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Integrative transcriptome and metabolome analysis identifies flavonoid biosynthesis as well as ascorbate and aldarate metabolism driving natural cold tolerance variation in dormant peach (Prunus persica)

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Abstract

Winter cold stress severely restricts the growth, overwintering survival, and geographical distribution of peaches (*Prunus persica*). Elucidating the key regulatory pathways and hub genes underlying genotypic divergence in cold tolerance is essential for the improvement of peach production. In this study, four cold-tolerant (T group) and four cold-sensitive (S group) cultivars were selected from 73 peach cultivars via field freeze-injury investigation and laboratory lethal temperature of 50% (LT_{50}) assays. Transcriptomic analyses were performed on dormant one-year-old branches of the T and S groups under 4°C control and − 25°C cold-stress treatments, and corresponding widely targeted metabolomic analyses were conducted under − 25°C cold-stress treatment. The results indicated that the MAPK signaling and plant hormone signal transduction pathway was universally activated by cold stress in both cold-tolerant and cold-sensitive peach groups. KEGG enrichment analysis revealed significant differences in gene expression associated with flavonoid biosynthesis and ascorbate aldarate metabolism between the T and S groups. Under control conditions, flavonoid biosynthesis-related DEGs exhibited distinct basal expression patterns between the two groups. Two L-ascorbate oxidase (AO) genes and one AO-like gene enriched in the ascorbate and aldarate metabolism pathway were markedly downregulated in the T group, while their associated metabolite, L-ascorbic acid-2-glucoside, was upregulated in the T group. Collectively, conserved cold-induced signaling pathways such as the MAPK cascade and plant hormone signal respond uniformly to cold stress in peaches, whereas constitutive differences in flavonoid biosynthesis and ascorbate and aldarate metabolism pathways constitute the key factors driving genotypic divergence in cold tolerance during dormancy. This study provides novel mechanistic insights into natural variation in peach cold tolerance and supplies valuable candidate genes and metabolites for the molecular breeding of cold-tolerant peach cultivars.

Introduction

Peach (*Prunus persica*), classified within the genus *Prunus* of the Rosaceae family, is the third most widely cultivated fruit crop globally, ranking only after apple and pear [1]. The global peach yield reached 27.89 million tons in 2024. China is the largest producer, with an annual yield of approximately 17 million tons (FAOSTAT; http://faostat.fao.org). Peach is a thermophilic fruit crop mainly grown in temperate and subtropical regions, and the LT_{50} values of peach germplasms range from − 17 ℃ to − 28 ℃ [2–4]. In recent years, extreme weather events have occurred frequently, and winter cold stress has become a key limiting factor restricting peach cultivation. Winter cold stress causes substantial yield losses in overwintering temperate tree species, while constraining their geographical distribution and growth performance [5]. Therefore, unraveling the regulatory mechanisms underlying cold tolerance and breeding cold-tolerant peach cultivars represent one of the most effective targets for the peach industry [6]. However, the molecular mechanisms governing cold tolerance in peach remain largely unclear to date. Generally, after thousands of years of cultivation and evolution, peach has harbored abundant genetic diversity. More than 1 ,000 peach cultivars have been developed worldwide, showing extensive phenotypic variation in fruit traits and environmental adaptability [7]. Accordingly, it is necessary to evaluate the cold-tolerance performance of diverse peach cultivars and characterize their physiological, metabolic, and transcriptomic responses under cold stress, so as to elucidate the regulatory mechanisms of cold adaptation in peach [8].

Plants have evolved multiple sophisticated mechanisms to adapt to cold stress at transcriptional, metabolic, physiological and morphological levels. Numerous studies have demonstrated that *CBF* (C-repeat binding factor) genes have played a critical role in plant cold-stress responses [9–11]. When plants encounter cold stress, *CBF* expression is directly modulated by a set of transcription factors, including positive regulators such as *ICE* (inducer of CBF expression) and *CAMTA* (calmodulin-binding transcription activator), as well as negative regulators including *MYB15* and *HOS1* (high expression of osmotically responsive gene 1) [12–14]. Subsequently, CBF transcription directly activates a suite of *COR* (cold-responsive) genes, leading to the enhancement of cold tolerance [15–17]. For example, in the model plant *Arabidopsis thaliana*, *ICE1* and *ICE2* activate *CBF1*, *CBF2*, and *CBF3* by binding to their promoters; defective *ICE1* and *ICE2* result in markedly reduced cold tolerance [18–20]. Moreover, *HOS1* mediates the degradation of *ICE1* and *ICE2* via the ubiquitin/proteasome pathway, acting as an attenuator of cold-signal transduction [18]. In peach, *CBFs* appear to function not only in cold tolerance but also in multiple other biological processes, such as regulating plant dormancy, bud formation, leaf senescence, and fruit sucrose accumulation. For instance, overexpressing peach *PpCBF1* in apple plants improved cold tolerance; furthermore, it repressed plant growth, promoted early bud formation and leaf senescence, delayed spring bud burst, and induced early entry into and late exit from dormancy [21]. In peach fruit, *PpCBF1* directly binds to the promoter of *PpVIN2* (vacuolar acid invertase-encoding gene) to activate its transcription, which accelerates sucrose catabolism and reduces fruit chilling tolerance [22] In contrast, *PpCBF6* represses *PpVIN2* promoter activity, thereby altering sucrose accumulation [23].

Although the CBF-dependent pathway has been the most thoroughly characterized cascade in cold-signal transduction, the effects of CBFs only account for 10–20% of the total *COR* genes regulatory network [13, 20]. Besides the CBF-dependent pathway, multiple alternative pathways contribute to plant cold-stress responses. Numerous transcription factors, including *ERF5*, *WRKY33*, *RAV1*, *BZR1* (*BRASSINAZOLERESISTANT 1*), *ZAT10* (*zincfinger protein 10*), *ZAT12*, *STZ1* (*salt-tolerance zincfinger*), *CZF2* (*C2H2 zincfinger protein 2*), *MYB73, PIF8* (*phytochromeinteracting transcription factor 8*), *HSFA1* (*heatshock transcription factor A1*) and *HSFC1*, can also trigger the expression of *COR* genes under cold stress [13, 24]. For example, the PeSTZ1 protein can directly bind to the promoter of *PeAPX2* and activate its transcription, which contributes to cold tolerance in *Populus euphratica* independently of the CBF-mediated cold-signaling cascade [25]

Over evolutionary time, plants have developed diverse metabolic regulatory mechanisms to adapt cold stress during dormancy. These adaptations involve the biosynthesis of protective compounds, including a series of osmoregulatory substances such as soluble sugars and free amino acids, secondary metabolites such as flavonoids, alkaloids, and terpenoids, as well as antioxidant substances such as ascorbic acid (AsA) and glutathione (GSH) [26, 27]. Upon exposure to cold stress, plants actively accumulate diverse organic and inorganic substances within cells to lower cellular osmotic potential, prevent excessive cellular water loss, and sustain proper intracellular osmotic concentration. The major osmotic-adjustment substances in plants fall into two groups: inorganic ions and organic solutes, which include soluble sugars, proline, soluble proteins, and polyamines [28]. Soluble sugars function as osmoprotectants in plants. Their cellular accumulation increases osmotic pressure, alleviates low-temperature-induced cell shrinkage, and thereby shields cell membranes against dehydration and freezing injury [29]. Transient expression of *PpBZR1* in peach elevates sucrose levels, which protects fruit against chilling injury [30]. *Arabidopsis thaliana* plants heterologously expressing *PpBZR1* show higher germination and seedling survival rates under low-temperature conditions, which may be attributed to increased sucrose accumulation and reduced oxidative damage [30]. Proline also serves as a key osmotic regulator in plants. It decreases osmotic potential, improves water retention and turgor maintenance, and helps stabilize proteins and membranes under dehydration triggered by extracellular ice formation [31–34]. For example, overexpression of *Rosa multiflora ZAT10* in tobacco leads to massive proline accumulation and enhanced cold tolerance, whereas VIGS-silenced plants display the opposite phenotype [35].

Cold stress disrupts normal reactive oxygen species (ROS) metabolism in plants. Excessive ROS accumulation damages intracellular proteins, carbohydrates and genetic materials, and further impairs plant growth. Accordingly, plants possess a complete antioxidant defense system to eliminate excess cellular ROS at any time [36, 37]. The ascorbic acid (AsA)-glutathione (GSH) cycle constitutes an indispensable pathway for hydrogen peroxide (H_2O_2) detoxification. Upon cold acclimation, cellular pools of AsA and GSH expand to strengthen ROS-scavenging capacity [29]. In kiwifruit, *AcePosF21* interacts with *AceMYB102* and upregulates *AceGGP3* (encoding GDPLgalactose phosphorylase 3), thereby boosting AsA biosynthesis to scavenge excess ROS [38]. Flavonoids, a large class of plant secondary metabolites, also act as ROS scavengers owing to hydroxyl groups on their ring skeletons, and can neutralize free radicals [39, 40]. In wild potato (Solanum commersonii), ScF3′H protein catalyzes hydroxylation of flavonoid ring structures to produce more downstream flavonoid derivatives, which enhances ROS-scavenging activity and ultimately improves cold tolerance [41].

