The Transcriptome and Metabolic Pathways of *Persea americana* under Drought and Low-temperature Stress

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Persea americana Mill. is an important cash crop that contains effective ingredients to reduce cholesterol and protect the cardiovascular system. Presently, the gene regulation mechanism and signal pathway of stress response in *P. americana* are unclear. To explore the gene expression changes of *P. americana* under drought and low-temperature stress, the transcripts of *P. americana* were sequenced under these conditions. The results produced 42,815,960 bp raw reads. Analysis of the related metabolic pathways and differentially expressed genes showed that under drought stress, the gene expression of beta-amylase 3, glyceraldehyde-3-phosphate dehydrogenase and hexokinase were upregulated, while the gene expression of UDP-glycosyltransferase superfamily protein isoform, glucose-1-phosphate adenylyltransferase, and glucose-6-phosphate 1-epimerase were downregulated. Under low-temperature stress, the expression of beta-amylase and shikimate O-hydroxycinnamoyl transferase genes was downregulated. In addition, WRKY, MYB, bHLH, and NAC transcription factors were expressed under drought and low-temperature stress. Finally, the RNA-Seq data were validated using real-time fluorescence quantitative analysis to identify the key genes of *P. americana* regulated at the transcriptional level under drought and low-temperature stress. This study provides a theoretical basis for the selection of drought-resistant and low-temperature tolerant *P. americana* varieties.

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INTRODUCTION

Persea americana Mill., also known as the 'alligator pear,' is a Lauraceae native to Mexico and Central America (Chanderbali *et al*. 2008; Bhuyan *et al*. 2019). It is one of the world's most important tropical/subtropical fruit crops and has high economic value (Kuhn *et al*. 2019). Its fruit is not only rich in protein and fat-soluble vitamins but also in tocopherols, lutein, and other carotenoids, which are widely used in the pharmaceutical and cosmetic industries (Duarte *et al*. 2016; Tabeshpour *et al*. 2017). In addition, the oil extracted from *P. americana* seeds can be used as an alternative biodiesel source for the

biofuel industry (Ge *et al*. 2021).

Because *P. americana* fruit benefits human health, there is an increased demand for its functional compounds in the market. Alkhalaf *et al*. (2019) evaluated the antioxidant, anti-inflammatory, and anti-cancer potential of each extract of *P. americana* fruit and seed. Tremocoldi *et al*. (2018) used HPLC-MS/MS to conduct a real-time analysis of phenolic compounds obtained from green extraction of *P. americana* by-products to determine their contribution to the total biological activity of *P. americana* by-products. Ibarra-Laclette *et al*. (2015) used a high-throughput sequencing platform to develop a gene expression map of the *P. americana* transcriptome. Specifically, they analyzed the expression of genes involved in acyl lipid metabolism, maturation process, and organ specificity, aiding further research on *P. americana* basic biology. Ge *et al*. (2019a) used molecular identification methods to study the different geographical sources of *P. americana* germplasm resources in southern China and conducted a supplementary analysis. Due to its high economic value, *P. americana* is widely cultivated and developed in some provinces in southern China, including Taiwan, Hainan, Guangxi, and Yunnan (Ge *et al*. 2017, 2019b). Yunnan Province is in the southwest of China, has a complex terrain, is affected by two Asian summer monsoons, and is extremely sensitive to climate change. According to previous research, extreme weather events (such as floods, low temperatures, and droughts) have often occurred in this province in recent decades (Shi and Chen 2018), so breeding *P. americana* with strong resistance is a key factor for the success of introduction.

Abiotic stresses such as drought and low temperature affect plant growth, development, and secondary metabolism (Li *et al*. 2021). Stress reduces net photosynthetic rate, destroys chloroplast structure, reduces reactive oxygen species clearance, and reduces carbohydrate metabolism (Valliyodan and Nguyen 2006; Maestrini *et al*. 2009; Abdel-Ghany *et al*. 2020). Moreover, it leads to changes in functional genes and transcription factors (TFs) involved in metabolic and physiological processes during transcription (Wang *et al*. 2017). Xu *et al*. (2021) used Illumina sequencing technology to analyze the transcriptome of *Salix cupularis* Rehd under drought stress and identified 4,289 differentially expressed genes (DEGs) – 2,340 were upregulated, and 1,949 were downregulated. Li *et al*. (2021) found that *Larix kaempferi* (Lamb.) Carr had 27 DEGs involved in drought stress. Under drought stress, *Prunus mahaleb* L is mainly involved in carbohydrate metabolism and hormone signal transduction (Feng *et al*. 2017). *Pugionium cornutum* (L.) Gaertn was found to be mainly involved in photosynthesis, nitrogen metabolism, and hormone signal transduction pathways (Wang *et al*. 2017). Under low-temperature stress, *Populus tomentosa* Carr is mainly involved in metabolic pathways such as sugar metabolism, antioxidant defense system, hormone signaling and photosynthesis (Yang *et al*. 2019). *Populus simonii* Carr is mainly involved in photosynthesis, abscisic acid (ABA) transport, and antioxidant defense system (Song *et al*. 2013). Wang *et al*. (2013) identified 1,770 DEGs from the whole genome of *Camellia sinensis* (L.) Kuntze, of which 1,168 genes were upregulated, mainly involved in carbohydrate metabolism and calcium signaling pathways, and 58 TFs families responded to low-temperature stress. Gong *et al*. (2018) identified 239 TFs that responded to low-temperature stress from the whole genome of *Hevea brasiliensis* (Willd. ex A. Juss.) Muell. Arg. Those TFs are involved in flavonoid biosynthesis, phenylalanine biosynthesis and metabolism, plant hormone signal transduction, and starch and sucrose metabolism. Therefore, this study was conducted to investigate the molecular response mechanisms made by *P. americana* under different stresses.

In this study, NovaSeq technology was used to sequence the participating transcriptome of *P. americana* and excavate the key genes and TFs related to the metabolic pathway of drought and low-temperature stress; the results were verified by qRT PCR. The results lay the foundation for the in-depth study of *P. americana* and provide a theoretical basis for breeding drought and low-temperature resistant varieties.

EXPERIMENTAL

Plant Materials

Annual *P. americana* seedlings with good growth conditions were planted in the greenhouse at the Bailong Campus of Southwest Forestry University (Kunming, China). In line with the material treatment reference (Wang *et al*. 2014; Wu *et al*. 2015), the treatment conditions of the control group (PAMZ) were as follows: the relative soil water content was kept at 80% of the field water capacity, and normal watering (five times/d) at room temperature (25 ℃). Drought treatment (PAMG): watering of seedlings at room temperature was terminated after sufficient water has been applied to saturate the soil with water, allowing the relative soil moisture content to fall to 40% of the field holding capacity, and sampling after the leaves had curled. Low temperature treatment: *P. americana* seedlings were placed in a low temperature incubator at a low temperature of 4 ℃ (PAM4), a low temperature of 15 ℃ (PAM5), and the rest of the incubation conditions were the same. Sampling was carried out after 6 h of normal watering, and the collected leaves were immediately snap-frozen in liquid nitrogen, stored at -80 ℃, and sent to Personalbio (Shanghai, China) for transcriptome sequencing.

RNA Extraction and Sequencing

Total RNA extracted from mature leaves tissue of *P. americana* was determined under different treatments using the Ultrapure RNA Kit (CWBIO, China). The concentration and quality of RNA were detected by NanoDrop 2000 (NanoDrop; Thermo Fisher Scientific, Inc., Wilmington, DE, USA) and 1.5% agarose gel electrophoresis, respectively. The mRNA with polyA structure was enriched by Oligo (dT) magnetic beads and broken into fragments of approximately 300 bp in length by ion interruption. The first strand of cDNA was synthesized with RNA as a template, using 6-base random primers, and reverse transcriptase. The second strand of cDNA was synthesized with the first cDNA as a template. The cDNA fragment with a specific length of 450 bp was recovered for PCR amplification and sequenced using the Illumina NovaSeq platform.

Transcriptome Data Processing, Gene Ontology (GO) Annotation, and KEGG Enrichment Analysis

The obtained raw data were filtered using Cutadapt v1.12 online software to remove joints at the 3' end and read with average mass fraction below Q20 to obtain Clean Reads, and the resulting Clean Reads were annotated in the following databases: NCBI Non-Redundant Protein Sequences (Nr), eggNOG, Swissport, Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Ontology (GO). GO and KEGG enrichment analyses were performed using topGO and clusterprofiler to identify GO terms and KEGG pathways that are significantly enriched for differential genes by calculating P-values (the criterion for significant enrichment is $P < 0.05$) by the hypergeometric distribution method.

Screening of Differentially Expressed Genes

HTSeq statistics were used to compare to Read Count values on each gene as the original expression of the gene, normalized gene expression using FPKM values (FPKM ＞1), and performed differentially expressed gene analysis by DESeq, screening differentially expressed genes with the following conditions: expression difference fold |log2FoldChange|>1, significant P- value<0.05.

