia*]	1.87	-0.26	1.66	3.25
TRINITY_DN1832_c1_g1_i1-s4	unnamed protein product [*Spirodela intermedia*] & gt; CAB1184584.1 unnamed protein product [*Spirodela intermedia*]	0.00	-1.76	-0.90	-2.62
TRINITY_DN1865_c0_g1_i12-s9	probable WRKY transcription factor 31 [*Phoenix dactylifera*]	3.63	0.94	-0.20	4.32

Table 9. (Cont'd.).

Gene ID	Description	log2 Fold change			
		Stage 1-vs-Stage 2	Stage 2-vs-Stage 3	Stage 3-vs-Stage 4	Stage 1-vs-Stage 4
TRINITY_DN1865_c0_g1_i5-s9	probable WRKY transcription factor 31 [*Phoenix dactylifera*]	4.87	1.20	-2.24	3.83
TRINITY_DN1865_c0_g1_i9-s9	WRKY transcription factor 42 [*Cocos nucifera*]	8.93	1.27	-2.82	7.37
TRINITY_DN1869_c0_g1_i1-s5	PREDICTED: probable WRKY transcription factor 40 [*Cucumis melo*] & gt; TYK27018.1 putative WRKY transcription factor 40 [*Cucumis melo* var. *makuwa*]	1.77	1.44	0.29	3.49
TRINITY_DN1888_c0_g1_i1-s12	unnamed protein product [*Spirodela intermedia*]	4.25	0.18	0.03	4.51
TRINITY_DN1888_c0_g1_i5-s12	unnamed protein product [*Spirodela intermedia*]	1.52	1.59	-0.66	2.47
TRINITY_DN1888_c0_g1_i7-s12	hypothetical protein BHM03_00000256 [*Ensete ventricosum*]	-1.28	2.51	1.71	2.93
TRINITY_DN1888_c0_g1_i8-s12	hypothetical protein B296_00052271 [*Ensete ventricosum*]	-3.49	4.31	2.81	3.65
TRINITY_DN1917_c0_g1_i1-s12	probable WRKY transcription factor 47 [*Phoenix dactylifera*]	7.64	-2.88	3.11	7.83
TRINITY_DN1917_c0_g1_i6-s12	probable WRKY transcription factor 47 [*Phoenix dactylifera*]	1.95	0.15	2.68	4.73
TRINITY_DN1934_c0_g1_i4-s7	probable WRKY transcription factor 31 [*Phoenix dactylifera*]	3.74	1.06	-0.65	4.12
TRINITY_DN19883_c0_g3_i1-s12	putative WRKY transcription factor 57, partial [*Cucurbita argyrosperma* subsp. *argyrosperma*]		1.77	2.46	4.59
TRINITY_DN2214_c0_g1_i13-s1	unnamed protein product [*Spirodela intermedia*]	-4.67	2.19	0.16	-2.34
TRINITY_DN236_c0_g1_i4-s6	unnamed protein product [*Spirodela intermedia*]	1.43	-1.05	1.73	2.15