Of note, most existing studies on peach cold-tolerance mechanisms focused on fruit chilling injury and cold tolerance during the growing season, whereas fewer investigations addressed cold tolerance in dormant peach plants. Moreover, most transcriptome-based studies aiming to identify cold-tolerance-related genes in peach have only used a limited set of cultivars (typically one cold-tolerant and one cold-sensitive genotype), which fails to reflect the generalizability of cold-tolerance. More cultivars with well-characterized cold-tolerance phenotypes should be included to accurately screen for cold-tolerance-related candidate genes. In the present study, eight cultivars with two contrasting extreme cold-tolerant phenotypes were selected from 73 cultivars, including four cold-tolerant cultivars formed the cold-tolerant group (T group) and four cold-sensitive cultivars formed the cold-sensitive group (S group). To identify the critical genes and metabolites related to cold stress, transcriptomic and metabolomic analyses were subsequently performed on dormant one-year-old branches from the T group and S group before and after − 25℃ cold treatment. Our findings help elucidate the molecular basis of peach cold tolerance and offer new insights into the regulatory network underlying cold-stress responses during peach dormancy.

Materials and mothods

Plant materials

A total of 73 peach cultivars were subjected to cold-tolerance classification and evaluation (Table S1). Twenty cultivars representing a gradient of cold tolerance (from high cold tolerance to cold susceptibility) were screened from this panel for subsequent determination of lethal temperature of 50% (LT_{50}). Based on LT_{50} values, eight cultivars with extreme cold-tolerant and cold-sensitive phenotypes were selected for subsequent transcriptomic and metabolomic analyses. The 8 cultivars include 4 cold-tolerant cultivars, namely ‘Hangongzhu’, ‘Huichun’, ‘Donghe No1’, and ‘Qiuyue’, formed the tolerant group (T group); 4 cold-sensitive cultivars, namely ‘21st Century’, ‘Zhonghuashoutao’, ‘Jinhuangjin’, and ‘Zhongtao 13’, formed the sensitive group (S group). All cultivars were 8-year-old trees grown in the field of Changli fruit tree research institute, Hebei academy of agricultrue and forestry sciences in Changli county, Hebei province of China (N: 39°42', E: 119°9') with conventional pest and disease control and field management.

Classification and evaluation on cold tolernce of 73 peach cultivars in the natural environment

The cold tolerance of 73 peach cultivars was evaluated in mid-March over two consecutive years. Surveys were performed of freeze-injury symptoms of branches and the cambium at the trunk base, with at least three trees surveyed for each cultivar. All cultivars were classified into 4 grades according to these symptoms: Grade 0 (no freeze injury), Grade 1 (slight freeze injury), Grade 2 (moderate freeze injury), and Grade 3 (severe freeze injury).

The determination of LT_{50} in one-year-old peach branches

One-year-old branches of each cultivar were collected from 3 individual trees in the winter of 2025 during the dormant period. Subsequently, the samples of each cultivar were divided equally into 5 subsamples, which were treated at 5 temperature gradients (4 °C as the control, -15 °C, -20 °C, -25 °C, -30 °C) for 12h in a temperature-humidity-controlled chamber at a cooling rate of 5 °C/h. Following cold treatment, all samples were cut into approximately 3-mm-thick slices, and divide into 3 parts for three biological replicates. Thereafter, 1 g of sliced sample was weighed and immersed in 25 mL of deionized water for a 2-h extraction at 25 °C. Initial electrolyte leakage (C_0) was determined with a conductivity meter (INESA (Leici) DDS-307A). The samples were then boiled in a water bath, after which the final electrolyte leakage (C_1) was measured. Relative electrolyte leakage was calculated using the formula: (C_0/C_1) × 100%.

LT_{50} was determined by fitting the relative electrolyte leakage data to a logistic sigmoidal curve. The logistic equation was defined as $y = K/(1 + e^{a-bx})$, where y indicates the relative electrolyte leakage (%), x indicates the treatment temperature (°C), a and b are the fitted constant parameters. The maximum saturation value K was fixed at 100 according to the theoretical maximum electrolyte release after boiling treatment. The LT value, corresponding to the temperature at the inflection point of the S-curve, was calculated as LT_{50} = a/b.

RNA-seq

The one-year-old branches of T group and S group were collected from 3 individual trees of each cultivar during the dormant period in winter 2025. Of the 2 groups, T group included 4 cold-tolerant cultivars and S group included 4 cold-sensitive cultivars, with the 4 cultivars within each group serving as 4 biological replicates. After collection, half of the branches of each cultivar were kept at 4℃ for 12 h as the control; while the other half were exposed to -25℃ for 2 h and then gradually returned to 4℃ with a changing rate of 5℃ per hour as the cold-stress treatment, totaling 12 h. A total of 16 samples were obtained after treatment and all were stored at −80°C for subsequent analysis.

Total RNA was extracted from approximately 0.5 g of one-year-old branches via the CTAB method, then quantified with a NanoDrop 2000 (Thermo Fisher Scientific). Sequencing libraries were prepared using NEBNext UltraTM RNA Library Prep Kit for Illumina (NEB). A total of 16 libraries were prepared from 8 cultivars including controls and treatments. Samples were sequenced at 150 bp paired-ends used Illumina platform (X-ten) by Metware Biotechnology (Wuhan, China), and raw data were subsequently generated. To obtain clean data, reads containing adapters, reads with poly-N content ≥ 10%, and low-quality reads (Q ≤ 20) were filtered out from raw data. After quality control, the clean reads were aligned to the peach reference genome (*Prunus persica* v2.0.a1, https://www.rosaceae.org/species/prunus_persica/genome_v2.0.a1) using Hisat2.

Differential expression analysis

Differential expression analysis was performed using DESeq2 based on the negative binomial distribution. Gene expression was quantified using fragments per kilobase of transcript per million mapped reads (FPKM). Genes with false discovery rate (FDR) < 0.05 and log_2fold change (log_2FC) ≥ 1.5 were identified as differentially expressed genes (DEGs). Gene functions were annotated based on the databases of NCBI non-redundant protein sequences (fttp://ncbi.nih.gov/blast/db/). Gene Ontology (GO) enrichment analysis was performed based on the GO database (http://www.geneontology.org/). Pathway enrichment analysis against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/) was conducted to explore pivotal metabolic and signaling pathways associated with these DEGs. Both GO terms and KEGG pathways were statistically assessed, and items with an adjusted P-value < 0.05 were regarded as significantly enriched.

Quantitative realtime quantitative PCR (qRT-PCR) analysis

Approximately 0.5 μg RNA was reverse transcribed into first-strand cDNA using HiScript II 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China). qRT-PCR was performed in the QuantStudio™ 6 Flex Real-Time PCR (Thermo Fisher Scientific, Waltham, USA) using NovoStrat SYBR qPCR SuperMix Plus (Novoprotein, Suzhou city, Jiangsu province, China). *PpActin* (*Prupe.5G137100*) was used as the internal reference gene for qRT-PCR. The relative expression levels were calculated using the 2^-ΔΔCT method. Three biological replicates were set up for each assay. All primers were designed by Primer-BLAST and listed in Table S2.

Metabolite extraction and UPLC-ESI-MS/MS detection

The samples used for widely-targeted metabolomics were consistent with those for RNA-seq. All samples used for metabolites extraction were exposed to -25℃ for 2 h, and then gradually returned to 4℃ a changing rate of 5℃ per hour. Sample metabolites were analyzed using the widely-targeted metabolomics platform from Metware Biotechnology (Wuhan, China). Samples were lyophilized in a Scientz100F freeze dryer and pulverized with a Retsch MM 400 mill (30 Hz, 1.5 min). Briefly, 30 mg powder was extracted with 1500 μL prechilled (−20 °C) 70% methanol containing internal standards. The mixture was intermittently vortexed. After centrifugation at 12, 000 rpm for 3 min, supernatants were filtered through 0.22 μm membranes for UHPLCMS/MS analysis.

Metabolite detection was performed on an ExionLC™ AD UHPLC coupled to a triplequadrupole linear iontrap mass spectrometer equipped with an Agilent SBC18 column (1.8 μm, 2.1 mm × 100 mm). Gradient elution used 0.1% formic acid water (A) and 0.1% formic acid acetonitrile (B). Flow rate was 0.35 mL/min, column temperature 40 °C, injection volume 2 μL. ESI source parameters were set for positive and negative ion modes. Metabolites were monitored in MRM mode with individually optimized declustering potential and collision energy for each analyte.