Quantitative Real-Time PCR (qRT-PCR) Analysis

To verify the results of transcriptome sequencing and the expression pattern of target genes, ten differentially expressed genes were randomly selected, and their gene expression was detected by qRT-PCR. Using the transcriptome data, primers were designed (Table 1) with 1-alpha (EF1a) gene as the internal regulator of gene normal expression. In addition, SYBR Green (Invitrogen Beijing) was used to detect the PCR products of specific primers in the ABI 7300 Real-Time PCR System (Applied Biosystems, Foster City, USA). The conditions of the PCR reactions were as follows: denaturation (94 °C, 2min), 40 cycles of quantitative amplification (94 °C - 15s; 60 °C - 15s; 72 °C - 45s), with a final extension step (72 °C, 7min).

Table 1. Primer Sequences

Primer name	Sequence (5'-3')
PaEF1aF	CTGAGATGAACAAGCGTT
PaEF1aR	GAATCAATAATAAGGACA
Pa0068F	ACGATCAGTACAAAGATGCA
Pa0068R	TTCACAAGTTACAATCTCAA
Pa0058F	ATAAGGAACTCTAGACATGA
Pa0058R	CATCAACCATCACACCTTCA
Pa0027F	AGATTGCTACAGCAGTGGCA
Pa0027R	AGGTTGAACAGCCATCAGAA
Pa0537F	CAAGTTCAAGTCGATTACAC
Pa0537R	CCTTGCTGATAGTTACCAGA
Pa0039F	GCCAACAGAATGCAAGAGCG
Pa0039R	TGATCTGTAAAGAAGATATC
Pa0005F	TCTACCAGTCCACCATCGAC
Pa0005R	AGCAGCCTCTTTCACTGCAA
Pa0674F	GAAACAAGTAGAGAGATGCA
Pa0674R	AGGTTCGTGGGGTCATGTGT
Pa0168F	GATTATGCATGAATATAGGC
Pa0168R	TCATTTCTGG GAGAGATTCG
Pa0149F	CTTGGTGCCAGAAGCATTGG
Pa0149R	TTGTATTCCTTGAACTTGAC

RESULTS AND DISCUSSION

Gene Sequence Assembly and Function Annotation

P. americana leaves after different treatments were analyzed using the platform Illumina NovaSeq platform, and the obtained data were uploaded to NCBI (https://www.ncbi.nlm.nih.gov/sra/SRX19802688). The results showed that the raw reads generated from each set of samples were at least 42,815,960 bp, and the clean reads obtained after filtering were at least 38,591,384 bp, and the total sequence comparison rate with the reference genome (*GCA_003546025.1*) was higher than 84.88%, Q20> 96.33% and Q30> 90.88%(Table 2). The above data indicated that the obtained *P. americana* transcriptome data were of high quality and could be analyzed in the next step.

Table 2. Statistical Results

Sample	Raw Reads	Clean Reads	Total Mapped	Q20(%)	Q30(%)
PAM4	46483170	41983952	37209140 (88.63%)	97.16	92.47
PAM5	45429866	41106282	34893004 (84.88%)	96.33	90.88
PAMG	46405380	42079586	37268575 (88.57%)	96.91	91.9
PAMZ	42815960	38591384	34285115 (88.84%)	97.26	92.65

The obtained Unigenes were subjected to BLASTx comparison of the following databases and the results showed (Fig. 1 and Table S1) that 38,178 genes were annotated in the NR database, 17,970 in the GO database, 17,816 in the KEGG database, 37,873 in the eggNOG database and 3,960 in the Swissprot database, with annotation in the NR database being the most numerous.

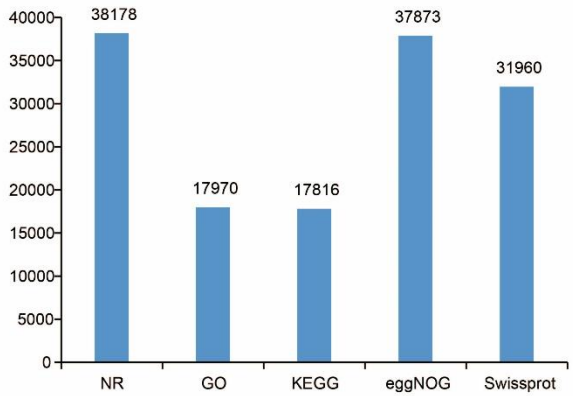

Fig. 1. Gene function annotation

Analysis of Metabolic Pathways

The results of GO analysis (Fig. 2 and Table S2) show that a total of 17,970 genes were divided into 92 functional groups, which came from three major categories: molecular function (MF), cellular component (CC), and biological process (BP). Most genes annotated for the PAMZ-PAMG comparison group were related to photosystem I and ion transport pathways: a total of 1,354 genes – including 406 MF (30%), 123 CC (9.1%), and 825 BP (60.9%). A total of 1341 genes were annotated for the PAMZ-PAM4 group, including 397 MF (29.6%), 170 (12.7%) CC, and 882 BP (65.8%) – mainly related to the inositol bisdiphosphate

tetrakisphosphate diphosphatase activity, inositol diphosphate pentakisphosphate diphosphatase activity, and photosynthesis metabolic pathways. A total of 1,508 genes were annotated for the PAMZ-PAM5 comparison group, including 456 MF (30.2%), 170 CC (11.3%), and 882 BP (58.5%). The gamma-aminobutyric acid transmembrane transporter activity was the latter group's most enriched metabolic pathway. A total of 1,387 genes were annotated in the PAMG-PAM4 comparison group, including 416 MF (30%), 149 CC (10.7%), and 912 BP (65.8%). The most enriched metabolic pathways for PAMG-PAM4 were xyloglucan: xyloglucosyl transferase activity and hemicellulose metabolic process. For the PAMG-PAM5 comparison group, 1,477 genes were annotated, including 416 MF (28.2%), 149 CC (10.1%), and 912 BP (61.7%). PAMG-PAM15 most enriched metabolic pathway was the xyloglucan metabolic process. A total of 1,581 genes were annotated in the PAM15-PAM4 comparison group, including 469 MF (29.7%), 160 CC (10.1%), and 952 BP (60.2%). The latter group's most enriched metabolic pathways were inositol bisdiphosphate tetrakisphosphate diphosphatase activity and photosynthesis.

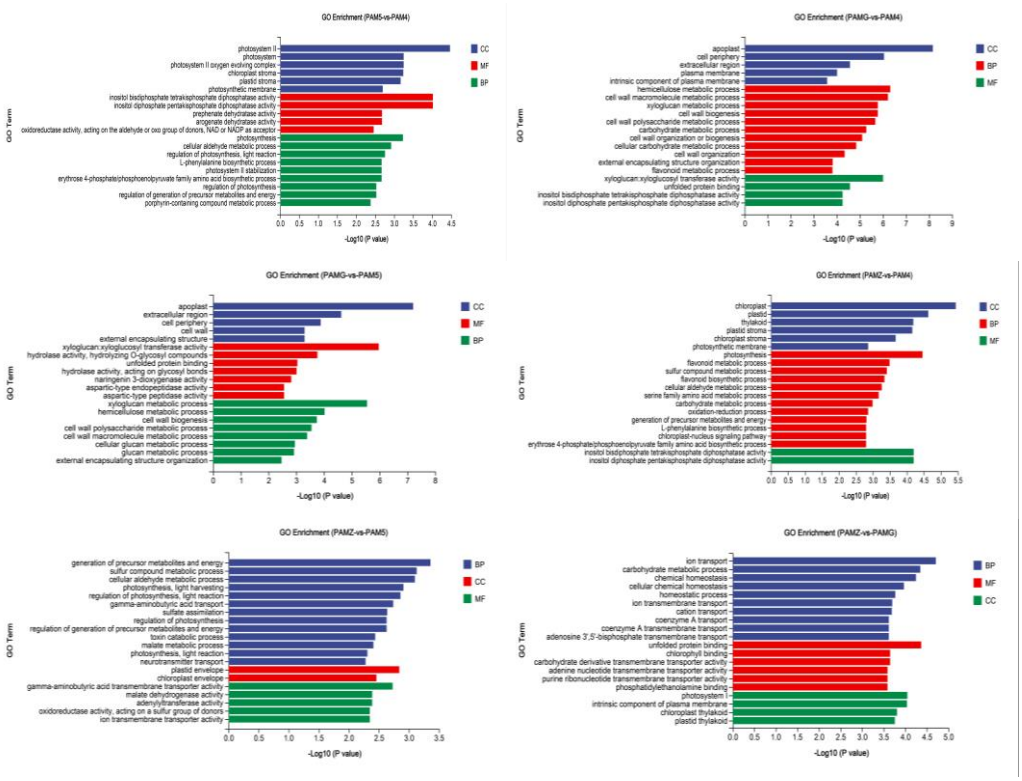

Fig. 2. GO enrichment of *P. americana* unigenes. The x-coordinate represents the GO term, and the ordinate is the -log10 (p-value) enriched by the GO Term.

The KEGG pathway analysis identified that a total of 23,224 genes were enriched into 10 KEGG metabolic pathways (Fig. 3 and Table S3). In addition, 1,442 DEGs were involved in metabolism, genetic information processing, environmental information processing, cell processes, and biological system pathways. Among those, the three most significantly enriched KEGG metabolic pathways were starch and sucrose metabolism, glycolysis/gluconeogenesis, and flavonoid biosynthesis. Therefore, it is speculated that drought and low-temperature stress impact *P. americana* gene expression.

