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**Comparative transcriptomics unveils the role of DELLA protein GAIP-B in cold
tolerance of mango (*Mangifera indica* L.)**

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Abstract

Background: Cold stress severely limits the productivity and geographical distribution of mango, a tropical fruit crop of significant economic importance. We combined transcriptomic profiling and functional genomics to investigate the molecular mechanisms underlying cold tolerance in mango.

Results: Comparative analysis of the cold-tolerant Jinhuang (JH) and cold-sensitive Guiqi (GQ) cultivars identified 7,757 differentially expressed genes, with significant enrichment in plant hormone signal transduction pathways. Notably, the DELLA protein GAIP-B (LOC123214451) was markedly upregulated in JH under cold stress. Functional validation through CRISPR/Cas9-mediated knockout and overexpression in *Nicotiana benthamiana* demonstrated that GAIP-B is both necessary and sufficient for cold tolerance. The knockout lines exhibit extreme cold sensitivity, reduced antioxidant enzyme activity, increased oxidative damage, and impaired osmotic adjustment. Conversely, the overexpression lines showed enhanced cold tolerance, with superior antioxidant capacity, maintenance of photosynthetic pigments, and increased soluble sugar accumulation.

Conclusions: These findings establish GAIP-B as a central regulator that integrates antioxidant defense, photosynthetic protection, and osmotic adjustment in the cold stress response. This study provides valuable insight into molecular breeding strategies aimed at enhancing cold tolerance in mango and other economically important fruit crops.

Keywords: Antioxidant defense; photosynthetic protection; osmotic adjustment; cold tolerance; fruit crop

Introduction

Mango (*Mangifera indica* L.), one of the most economically important tropical fruit crops worldwide, is valued for its excellent flavor, nutritional richness, and versatility in food processing [1]. Mango trees are grown in the tropical and subtropical regions of more than 100 countries between 30°S and 30°N, stretching from as far north as southern Sichuan in China, the southern islands of Japan, and as far south as southern Africa (<http://www.fao.org/faostat>) [2]. China is the third largest producer of mangoes, after India and Indonesia (www.worldpopulationreview.com). Mango is widely planted in tropical and subtropical regions of China, including Hainan, Guangdong, Guangxi, Fujian, Yunnan, Guizhou, and Sichuan [3].

In nature, mango is a tropical evergreen fruit tree that prefers high temperatures (25–35 °C), and its optimum growth temperature range is 24–27 °C. Mango trees are highly sensitive to low temperatures, which can severely impair their growth, yield, and fruit quality [4]. Cold stress poses a major constraint on mango cultivation, particularly in subtropical regions, where occasional frost or prolonged chilling can cause irreversible damage [5]. Young mango trees are especially vulnerable, with temperatures below 5 °C inducing cellular damage, while mature trees can only briefly tolerate temperatures near 0 °C before suffering from bark splitting, root injury, and leaf necrosis [6]. These physiological disruptions lead to reduced photosynthesis, impaired nutrient transport, and increased susceptibility to secondary infections, ultimately threatening orchard productivity [7].

However, tolerance or sensitivity to these abiotic factors is a complex phenomenon involving responses at the molecular, biochemical, and physiological levels [8]. In recent years, high-throughput omics analyses, such as genomics, transcriptomics, proteomics, and metabolomics, have been widely used to study various types of abiotic stress in plants, deepening our understanding of different biological regulation pathways. Transcriptome high-throughput sequencing, a genetic research tool, is widely used to study plant responses to various stressors, revealing changes in the expression of various genes during exposure to environmental extremes [9]. Many studies have been conducted on the transcriptional response of plants, including banana [10], litchi [11], pineapple [12], and other tropical crops, to low-temperature stress. Several studies have revealed the molecular basis of cold stress response in mango trees. Some ethylene-responsive transcription factor (ERFs) [3] and cyclic nucleotide-gated ion channel

(CNGC) genes [13] may play important regulatory roles in cold resistance in plants.

Currently, more than 80 mango varieties are cultivated in China [14]. Guiqi Mango, a prestigious cultivated variety from Guangxi, China, is often hailed as the “King of Mangoes,” possessing strong natural resistance to biotic stressors such as powdery mildew, anthracnose, and fruit borers, making it a valuable germplasm resource for disease-resistance breeding. Despite these advances, the Guiqi cultivar is grown predominantly in Guangxi, where temperatures rarely drop to critical thresholds, suggesting a narrower climatic adaptation range. Correspondingly, the Jinhuang cultivar, widely cultivated across diverse climatic zones in China, including Taiwan, Hainan, Yunnan, and Guangdong, exhibits robust performance even under suboptimal soil conditions. Although plants can evolve cold-resistant pathways to adapt to low-temperature and cold environments through long-term genetic variation and natural selection [15], it is difficult to breed varieties adapted to both low temperature and cold conditions. Understanding the molecular mechanisms of cold tolerance in these mango cultivars will provide significant support for the future cultivation of excellent varieties.

Currently, research on cold stress resistance in mango plants primarily encompasses several key areas: the identification and evaluation of stress tolerance across diverse germplasm resources, monitoring changes in physiological and biochemical indicators, mining cold resistance-related genes and pathways, and developing measures to prevent and mitigate chilling injury [3, 13, 16-18]. However, the molecular mechanisms underlying cold resistance in mango remain largely unknown. In this study, we selected the Guiqi and Jinhuang cultivars to investigate transcriptomic changes under cold stress conditions. By identifying key cold-responsive genes, we aimed to identify potential candidates associated with cold resistance. Transgenic tobacco, which can be used for the functional verification of potential genes, was used to elucidate the function of the cold-resistance genes. This research not only offers a new perspective on the molecular adaptation of mango to low temperatures but also contributes to the genetic improvement of stress-resistant varieties, facilitating the development of strategies to mitigate cold damage in tropical fruit production.

Materials and Methods

Plant materials

Two different mango cultivars, Jinhuang (JH) and Guiqi (GQ), collected from the Mango Germplasm Repository at the Subtropical Crops Research Institute of the Guangxi Zhuang Autonomous Region, were used to analyze resistance mechanisms against cold stress. The branches of two-year-old trees were placed in RR-CTC806C incubators (Rainroot Scientific, Beijing, China) for cold treatment at 7 °C for 24 h. The two control groups were maintained in the greenhouse at 24 °C for 24 h. Leaves from two branches were pooled as one sample and each sample was repeated three times as a biological replicate (Fig. 1). All the samples were stored at –80 °C until analysis.



Fig. 1 Phenotypic symptoms responses of Jinhuang (JH) and Guiqi (GQ) mango leavers responding to cold stress and untreated group. A: JH (Left), GQ (Right); B: GQ (Left), JH (Right).

Tobacco (*Nicotiana benthamiana*) was used as the background for the genetic transformation. The following four transgenic lines were generated: (1) WT (non-transformed control), (2) *GAIP-B* overexpression (OE), (3) *GAIP-B* knockdown (KO), and (4) *GAIP-B* knockout (KO-OE). *N. benthamiana* seeds were surface-sterilized and sown on half-strength Murashige and Skoog ($\frac{1}{2}$ MS) medium. All tobacco plants were grown under standard conditions (25 °C, 16 h light / 8 h dark cycle) until the six-leaf stage, then subjected to 7 °C cold treatment.