Differentially accumulated metabolites (DAMs) screening and functional annotation

DAMs were screened using different thresholds according to comparison types. For pairwise comparisons, metabolites with Variable Importance in the Projection (VIP) > 1 and $|Log_2FC| \geq 1.0$ were identified as DAMs. Orthogonal partial least squares discriminant analysis (OPLS-DA) was performed with the R package *MetaboAnalystR*, and VIP values were extracted from the established model. A total of 200 permutation tests were carried out to avoid model overfitting and validate the stability of OPLS-DA results. All detected metabolites were first annotated against the KEGG Compound database, and the annotated metabolites were further mapped to the KEGG Pathway database for functional enrichment analysis. The correlation coefficient between DEGs and DAMs were further calculated. Network visualization of correlative DEGs and DAMs was performed using Cytoscape v3.10.4.

Result

Cold tolerance assessment of 73 peach cultivars

Cold tolerance of 73 peach cultivars was assessed, and they were assigned to Grades 0-3 based on freeze-injury symptoms under natural-field conditions (Table S1). The number of cultivars in Grades 0, 1, 2 and 3 was 33, 13, 14 and 13, respectively. This distribution revealed that most cultivars had strong or moderate cold tolerance, while only a small number exhibited poor cold tolerance.

To screen cultivars exhibiting gradient cold tolerance phenotypes for the identification of cold tolerance-related genes, 20 cultivars were selected from the 73 accessions, with 5 cultivars chosen from each grade. These 20 cultivars were then used for LT_{50} measurements. Results show that the LT_{50} of the 20 cultivars ranging from -20.93 to -12.85 °C, with an average value of -16.06 °C (Table1). Four cultivars with the lowest LT_{50} values ('Hangongzhu', 'Huichun', 'Donghe No1', 'Qiuyue') and 4 with the highest LT_{50} values ('21th Century', 'Zhonghuashoutao', 'Jinhuangjin', 'Zhongtao 13') were identified, and they were selected as the cold-tolerant group (T group) and cold-sensitive group (S group) for subsequent RNA-seq and metabolomic analysis to explore candidate cold tolerance-related genes. These findings demonstrated that peach cold tolerance is a quantitative trait with continuous phenotypic variation.

Table 1. Logistic equations and LT50 of 20 peach cultivars

cultivars	logistic regression equation	LT50/℃	R2
Hangongzhu	y = 100/(1+e0.98-0.047x)	-20.93	0.77
Donghe No1	y = 100/(1+e1.04-0.051x)	-20.58	0.76
Huichun	y = 100/(1+e1.05-0.052x)	-20.33	0.84
Qiuyue	y = 100/(1+e1.09-0.057x)	-19.09	0.86
Jinbao	y = 100/(1+e0.99-0.054x)	-18.14	0.87
Xiahui 6	y = 100/(1+e0.97-0.057x)	-17.12	0.84
Beijingwanmi	y = 100/(1+e0.95-0.057x)	-16.55	0.95
Zhongyou 21	y = 100/(1+e0.96-0.06x)	-16.14	0.87
Qiuyan	y = 100/(1+e0.89-0.057x)	-15.71	0.99
Qiuyi	y = 100/(1+e0.95-0.062x)	-15.40	0.98
Xiufeng	y = 100/(1+e1-0.066x)	-15.27	0.95
Okubo	y = 100/(1+e0.89-0.059x)	-15.04	0.93
Yantehong	y = 100/(1+e0.91-0.061x)	-14.92	0.93
Zhongyoupan 7	y = 100/(1+e0.85-0.06x)	-14.19	0.92
Zhongyou 8	y = 100/(1+e0.83-0.059x)	-14.09	0.96
Cuibao	y = 100/(1+e0.85-0.062x)	-13.85	0.98
21th Century	y = 100/(1+e0.89-0.065x)	-13.77	0.93
Jinhuangjin	y = 100/(1+e0.87-0.063x)	-13.65	0.91
Zhongtao 13	y = 100/(1+e0.90-0.066x)	-13.56	0.95
Zhonghuashoutao	y = 100/(1+e0.87-0.068x)	-12.85	0.98

Overview of RNA-seq sequencing data

Four cold-tolerant peach cultivars (T group, No. 1-4) and 4 cold-sensitive peach cultivars (S group, No. 5-8) were used for RNA-seq. Samples were classified into 4 categories: TC (cold-tolerance control), SC (cold-sensitive control), TT (cold-tolerance treatment), and ST (cold-sensitive treatment). Library construction was successfully performed for a total of 16 libraries corresponding to control and cold treatments of one-year-old branches collected from eight peach cultivars. After filtering raw data, the clean data of each sample ranged from 8.24 Gb to 10.79 Gb, with Q30 values between 95.02% and 96.71%. Reads were mapped to the peach reference genome (*Prunus persica* v2.0.a1). The proportion of uniquely mapped reads for all samples was 90.68%-94.38%, whereas multiply mapped reads accounted for 3.30%-6.33%. Detailed sequencing statistics for all samples are summarized in Table S3. All RNA-seq data have been deposited in NCBI under the BioProject accession number PRJNA1539684. Gene expression levels were calculated as FPKM values. In total, 25,282 genes were detected in cold-treated and control groups, of which 24,411 were annotated based on the reference genome and 871 were novel genes. These results demonstrated that the RNA-seq data were of high quality, and the majority of clean reads were successfully mapped to the peach reference genome.

The identified and enriched analysis of genes responding to cold stress

Pairwise differential expression analyses between control and cold treatments (TC vs TT and SC vs ST) were conducted to screen cold-responsive genes in peach. Under cold treatment, 119 DEGs were up-regulated and 91 were down-regulated in the comparison TC vs TT (Figure 1A), while 171 DEGs were up-regulated and 215 were down-regulated in SC vs ST (Figure 1B). In total, 438 DEGs were identified in the two comparisons, among which 52 were specific to TC vs TT, 228 were specific to SC vs ST, and 158 were common to both comparisons (Figure 1C).

To identify core biological processes related to cold stress, DEGs obtained from TC vs TT and SC vs ST were subjected to GO functional enrichment analysis. Adjusted *P*-values of all GO terms were calculated to filter significantly enriched entries (Adjusted *P*-values < 0.05). The significant GO terms were separately extracted from TC vs TT and SC vs ST, all of them fell under biological processes (Figure 2 A,B). Among these GO terms, nine were significantly enriched in both the TC vs TT and SC vs ST comparisons, including response to hypoxia (GO:0001666), abscisic acid-activated signaling pathway (GO:0009738), regulation of signal transduction(GO:0009966), ethylene-activated signaling pathway (GO:0009873), phosphorelay signal transduction system (GO:0000160), positive regulation of signal transduction (GO:0009967), positive regulation of response to stimulus (GO:0048584), S-glycoside metabolic process (GO:0016143), and response to water deprivation (GO:0009414). These shared functional pathways are presumed to exert critical functions during cold stress. Furthermore, thirteen terms were significantly enriched in only one of the two comparisons (TC vs TT or SC vs ST), which may partly explain the differences in cold tolerance between T and S groups. KEGG pathway enrichment analysis of cold-responsive DEGs was performed to identify pivotal metabolic and signal-transduction pathways responding to cold stress. Two KEGG pathways, namely MAPK signaling pathway-plant (ko04016) and plant hormone signal transduction (ko04075), were significantly enriched (adjusted P-value < 0.05) in both the TC vs TT and SC vs ST comparisons (Figure 2 C,D). These results indicated that these 2 pathways were early signaling pathways rapidly activated when plants were exposed to cold stress.

DEGs underlying cold tolerance differences between cold-tolerant and cold-sensitive peach cultivars

To screen key genes responsible for cold tolerance divergence, pairwise differential expression analysis was carried out between T and S groups at both control and low-temperature stages (TC vs SC and TT vs ST). For the control group (TC vs SC), 66 DEGs were upregulated and 94 were downregulated in T group relative to S group (Figure 3A). In the cold treatment (TT vs ST), 49 DEGs were upregulated and 60 DEGs were downregulated in T group relative to S group (Figure 3B). In total, 199 DEGs were obtained from the comparisons. Among them, 90 DEGs were unique to the control comparison (TC vs SC), 39 DEGs were unique to the cold-treatment comparison (TT vs ST), and 70 DEGs overlapped in both two comparisons (Figure 3C).

To uncover functional biological processes responsible for cold-tolerance disparities, GO and KEGG enrichment analyses were conducted using DEGs from the two comparisons (TC vs SC and TT vs ST). The numbers of GO terms met the significance threshold of TC vs SC and TT vs ST were 5 and 3 respectively (Figure 4 A,B). Among these 8 GO terms, ADP binding (GO:0043531) exhibited the highest statistical significance in both two comparisons, implying its vital function in peach cold tolerance. KEGG enrichment results revealed that 3 pathways met the significance threshold in at least one of the two comparisons, namely plant-pathogen interaction (ko04626), flavonoid biosynthesis (ko00941), and ascorbate and aldarate metabolism (ko00053) (Figure4 C,D). Among the 3 pathways, plant-pathogen interaction was the only pathway significantly enriched in both the control comparison (TC vs SC) and cold-stress comparison (TT vs ST). By contrast, the ascorbate and aldarate metabolism pathway was only enriched in the cold-stress comparison (TT vs ST), while the flavonoid biosynthesis pathway was only enriched under control conditions. These results indicate that the genes within the above-mentioned 8 GO terms and 3 KEGG pathways have a close association with cold-tolerance differences in peach.