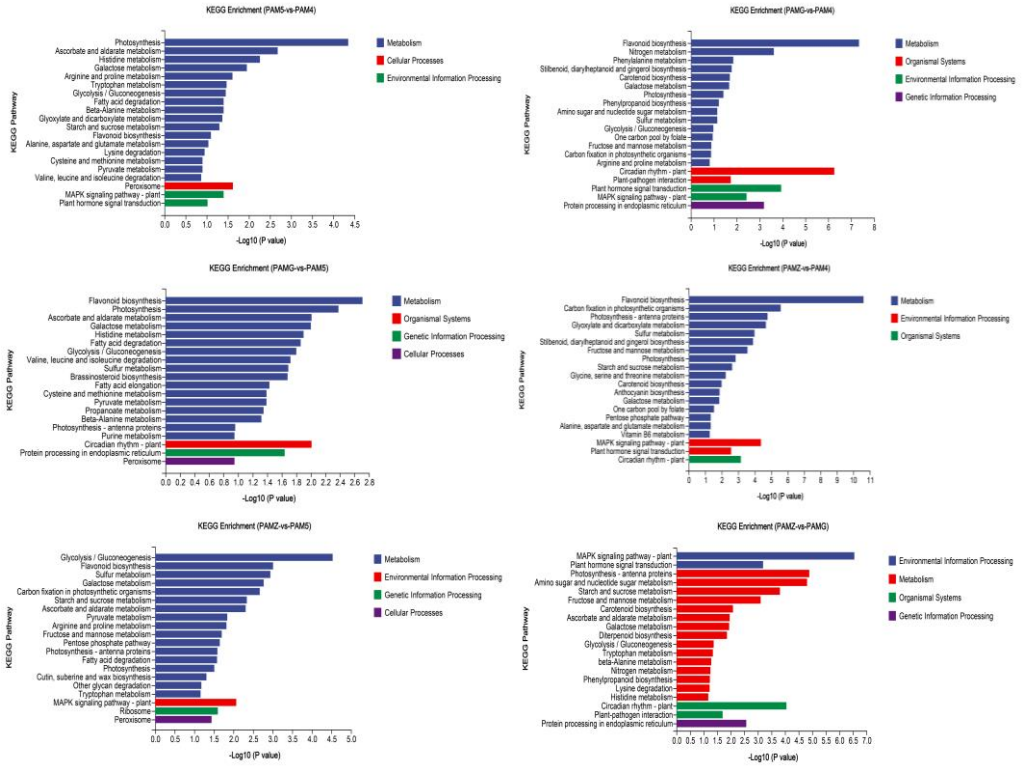

Fig. 3. KEGG enrichment of *P. americana* unigenes. The x-coordinate is KEGG Term, and the ordinate is -log10 (p-value) enriched by KEGG Term.

Identification of Differentially Expressed Genes

Differential analysis of gene expression using DESeq identified a total of 5,171 differential genes (Table 3), of which 800 were identified for PAMG_vs_PAM4 (280 upregulated, 520 downregulated), 797 for PAMG_*vs.*_PAM5 (203 upregulated, 594 downregulated), 991 for PAM5_vs_PAM4 (714 upregulated, 277 downregulated), 850 for PAMZ_vs_PAM5 (271 upregulated, 579 downregulated), 866 for PAMZ_vs_PAMG (556 upregulated, 310 downregulated) and 867 for PAMZ_vs_PAM4 (458 upregulated, 409 downregulated).

Table 3. The Number of Differentially Expressed Genes (DEGs)

Control_vs_Treat	Up regulated	Downregulated	Total
PAMG_vs_PAM4	280	520	800
PAMG_vs_PAM5	203	594	797
PAM5_vs_PAM4	714	277	991
PAMZ_vs_PAM5	271	579	850
PAMZ_vs_PAMG	556	310	866
PAMZ_vs_PAM4	458	409	867

Three treatment groups, PAMZ-vs-PAMG, PAMZ-vs-PAM4 and PAMG-vs-PAM4, were selected to produce a Venn diagram (Fig. 4), which showed that the number of shared unique differential genes between the three treatment groups was 27 (22

upregulated and 5 downregulated), further revealing that drought and low temperature stress affects gene expression in *P. americana* and regulates it through DEGs.

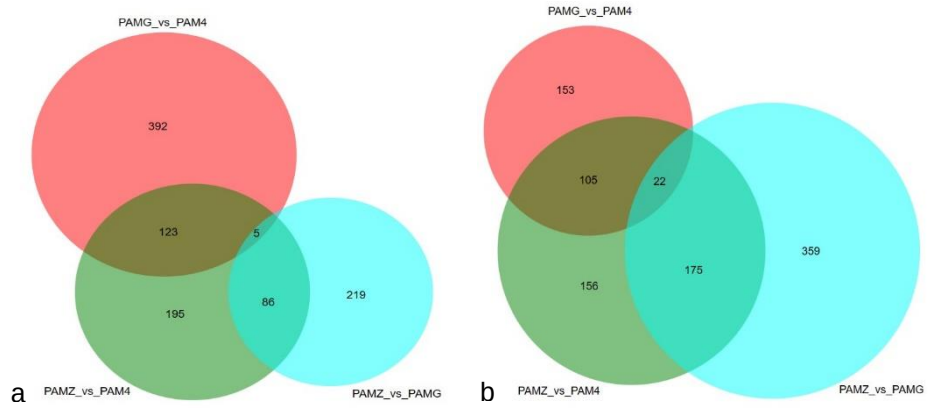

Fig. 4. Venn diagrams showing the number of differentially expressed genes (DEGs). (a) Venn diagram showing the upregulated DEGs; (b) Venn diagram showing the downregulated DEGs

Transcription Factors with High Transcript Levels

Under drought and low-temperature stress, 5,290 differentially expressed genes were found in *P. americana*, belonging to 53 TF families, of which the bHLH family was the largest, with 525 participating genes, followed by NAC and ERF, with 406 and 347 genes involved, respectively (Fig. 5). Moreover, C2H2, MYB, and WRKY family members were also involved in different biological stress responses. Taking the normal treatment as the control, at least 28 TF families were identified to be differentially expressed under drought and low-temperature stress. It was found that 25 DEGs of ERF were upregulated under drought and low-temperature stress, MYB was concentrated under drought stress, and WRKY was concentrated under low-temperature stress.

Analysis of Gene Expression in *P. americana* under Drought And Low-Temperature Stress

The online software heatmap (V1.0.8) was used to draw gene expression heat maps, and to evaluate the effects of drought and low-temperature stress on preliminary analysis of gene expression of the americana metabolic pathway and transcription factor family (Fig. 6 and Table. S4, Table. S5). The results revealed that three metabolic pathways, starch and sucrose metabolism, sugar degradation and sugar metabolic synthesis and flavonoid biosynthesis were the most significantly enriched under drought and low temperature stress, with a total of 19 genes differentially expressed. The expression of *Pa0058*, *Pa0202* and *Pa0748* genes, which were significantly enriched in starch and sucrose metabolism and sugar degradation and sugar metabolism anabolic pathways, were upregulated under drought stress, while *Pa0001*, *Pa0068*, *Pa0249* and *Pa0571* genes were downregulated. The expression of *Pa0822* gene, which was significantly enriched in flavonoid biosynthesis, was upregulated and *Pa0005*, *Pa0058* and *Pa1187* genes were downregulated under low temperature stress, presumably the flavonoid biosynthesis pathway was the main biosynthetic pathway in avocados under low temperature stress.