Physical and chemical characteristic measurement

Samples of the two cultivars (JH and GQ) and four tobacco lines were collected at 0, 6, 12, 18, and 24 h of cold-stress treatment for physical analysis and measurements of peroxidase (POD) activity, catalase (CAT) activity, superoxide dismutase (SOD) activity, H₂O₂ content, malondialdehyde (MDA) content, photosynthetic pigments, and total soluble carbohydrate content. SOD, CAT, and POD activities were measured using kits provided by Suzhou Grace Biotechnology Co., Ltd. (Catalog No. G0107F, Grace, Suzhou, China). H₂O₂ content was measured using the Amplex UltraRed fluorescent probe method (Invitrogen, A36006; Thermo Fisher Scientific, Waltham, MA, USA). MDA accumulation was evaluated using a thiobarbituric acid reactive substance assay [19]. Photosynthetic pigments and total soluble carbohydrate content were measured as previously described [20]. Statistical significance was determined using Student's *t*-tests. Differences were considered significant at $P < 0.05$.

RNA extraction and RNA-seq

Total RNA content was extracted from mango leaves using the TRIzol reagent kit (Invitrogen), according to the manufacturer's instructions. RNA integrity was evaluated on a 1.0% agarose gel and quantified using a Qubit 4.0 fluorometer (Invitrogen). Purity was tested using a NanoDrop spectrophotometer (Thermo Fisher Scientific). cDNA library products of each sample were generated via six steps: mRNA purification, first-strand cDNA synthesis, second-strand cDNA synthesis, cDNA fragment purification, end repair, adaptor ligation, and addition of index codes. Finally, the cDNA libraries were amplified and purified for quality inspection using a Qsep400 standard DNA clip kit on a Qsep400 Bio-Fragment Analyzer (BioOptic, New Taipei City, Taiwan, China). The cDNA, ca. 200 bp in length, was sequenced on an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA).

The raw sequencing data were initially processed to remove low-quality reads, adapter sequences, and contaminants using fastq (Version 0.19.3) [21], generating high-quality Clean Data. The filtered reads were then aligned to the reference genome (NCBI accession number: GCF_011075055.1) using the splice-aware aligner HISAT2 v2.1.0 [22], with alignment rates >85% for all samples. For visualization, the aligned reads were inspected using Integrative Genomics Viewer (IGV) v2.11.1 [23].

Bioinformatic analysis

The novel transcripts were assembled using StringTie v2.1.4 [24] and compared against the reference annotation using GFFcompare v0.12.1 [25], with the results stored in GTF format. The corresponding genomic sequences of the novel genes were extracted using gffread and subjected to a comprehensive functional annotation. Sequence homology searches were performed against KEGG pathway, Gene Ontology (GO), NR, Swiss-Prot, TrEMBL, and KOG databases using DIAMOND v2.0.13 [26] with stringent parameters ($E\text{-value} \leq 1e-5$, maximum target sequences = 1). For transcription factor prediction, we employed iTAK v1.7 [27], which integrates two authoritative plant-specific databases, PlnTFDB v3.0 [28] and PlantTFDB v5.0 [29], enabling systematic classification of transcription factors based on conserved domain architecture and phylogenetic relationships.

Gene expression was quantified using Fragments Per Kilobase of transcript per million fragments mapped (FPKM) values, which normalize read counts based on both transcript length and sequencing depth, enabling cross-sample comparisons [30]. Differential expression analyses of the two samples was performed using DESeq2/edgeR (version 1.22.1) [31]. P -values were adjusted using the Benjamini–Hochberg method. Significantly differentially expressed unigenes were identified based on the thresholds of a false discovery rate (FDR) ≤ 0.05 and an absolute value of $\log_2(\text{fold change}) \geq 1$.

qRT-PCR analysis

Quantitative reverse transcription real-time PCR (qRT-PCR) was performed to quantify the expression of *GAIP-B*. Total RNA was isolated from JH and GQ samples, as described under the heading “RNA extraction and RNA-seq.” For RT-qPCR validation, cDNA was synthesized from ca. 2 μg RNA using the SuperScript III First-Strand Synthesis System (Invitrogen). Reactions were performed on a LightCycler 480 II (Roche, Basel, Switzerland) using SYBR Green qPCR Master Mix (Thermo Fisher Scientific), with three biological and technical replicates per gene. The DELLA protein *GAIP-B* gene served as an internal reference for normalization. Relative expression was calculated via the $2^{-\Delta\Delta C_t}$ method, with MD2T0 as the reference sample. Statistical significance ($p < 0.05$) was determined using GraphPad Prism 9 (DotMatics, Boston, MA, USA). Primers were designed using NCBI Primer-BLAST (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>), and the sequences are reported in Supplementary Table S1.

Gene editing of GAIP-B in *Nicotiana benthamiana*

To investigate GAIP-B function, transgenic *N. benthamiana* lines were used and the CRISPR/Cas9 system was applied, as previously described [32]. For knockout (KO) constructs, two specific sgRNAs targeting conserved regions of *GAIP-B* (NCBI accession: 107583202) were designed using CRISPR-P 2.0 and were cloned into the pYLCRISIPR/Cas9 binary vector [33]. For overexpression (OE), full-length GAIP-B CDS was amplified and inserted into the p1301S vector under the control of the CaMV 35S promoter. *Agrobacterium tumefaciens* GV3101 harboring these constructs was used to transform *N. benthamiana* wild-type (WT) plants via leaf disc infiltration. Transgenic lines were selected on kanamycin (50 mg/L) and validated through (1) PCR genotyping, (2) a T7E1 endonuclease assay for indel mutations in KO lines, and (3) Sanger sequencing of the target sites. The OE and KO-OE (knockout followed by complementation) lines were further confirmed via RT-qPCR using *NbActin* as a reference gene. Homozygous T2 plants of each genotype (WT, KO, OE, and KO-OE) were subjected to phenotypic characterization under controlled growth conditions. The primers used for each gRNA sequence are listed in Supplementary Table S1. All experiments included three biological replicates, each with three technical repeats.

Subcellular localization of GAIP-B

The full-length GAIP-B open reading frame was amplified from the pMD18-T-GAIP-B template using specific primers designed using CE II Design software. The pCAMBIA1300-GFP vector was double-digested using the KpnI and BamHI restriction enzymes. The purified PCR product was recombined with the linearized vector to generate the pCAMBIA1300-GAIP-B-GFP construct. The recombinant plasmid was transformed into *Escherichia coli* TG1 competent cells and plated on a selective medium for 3 d. Positive clones were verified by colony PCR and sequencing. The confirmed plasmid was introduced into *Agrobacterium tumefaciens* GV3101. For the co-localization analysis, the transformed agrobacteria containing pCAMBIA1300-GAIP-B-GFP were mixed at a 1:1 ratio with the cytoplasmic marker pCAMBIA1300-35S-UGPase-cherry-NOS and infiltrated into *N. benthamiana* leaves. The empty vector served as the negative control. After 2 d of dark incubation, subcellular localization was observed using confocal microscopy (LSM880; Zeiss; Oberkochen, Germany) with excitation and emission settings of 488 nm/500–550 nm for GFP and 583 nm/600–650 nm for cherry fluorescence. Images were processed using ZEN 3.0 and ImageJ software.