TRINITY_DN2407_c0_g1_i10-s8	probable WRKY transcription factor 31 [*Phoenix dactylifera*]	1.51	1.74	1.39	4.60
TRINITY_DN2600_c0_g2_i1-s10	putative WRKY transcription factor 11 [*Apostasia shenzhenica*]	-0.35	1.34	1.78	2.78
TRINITY_DN27822_c0_g1_i1-s5	unnamed protein product [*Spirodela intermedia*]	3.82	-4.12	3.77	3.45
TRINITY_DN3099_c0_g1_i6-s5	WRKY transcription factor WRKY51-like [*Phoenix dactylifera*]	3.46	0.81	-1.59	2.68
TRINITY_DN3117_c0_g1_i6-s5	WRKY1 [*Anthurium andraeanum*]	1.95	1.20	-0.54	2.58
TRINITY_DN3542_c0_g1_i6-s6	probable WRKY transcription factor 70 [*Elaeis guineensis*]	6.18	-2.88	-0.99	2.32
TRINITY_DN3560_c0_g2_i3-s12	unnamed protein product [*Spirodela intermedia*]	1.48	0.23	0.59	2.29
TRINITY_DN380_c0_g2_i3-s9	hypothetical protein RHGRI_023372 [*Rhododendron griersonianum*]	0.82	1.94	-0.38	2.39
TRINITY_DN380_c1_g1_i2-s9	WRKY transcription factor 22 [*Dendrobium catenatum*]	3.54	0.80	-1.55	2.78
TRINITY_DN382_c0_g1_i10-s10	WRKY transcription factor WRKY24 [*Cocos nucifera*]	2.09	1.36	1.07	4.50
TRINITY_DN39_c0_g1_i6-s8	transcription factor WRKY33 [*Lindera glauca*]	1.72	1.34	-0.13	2.90
TRINITY_DN393_c0_g1_i1-s12	WRKY transcription factor WRKY51 [*Elaeis guineensis*]	0.82	0.62	2.19	3.66
TRINITY_DN393_c0_g1_i2-s12	PREDICTED: probable WRKY transcription factor 17 [*Nelumbo nucifera*]	0.66	2.10	2.31	5.07
TRINITY_DN393_c0_g1_i4-s12	putative WRKY transcription factor 11 [*Apostasia shenzhenica*]	-0.18	1.94	1.49	3.22
TRINITY_DN393_c0_g1_i5-s12	PREDICTED: probable WRKY transcription factor 17 [*Nelumbo nucifera*]	-0.41	-0.30	2.98	2.26
TRINITY_DN393_c0_g1_i6-s12	PREDICTED: probable WRKY transcription factor 17 [*Nelumbo nucifera*]	8.15	1.08	1.54	10.74
TRINITY_DN393_c0_g1_i9-s12	WRKY transcription factor WRKY51 [*Elaeis guineensis*]	8.36	1.51	0.77	10.60
TRINITY_DN393_c0_g3_i1-s12	WRKY transcription factor 22 [*Elaeis guineensis*]	0.94	1.88	-0.33	2.48