The analysis of DAMs between cold-tolerant and cold-sensitive cultivars

To uncover vital metabolites responsible for cold tolerance divergence between cold-tolerant and cold-sensitive peach cultivars, widely-targeted metabolomics was performed on samples from the cold-treatment group (TT vs ST) for metabolite detection and quantification. In total, 3,265

metabolites were detected; 438 of these were identified as DAMs based on the thresholds VIP > 1 and $|Log_2FC| > 1$. Among the 438 DAMs, 358 were up-regulated and 80 were down-regulated in T group relative to S group (Figure 5A).

KEGG functional enrichment was carried out using DAMs from the comparison of T group vs S group under cold treatment (TT vs ST). In total, 663 out of 3,265 detected metabolites were annotated and mapped to KEGG pathways, among which 105 belonged to the 438 identified DAMs. Only two pathways met the significance criterion (adjusted *P*-value < 0.05): flavonoid biosynthesis (ko00941) and isoflavonoid biosynthesis (ko00943) (Figure 5B). These 2 pathways are core branches of phenylpropanoid metabolism, and their metabolic products are collectively termed flavonoid polyphenols. Notably, all DAMs enriched in these 2 pathways accumulated at significantly higher levels in cold-tolerant cultivars than in cold-sensitive cultivars. Collectively, these results supported the KEGG pathway enrichment analysis of DEGs from RNA-seq data, which revealed that the levels of flavonoids and isoflavonoids have a positive correlation with peach cold tolerance.

Identification of DEGs in the flavonoid biosynthesis pathway and screening of core candidate genes for cold-tolerance divergence in peach

Since the flavonoid biosynthesis pathway showed significant enrichment at the transcriptional level under normal control conditions and at the metabolic level under cold stress in the comparison between the T group and S group, we characterized the functional features and expression profiles of DEGs associated with flavonoid biosynthesis to screen core genes responsible for genotypic divergence in intrinsic cold tolerance. A total of 6 DEGs were annotated in the flavonoid biosynthesis pathway (Table 2). Among these 6 DEGs, *Prupe.3G185000* encodes UDP-glycosyltransferase 71A16 (UGT71A16), which was significantly upregulated in T group relative to S group. This gene exhibited the most significant statistical difference among the 6 DEGs, with an adjusted *P*-value of 1.10×10^{-8} and a log_2FC of 1.43. *UGT71A16* encodes a 2'-O-glycosyltransferase with catalytic activity toward chalcones and flavonols; it can convert phloretin into phlorizin (phloretin 2'-O-glucoside), which is a powerful antioxidant metabolite. The remaining 5 DEGs all encode structural enzymes in the flavonoid pathway, with homologous annotations corresponding to leucoanthocyanidin dioxygenase, polyketide synthase 5, chalcone isomerase-like protein 2, cytochrome P450 98A2 and naringenin 2-oxoglutarate 3-dioxygenase, respectively. Notably, these 5 genes were significantly downregulated in T group compared with S group. Collectively, these results indicated that the flavonoid biosynthesis pathway undergoes distinct genotype-specific transcriptional regulation in peach under cold stress, and *Prupe.3G185000* (*UGT71A16*) is identified as a core candidate gene responsible for genotypic cold tolerance divergence in peach.

Table 2 DEGs involved in the flavonoid biosynthesis pathway (ko00941) between T and S group

gene ID	log_2FC	regulated	gene annotation
Prupe.3G185000	1.43	up	UDP-glycosyltransferase 71A16 (U7A16)
Prupe.5G086700	-1.45	down	Leucoanthocyanidin dioxygenase (LDOX)
Prupe.1G002900	-1.25	down	Polyketide synthase 5 (PKS5)
Prupe.2G263900	-1.01	down	Chalcone isomerase-like protein 2 (CHIL2)
Prupe.1G580200	-1.02	down	Cytochrome P450 98A2 (C98A2)
Prupe.7G168300	-1.09	down	Naringenin,2-oxoglutarate 3-dioxygenase (FL3H

up: DEG up-regulated in T group relative to S group

down: DEG down-regulated in T group relative to S group

Identification of DEGs involved in ascorbate and aldarate metabolism associated with genotypic cold-tolerance divergence in peach

L-ascorbic acid (AsA) is a central metabolite participating in plant antioxidant defense, which scavenges excess reactive oxygen species (ROS) accumulated under cold stress. KEGG enrichment analysis revealed that the ascorbate and aldarate metabolism pathway (ko00053) was significantly enriched at the transcriptional level between T group and S group under cold stress. A total of 3 DEGs were annotated in this pathway, and their functional characteristics and expression profiles were analyzed to identify key genes driving genotypic variation in intrinsic cold tolerance (Table 3). Notably, two of the 3 DEGs encode L-ascorbate oxidase (AO), and the remaining one is an AO-like gene, suggesting that these genes may share related biological functions. AO is a redox enzyme that catalyzes the oxidation of AsA into monodehydroascorbate (MDHA). Moreover, these 3 genes were significantly downregulated in cold-tolerant cultivars relative to cold-sensitive cultivars. Among them, *Prupe.2G077800* showed the most dramatic expression difference, with an adjusted *P*-value of 5.39×10^{-15} and a log_2FC of -9.84. This gene had an FPKM value of 4.88 in T group but exhibited undetectable expression in S group. In parallel, L-ascorbic acid-2-glucoside (AA2G), the stable glycosylated storage form of AsA, was significantly more abundant in cold-tolerant cultivars ($log_2FC = 1.03$). Collectively, these results suggest that the strong downregulation of AO and AO-like genes in cold-tolerant peaches may elevate endogenous AsA pools. Greater AsA availability enhances cellular antioxidant capacity, which may constitute a key factor that reinforces the intrinsic cold tolerance of cold-tolerant peach cultivars.

Table 3. DEGs involved in ascorbate and aldarate metabolism pathway (ko00053) between T and S group

gene ID	log_2FC	regulated	gene annotation
Prupe.2G077800	-9.84	down	L-ascorbate oxidase (AO)
Prupe.6G155100	-2.95	down	L-ascorbate oxidase (AO)
Prupe.1G357100	-1.09	down	L-ascorbate oxidase-like (AO-like)

up: DEG up-regulated in T group relative to S group

down: DEG down-regulated in T group relative to S group

Analysis of DEGs involved in the plant-pathogen interaction pathway underlying genotypic cold-tolerance divergence in peach

The plant-pathogen interaction pathway (ko04626) acts as a core upstream signaling hub that mediates cross-tolerance to biotic and abiotic stresses in plants. This pathway exhibited significant transcriptional enrichment between T group and S group under normal control and cold-stress treatment. However, no metabolites were detected in our metabolome dataset annotated to this pathway. In total, twenty-seven DEGs assigned to ko04626 were identified between the T and G groups (Table 4). Eight DEGs were simultaneously differentially expressed under both control and cold stress, and all 8 shared identical expression change trends across the two conditions. Five of these 8 DEGs encoded disease-tolerance proteins: *Prupe.2G057100*, *Prupe.4G284000*, *Prupe.5G019000*, *Prupe.4G285500* and *Prupe.4G284800*. These DEGs were significantly upregulated in T group relative to S group, except *Prupe.2G057100*, which was downregulated in T group. The remaining 3 shared DEGs were annotated to chitin elicitor receptor kinase 1, wall-associated receptor kinase-like 1 and rust-tolerance kinase Lr10, corresponding to *Prupe.3G213000*, *Prupe.3G181400* and *Prupe.2G047500*, respectively. Aside from the 8 DEGs shared by both two treatments, 19 DEGs were treatment-specific. Among them, 12 DEGs were detected only under control conditions, and 7 DEGs were unique to cold stress. Notably, the 8 shared DEGs exhibited lower adjusted *P*-values than 19 treatment-specific DEGs, indicating a closer association with cold tolerance in peach.