Statistical analysis

Differences in *GALP-B* expression, plant height, stem diameter, leaf length, leaf width, leaf thickness, and differences in physiological indicators between the two control samples and the transgenic tobacco plants were analyzed using Student's *t*-tests.

Results

Physical and chemical characteristic changes in mango leaves under cold stress

At the physiological level, POD, CAT, and SOD activities were higher in JH plants than in GQ plants under cold stress. POD activity differed significantly between the JH and GQ plants after 7 °C treatment for 6 h (Fig. 2A), and CAT activity differed significantly between the JH and GQ plants after 7 °C treatment for 18 h (Fig. 2B). Similar results were observed for SOD activity (Figure 2C). Under cold stress, the H₂O₂ content of the cold-sensitive GQ increased from 12.09 µmol/g at 0 h to a peak of 17.16 µmol/g at 24 h (Fig. 2D). In contrast, in the cold-tolerant JH cultivar, this increase was gradual and restrained, reaching only 12.4 µmol/g at 24 h (27.7% lower than in GQ at the same time point; $p < 0.01$) (Fig. 2D). A similar difference was observed for MDA content. In GQ, MDA levels increased rapidly from 23.6 nmol/g at 0 h to 44.8 nmol/g at 24 h, indicating severe membrane damage. Conversely, in JH, MDA accumulation was significantly mitigated, rising to only 29.6 nmol/g at 24 h (33.9% lower than GQ; $p < 0.01$) (Fig. 2E). JH exhibited superior physiological resilience: its chlorophyll content decreased slowly from 97.3 mg/g to 90.3 mg/g (a 7.2% loss) over the 24-h period. In stark contrast, GQ suffered a rapid decline in chlorophyll content, from 113.7 mg/g to 41.9 mg/g (a 63.1% loss) (Fig. 2F), exhibiting pronounced chlorosis. JH maintained a significantly higher level of soluble sugars, which increased from 26.1 mg/g to 44.9 mg/g over 24 h, while in GQ they increased from 21.6 mg/g to 33.6 mg/g (Fig. 2G).

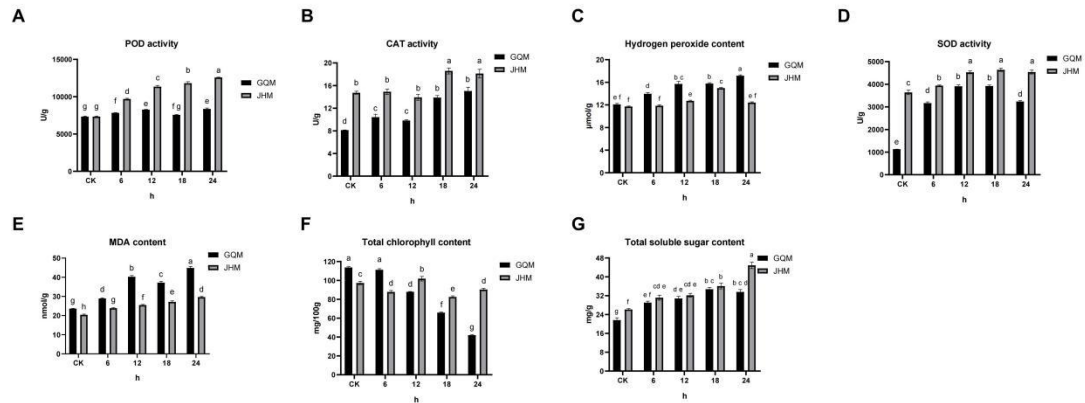


Figure 2 Physiological responses of Jinhuang (JH) and Guiqi (GQ) mango cultivars to cold stress. (A) Superoxide dismutase (POD) activity, (B) Catalase (CAT) activity, (C) Superoxide dismutase (SOD) activity, (D) H₂O₂ content, (E) Malondialdehyde (MDA) content, (F) Photosynthetic pigments, and (G) Total soluble carbohydrate content. *** $p \leq 0.01$, * $p \leq 0.05$; ns, non-significant.

Differential gene expression in mango leaves under cold stress

The raw-data quality assessment of the JH and GQ libraries for the control and cold treatments is presented in Supplementary Table S2. Overall, the sequencing process generated 741,405,192 raw reads across all samples, with an average of 61,783,766 raw reads per sample. After stringent quality filtering, we retained an average of 60,738,067 high-quality reads per sample, representing 98.31% of original reads. The filtered reads exhibited excellent base-call accuracy, with Q20 scores (1% error rate) ranging from 96.70% to 97.59%, and Q30 scores (0.1% error rate) between 90.53% and 92.65% across all samples. The GC content distribution remained consistent among the samples, ranging from 44.59% to 47.26%, which is within the expected range for plant transcriptomes. Consequently, 92.65% of the clean reads could be aligned to the reference genome (GCF_011075055.1_CATAS_Mindica_2.1_genomic). The total alignment rates ranged from 91.36% to 93.67%, and uniquely mapped reads accounted for 84.66% to 88.75% of the total mapped reads (Supplementary Table S2).

PCA revealed that the treatments tended to group together (Fig. 3A). PC1 and PC2 explained 26.21% and 23.05% of variation, respectively. After low-temperature treatment, a large number of differentially expressed genes (DEGs) were identified in both varieties (Fig. 3B). Using DESeq2, we identified 11,294 DEGs (5,709 upregulated and 5,585 downregulated) between the JH control (jhm-ck) and JH 24 H treatment (jhm-24) groups, based on FC and FDR values; 6,546 DEGs (3,625 upregulated and 2,921

downregulated) were identified between the GC control (gqm-ck) and GQ 24 H treatment (gqm-24) groups; 7,948 DEGs (4,791 upregulated and 3,157 downregulated) were identified between the jhm-ck and gqm-ck groups; and 4,156 DEGs (2,565 up-regulated and 1,591 down-regulated) were identified between the jhm-24 and gqm-24 groups. The majority of the DEGs identified here are related to categories such as regulation of transcription, response to stress, transport, protein modification, nucleosome assembly, cell wall organization and biogenesis, signal transduction, oxidation reduction, RNA modification, and cell redox homeostasis.

Gene ontology (GO) enrichment analysis of differentially expressed genes (DEGs) was performed. The number of DEGs differed between the two cultivars, and most of these participated in the biological processes of mango development, followed by molecular function and cellular component. Functional analysis of the DEGs revealed that 46 GO terms ($p < 0.05$) annotated to the DEGs identified between the gqm-ck and gqm-24 groups (Fig. 3C); 30 GO terms were annotated to the DEGS identified between the jhm-ck and jhm-24 groups, and of these, only five (16.7%) overlapped with the 46 terms for the gqm-ck vs. gqm-24 comparison (Fig. 3C). The DEGs identified between gqm-ck and gqm-24 were involved in positive regulation of immune system processes, positive regulation of response to stimulus, biological adhesion, activation of immune response, and positive regulation of immune response (Fig. 3C). DEGs identified between jhm-ck and jhm-24 were involved in positive regulation of the immune system process, defense response, positive regulation of response to stimulus, and endothelial cell migration (Fig. 3C). Similarly, DEGs identified between jhm-ck and gqm-ck and between gqm-24 and gqm-24 were involved in the carbohydrate metabolic process, cellular glucan metabolic process, protein phosphorylation, nucleotide–sugar metabolic process, response to wounding, carbohydrate biosynthetic process, pectin metabolic process, cellular response to alkaline pH, photosystem, and phosphoglycerate dehydrogenase activity.