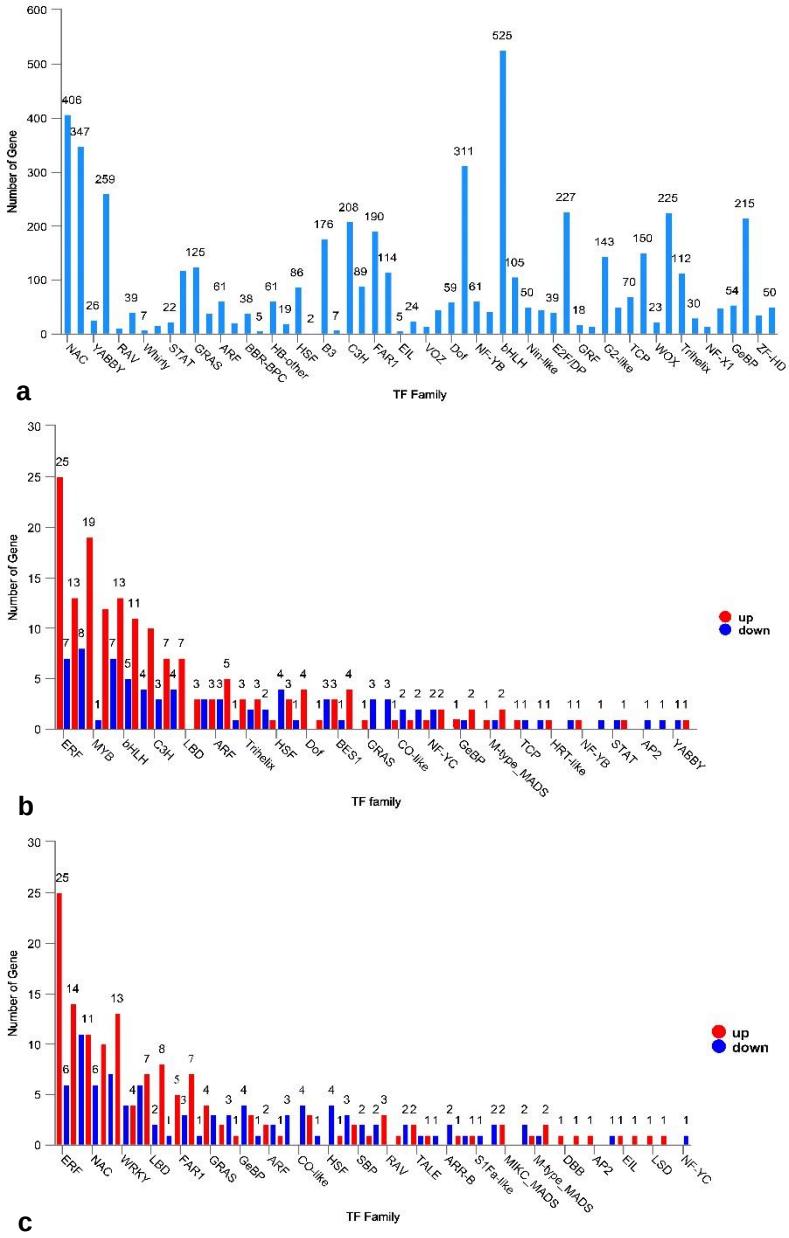

Fig. 5. Analysis of differentially expressed transcription factor genes in *P. americana* under drought and cold stress. (a) Distribution of *P. americana* transcription factor family. (b) PAMZ_vs_PAMG; (c) PAMZ_vs_PAM4. The x-coordinate is a different transcription factor family, and the ordinate is the number of different genes falling into this transcription factor family.

A total of 28 transcription factors in the transcription factor family were highly expressed under drought and low temperature stress, including the bHLH, NAC, ERF, MYB and WRKY transcription factor families. 40 DEGs encoding highly expressed transcription factors were selected for heat map analysis. The results showed that under drought stress, the expression of eight genes encoding ERF (*pa0027*, *pa0035*, *pa0039*, *pa0157*, *pa0822*) and NAC (*pa0036*, *pa0168*, *pa0553*) was upregulated, and the expression of genes encoding ERF (*pa0149*), NAC (*pa0005*, *pa1027*) and WRKY (*pa0674*) was downregulated. Under low-temperature stress, the expression of 5 DEGs of bHLH (*pa0131*, *pa00204*, *pa0335*), WRKY (*pa0537*, *pa0889*), and ERF (*pa0822*) transcription factors

were upregulated, of which the expression of bHLH and WRKY transcription factor family was significantly different, and the expression of 3 DEGs of ERF (*pa0027*, *pa0035*) and NAC (*pa0014*) transcription factors was downregulated.

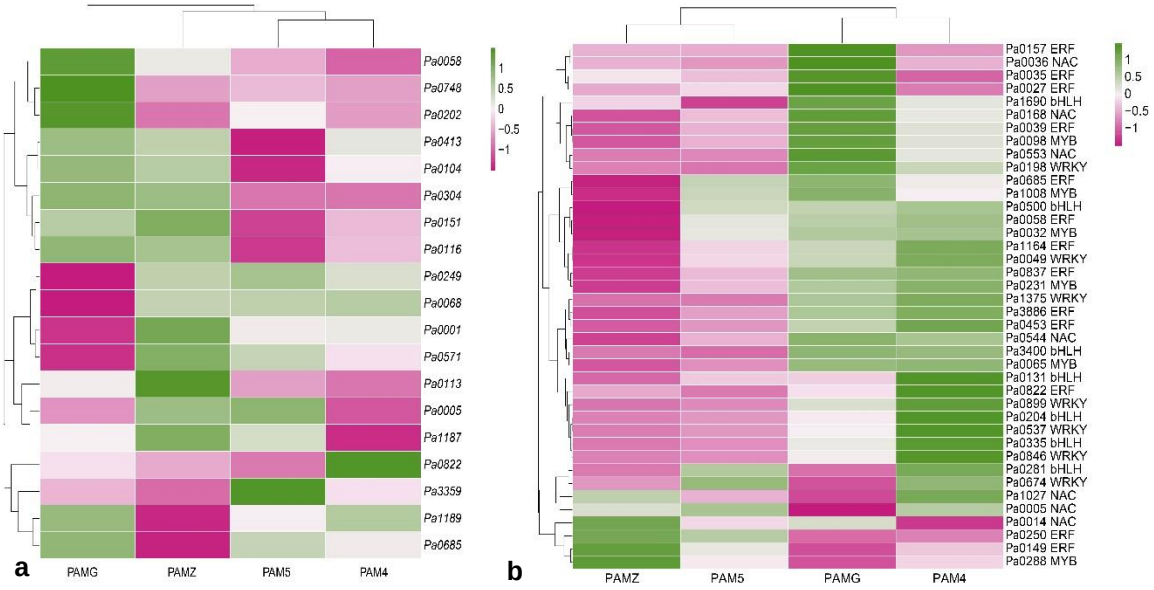

Fig. 6. (a): Heat map of 19 differentially expressed genes; (b): Heat map of transcription factor family gene expression. Horizontally represent genes, and each column represents a sample. Green and pink color marks indicate up-regulation and down-regulation of gene expression, respectively

Validation of Transcriptome Data by qRT-PCR Analyses

To verify the accuracy of the transcriptome data, nine differential genes were randomly selected for qRT-PCR analysis (Fig. 7). The results showed that the expression of *Pa0058*, *Pa0027*, *Pa0039* and *Pa0168* genes were upregulated, *Pa0068* and *Pa0005* genes were downregulated under drought stress; the expression of Pa0068, *Pa0537* and *Pa0005* genes were upregulated; and *Pa0149* gene expression was downregulated under low temperature stress. The *P. americana* data indicates that several key genes related to drought and low temperature stress in *P. americana* were regulated at the transcriptional level. The gene expression levels obtained by qRT-PCR analysis were generally consistent with the trend of transcriptome expression profiling, indicating that the transcriptome sequencing results were reliable.

When plants are under stress, genes' expression type and expression level will change significantly at the transcriptional level. The response mechanism of plants to drought and low temperature is a complex process in which multiple genes and metabolic pathways participate in regulation and interact with each other (Hwang *et al*. 2018). To further understand the gene expression of *P. americana* under drought and low-temperature stress, this study used Illumina high-throughput sequencing technology to sequence the transcriptome of *P. americana* leaves with different drought and low-temperature treatments. A total of 24,616 genes were obtained, and 5,171 differential genes were screened, including 2,482 upregulated genes and 2,689 downregulated genes. Based on KEGG pathway analysis, it was shown that starch and sucrose metabolism, flavonoid biosynthesis, and transcription factors are critically involved in the response of *P. americana* to drought and low temperature. Meanwhile, the expression changes of these

genes and their putative functions were analyzed to provide new insights into the molecular mechanisms of *P. americana* related resistance.

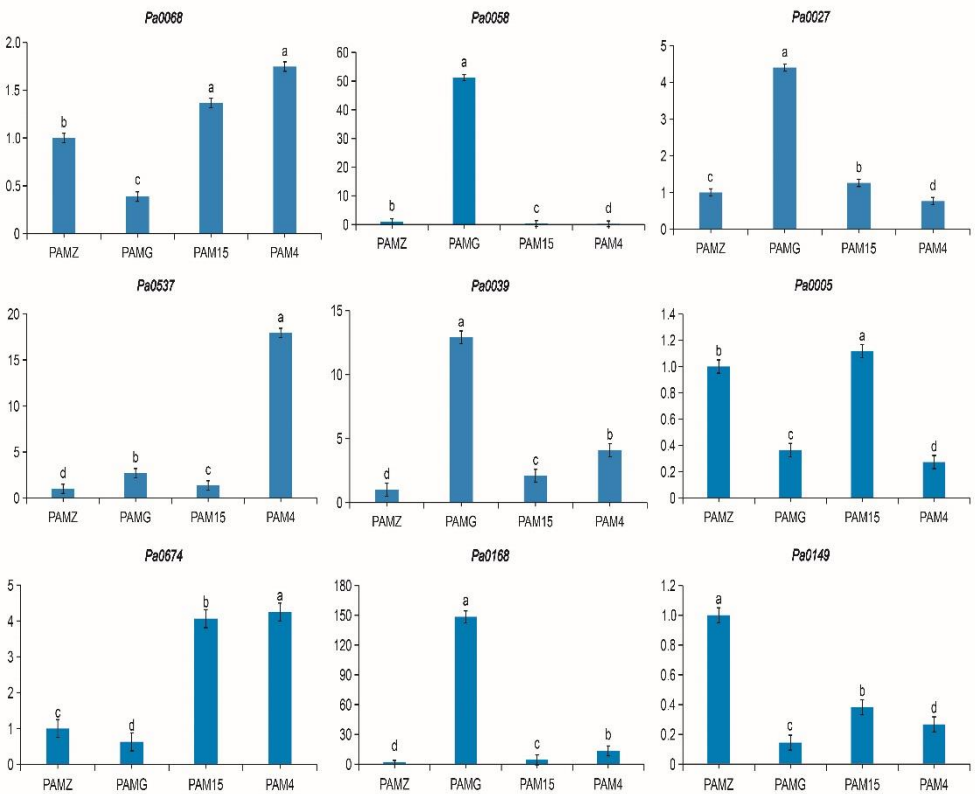

Fig. 7. The expression profiles of nine transcripts in *P. americana* by qRT-PCR. The x-coordinate is a different treatment group, and the ordinate is DEGs expression changes.