Table 4. DEGs involved in the plant-pathogen interaction pathway (ko04626) between T and S group under control and cold-stress conditions

	TC vs SC		TT vs ST		
gene ID	log_2FC	regulated	log_2FC	regulated	gene annotation
Prupe.3G213000	-9.93	down	-9.76	down	Chitin elicitor receptor kinase 1 (CERK1)
Prupe.3G181400	-7.84	down	-9.88	down	Wall-associated receptor kinase-like 1 (WAKLA)
Prupe.2G057100	-3.26	down	-3.88	down	Disease resistance protein RPV1 (RPV1)
Prupe.4G284000	4.65	up	4.77	up	Putative disease resistance protein RGA3 (RGA3)
Prupe.5G019000	2.58	up	2.43	up	Disease resistance protein Roq1 (ROQ)
Prupe.4G285500	8.74	up	9.46	up	Putative disease resistance protein RGA3 (RGA3)
Prupe.4G284800	5.80	up	4.82	up	Putative disease resistance protein RGA3 (RGA3)
Prupe.2G047500	1.43	up	1.38	up	Rust resistance kinase Lr10 (LRK10)
Prupe.2G043200	1.27	up	-	-	Putative disease resistance RPP13-like protein 1 (R13L1)
Prupe.5G117000	1.16	up	-	-	Probable WRKY transcription factor 53 (WRK53)
Prupe.8G077100	-2.30	down	-	-	Putative disease resistance protein RGA4 (RGA4)
Prupe.2G087200	-1.71	down	-	-	Rust resistance kinase Lr10 (LRK10)
Prupe.2G045200	1.38	up	-	-	TMV resistance protein N (TMVRN)
Prupe.6G274900	1.40	up	-	-	Probable LRR receptor-like serine/threonine-protein kinase (Y3475)
Prupe.4G233300	-6.82	down	-	-	Probable strigolactone esterase DAD2 (DAD2)
Prupe.2G046900	1.32	up	-	-	Putative disease resistance RPP13-like protein 1 (R13L1)
Prupe.2G307400	1.08	up	-	-	Probable WRKY transcription factor 53 (WRK53)
Prupe.4G029800	-2.37	down	-	-	Calcium/calmodulin-regulated receptor-like kinase 1 (CRLK1)
Prupe.8G237200	1.00	up	-	-	Disease resistance-like protein DSC1 (DSC1)
Prupe.1G550800	-1.11	down	-	-	TMV resistance protein N (TMVRN)
Prupe.2G014100	-	-	-3.12	down	Disease resistance protein RGA2 (RGA2)
Prupe.2G077400	-	-	2.06	up	Receptor-like protein EIX2 (EIX2)
Prupe.2G055700	-	-	-2.11	down	TMV resistance protein N (TMVRN)
Prupe.2G057800	-	-	-2.31	down	TMV resistance protein N (TMVRN)
Prupe.5G025400	-	-	1.33	up	Disease resistance protein RPM1 (RPM1)
Prupe.2G054300	-	-	1.39	up	Putative disease resistance RPP13-like protein 1 (R13L1)
Prupe.5G025300	-	-	1.18	up	Disease resistance protein RPM1 (RPM1)

TC vs SC: T group compared with S group under normal control conditions; TT vs ST: T group compared with S group under cold-stress conditions. up: DEG upregulated in T group relative to S group; down: DEG downregulated in T group relative to S group. "-" indicates no significant differential expression.

Correlation-network analysis of DEGs and DAMs associated with cold-tolerance divergence in peach

To further mine the genes related to cold-tolerance-associated metabolites, correlation analysis was performed on the DEG and DAM datasets between the T group and S group. Correlation coefficients for DEG-DEG and DEG-DAM pairs were calculated, and the network was constructed using Cytoscape software (v3.10.4). Only correlation pairs with a Pearson correlation coefficient ≥ 0.9 and an adjusted *P*-value ≤ 0.05 were visualized in the network (Figure 6). The visualized network contained 88 nodes connected by 93 edges. These 88 nodes included 53 genes and 35 metabolites. Furthermore, 83 pairs showed positive correlations, whereas 5 pairs exhibited negative correlations. Among the 53 genes within the network, four genes belonged to the flavonoid biosynthesis pathway (ko00941) and clustered together. *Prupe.5G086700* was the center of this sub-network and connected with chrysin-5-O-glucoside (toringin) and hispidulin-8-C-glucoside, which are secondary metabolites derived from the flavone backbone sub-branch. Moreover, *Prupe.2G077800*, which belonged to the ascorbate and aldarate metabolism pathway (ko00053), was

correlated with two chromosomally adjacent genes (*Prupe.2G078200* and *Prupe.2G078300*) as well as one metabolite (6a-hydroxy-3,6-dimethyl-1,2,3,3a,4,6a-hexahydropentalen-2-one. Nevertheless, these two genes and this metabolite did not belong to the ko00053 pathway, implying potential unknown regulatory mechanisms among them. These results indicated that multiple DEGs were highly correlated with their corresponding metabolites, especially genes involved in flavonoid biosynthesis.

Based on the DEG-metabolite correlation network and the KEGG enrichment results of DEGs and DAMs between T and S groups, the flavonoid biosynthesis and ascorbate and aldarate metabolism pathways were identified as crucial pathways contributing to the natural variation in cold tolerance. In total, nine genes from these two pathways were regarded as candidate genes that contributed to cold-tolerance divergence in peach cultivars, and their expression levels were further analyzed in the T and S groups under both control and cold-stress conditions (Figure 7). qRT-PCR validation showed that the expression patterns of most genes were highly consistent with the RNA-Seq results, supporting the reliability of the RNA-Seq data obtained in this study.

Discussion

Peach is a thermophilic fruit crop whose growth and geographical distribution are strongly constrained by winter cold stress during dormancy. However, the key genes and molecular mechanisms underlying peach cold tolerance are still poorly understood. Here, two peach groups with contrasting cold-tolerance performance (T and S groups) were analyzed via transcriptomics (control and coldstress conditions) and metabolomics (coldstress condition only). Cross-treatment comparisons enabled identification of cold-responsive genes, whereas inter-group comparisons at identical temperatures uncovered candidate genes and metabolites responsible for their distinct cold-tolerance capabilities.

The MAPK signaling cascade is strongly cold-responsive but does not contribute to phenotypic divergence in cold tolerance

The MAPK signaling cascade is central to plant cellular responses to environmental stresses, including freezing injury [42]. This cascade comprises three sequentially acting protein kinases: MAP kinase kinase kinases (MAPKKK/MAP3K/MEKK), MAP kinase kinases (MAP2K/MKK/MEK), and MAP kinases (MAPK). Cold stress can activate MKK2 and the MAPKKK type kinase MEKK1, and activated MKK2 further promotes *COR* genes expression to enhance plant cold tolerance [43]. In our study, the MAPK signaling pathway (ko04016) represented the most significantly enriched KEGG pathway in both TC vs TT and SC vs ST comparisons, suggesting that MAPK cascade are cold-inducible. Cold-triggered activation of the MAPK cascade has also been documented in *Rhododendron chrysanthum* Pall., *Brassica napus* L., almond, and banana [44-46]. Among the all 22 DEGs assigned to this pathway, nine were differentially expressed in both TC vs TT and SC vs ST, and exhibited consistent expression trends under cold stress in the two groups. This result indicated that these nine genes mediate largely conserved cold-response mechanisms in both T and S groups. Notably, none of these 22 genes were differentially expressed between T and S groups under control or cold-stress treatment (TC vs SC and TT vs ST). Therefore, we infer that MAPK-signaling related genes are responsive to cold stress, but do not underpin the phenotypic divergence in cold tolerance between T and S groups.

Flavonoid metabolism contributes to genotypic divergence in peach cold tolerance

Flavonoids are polyphenolic secondary metabolites produced via the phenylpropanoid pathway, which positively respond to cold stress in plants [47]. Owing to hydroxyl groups on their aromatic rings, flavonoids can scavenge ROS, and thus promote plant cold acclimation [39,41]. Increased flavonoid biosynthesis is associated with enhanced cold tolerance across several plant species. In rice, *OsJRL* knockout lines exhibited higher phenylalanine ammonialyase (PAL) activity and flavonoid abundance under cold stress, accompanied by lower ROS levels and superior cold tolerance relative to wild type [48]. Likewise, tobacco *NtMSI4c* acts as a negative regulator of cold tolerance by repressing flavonoid biosynthesis; the msi4c mutant displayed elevated flavonoid contents, reduced ROS accumulation and improved cold tolerance compared with wild type [49]. In tomato, CPK27 phosphorylates ELONGATED HYPOCOTYL 5 (HY5), which binds to the promoters of flavonoid biosynthetic genes and activates their transcription in a Ca^{2+}dependent manner, thereby promoting flavonoid production and cold tolerance [50]. Collectively, these findings highlight a conserved mechanism: flavonoids alleviate oxidative damage to support plant cold acclimation.

In this study, the flavonoid biosynthesis pathway showed significant enrichment for DEGs when comparing the T and S groups under control conditions. Moreover, the same pathway exhibited significant enrichment of DAMs under cold-stress conditions, which is consistent with our previous metabolomic study [51]. Six DEGs were assigned to the flavonoid biosynthesis pathway, including *Prupe.3G185000*, *Prupe.5G086700*, *Prupe.1G002900*, *Prupe.2G263900*, *Prupe.1G580200* and *Prupe.7G168300*; all encode enzymes participating in flavonoid biosynthesis. Only *Prupe.3G185000* was upregulated, while the other 5 DEGs were downregulated in T group relative to S group. Moreover, these 6 genes exhibited no significant differential expression between control and cold-stress treatments in either the T or S groups (SC vs ST and TC vs TT), indicating that they are not cold-induced genes. Most DAMs within the flavonoid biosynthesis pathway (131 out of 159) were upregulated in T group compared with S group. Based on these results, we hypothesize that the T group accumulates more flavonoids than the S group under nonstress conditions, which contributes to its superior cold tolerance via the ROS-scavenging activities of flavonoids. Differential expression of these flavonoid biosynthesis-related DEGs may occur prior to cold stress, rather than being triggered by cold stress during peach dormancy. The uniquely upregulated flavonoid biosynthesis-related DEG, *Prupe.3G185000*, is homologous to the *AtUGT* gene, which encodes an enzyme that transfers

sugar moieties from UDP-sugars to flavonoids [52]. Recent studies have shown that *UGTs* positively regulate plant cold tolerance [53,54]. For instance, overexpression of *ScUGT73B4* from wild potato (*Solanum chacoense*) in potato (*Solanum tuberosum* L.) plantlets enhanced glycosylated flavonoid accumulation and strengthened antioxidant capacity, thereby improving freezing tolerance [55]. In our study, *Prupe.3G185000* exhibited significantly higher expression in T group than in S group, with the highest $\log_2$FC value (1.43) among these 6 flavonoid biosynthesis-related DEGs. We therefore propose that this gene represents a promising candidate for modulating peach cold tolerance via flavonoid metabolism.