KEGG enrichment annotation analysis was performed on the DEGs identified in the four comparisons: gqm-ck vs. gqm-24, jhm-24 vs. gqm-24, jhm-ck vs. gqm-ck, and jhm-ck vs. jhm-24: for these comparisons, 19, 16, 25, and 19 significant pathways were annotated, respectively (Fig. 3D). KEGG pathway enrichment analysis revealed that the DEGs identified between the two cultivars were enriched predominantly in pathways such as alanine, aspartate and glutamate metabolism pathway,

270 phenylalanine, tyrosine and tryptophan biosynthesis pathway, phenylpropanoid biosynthesis pathway,
 271 flavonoid biosynthesis pathway, flavone and flavonol biosynthesis pathway, pentose and glucuronate
 272 interconversions pathway, glycolysis/gluconeogenesis pathway, ascorbate and aldarate metabolism
 273 pathway, photosynthesis pathway, photosynthesis–antenna proteins pathway, plant–pathogen interaction
 274 pathway, protein processing in endoplasmic reticulum pathway, amino sugar and nucleotide sugar
 275 metabolism pathway, ubiquinone and other terpenoid–quinone biosynthesis pathway, ribosome
 276 biogenesis in eukaryotes pathway, chloroalkane and chloroalkene degradation pathway (Fig. 3D).
 277 Furthermore, significant enrichment in pathways was found in gqm-ck vs. gqm-24, including alanine,
 278 aspartate, and glutamate metabolism pathways; glycine, serine, and threonine metabolism pathway;
 279 cysteine and methionine metabolism pathway; phenylalanine, tyrosine, and tryptophan biosynthesis
 280 pathway; monobactam biosynthesis pathway; circadian rhythm-plant pathway; amino sugar and
 281 nucleotide sugar metabolism pathway; and ubiquinone and other terpenoid-quinone biosynthesis
 282 pathways. In contrast, for the DEGs identified between gqm-ck and gqm-24, KEGG analysis identified
 283 six annotated pathways: the glycolysis/gluconeogenesis pathway, photosynthesis-antenna protein
 284 pathway, protein processing in the endoplasmic reticulum pathway, fatty acid degradation pathway,
 285 limonene degradation pathway, and ribosome biogenesis in eukaryotes.

biosynthesis pathway, and the plant hormone signal transduction pathway. Therefore, we constructed a
 gene regulatory network that included the DEGs in these pathways. A considerable number of genes were
 enriched in the terpenoid backbone biosynthesis pathway in the JH cultivar. Notably, these genes were
 downregulated under cold stress, specifically in JH, leading to suppression of the gibberellic acid (GA)-
 related biosynthetic pathway. Correspondingly, multiple genes involved in GA downstream signaling,
 including *GIDI*, *DELLA*, and PIF, were significantly upregulated under cold stress in JH but not in GQ.
 Enhanced ubiquitination of the DELLA protein (GAIP-B) is suggested to be a key factor contributing to
 the maintained growth performance of JH under cold-stress (Fig. 4B). To validate the reliability of the
 transcriptome sequencing results, *GAIP-B* expression levels were confirmed by qRT-PCR: *GAIP-B*
 expression was significantly higher in jhm-24 than in the other groups (Supplementary Fig. 1). The high
 concordance between the two methodological approaches confirms the accuracy of the transcriptomic
 findings and supports the use of the RNA-Seq dataset for subsequent functional analyses.

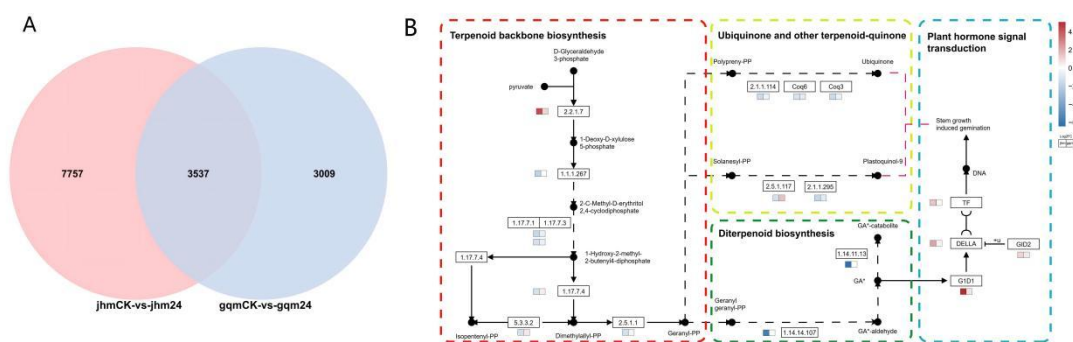


Figure 4 Analysis of interactions among the DEGs between the comparison groups. (A) Venn analysis of DEGs identified between the experimental groups, revealing both unique and shared cold-stress genes; (B) Schematic representation of the pathways involved in gibberellin synthesis, which is associated with dwarfism, including terpenoid backbone biosynthesis and diterpenoid biosynthesis. +ub, ubiquitination.

Characterization of mango GAIP-B

PCR amplification and sequencing identified the mango DELLA protein *GAIP-B*, which contains a
 1,662 bp open reading frame encoding a 553-amino acid protein (molecular weight: 61.08 kDa;

theoretical pI: 5.49). BLAST analysis of DELLA proteins from ryegrass, cotton, and *Amomum villosum* confirmed their identity as mango DELLA homologs (Fig. 5A). The DELLA protein is rich in Ala, Leu, Ser, Glu, and Gly, with 68 acidic (Asp + Glu) and 51 basic (Arg + Lys) residues (Fig. 5B). Secondary structure prediction (using DNAMAN) revealed 46.11% α -helices, 7.59% β -sheets, and 46.29% random coils (Fig. 5C). Hydrophobicity analysis identified Glu4 as the most hydrophilic residue (score: -3.75) and Ala511 as the most hydrophobic (score: 2.96) (Fig. 5D). Analysis using Phobius confirmed the absence of signal peptides and transmembrane domains, suggesting that DELLA is a non-cytoplasmic protein without membrane associations (Fig. 5E). Tertiary structure prediction showed a composition of α -helices, β -turns, and random coils, consistent with the secondary-structure results (Fig. 5F). Phylogenetic analysis revealed its closest homology to *Pistacia vera* DELLA (XP_031271042.1), followed by DELLA proteins from *Acer negundo* (KAI9170197.1), *Acer yangbiense* (TXG57155.1), *Dimocarpus longan* (AUF71968.1), *Litchi chinensis* (QAT94458.1), and *Citrus* spp. (XP_006448223.1, KAH9794669.1, KDO64603.1, XP_006469195.1, GAY58951.1) (Fig. 5G).