Starch is the main carbohydrate in plants, releasing energy, sugars, and secondary metabolites through its own starch reserves under abiotic forcing (Thalmann and Santelia 2017). When subjected to drought and low-temperature stress, plants receive external stress signals for physiological resistance responses. As drought and low-temperature stress intensify, the production of intracellular reactive oxygen species and extracellular electrolyte leakage increase, causing damage to their biomolecules. Therefore, plants alleviate osmotic stress by increasing the content of small molecules or soluble proteins, while reducing the intensity of respiration, slowing down metabolic activity, increasing photosynthesis, and accumulating sugars, thus reducing the negative effects of stress (Thalmann *et al*. 2016; Zanella *et al*. 2016; Li *et al*. 2017). β-amylase (BAM) is the main enzyme of starch catabolism metabolism and is involved in the response of different plants to a variety of abiotic stresses (Sørensen *et al*. 2012). For example, the expression of the gene encoding BAM was upregulated under drought stress in *Millet* (Cao *et al*. 2022), and the gene encoding BAM, *VvBAM1*, was significantly upregulated under low-temperature stress in tomato (Liang *et al*. 2021). In the study of *Pyrus bretschneideri*, three genes encoding BAM were upregulated in expression under both drought and low temperature stresses (Zhao *et al*. 2019). In the present study, the gene encoding BAM, *Pa0058*, was upregulated under drought stress and downregulated under low-temperature stress, in general agreement with the results of the former study under drought stress. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is an essential enzyme in the

glycolytic metabolic pathway and plays an important role in carbon metabolism, plant development, and stress tolerance (Wei *et al*. 2022). In the present study, *Pa0202*, the gene encoding GAPDH, was expressed upregulated under both drought and low-temperature stress, which is similar to the results of upregulated of the expression of genes encoding GAPDH in banana under mild and moderate low-temperature stress (Liu *et al*. 2020), and upregulated of the expression of most genes encoding GAPDH in *Triticum aestivum* roots under drought stress (Zeng *et al*. 2016), suggesting that GAPDH is a key factor in plant response to abiotic stresses.

Flavonoids, as important components of plant secondary metabolites, have important roles in microbial signaling, protection against pathogens and pests, UV protection, growth hormone transport regulation, and pigmentation (Han *et al*. 2019). The flavonoid biosynthetic pathway is one of the best known secondary metabolic pathways in plants, and many key genes have been studied in response to environmental stresses (Saito *et al*. 2013). Flavanone 3-hydroxylase (F3H) is a key enzyme in the flavonoids biosynthesis pathway and plays an important role in biotic and abiotic stress (Ma *et al*. 2014). It was reported that the transcript of F3H was significantly enhanced in *Reaumuria soongorica* under drought stress (Liu *et al*. 2013), and in the present study, one gene encoding F3H was also significantly enhanced under drought stress, and the results of both studies were generally consistent. Chalcone synthase (CHS) is the key enzyme in the first committed step of the flavonoid biosynthetic pathway and catalyzes the stepwise condensation of 4-coumaroyl-CoA and malonyl-CoA to naringenin chalcone (Deng *et al*. 2014). In the study of Hybrid Poplar (*P. tremula* × *P. alba*), the expression of *PtCHS*, a gene encoding CHS, gradually increased with increasing time of drought stress (Ahmed *et al*. 2021), and in the present study, the expression of two genes encoding CHS was upregulated under drought stress, in agreement with the previous study the results were consistent with the previous study. In the study of tobacco, the gene *Nitab4.5_0001066g0070* encoding CHS in cultivars Xiangyan7 was downregulated under low-temperature stress (Hu *et al*. 2022), and in the present study, the gene encoding CHS was downregulated under low-temperature stress, and both results were consistent. The above results suggest that flavonoids have protective effects against drought and low temperature stresses, and different varieties may have different response mechanisms to different stresses.

Transcription factors play an important regulatory role in plant growth, development, metabolism, and other functions (Fan *et al*. 2021). They regulate transcription through the expression of other transcription factors, synthesizing the corresponding proteins to guide the generation of metabolites and respond to drought and low-temperature stress. In the present work, 53 transcription factor families were identified related to drought and cold stress response through genome-wide analysis, of which bHLH, NAC, ERF, MYB, and WRKY family members were the most representative. At present, a large number of studies have shown that transcription factor families such as the bZIP, AP2/ERE, WRKY, NAC, and MYB are closely related to the expression and regulation of genes under drought and low-temperature stress (Xu *et al*. 2011; Wang *et al*. 2020). Among them, the present study found that the expression of 25 DEGs in the ERF family was significantly altered: five DEGs were upregulated, one DEG was downregulated under drought stress, and two DEGs were downregulated under low-temperature stress. For WRKY members, 13 DEGs were upregulated under low-temperature stress. In a study of *Ammopiptanthus nanus*, Cao *et al*. (2020) found that DREB responds to low-temperature and drought stress by binding to WRKY. In a study of *Salix matsudana* leaves, Xu *et al*. (2021) found that the expression of five genes encoding WRKY transcription factors was

upregulated under drought stress. In a study of tea, Wang *et al*. (2013) found that the expression of five genes encoding WRKY transcription factors was upregulated in three and downregulated in two under low temperature stress. Chen *et al*. (2014) found that under low-temperature stress, the expression of DEGs encoding AP2/ERF, WRKY, and NAC transcription factors in *Populus euphratica* was upregulated, indicating that the three transcription factor families play a key role in the low-temperature response of *P. euphratica*. The results of the transcription factors in this study were generally consistent with those of previous studies, which strongly suggest that ERF and WRKY transcription factors are associated with drought and low temperature in plants and play important roles in drought and low-temperature stresses.

This study preliminarily explored the key genes and transcription factor families of *P. americana*-related metabolic pathways under drought and low-temperature stress, laying the foundation for further research on biosynthesis, secondary metabolic pathways, and transcriptome. Moreover, it provides a theoretical basis for breeding *P. americana* varieties with drought and low-temperature resistance. Further functional characterization of DEGs and their pathways are needed because they potentially serve as targets for marker-assisted selection or transgenes to enhance stress tolerance.

CONCLUSIONS

Abiotic stresses such as drought and low temperature limit the acreage of *P. americana* in Yunnan, China. In this study, transcriptome analysis of *P. americana* was performed under drought and low-temperature stress. The result of transcriptome analysis showed that the three most significantly enriched KEGG metabolic pathways responded to drought and low temperature were starch and sucrose metabolism, glycolysis/gluconeogenesis, and flavonoid biosynthesis. The gene expression of beta-amylase, glyceraldehyde-3-phosphate dehydrogenase and hexokinase were upregulated under drought stress, while the gene expression of UDP-glycosyltransferase superfamily protein isoform, glucose-1-phosphate adenylyltransferase and glucose-6-phosphate 1-epimerase were downregulated. The gene expression of beta-amylase and shikimate O-hydroxycinnamoyl transferase was downregulated under low-temperature stress. WRKY, MYB, bHLH, and NAC transcription factors also were involved in responding to drought and low-temperature stress.