Ascorbate aldarate metabolism contributes to genotypic divergence in cold tolerance through modulating AsA redox state

AsA is an important cellular antioxidant that scavenges ROS triggered by various environmental stresses and protects plant tissues from oxidative damage [56,57]. A growing number of studies have reported that AsA metabolism is associated with plant cold tolerance. For example, overexpression of *CsCIPK20* in *Arabidopsis* elevated AsA levels and was accompanied by enhanced plant cold tolerance, whereas its transient knockdown in tea plants produced the opposite phenotype [58]. In *Pyrus betulaefolia*, *PbrMYB5* promotes AsA biosynthesis and exerts a positive effect on chilling tolerance [59]. Furthermore, heterologous overexpression of the tomato Dgalacturonic acid reductase gene *FaGalUR* resulted in increased AsA levels, thereby improving plant tolerance to abiotic stresses including oxidative stress, salinity and cold [56].

In this study, KEGG enrichment analysis revealed that DEGs from T and S group under cold-stress treatment (TT vs ST) were significantly enriched in the ascorbate and aldarate metabolism pathway. Moreover, L-Ascorbic acid-2-glucoside, a characteristic metabolite of this pathway that partially reflects sample AsA content, was significantly upregulated in T group compared with S group, with a VIP value of 1.39. These results suggest that AsA metabolism is closely linked to cold tolerance in peach. Three DEGs mapped to ascorbate and aldarate metabolism pathway: *Prupe.2G077800*, *Prupe.6G155100* and *Prupe.1G357100*. Interestingly, all 3 DEGs were downregulated in T group relative to S group and are homologous to Lascorbate oxidase (AO). AO is a cell-wall-localized enzyme that catalyzes the oxidation of AsA into unstable MDHA. MDHA subsequently undergoes disproportionation to form DHA, thereby modulating the AsA redox state and maintaining ROS homeostasis [60,61]. Multiple studies have shown that *AO* genes regulate AsA accumulation and further influence plant stress responses. Silencing of the *AO* gene *Solyc04g054690* in tomato increases AsA abundance and improves salt tolerance [62]. On the contrary, heterologous overexpression of *AnAO5*, an *AO* gene from *Ammopiptanthus nanus*, enhances cold tolerance in *Arabidopsis* seedlings [63]. We hypothesize that *AOs* act as repressors of AsA accumulation in peach. Lower transcript abundance of *AO* genes in the T group compared with the S group leads to higher AsA content. The enlarged AsA pool strengthens ROS-scavenging capacity, which ultimately contributes to the superior cold tolerance observed in the T group.

Conclutions

In this study, we integrated transcriptomic and widely targeted metabolomic analyses to systematically dissect the molecular and metabolic mechanisms underlying natural variation in cold tolerance of dormant peach cultivars. The results demonstrated that cold stress universally activates conserved upstream signaling pathways, including the MAPK signaling pathway and plant hormone signal transduction pathway, in both cold-tolerant and cold-sensitive peach cultivars. The distinct regulatory patterns of flavonoid biosynthesis and ascorbate and aldarate metabolism are the key determinants of differential cold tolerance in dormant peaches. Under control conditions, flavonoid biosynthesis-related DEGs exhibited distinct basal expression patterns between the two groups. Two L-ascorbate oxidase (AO) genes and one AO-like gene enriched in the ascorbate and aldarate metabolism pathway were markedly downregulated in the cold-tolerant cultivars, while their associated metabolite, L-ascorbic acid-2-glucoside, was upregulated in the cold-tolerant cultivars. Collectively, this study reveals new mechanistic insights into natural variation in cold tolerance in peach and identifies promising candidate genes and metabolites for the dissection of the genetic mechanism underlying cold tolerance in peach.

Declarations

Ethics approval and consent to participate

Not applicable. All peach cultivars used in this study were publicly available and commercially cultivated germplasms. No human participants, human data, or animal samples were involved in the present study.

Consent for publication

Not applicable.

Availability of data and materials

The raw transcriptome sequencing data generated in this study have been deposited in the Sequence Read Archive (SRA) database of NCBI under the accession number PRJNA1539684. The metabolomics raw data and all datasets supporting the conclusions of this study are available from the corresponding author upon reasonable request.

Competing interests

Not applicable.

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Authors' contributions

Zhendong Tian and Yonghong Li conceived and designed the research. Zhendong Tian, Jie Li, Qihang Tian , Zhaoyuan Wang, Yu Wang performed experiments. Guojian Liu and Ruifeng Chang participated in data statistics. Zhendong Tian drafted the manuscript. All authors have read and approved the final version of the manuscript.

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Figures

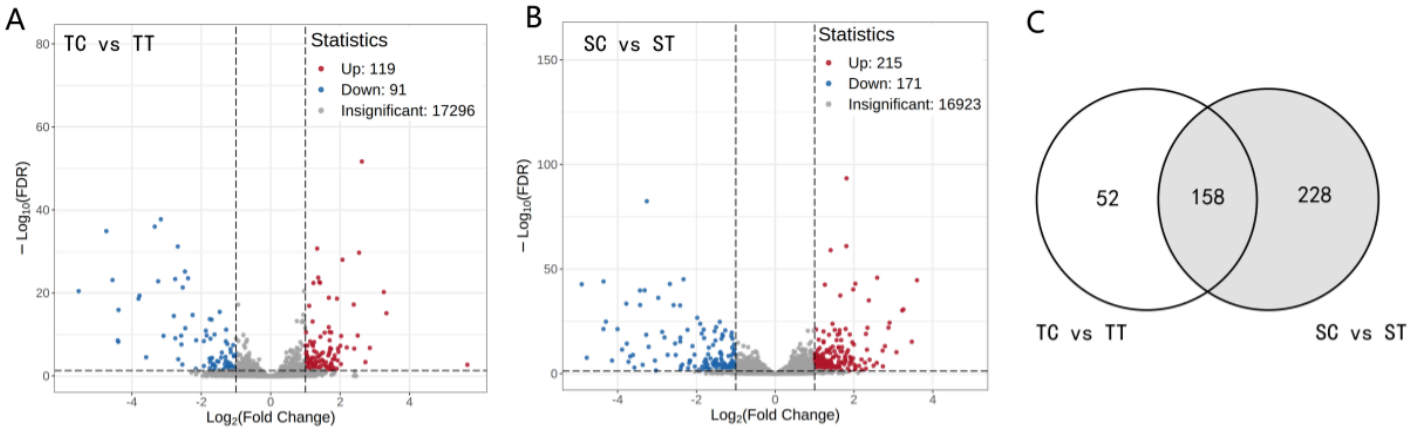


Figure 1

Overview of treatment-specific DEGs in T and S groups.

(A) Volcano plot of DEGs for TC vs TT. Red dots represent significantly up-regulated genes, blue dots represent significantly down-regulated genes, and gray dots indicate non-differentially expressed genes. (B) Volcano plot of DEGs for SC vs ST. (C) Venn diagram showing the overlap of DEGs identified from TC vs TT and SC vs ST.