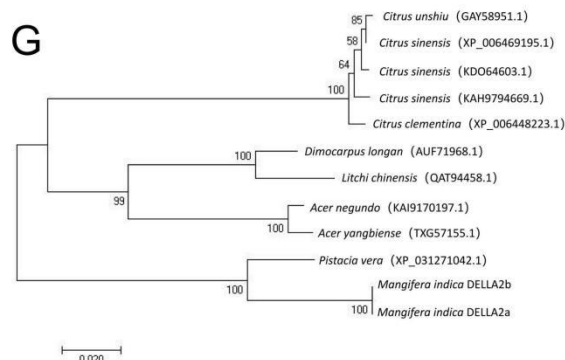
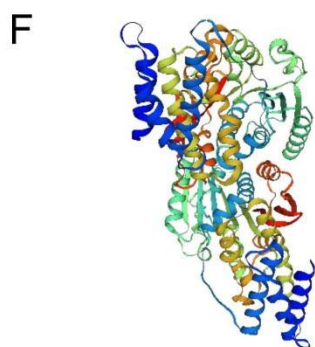
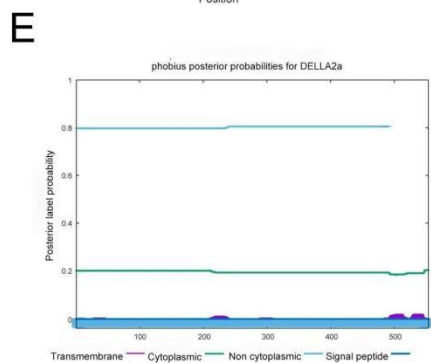
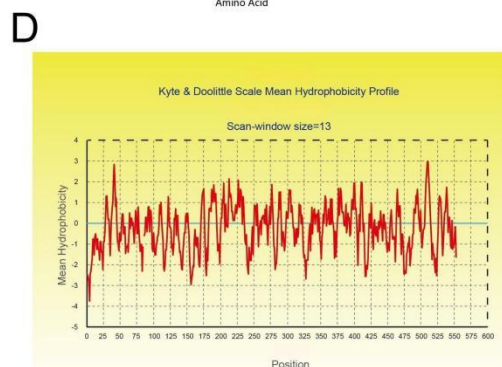
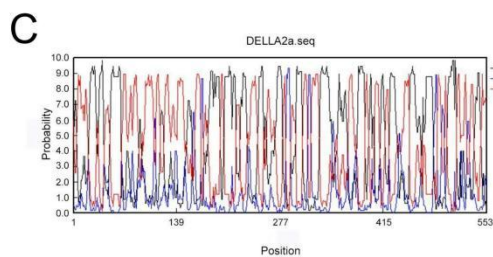
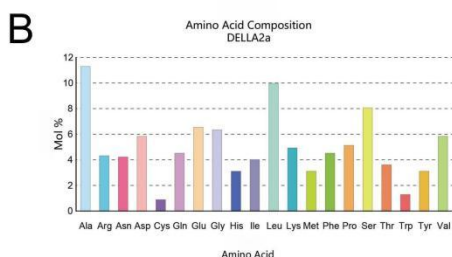
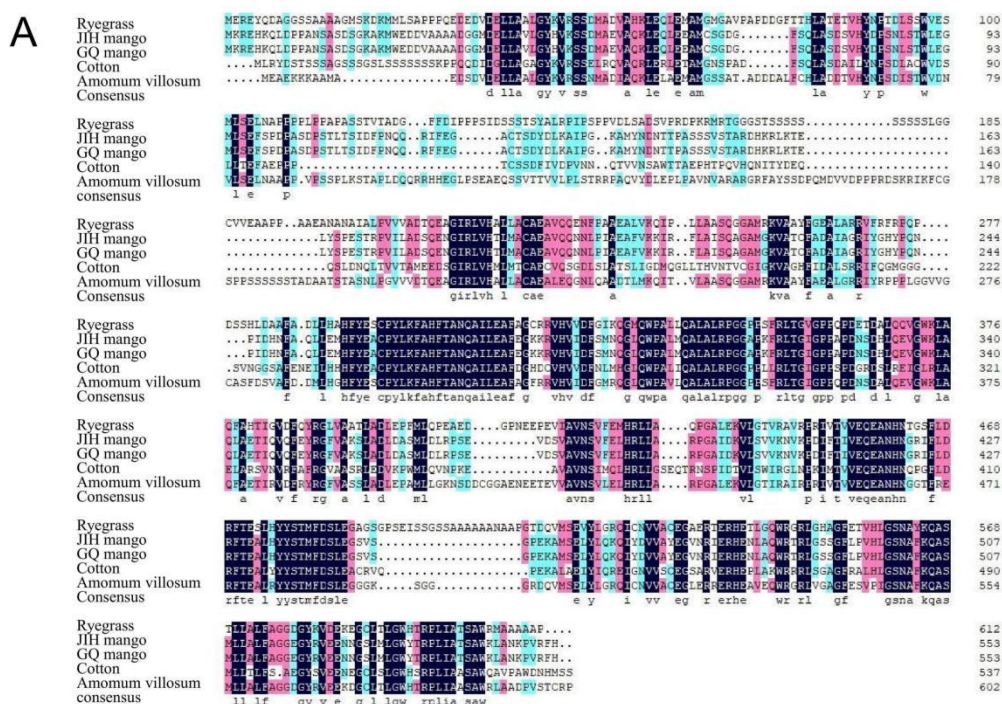


Figure 5 Molecular characterization of GAIP-B in mango. (A) BLAST analysis against DELLA proteins from ryegrass, cotton, and *Amomum villosum* confirmed its identity as a mango DELLA homolog; (B) Amino acid composition of DELLA; (C) Secondary structure prediction of DELLA, using DNAMAN; (D) Hydrophobicity analysis of DELLA; (E) Phobius-derived posterior probabilities for DELLA; (F) Tertiary structure prediction of DELLA; (G) Phylogenetic analysis of DELLA, revealing its relationships to homologous genes.

Gene editing of GAIP-B in tobacco altered its cold tolerance

The mango *GAIP-B* gene was successfully introduced into *Nicotiana benthamiana* via *Agrobacterium*-mediated transformation. qPCR analysis confirmed the genomic integration of the *GAIP-B* transgene in both overexpression and CRISPR/Cas9-edited lines (Supplementary Fig. 2). The target locus was successfully edited in three independent knockout lines (KO-1, KO-2, and KO-3), and the two overexpression lines (OE-1 and OE-2) exhibited strong transgene expression. Sequencing analysis confirmed the presence of frameshift mutations, resulting in premature stop codons, in all knockout lines.

Under normal growth conditions (25 °C), all transgenic and WT lines showed comparable morphological and growth characteristics (Fig. 6A). However, after 24 h of cold treatment at 7 °C, dramatic phenotypic differences emerged (Fig. 6B). The WT plants exhibited moderate wilting and slight chlorosis. The *GAIP-B* overexpression lines showed significantly enhanced cold tolerance, maintenance of turgor, and green coloration, with minimal visible damage. In contrast, the *GAIP-B*-KO lines exhibited severe cold sensitivity, with extensive wilting, necrosis, and the complete collapse of leaf structures in the most severely affected plants.