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REFERENCES CITED

Abdel-Ghany, S. E., Ullah, F., Ben-Hur, A., and Reddy, A. S. (2020). "Transcriptome analysis of drought-resistant and drought-sensitive sorghum (*Sorghum bicolor*) genotypes in response to PEG-induced drought stress," *International Journal of Molecular Sciences* 21(3), 772. DOI: 10.3390/ijms21030772

Ahmed, U., Rao, M. J., Qi, C., Xie, Q., Noushahi, H. A., Yaseen, M., Shi, X., and Zheng, B. (2021). "Expression profiling of flavonoid biosynthesis genes and secondary metabolites accumulation in populus under drought stress," *Molecules* 26(18), 5546. Doi: 10.3390/molecules26185546

Alkhalaf, M. I., Alansari, W. S., Ibrahim, E. A., and ELhalwagy, M. E. (2019). "Anti-oxidant, anti-inflammatory and anti-cancer activities of avocado (*Persea americana*) fruit and seed extract," *Journal of King Saud University-Science* 31(4), 1358-1362. DOI: 10.1016/j.jksus.2018.10.010

Bhuyan, D. J., Alsherbiny, M. A., Perera, S., Low, M., Basu, A., Devi, O. A., Barooah, M. S., Li, C. G., and Papoutsis, K. (2019). "The odyssey of bioactive compounds in avocado (*Persea americana*) and their health benefits," *Antioxidants* 8(10), 426. DOI: 10.3390/antiox8100426

Cao, S., Wang, Y., Li, X., Gao, F., Feng, J., and Zhou, Y. (2020). "Characterization of the AP2/ERF transcription factor family and expression profiling of DREB subfamily under cold and osmotic stresses in *Ammopiptanthus nanus*," *Plants* 9(4), 455. DOI: 10.3390/plants9040455

Cao, X., Hu, Y., Song, J., Feng, H., Wang, J., Chen, L., Wang, L., Diao, X., Wan, Y., Liu, S., et al. (2022). "Transcriptome sequencing and metabolome analysis reveals the molecular mechanism of drought stress in *Millet*," *International Journal of Molecular Sciences* 23(18), article 10792. DOI: 10.3390/ijms231810792

Chanderbali, A. S., Albert, V. A., Ashworth, V. E., Clegg, M. T., Litz, R. E., Soltis, D. E., and Soltis, P. S. (2008). "*Persea americana* (avocado): Bringing ancient flowers to fruit in the genomics era," *BioEssays* 30(4), 386-396. DOI: 10.1002/bies.20721

Chen, J., Tian, Q., Pang, T., Jiang, L., Wu, R., Xia, X., and Yin, W. (2014). "Deep-sequencing transcriptome analysis of low temperature perception in a desert tree, *Populus euphratica*," *BMC Genomics* 15(1), 1-15. DOI: 10.1186/1471-2164-15-326

Deng, X., Bashandy, H., Ainasoja, M., Kontturi, J., Pietiäinen, M., Laitinen, R. A., Albert, V., Valkonen, J. T., Elomaa, P., and Teeri, T. H. (2014). "Functional diversification of duplicated chalcone synthase genes in anthocyanin biosynthesis of *Gerbera hybrida*," *New Phytologist* 201(4), 1469-1483. Doi: 10.1111/nph.12610

Duarte, P. F., Chaves, M. A., Borges, C. D., and Mendonça, C. R. B. (2016). "Avocado: Characteristics, health benefits and uses," *Ciencia Rural* 46, 747-754. DOI: 10.1590/0103-8478cr20141516

Fan, Y., Yang, H., Lai, D., He, A., Xue, G., Feng, L., Chen, L., Cheng, X., Ruan, J., Yan. J., *et al*. (2021). "Genome-wide identification and expression analysis of the bHLH transcription factor family and its response to abiotic stress in sorghum [*Sorghum bicolor* (L.) Moench]," *BMC Genomics* 22(1), 1-18. DOI: 10.21203/rs.3.rs-150639/v1

Feng, Y., Liang, C., Li, B., Wan, T., Liu, T., and Cai, Y. (2017). "Differential expression profiles and pathways of genes in drought resistant tree species *Prunus mahaleb* roots and leaves in response to drought stress," *Scientia Horticulturae* 226, 75-84. DOI: 10.1016/j.scienta.2017.07.057

Ge, Y., Dong, X., Liu, Y., Yang, Y., and Zhan, R. (2021). "Molecular and biochemical

analyses of avocado (*Persea americana*) reveal differences in the oil accumulation pattern between the mesocarp and seed during the fruit developmental period," *Scientia Horticulturae* 276, article 109717. DOI: 10.1016/j.scienta.2020.109717

Ge, Y., Hu, F., Tan, L., Wu, B., Wang, T., Zhang, T., Ma, F., Cao, J., Xu, Z., and Zhan, R. (2019a). "Molecular diversity in a germplasm collection of avocado accessions from the tropical and subtropical regions of China," *Crop Breeding and Applied Biotechnology* 19, 153-160. DOI: 10.1590/1984-70332019v19n2a22

Ge, Y., Si, X.Y., Lin, X.E., Wang, J.S., Zang, X.P., and Ma, W. H. (2017). "Advances in avocado (*Persea americana* Mill.)," *South China Fruit* 46, 63-70. DOI: 10.13938/j.issn.1007-1431.20160156

Ge, Y., Zang, X., Tan, L., Wang, J., Liu, Y., Li, Y., Wang, N., Chen, D., Zhan, R., and Ma, W. (2019b). "Single-molecule long-read sequencing of avocado generates microsatellite markers for analyzing the genetic diversity in avocado germplasm," *Agronomy* 9(9), 512.DOI: 10.3390/agronomy9090512

Gong, X. X., Yan, B. Y., Hu, J., Yang, C. P., Li, Y. J., Liu, J. P., and Liao, W. B. (2018). "Transcriptome profiling of rubber tree (*Hevea brasiliensis*) discovers candidate regulators of the cold stress response," *Genes & Genomics* 40(11), 1181-1197. DOI: 10.1007/s13258-018-0681-5

Hu, Z., Yan, W., Yang, C., Huang, X., Hu, X., Li, Y., Yang, J., Xiang, S., Yi, P., and Hu, R. (2022). "Integrative analysis of transcriptome and metabolome provides insights into the underlying mechanism of cold stress response and recovery in two tobacco cultivars," *Environmental and Experimental Botany* 200, article 104920. DOI: 10.1016/j.envexpbot.2022.104920

Hwang, J. E., Kim, Y. J., Shin, M. H., Hyun, H. J., Bohnert, H. J., and Park, H. C. (2018). "A comprehensive analysis of the Korean fir (*Abies koreana*) genes expressed under heat stress using transcriptome analysis," *Scientific Reports* 8(1), 1-11. DOI: 10.1038/s41598-018-28552-1

Ibarra-Laclette, E., Mendez-Bravo, A., Perez-Torres, C. A., Albert, V. A., Mockaitis, K., Kilaru, A., López-Gómez, R., Cervantes-Luevano, J. I., and Herrera-Estrella, L. (2015). "Deep sequencing of the Mexican avocado transcriptome, an ancient angiosperm with a high content of fatty acids," *BMC Genomics* 16(1), 1-18. DOI: 10.1186/s12864-015-1775-y

Kuhn, D. N., Livingstone III, D. S., Richards, J. H., Manosalva, P., Van den Berg, N., and Chambers, A. H. (2019). "Application of genomic tools to avocado (*Persea americana*) breeding: SNP discovery for genotyping and germplasm characterization," *Scientia Horticulturae* 246, 1-11. DOI: 10.1016/j.scienta.2018.10.011

Li, N., Zheng, Y. Q., Ding, H. M., Liu, X. H., Sheng, W. T., Jiang, B., and Li, H. B. (2017). "Analysis on differential expression of cold resistance related genes of *Casuarina equisetifolia* under low temperature stress," *Scientia Silvae Sinicae* 53(7), 62-71. DOI: 10.11707/j.1001-7488.20170707

Li, W., Lee, J., Yu, S., Wang, F., Lv, W., Zhang, X., Li, C., and Yang, J. (2021). "Characterization and analysis of the transcriptome response to drought in *Larix kaempferi* using PacBio full-length cDNA sequencing integrated with de novo RNA-seq reads," *Planta* 253(2), 1-13. DOI: 10.1007/s00425-020-03555-3

Liang, G., He, H., Nai, G., Feng, L., Li, Y., Zhou, Q., Ma, Z., Yue, Y., Chen, B., and Mao, J. (2021). "Genome-wide identification of BAM genes in grapevine (*Vitis vinifera* L.) and ectopic expression of VvBAM1 modulating soluble sugar levels to improve low-

temperature tolerance in tomato," *BMC Plant Biology* 21, 1-15. DOI: 10.1186/s12870-021-02916-8

Liu, J., Takáč, T., Yi, G., Chen, H., Wang, Y., Meng, J., Yuan, W., Tan, Y., Ning, T., He, Z., *et al.* (2020). "Acceleration of carbon fixation in chilling-sensitive banana under mild and moderate chilling stresses," *International Journal of Molecular Sciences* 21(23), 9326. DOI: 10.3390/ijms21239326

Liu, M., Li, X., Liu, Y., and Cao, B. (2013). "Regulation of flavanone 3-hydroxylase gene involved in the flavonoid biosynthesis pathway in response to UV-B radiation and drought stress in the desert plant, *Reaumuria soongorica*," *Plant Physiology and Biochemistry* 73, 161-167. DOI: 10.1016/j.plaphy.2013.09.016

Ma, D., Sun, D., Wang, C., Li, Y., and Guo, T. (2014). "Expression of flavonoid biosynthesis genes and accumulation of flavonoid in wheat leaves in response to drought stress," *Plant Physiology and Biochemistry* 80, 60-66. DOI: 10.1016/j.plaphy.2014.03.024

Maestrini, P., Cavallini, A., Rizzo, M., Giordani, T., Bernardi, R., Durante, M., and Natali, L. (2009). "Isolation and expression analysis of low temperature-induced genes in white poplar (*Populus alba*)," *Journal of Plant Physiology* 166(14), 1544-1556. DOI: 10.1016/j.jplph.2009.03.014