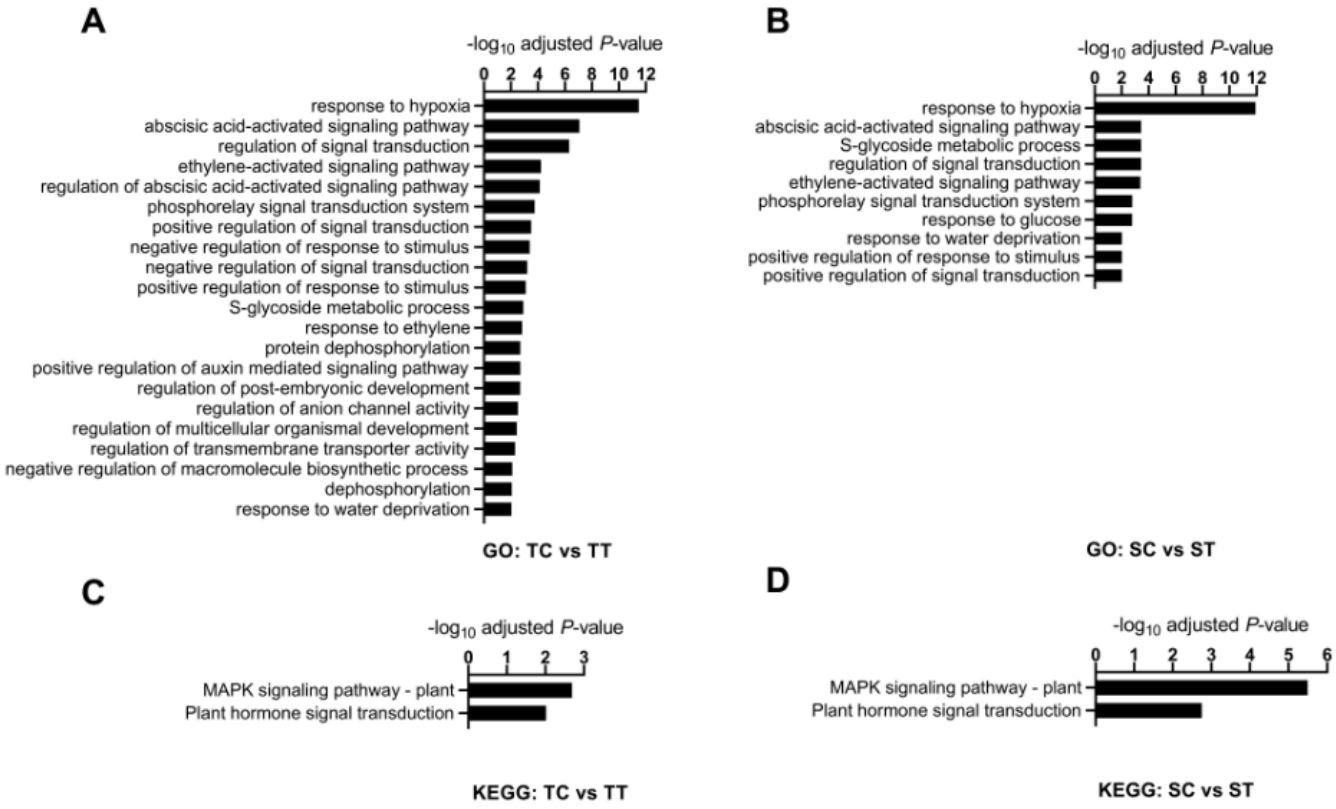


Figure 2

GO and KEGG enrichment analyses of DEGs for TC vs TT and SC vs ST.

Bar plots showing enriched GO terms (A,B) and KEGG pathways (C,D) for the comparisons TC vs TT (A,C) and SC vs ST (B,D). Significant GO terms and KEGG pathways were identified with adjusted P-value < 0.05

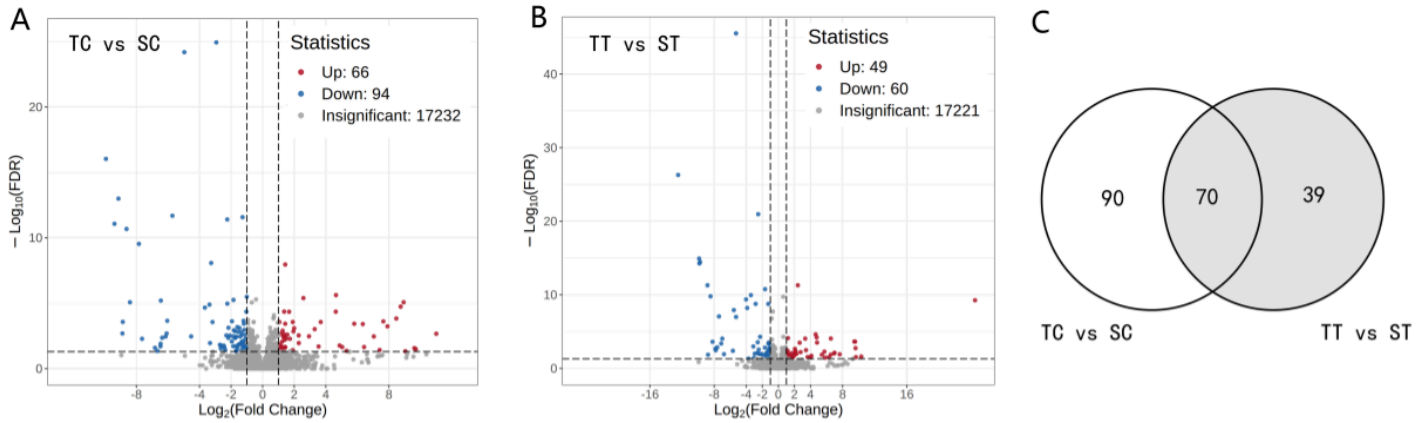


Figure 3

Overview of genotype-specific DEGs under control and cold-stress conditions.

(A) Volcano plot of DEGs for TC vs SC. Red dots represent significantly up-regulated genes, blue dots represent significantly down-regulated genes, and gray dots indicate non-differentially expressed genes. (B) Volcano plot of DEGs for TT vs ST. (C) Venn diagram showing the overlap of DEGs identified from TC vs SC and TT vs ST.

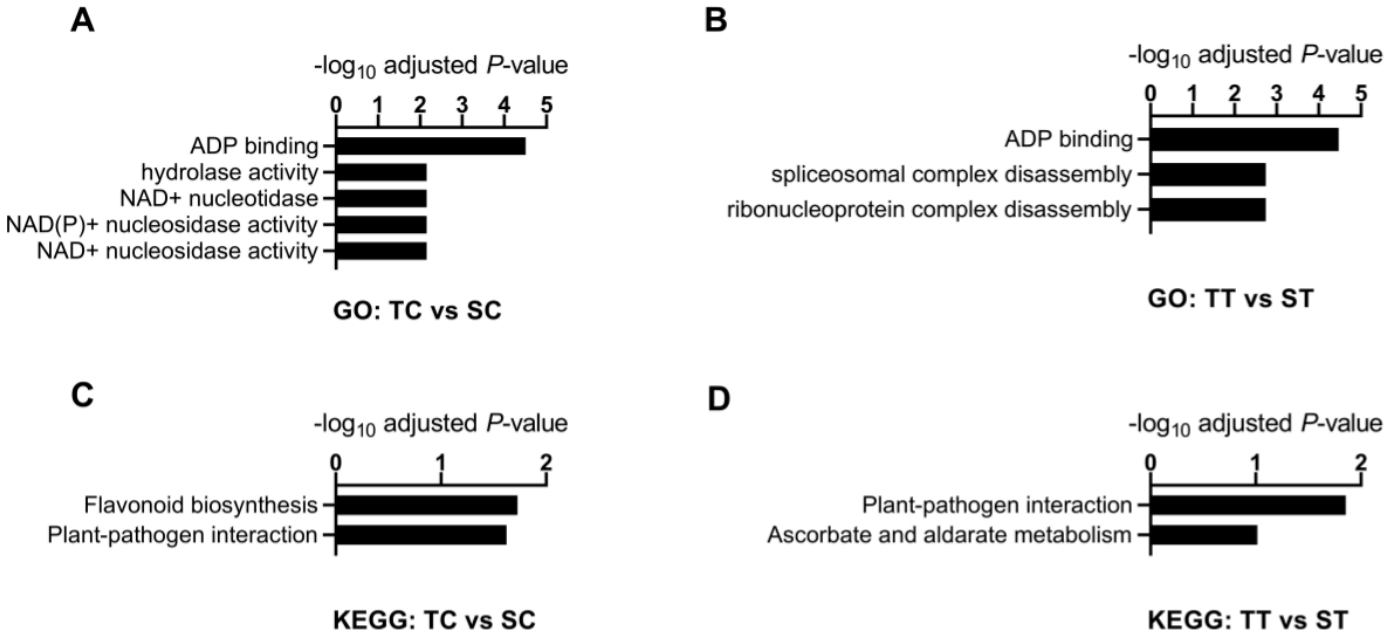


Figure 4

GO and KEGG enrichment analyses of DEGs for TC vs SC and TT vs ST.

Bar plots showing enriched GO terms (A,B) and KEGG pathways (C,D) for the comparisons TC vs SC (A,C) and TT vs ST (B,D). Significant GO terms and KEGG pathways were identified with adjusted P-value < 0.05.