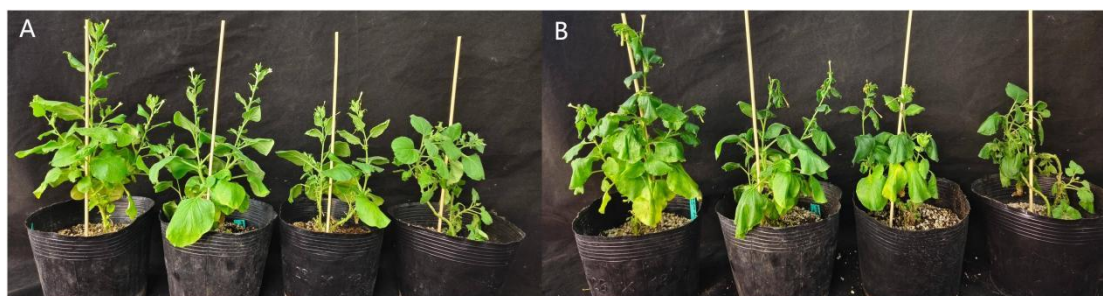


Figure 6 Photographs showing the morphology and growth characteristics of all transgenic and wild-type tobacco. (A) Normal growth conditions (25 °C). Left to right: Overexpression (OE), knockout (KO)–OE, control (CK), and KO; (B) 24 h cold treatment at 7 °C. From left to right, OE, KO–OE, CK, and KO.

The activities of the key antioxidant enzymes were significantly altered in the edited lines under cold stress (Fig. 7). The OE lines showed enhanced antioxidant capacity, with POD activity increasing to 10819.5 U/g, SOD activity to 2638.8 U/g, and CAT activity to 3.45 U/g ($p < 0.01$). Conversely, the KO lines showed significantly reduced enzyme activities: POD at 7709.1 U/mg (Fig. 7A), SOD at 1637.2 U/mg (Fig. 7B), and CAT at 2.51 U/g (Fig. 7C). Consistent with these enzymatic changes, the oxidative damage markers showed the opposite trend. In the OE lines, H₂O₂ content was maintained at 1.31 µmol/g, while in the KO lines it accumulated to 2.28 µmol/g (Fig. 7D). Similarly, MDA content, indicating membrane lipid peroxidation, was only 8.83 nmol/g in the OE lines but reached 15.33 nmol/g in the KO lines ($p < 0.01$) (Fig. 7E). Cold stress induced chlorophyll degradation in all lines, but to markedly different extents. The OE lines maintained a total chlorophyll content of 105.31 mg/g, whereas in the KO lines it declined to 86.15 mg/g (Fig. 7F). Total soluble carbohydrate accumulation under cold stress differed significantly between the lines, accumulating to 3.33 mg/g in the OE lines, but reaching only 2.33 mg/g in the KO lines ($p < 0.01$) (Fig. 7G).

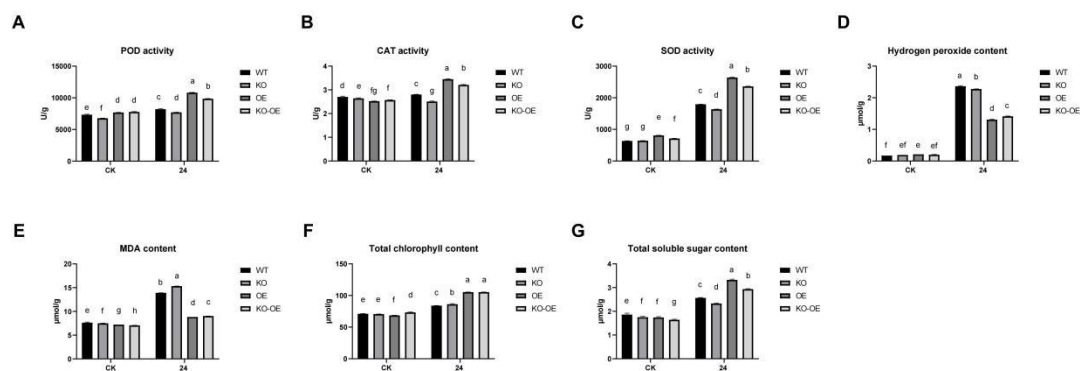


Figure 7 Physiological responses of the four tobacco lines (wild-type, WT; overexpression, OE; knockout, KO; and KO–OE) to cold stress. (A) Superoxide dismutase (POD) activity, (B) Catalase (CAT) activity, (C) Superoxide dismutase (SOD) activity, (D) H₂O₂ content, (E) Malondialdehyde (MDA)

content, (F) Photosynthetic pigments, and (G) Total soluble carbohydrate content. *** $p \leq 0.01$, * $p \leq 0.05$; ns, non-significant.

Discussion

This study clearly reveals the superior cold tolerance of the JH mango cultivar over the GQ cultivar. Exposure to cold stress induced pronounced physiological alterations in both cultivars, but the extent of these changes was markedly dependent on genotype. The initial line of defence against chilling-induced reactive oxygen species (ROS) is the antioxidant enzyme system [34]. While the activities of SOD, POD, and CAT were generally higher in the JH cultivar, significant divergence was only evident at specific time points (e.g., for CAT at 18 h). This suggests that the superiority of JH may lie not in the absolute maximum capacity of these enzymes, but in the kinetics and efficiency of their activation, preventing the early accumulation of ROS to a critical threshold. This was strongly evidenced by the drastically lower levels of H₂O₂ and MDA in the JH cultivar, indicating the successful containment of the oxidative burst and minimal membrane lipid peroxidation, a hallmark of cold stress injury [34, 35].

These physiological data were strongly supported by our transcriptome analysis. The RNA-Seq data reveal that the JH cultivar exhibited more rapid and pronounced upregulation of genes encoding key antioxidant enzymes, including glutathione S-transferases (*GST*) and ascorbate peroxidases (*Apx*), often within the first 6–12 h of cold stress. Early transcriptional reprogramming likely primes the antioxidant system for efficient ROS scavenging. Furthermore, the JH cultivar showed enhanced expression of transcription factors belonging to the DREB/CBF, MYB, and NAC families, which are master regulators of cold-responsive gene networks [36, 37]. This suggests that the JH cultivar possesses a more robust regulatory circuitry to initiate cold acclimation. The significantly higher accumulation of soluble sugars in the JH cultivar is a critical adaptive response. Sugars, such as sucrose and raffinose, act as osmoprotectants, stabilizing membrane and protein structures, and as energy sources for stress responses [38]. Our transcriptome data provides a mechanistic explanation: genes involved in starch degradation and sucrose biosynthesis such as *BAM1* (encoding β -amylases) [39], *mex1* (encoding maltose transporter) [40], *spsA* (encoding sucrose-phosphate synthases) were significantly more upregulated in the JH cultivar. This indicates that the JH cultivar efficiently redirects carbon resources towards the production of these protective compounds, a strategy less efficiently employed in the GQ

cultivar. The slower degradation of chlorophyll in JH was a consequence of reduced oxidative damage and improved energy status. Transcriptomic evidence has indicated lower expression of chlorophyll degradation genes (e.g., pheophorbide a oxygenase, PaO) and sustained expression of photosynthesis-associated genes in JH, consistent with its ability to maintain photosynthetic functions under stress [41]. The significant enrichment of GO terms related to protein kinase activity, transferase activity, protein phosphorylation, and ATP binding is highly indicative of the activation of a massive signaling cascade. This is a classic and crucial response to cold stress, because kinase-mediated protein phosphorylation is the primary mechanism for transducing cold signals and amplifying downstream adaptive responses [42]. The requirement for ATP-binding underscores the high-energy demand necessary to fuel these extensive biochemical adjustments, consistent with the observed metabolic shifts. Notably, KEGG pathway analysis identified several key metabolic pathways central to cold adaptation. Enrichment in the terpenoid backbone, ubiquinone, and diterpenoid biosynthesis was particularly significant. Terpenoids, including diterpenoids, play vital roles in membrane stabilization and as antioxidants, helping to maintain membrane fluidity and integrity under cold stress [43]. Ubiquinone (coenzyme Q) is essential for mitochondrial electron transport and serves as a lipid-soluble antioxidant that protects cells from oxidative damage [44]. The concurrent upregulation of these pathways in JH suggests a concerted effort to protect cellular structures against chilling-induced damage and enhance antioxidant capacity at the metabolic level.