Saito, K., Yonekura-Sakakibara, K., Nakabayashi, R., Higashi, Y., Yamazaki, M., Tohge, T., and Fernie, A. R. (2013). "The flavonoid biosynthetic pathway in *Arabidopsis*: Structural and genetic diversity," *Plant Physiology and Biochemistry* 72, 21-34. DOI: 10.1016/j.plaphy.2013.02.001

Shi, H., and Chen, J. (2018). "Characteristics of climate change and its relationship with land use/cover change in Yunnan Province, China," *International Journal of Climatology* 38(5), 2520-2537. DOI: 10.1002/joc.5404

Song, Y., Chen, Q., Ci, D., and Zhang, D. (2013). "Transcriptome profiling reveals differential transcript abundance in response to chilling stress in *Populus simonii*," *Plant Cell Reports* 32(9), 1407-1425. DOI: 10.1007/s00299-013-1454-x

Sorensen, A., Ahring, B. K., Lubeck, M., Ubhayasekera, W., Bruno, K. S., Culley, D. E., and Lübeck, P. S. (2012). "Identifying and characterizing the most significant β-glucosidase of the novel species *Aspergillus saccharolyticus*," *Canadian Journal of Microbiology* 58(9), 1035-1046. DOI: 10.1139/w2012-076

Tabeshpour, J., Razavi, B. M., and Hosseinzadeh, H. (2017). "Effects of avocado (*Persea americana*) on metabolic syndrome: A comprehensive systematic review," *Phytotherapy Research* 31(6), 819-837. DOI:10.1002/ptr.5805

Thalmann, M., and Santelia, D. (2017). "Starch as a determinant of plant fitness under abiotic stress," *New Phytologist* 214(3), 943-951. DOI: 10.1111/nph.14491

Thalmann, M., Pazmino, D., Seung, D., Horrer, D., Nigro, A., Meier, T., Kolling, K., Pfeifhofer, H. W., Zeeman, S. C., and Santelia, D. (2016). "Regulation of leaf starch degradation by abscisic acid is important for osmotic stress tolerance in plants," *The Plant Cell* 28(8), 1860-1878. DOI: 10.1105/tpc.16.00143

Tremocoldi, M. A., Rosalen, P. L., Franchin, M., Massarioli, A. P., Denny, C., Daiuto, É. R., Paschoal, J. A. R., Melo, P. S., and Alencar, S. M. D. (2018). "Exploration of avocado by-products as natural sources of bioactive compounds," *PloS One* 13(2), e0192577. DOI: 10.1371/journal.pone.0192577

Valliyodan, B., and Nguyen, H. T. (2006). "Understanding regulatory networks and engineering for enhanced drought tolerance in plants," *Current Opinion in Plant Biology* 9(2), 189-195. DOI: 10.1016/j.pbi.2006.01.019

Wang, X. C., Zhao, Q. Y., Ma, C. L., Zhang, Z. H., Cao, H. L., Kong, Y. M., Yue, C., Hao, X. Y., Chen, L., Ma, J. Q., *et al*. (2013). "Global transcriptome profiles of *Camellia sinensis* during cold acclimation," *BMC Genomics* 14(1), 1-15. DOI: 10.1186/1471-2164-14-415

Wang, N., Yao, F., Yuan, M. Li., and Su, J. L. (2014). "Analysis on cold tolerance of five camphor tree species during the natural drop in temperature process," *Journal of Henan Agricultural University* 48(03), 302-309. DOI: CNKI:SUN:NNXB.0.2014-03-008

Wang, P., Wang, F., and Yang, J. (2017). "De novo assembly and analysis of the *Pugionium cornutum* (L.) Gaertn. transcriptome and identification of genes involved in the drought response," *Gene* 626, 290-297. DOI: 10.1016/j.gene.2017.05.053

Wang, B., Chen, M. D., Lin, L., Ye, X. R., Zhu, H. H., and Wen, Q. F. (2020). "Signaling pathways and related transcription factors of drought stress in plants," *Acta Botanica Boreali-Occidentalia Sinica* 40(10), 1792-1806. DOI: 10.7606/j.issn.1000-4025.2020.10.1792

Wei, H., Movahedi, A., Yang, J., Zhang, Y., Liu, G., Zhu, S., Yu, C., Chen, Y., Zhong, F., and Zhang, J. (2022). "Characteristics and molecular identification of glyceraldehyde-3-phosphate dehydrogenases in poplar," *International Journal of Biological Macromolecules* 219, 185-198. DOI: 10.1016/j.ijbiomac.2022.08.001

Wu, F. Z., Wang, H. X., Xu, G. H., and Zhang, Z. C. (2015). "Research progress on the physiological and molecular mechanisms of woody plants under low temperature stress," *Scientia Silvae Sinicae* 51(07), 116-128. DOI: 10.11707/j.1001-7488.20150713

Xu, D., Li, J., Zhu, T., Yang, H., and Zhuo, Z. (2021). "Comparative transcriptome analysis of *Salix cupularis* under drought stress," *Global Ecology and Conservation* 27, e01532. DOI: 10.1016/j.gecco.2021.e01532

Xu, Z. S., Chen, M., Li, L. C., and Ma, Y. Z. (2011). "Functions and application of the AP2/ERF transcription factor family in crop improvement F," *Journal of Integrative Plant Biology* 53(7), 570-585. DOI: 10.1016/S0735-1097(13)61853-7

Yang, X., Zhao, T., Rao, P., Gao, K., Yang, X., Chen, Z., and An, X. (2019). "Transcriptome profiling of *Populus tomentosa* under cold stress," *Industrial Crops and Products* 135, 283-293. DOI: 10.1016/j.indcrop.2019.04.056

Zanella, M., Borghi, G. L., Pirone, C., Thalmann, M., Pazmino, D., Costa, A., Santelia, D., Trost, P., and Sparla, F. (2016). "β-amylase 1 (BAM1) degrades transitory starch to sustain proline biosynthesis during drought stress," *Journal of Experimental Botany*, 67(6), 1819-1826. DOI: 10.1093/jxb/erv572

Zeng, L., Deng, R., Guo, Z., Yang, S., and Deng, X. (2016). "Genome-wide identification and characterization of Glyceraldehyde-3-phosphate dehydrogenase genes family in wheat (*Triticum aestivum*)," *BMC Genomics* 17(1), 1-10. DOI: 10.1186/s12864-016-2527-3

Zhao, L., Gong, X., Gao, J., Dong, H., Zhang, S., Tao, S., and Huang, X. (2019). "Transcriptomic and evolutionary analyses of white pear (*Pyrus bretschneideri*) β-amylase genes reveals their importance for cold and drought stress responses," *Gene* 689, 102-113. DOI: 10.1016/j.gene.2018.11.092

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APPENDIX

Supplemental Material

Table S1. Statistics of Gene Function Annotation

Annotation Genes	GO	KEGG	eggNOG	Swissprot	Nr
24616	17970	17816	37873	31960	38178

Table S2. GO Enrichment of Unigenes

PG	GO.ID	Category	Term	Up	Down	DEG
PAMZ-vs-PAMG	GO:0006811	BP	ion transport	12	19	31
	GO:0051082	MF	unfolded protein binding	10	0	10
	GO:0005975	BP	carbohydrate metabolic process	27	10	37
	GO:0048878	BP	chemical homeostasis	8	4	12
	GO:0009522	CC	photosystem I	0	6	6
	GO:0031226	CC	intrinsic component of plasma membrane	8	4	12
	GO:0055082	BP	cellular chemical homeostasis	6	3	9
	GO:0009534	CC	chloroplast thylakoid	3	8	11
	GO:0042592	BP	homeostatic process	10	6	16
	GO:0031976	CC	plastid thylakoid	3	8	11
	GO:0034220	BP	ion transmembrane transport	6	15	21
	GO:0006812	BP	cation transport	8	12	20
	GO:0016168	MF	chlorophyll binding	0	5	5
	GO:1901505	MF	carbohydrate derivative transmembrane transporter activity	2	3	5
	GO:0015880	BP	coenzyme A transport	0	3	3
	GO:0035349	BP	coenzyme A transmembrane transport	0	3	3
	GO:0071106	BP	adenosine 3',5'-bisphosphate transmembrane transport	0	3	3
	GO:0000295	MF	adenine nucleotide transmembrane transporter activity	0	3	3
	GO:0005346	MF	purine ribonucleotide transmembrane transporter activity	0	3	3
	GO:0008429	MF	phosphatidylethanolamine binding	0	3	3
PAMG-vs-PAM4	GO:0048046	CC	apoplast	0	13	13
	GO:0010410	BP	hemicellulose metabolic process	0	9	9
	GO:0044036	BP	cell wall macromolecule metabolic process	0	11	11
	GO:0071944	CC	cell periphery	11	32	43
	GO:0016762	MF	xyloglucan:xyloglucosyl transferase activity	0	7	7
	GO:0010411	BP	xyloglucan metabolic process	0	7	7
	GO:0042546	BP	cell wall biogenesis	0	10	10
	GO:0010383	BP	cell wall polysaccharide metabolic process	0	9	9
	GO:0005975	BP	carbohydrate metabolic process	7	30	37
	GO:0071554	BP	cell wall organization or biogenesis	1	18	19