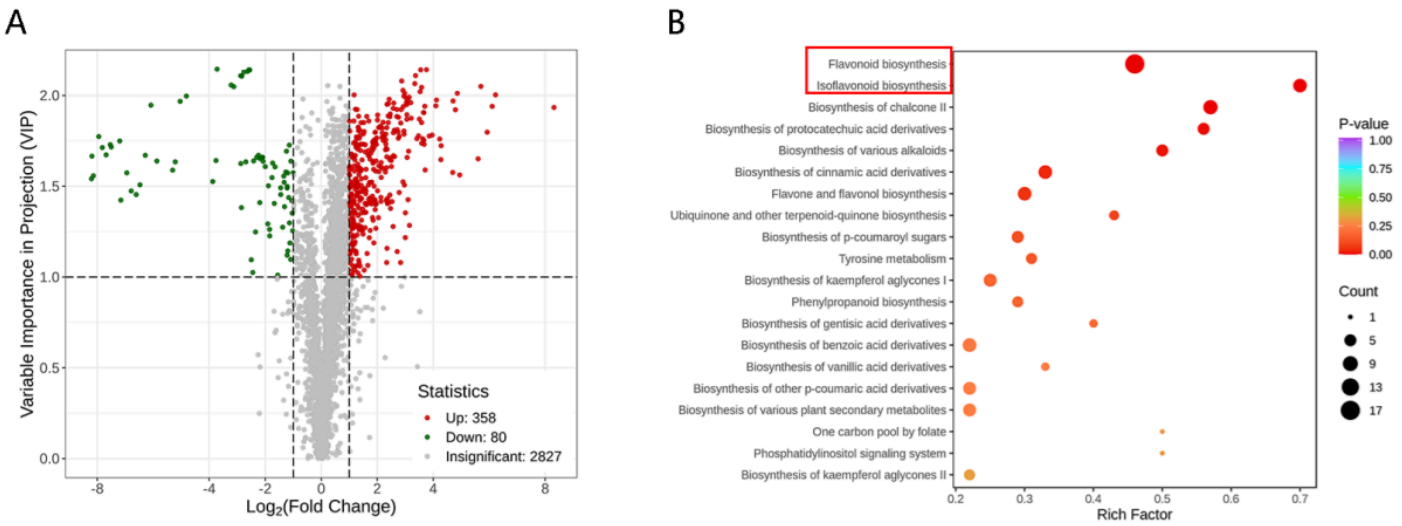


Figure 5

Identification of DAMs between two peach genotypes under control conditions and KEGG

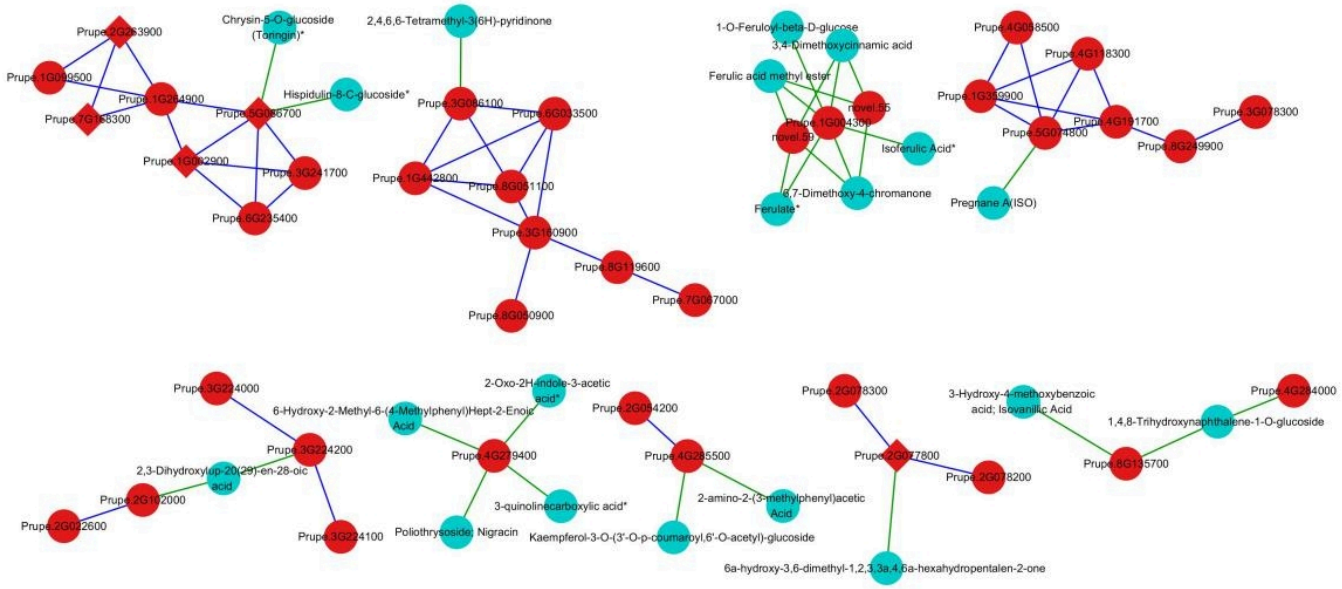


Figure 6

Correlation network of DEGs and DAMs between the T group and S group.

Red nodes represent DEGs, and cyan nodes represent DAMs. Green lines indicate correlations between DEGs and DAMs; purple lines indicate correlations between DEGs and DEGs. Rhombus nodes represent candidate genes.

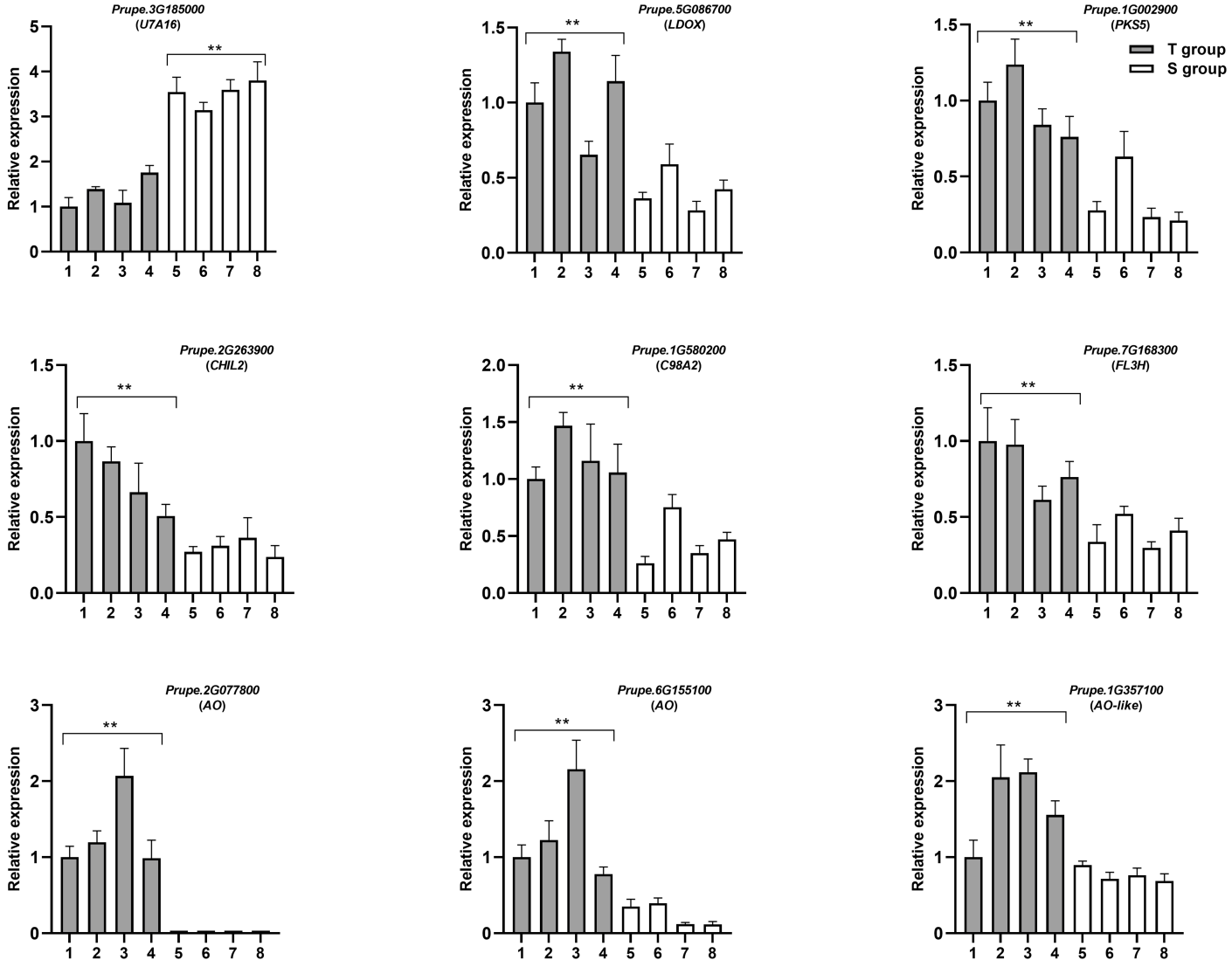


Figure 7

Relative expression of 9 candidate genes involved in flavonoid biosynthesis and ascorbate and aldarate metabolism pathways that contributed to cold-tolerance divergence in peach cultivars.

All data are shown as the mean of three biological replicates, error bars indicate standard deviation. * , Significant difference between the two peach cultivars at the same time-point ($P < 0.05$); ** , Highly significant difference at $P < 0.01$.

Supplementary Files

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- TableS.docx