This analysis identified many DEGs in the two cultivars. KEGG annotation identified a key pathway in response to low-temperature induction, namely the plant hormone signal transduction pathway, which was associated with marked upregulation of the LOC123214451 (DELLA protein GAIP-B-like) gene; this presents a compelling regulatory mechanism for the cold tolerance of JH. The upregulation of *GAIP-B* in JH suggests that this cultivar has an enhanced ability to initiate this protective response. DELLA proteins, a well-conserved subfamily of GRAS domain-containing transcriptional regulators, function as key negative regulators of the gibberellin (GA) signaling pathway [45]. In the presence of bioactive GA, GID1 receptors perceive hormones and undergo conformational changes that promote their interactions with DELLA proteins. This complex facilitates ubiquitination and subsequent proteasomal degradation of DELLA proteins via the SCF E3 ubiquitin ligase complex, thereby releasing their repression and

allowing GA-mediated growth responses (e.g., cell elongation and seed germination) to proceed [46]. However, under abiotic stress conditions, such as chilling, this degradation is inhibited. The consequent accumulation of DELLA proteins acts as a central switch, repressing energy-intensive growth processes and diverting cellular resources towards activation of a comprehensive stress defense program [47, 48]. Thus, we conclude that *GAIP-B* serves as a master regulator of cold tolerance in the cold-resistant mango cultivar.

Combined with functional validation in *Nicotiana benthamiana*, this study provides compelling evidence that GAIP-B functions as an evolutionarily conserved regulator of cold tolerance in plants. This demonstrates that genetic manipulation of *GAIP-B* expression in *Nicotiana benthamiana* directly alters cold tolerance by modulating antioxidant defenses, membrane stability, and osmotic adjustment mechanisms. Here, CRISPR/Cas9-mediated knockout of *GAIP-B* abolished cold tolerance, with the mutant plants exhibiting reduced antioxidant enzyme activity; this reduction was directly correlated with the massive accumulation of ROS, as evidenced by the increase in H₂O₂ content. In *Arabidopsis*, accumulated DELLAs proteins stabilize the ICE1 transcription factor, a key regulator of the C-repeat binding factor (CBF) pathway, which in turn upregulates a suite of cold-responsive genes, including those encoding SOD, POD, and CAT [49]. By restraining growth, DELLA proteins help to conserve carbon and energy; further, they contribute to the protection of the photosynthetic apparatus under stress. The observed slower degradation of chlorophyll in JH could be a direct result of DELLA activity, which may suppress the expression of chlorophyll catabolic genes (e.g., PAO and SGR) and support the maintenance of photosynthetic capacity during stress, ensuring a continued energy supply for defense reactions [50].

Conversely, *GAIP-B* overexpression conferred enhanced cold tolerance via multiple protective mechanisms. The significant enhancement of antioxidant enzyme activities in the OE lines effectively contained oxidative damage, maintaining H₂O₂ and MDA levels, respectively. This robust antioxidant capacity likely contributes to the remarkable preservation of photosynthetic pigments, with the OE lines maintaining higher chlorophyll content under cold stress than the WT plants. The importance of DELLA-mediated antioxidant defenses has been demonstrated in various plant species under abiotic stress [51]. Here, notably, *GAIP-B* overexpression dramatically enhanced the osmotic adjustment capacity, with

soluble sugar accumulation reaching higher levels than in the wild type, consistent with previous findings that DELLA proteins regulate carbohydrate metabolism and allocation under stress conditions [52]. The accumulated compatible solutes likely contribute to membrane stabilization and maintenance of cellular homeostasis, as evidenced by the reduced MDA levels in the OE lines.

Our findings are particularly significant in the context of crosstalk between plant hormone signaling pathways. DELLA proteins are known to integrate signals from multiple hormone pathways, including gibberellin, abscisic acid, and jasmonic acid signaling [45, 53]. Enhanced cold tolerance in the *GAIP-B*-OE lines may involve coordination of these hormonal pathways to activate comprehensive defense responses. This integrative function positions GAIP-B as a central regulator of the cold-stress signaling network. In conclusion, our results establish that GAIP-B is both necessary and sufficient for cold tolerance in plants. Through the coordinated regulation of antioxidant defense, photosynthetic protection, and osmotic adjustment, GAIP-B orchestrates a comprehensive cold adaptation strategy. These findings provide valuable insights into molecular breeding approaches aimed at enhancing cold tolerance in economically important crops through the manipulation of DELLA protein function.

Conclusions

In conclusion, these transcriptomic findings provide profound molecular insights into the sophisticated defense mechanisms underlying cold tolerance in the JH mango cultivar. Our analysis of differential gene expression not only identified unique cold stress-responsive genes in the tolerant cultivar but also highlighted a shared core set of stress-related genes, suggesting a conserved basal response that is further refined and enhanced in the JH cultivar. Our study provides compelling evidence that GAIP-B functions as a master regulator of cold tolerance in plants through its multifaceted role in coordinating the physiological and molecular responses to low-temperature stress. Genetic manipulation of GAIP-B expression in *Nicotiana benthamiana* demonstrated that this gene is both necessary and sufficient for cold adaptation, with the KO lines exhibiting extreme cold sensitivity and the OE lines showing significantly enhanced cold tolerance. Thus, GAIP-B represents a promising target for genetic engineering and marker-assisted selection programs aimed at enhancing crop resilience to low-temperature stress. Future research should focus on identifying downstream targets of GAIP-B and

exploring its interactions with other stress-responsive pathways to develop comprehensive strategies for crop improvement under changing climatic conditions.

Supplementary Information

The online version contains supplementary material available at
Figure S1 The qRT-PCR detection of *GAIP-B* gene expression at the JH mango and GQ mango for 24 h cold treatment
Figure S2 The qPCR analysis using gene-specific primers confirmed the genomic integration of the *GAIP-B* transgene in both overexpression and CRISPR/Cas9-edited lines.
Table S1 The primers sequences used in this study
Tables S2 The raw RNA sequencing data

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Not applicable

Authors' Contributions

Conceived and designed for study: Y.Z. and Z.Z.; Experimental work: Y.Z., M.L.; Collected data and literature: Y.Z., Y.T.; Drafted the manuscript: Y.Z.; Corrected the manuscript: Y.Z., Z.Z., and J.Z.; Project fund management: Y.Z. All authors have read and agreed to the published version of the manuscript. All authors read and approved the final manuscript.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare no conflict of interest.