	GO:0044262	BP	cellular carbohydrate metabolic process	2	17	19
	GO:0051082	MF	unfolded protein binding	0	10	10
	GO:0005576	CC	extracellular region	2	15	17
	GO:0071555	BP	cell wall organization	1	13	14
	GO:0052841	MF	inositol bisdiphosphate tetrakisphosphate diphosphatase activity	3	0	3
	GO:0052842	MF	inositol diphosphate pentakisphosphate diphosphatase activity	3	0	3
	GO:0005886	CC	plasma membrane	9	22	31
	GO:0045229	BP	external encapsulating structure organization	1	13	14
	GO:0009812	BP	flavonoid metabolic process	0	9	9
	GO:0031226	CC	intrinsic component of plasma membrane	2	9	11

Table S3. KEGG Enrichment of Unigenes

PG	PathwayID	Pathway	Level1	level2	Up	Down	DEG
PAMZ-vs-PAMG	ko04016	MAPK signaling pathway - plant	Environmental Information Processing	Signal transduction	19	0	19
	ko00196	Photosynthesis - antenna proteins	Metabolism	Energy metabolism	0	6	6
	ko00520	Amino sugar and nucleotide sugar metabolism	Metabolism	Carbohydrate metabolism	15	3	18
	ko04712	Circadian rhythm - plant	Organismal Systems	Environmental adaptation	1	8	9
	ko00500	Starch and sucrose metabolism	Metabolism	Carbohydrate metabolism	9	7	16
	ko04075	Plant hormone signal transduction	Environmental Information Processing	Signal transduction	19	2	21
	ko00051	Fructose and mannose metabolism	Metabolism	Carbohydrate metabolism	8	2	10
	ko04141	Protein processing in endoplasmic reticulum	Genetic Information Processing	Folding, sorting and degradation	17	1	18
	ko00906	Carotenoid biosynthesis	Metabolism	Metabolism of terpenoids and polyketides	4	2	6
	ko00053	Ascorbate and aldarate metabolism	Metabolism	Carbohydrate metabolism	5	1	6
	ko00052	Galactose metabolism	Metabolism	Carbohydrate metabolism	7	0	7
	ko00904	Diterpenoid biosynthesis	Metabolism	Metabolism of terpenoids	2	2	4

				and polyketides			
	ko04626	Plant-pathogen interaction	Organismal Systems	Environmental adaptation	12	1	13
	ko00010	Glycolysis / Gluconeogenesis	Metabolism	Carbohydrate metabolism	8	2	10
	ko00380	Tryptophan metabolism	Metabolism	Amino acid metabolism	3	2	5
	ko00410	beta-Alanine metabolism	Metabolism	Metabolism of other amino acids	3	2	5
	ko00910	Nitrogen metabolism	Metabolism	Energy metabolism	1	3	4
	ko00940	Phenylpropanoid biosynthesis	Metabolism	Biosynthesis of other secondary metabolites	7	4	11
	ko00310	Lysine degradation	Metabolism	Amino acid metabolism	4	0	4
	ko00340	Histidine metabolism	Metabolism	Amino acid metabolism	3	0	3
PAMG-vs-PAM4	ko00941	Flavonoid biosynthesis	Metabolism	Biosynthesis of other secondary metabolites	1	11	12
	ko04712	Circadian rhythm - plant	Organismal Systems	Environmental adaptation	8	3	11
	ko04075	Plant hormone signal transduction	Environmental Information Processing	Signal transduction	10	11	21
	ko00910	Nitrogen metabolism	Metabolism	Energy metabolism	3	4	7
	ko04141	Protein processing in endoplasmic reticulum	Genetic Information Processing	Folding, sorting and degradation	1	17	18
	ko04016	MAPK signaling pathway - plant	Environmental Information Processing	Signal transduction	5	6	11
	ko00360	Phenylalanine metabolism	Metabolism	Amino acid metabolism	1	4	5
	ko00945	Stilbenoid, diarylheptanoid and gingerol biosynthesis	Metabolism	Biosynthesis of other secondary metabolites	1	3	4
	ko04626	Plant-pathogen interaction	Organismal Systems	Environmental adaptation	5	7	12
	ko00906	Carotenoid biosynthesis	Metabolism	Metabolism of terpenoids and polyketides	1	4	5
	ko00052	Galactose metabolism	Metabolism	Carbohydrate metabolism	1	5	6

	ko00195	Photosynthesis	Metabolism	Energy metabolism	0	5	5
	ko00940	Phenylpropanoid biosynthesis	Metabolism	Biosynthesis of other secondary metabolites	3	7	10
	ko00520	Amino sugar and nucleotide sugar metabolism	Metabolism	Carbohydrate metabolism	5	4	9
	ko00920	Sulfur metabolism	Metabolism	Energy metabolism	1	2	3
	ko00010	Glycolysis / Gluconeogenesis	Metabolism	Carbohydrate metabolism	1	7	8
	ko00670	One carbon pool by folate	Metabolism	Metabolism of cofactors and vitamins	0	2	2
	ko00051	Fructose and mannose metabolism	Metabolism	Carbohydrate metabolism	1	4	5
	ko00710	Carbon fixation in photosynthetic organisms	Metabolism	Energy metabolism	1	4	5
	ko00330	Arginine and proline metabolism	Metabolism	Amino acid metabolism	3	1	4

Table S4. Cross-expression of 19 Genes

Gene_ID	PAMZ:fpkm	PAMG:fpkm	PAM5:fpkm	PAM4:fpkm
Pa0068	113.76	3.65	119.39	135.55
Pa0058	9.96	509.68	1.47	0.28
Pa0202	8.14	32.35	13.91	9.52
Pa0748	15.46	69.74	17.58	15.59
Pa0001	421.76	129.33	253.32	256.66
Pa0249	212.77	80.03	238.81	190.63
Pa0571	121.27	33.63	88.60	65.51
Pa0822	35.70	51.19	25.65	226.52
Pa0005	1770.23	634.71	1903.05	476.11
Pa1187	89.73	32.50	44.64	8.18
Pa0413	11.30	80.94	0.00	1.56
Pa0104	88.43	167.59	1.33	25.25
Pa0304	143.72	155.85	43.39	43.45
Pa0151	77.40	22.20	0.17	1.66
Pa0116	47.32	71.45	0.67	5.04
Pa0113	44.57	10.19	5.37	3.82
Pa3359	19.21	27.38	99.41	34.10
Pa1189	23.61	106.03	60.52	90.00
Pa0685	270.93	1250.54	927.08	721.12

Table S5. Analysis of Differentially Expressed Transcription Factor Genes in Avocado under Drought and Cold Stress

Gene ID	PAMZ:fpkm	PAMG:fpkm	PAM15:fpkm	PAM4:fpkm
Pa3886	ERF	101.91	18.78	178.70
Pa1164	ERF	189.93	76.89	508.97
Pa0837	ERF	114.44	4.89	160.50
Pa0822	ERF	51.19	25.65	226.52
Pa0685	ERF	1250.54	927.08	721.12
Pa0453	ERF	489.55	77.19	1578.76
Pa0250	ERF	711.61	1552.07	769.75
Pa0157	ERF	244.50	19.60	15.96
Pa0149	ERF	335.52	931.95	662.97
Pa0058	ERF	170.64	63.90	305.76
Pa0039	ERF	1111.26	172.40	344.90
Pa0035	ERF	454.25	91.38	50.25
Pa0027	ERF	1101.20	323.48	198.80
Pa3400	bHLH	81.46	17.66	74.89
Pa1690	bHLH	83.61	2.33	17.66
Pa0500	bHLH	183.35	156.02	250.19
Pa0335	bHLH	34.51	18.22	100.80
Pa0281	bHLH	33.00	82.59	112.59
Pa0204	bHLH	36.33	24.19	109.68
Pa0131	bHLH	71.43	70.50	185.26
Pa1008	MYB	47.60	15.86	7.42
Pa0288	MYB	34.66	62.53	57.22
Pa0231	MYB	95.84	28.17	158.08
Pa0098	MYB	131.07	18.25	39.62
Pa0065	MYB	126.14	21.80	132.60
Pa0032	MYB	266.37	143.89	298.55
Pa1027	NAC	55.13	100.73	355.91
Pa0553	NAC	466.04	72.87	163.09
Pa0544	NAC	717.84	31.25	387.33
Pa0168	NAC	511.64	16.10	46.01
Pa0036	NAC	125.94	21.70	25.73
Pa0014	NAC	787.77	591.41	298.68
Pa0005	NAC	11.70	321.98	261.69
Pa1375	WRKY	143.72	29.48	227.64
Pa0899	WRKY	40.35	12.81	139.64
Pa0846	WRKY	61.56	22.32	415.69
Pa0674	WRKY	47.56	306.47	321.74
Pa0537	WRKY	139.66	63.55	923.38
Pa0198	WRKY	79.45	17.79	44.62
Pa0049	WRKY	18.18	6.20	62.54