Table 9. (Cont'd.).

Gene ID	Description	log2 Fold change			
		Stage 1-vs-Stage 2	Stage 2-vs-Stage 3	Stage 3-vs-Stage 4	Stage 1-vs-Stage 4
TRINITY_DN409_c0_g1_i1-s11	WRKY transcription factor WRKY24 [*Cocos nucifera*]	1.90	1.84	1.14	4.85
TRINITY_DN409_c0_g1_i3-s11	WRKY transcription factor WRKY24 [*Cocos nucifera*]	1.24	1.18	0.95	3.35
TRINITY_DN409_c0_g1_i4-s11	WRKY transcription factor WRKY24 [*Cocos nucifera*]	-2.36	3.69	1.33	2.60
TRINITY_DN409_c0_g1_i7-s11	WRKY transcription factor WRKY24 [*Cocos nucifera*]	3.39	2.22	0.10	5.70
TRINITY_DN409_c0_g1_i9-s11	unnamed protein product [*Spirodela intermedia*]	1.80	1.14	1.23	4.14
TRINITY_DN421_c1_g1_i9-s7	WRKY transcription factor WRKY51-like [*Phoenix dactylifera*]	1.96	0.45	-0.25	2.17
TRINITY_DN4274_c0_g1_i6-s10	unnamed protein product [*Ananas comosus* var. *bracteatus*]	1.35	1.93	0.26	3.52
TRINITY_DN4942_c0_g1_i1-s7	probable WRKY transcription factor 4 [*Phoenix dactylifera*] & gt; XP_008787094.2 probable WRKY transcription factor 4 [*Phoenix dactylifera*] > XP_008787095.2 probable WRKY transcription factor 4 [*Phoenix dactylifera*] & gt; XP_008787096.2 probable WRKY transcription factor 4 [*Phoenix dactylifera*] & gt; XP_038983447.1 probable WRKY transcription factor 4 [*Phoenix dactylifera*]	1.96	0.28	-0.12	2.13
TRINITY_DN521_c0_g1_i8-s5	WRKY transcription factor WRKY24-like [*Phoenix dactylifera*]	2.49	0.57	-0.79	2.24
TRINITY_DN5253_c1_g2_i2-s2	unnamed protein product [*Spirodela intermedia*]		6.05	-1.99	4.45
TRINITY_DN5253_c1_g2_i4-s2	unnamed protein product [*Spirodela intermedia*]	0.73	0.47	0.81	2.01
TRINITY_DN5462_c0_g1_i4-s8	probable WRKY transcription factor 51 [*Populus alba*] & gt; TKR78686.1 putative WRKY transcription factor 51 [*Populus alba*]	7.93	-0.70	-1.49	5.67
TRINITY_DN548_c0_g1_i14-s9	WRKY transcription factor WRKY24-like [*Phoenix dactylifera*]	2.72	1.46	-0.67	3.48
TRINITY_DN548_c0_g1_i2-s9	WRKY transcription factor WRKY24-like [*Phoenix dactylifera*]	10.02	0.59	-1.09	9.53
TRINITY_DN548_c0_g1_i4-s9	WRKY transcription factor WRKY24-like [*Phoenix dactylifera*]	0.60	1.97	0.51	3.05
TRINITY_DN5536_c0_g1_i5-s8	unnamed protein product [*Spirodela intermedia*]	-0.21	2.22	0.15	2.17
TRINITY_DN5590_c0_g1_i4-s2	protein WRKY1-like isoform X1 [*Phoenix dactylifera*]	-1.47	-1.20	-1.23	-3.88
TRINITY_DN5665_c0_g1_i3-s8	WRKY transcription factor WRKY24 [*Elaeis guineensis*]	3.54	1.42	-2.15	2.85
TRINITY_DN573_c0_g1_i3-s7	WRKY transcription factor WRKY24 [*Cocos nucifera*]	1.86	1.03	0.44	3.32
TRINITY_DN573_c0_g1_i5-s7	WRKY transcription factor WRKY24 [*Cocos nucifera*]	1.34	1.26	0.33	2.92
TRINITY_DN573_c0_g1_i6-s7	WRKY transcription factor WRKY24 [*Cocos nucifera*]	2.25	1.67	-0.23	3.67
TRINITY_DN5780_c0_g1_i3-s11	hypothetical protein F0562_000764 [*Nyssa sinensis*]	-3.39	4.22	-4.30	-3.43
TRINITY_DN6334_c0_g4_i1-s5	probable WRKY transcription factor 57 [*Vitis riparia*]	0.30	-0.54	-5.81	-6.08
TRINITY_DN647_c0_g1_i8-s8	unnamed protein product [*Spirodela intermedia*]	0.11	3.58	-0.23	3.44
TRINITY_DN66_c0_g1_i7-s12	WRKY transcription factor WRKY76-like [*Phoenix dactylifera*]	0.32	2.52	1.92	4.72
TRINITY_DN6637_c0_g1_i4-s11	WRKY transcription factor 5 [*Zostera marina*]	0.31	1.38	0.49	2.16
TRINITY_DN6664_c0_g2_i1-s4	hypothetical protein DKX38_012671 [*Salix brachista*]	5.54	-1.71	-1.87	2.01
TRINITY_DN684_c0_g1_i3-s12	unnamed protein product [*Spirodela intermedia*] & gt; CAB1184584.1 unnamed protein product [*Spirodela intermedia*]		3.77	0.46	4.59

Table 9. (Cont'd.).

Gene ID	Description	log2 Fold change			
		Stage 1-vs-Stage 2	Stage 2-vs-Stage 3	Stage 3-vs-Stage 4	Stage 1-vs-Stage 4
TRINITY_DN684_c0_g1_i4-s12	unnamed protein product [*Spirodela intermedia*] & gt; CAB1184584.1 unnamed protein product [*Spirodela intermedia*]	2.06	-0.01	0.05	2.11
TRINITY_DN6999_c0_g1_i3-s1	protein WRKY1 isoform X1 [*Elaeis guineensis*] & gt; XP_019706573.1 protein WRKY1 isoform X1 [*Elaeis guineensis*]	-0.85	-4.02	1.50	-3.34
TRINITY_DN6999_c0_g1_i7-s1	protein WRKY1 isoform X1 [*Elaeis guineensis*] & gt; XP_019706573.1 protein WRKY1 isoform X1 [*Elaeis guineensis*]	-2.30	-0.32	-0.19	-2.80
TRINITY_DN7178_c0_g1_i6-s8	unnamed protein product [*Spirodela intermedia*]	-0.08	1.05	1.14	2.12
TRINITY_DN7370_c0_g1_i3-s10	PREDICTED: probable WRKY transcription factor 70 [*Musa acuminata* subsp. *malaccensis*] & gt; CAG1846540.1 unnamed protein product [*Musa acuminata* subsp. *malaccensis*]	5.08	-0.27	-0.02	4.85
TRINITY_DN7602_c0_g1_i2-s10	hypothetical protein CIPAW_03G133500 [*Carya illinoinensis*]	4.86	-2.73	4.60	6.75
TRINITY_DN8118_c0_g1_i1-s2	probable WRKY transcription factor 7 [*Prunus dulcis*] & gt; VVA17281.1 PREDICTED: probable WRKY mRNAion factor [*Prunus dulcis*]	-1.11	-2.07	0.99	-2.16
TRINITY_DN8118_c0_g1_i4-s2	hypothetical protein RHGRI_023372 [*Rhododendron griersonianum*]	1.30	0.01	0.88	2.16
TRINITY_DN8158_c0_g2_i1-s1	hypothetical protein ZIOFF_059052 [*Zingiber officinale*]	1.84	-1.58	2.25	2.54
TRINITY_DN822_c0_g1_i12-s12	WRKY transcription factor 42 [*Cocos nucifera*]	2.65	2.27	1.12	6.01
TRINITY_DN822_c0_g1_i20-s12	probable WRKY transcription factor 31 [*Phoenix dactylifera*]	2.04	0.51	2.39	4.89
TRINITY_DN822_c0_g1_i2-s12	unnamed protein product [*Spirodela intermedia*]	1.89	1.32	0.40	3.57
TRINITY_DN8451_c0_g1_i3-s7	PREDICTED: probable WRKY transcription factor 7 [*Nicotiana attenuata*] & gt; OIT30401.1 putative wrky transcription factor 7 [*Nicotiana attenuata*]	-3.02	6.15	-0.96	2.18
TRINITY_DN852_c0_g1_i13-s9	unnamed protein product [*Ananas comosus* var. *bracteatus*]	0.90	2.27	0.39	3.53
TRINITY_DN852_c0_g1_i4-s9	unnamed protein product [*Ananas comosus* var. *bracteatus*]	1.99	1.38	-1.11	2.25
TRINITY_DN852_c0_g1_i7-s9	DNA-binding WRKY [*Macleaya cordata*]	1.63	2.41	-0.84	3.20
TRINITY_DN8567_c0_g1_i6-s11	probable WRKY transcription factor 27 [*Elaeis guineensis*]			2.44	2.72
TRINITY_DN9165_c0_g1_i4-s5	WRKY transcription factor WRKY76 isoform X2 [*Elaeis guineensis*]	7.42	-8.06	3.17	2.49
TRINITY_DN9165_c0_g2_i1-s5	probable WRKY transcription factor 40 [*Manihot esculenta*] & gt; AMO00447.1 WRKY transcription factor 79 [*Manihot esculenta*] & gt; OAY36998.1 hypothetical protein MANES_11G066500 [*Manihot esculenta*]	6.39	-6.08	1.85	2.13
TRINITY_DN9453_c0_g1_i1-s12	WRKY transcription factor 22 [*Dendrobium catenatum*]			3.85	4.13
TRINITY_DN953_c0_g1_i5-s11	WRKY1 [*Anthurium andraeanum*]	1.32	1.15	0.93	3.37
TRINITY_DN9766_c0_g2_i1-s12	unnamed protein product [*Spirodela intermedia*]	2.26	2.58	1.82	6.64
TRINITY_DN9915_c0_g2_i1-s1	probable WRKY transcription factor 30 [*Gossypium hirsutum*] & gt; AIE43839.1 WRKY transcription factor 59 [*Gossypium hirsutum*]	-2.74	0.87	-0.94	-2.81

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