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529 **List of abbreviations**

530 ERFs: Ethylene-responsive transcription factor

531 CNGC: Cyclic nucleotide-gated ion channel

532 POD: Peroxidase

533 CAT: Catalase

534 SOD: Superoxide dismutase

535 MDA: Malondialdehyde

536 H₂O₂ :Hydrogen peroxide
 537 GO: Gene Ontology
 538 FDR: False discovery rate
 539 OE: Overexpression
 540 KO: Knockout
 541 DEGs: Differentially expressed genes

542 Reference

- 543 1. Tharanathan RN, Yashoda HM, Prabha TN: **Mango (*Mangifera indica* L.), “The**
 544 **King of Fruits”—An Overview.** *Food Reviews International* 2006, **22**(2):95-123.
- 545 2. Ma X, Wu H, Liu B, Wang S, Zhang Y, Su M, Zheng B, Pan H, Du B, Wang J *et al*:
 546 **Genomic diversity, population structure, and genome-wide association reveal**
 547 **genetic differentiation and trait improvements in mango.** *Horticulture Research*
 548 2024, **11**(7):uhae153.
- 549 3. Wang JH, Feng X, Ashraf MA, Li Y, Kong Y, Cai Q, Xian S, Yin H, Yu NT:
 550 **Identification of common and specific cold resistance pathways from cold tolerant**
 551 **and non-cold tolerant mango varieties.** *PeerJ* 2024.
- 552 4. Abdel-Aziz HF, Hamdy AE, Sharaf A, Abd El-wahed AE-wN, Elnaggar IA, Seleiman
 553 MF, Omar M, Al-Saif AM, Shahid MA, Sharaf M: **Effects of fogging system and**
 554 **nitric oxide on growth and yield of ‘Naomi’ mango trees exposed to frost stress.**
 555 *Life* 2023, **13**(6):1359.
- 556 5. Sukhvibul N, Whiley AW, Smith M, Hetherington SE: **Susceptibility of mango**
 557 **(*Mangifera indica* L.) to cold-induced photoinhibition and recovery at different**
 558 **temperatures.** *Crop & Pasture Science* 2000, **51**:503-513.
- 559 6. Litz RE: **The Mango: Botany, Production and Uses.** 1997.
- 560 7. Kong Y, Hou X, Liu Z, Li Y: **Cold-stress induced metabolomic and transcriptomic**
 561 **changes in leaves of three mango varieties with different cold tolerance.** *BMC Plant*
 562 *Biology* 2024, **24**(1):266.
- 563 8. do Amaral MN, Arge LWP, Benitez LC, Danielowski R, Silveira SFdS, Farias DdR, de
 564 Oliveira AC, da Maia LC, Braga EJB: **Comparative transcriptomics of rice plants**
 565 **under cold, iron, and salt stresses.** *Functional & Integrative Genomics* 2016,
 566 **16**(5):567-579.
- 567 9. Imadi SR, Kazi AG, Ahanger MA, Gucl S, Ahmad P: **Plant transcriptomics and**
 568 **responses to environmental stress: an overview.** *Journal of Genetics* 2015.
- 569 10. Yang Q-S, Gao J, He W-D, Dou T-X, Ding L-J, Wu J-H, Li C-Y, Peng X-X, Zhang S,
 570 Yi G-J: **Comparative transcriptomics analysis reveals difference of key gene**
 571 **expression between banana and plantain in response to cold stress.** *BMC Genomics*
 572 2015, **16**(1):446.

- 573 11. Zhang H, Shen J, Wei Y, Chen H: **Transcriptome profiling of litchi leaves in response**
574 **to low temperature reveals candidate regulatory genes and key metabolic events**
575 **during floral induction.** *BMC Genomics* 2017, **18**(1):363.
- 576 12. Yow AG, Laosuntisuk K, Young RA, Doherty CJ, Gillitt N, Perkins-Veazie P, Jenny
577 Xiang Q-Y, Iorizzo M: **Comparative transcriptome analysis reveals candidate**
578 **genes for cold stress response and early flowering in pineapple.** *Scientific Reports*
579 2023, **13**(1):18890.
- 580 13. Zhang Y, Li Y, Yang J, Yang X, Chen S, Xie Z, Zhang M, Huang Y, Zhang J, Huang X:
581 **Genome-Wide Analysis and Expression of Cyclic Nucleotide–Gated Ion Channel**
582 **(CNGC) Family Genes under Cold Stress in Mango (*Mangifera indica*).** *Plants*
583 (2223-7747) 2023, **12**(3).
- 584 14. Chen QB: **Perspectives on the mango industry in mainland China.** In: 2010. 25-36.
- 585 15. Sasaki K, Imai R: **Mechanisms of cold-induced immunity in plants.** *Physiologia*
586 *Plantarum* 2023, **175**(1):e13846.
- 587 16. Sudheeran PK, Feygenberg O, Maurer D, Alkan N: **Improved Cold Tolerance of**
588 **Mango Fruit with Enhanced Anthocyanin and Flavonoid Contents.** In: *Molecules.*
589 vol. 23; 2018.
- 590 17. Yamanaka S, Hosaka F, Matsumura M, Onoue-Makishi Y, Nashima K, Urasaki N,
591 Ogata T, Shoda M, Yamamoto T: **Genetic diversity and relatedness of mango**
592 **cultivars assessed by SSR markers.** *Breeding science* 2019, **69**(2):332-344.
- 593 18. Gafni I, Rai AC, Halon E, Zviran T, Sisai I, Samach A, Irihimovitch V: **Expression**
594 **Profiling of Four Mango FT/TFL1-Encoding Genes under Different Fruit Load**
595 **Conditions, and Their Involvement in Flowering Regulation.** In: *Plants.* vol. 11;
596 2022.
- 597 19. Hodges DM, DeLong JM, Forney CF, Prange RK: **Improving the thiobarbituric**
598 **acid-reactive-substances assay for estimating lipid peroxidation in plant tissues**
599 **containing anthocyanin and other interfering compounds.** *Planta* 1999,
600 **207**(4):604-611.
- 601 20. Kumari S, Singh AK, Kumar A, Singh KP, Bains G: **Evaluating the efficacy of**
602 **chitosan and salicylic acid on photosynthetic pigments and antioxidant enzymes**
603 **towards resistance of mango malformation.** *Scientia Horticulturae* 2021,
604 **285**:110160.
- 605 21. Chen S, Zhou Y, Chen Y, Gu J: **fastp: an ultra-fast all-in-one FASTQ preprocessor.**
606 *Bioinformatics* 2018, **34**(17):i884-i890.
- 607 22. Kim D, Langmead B, Salzberg SL: **HISAT: a fast spliced aligner with low memory**
608 **requirements.** *Nature Methods* 2015, **12**(4):357-360.
- 609 23. Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov
610 JP: **Integrative genomics viewer.** *Nature Biotechnology* 2011, **29**(1):24-26.
- 611 24. Pertea M, Pertea GM, Antonescu CM, Chang T-C, Mendell JT, Salzberg SL: **StringTie**
612 **enables improved reconstruction of a transcriptome from RNA-seq reads.** *Nature*
613 *Biotechnology* 2015, **33**(3):290-295.
- 614 25. Pertea G, Pertea M: **GFF Utilities: GffRead and GffCompare.** *F1000 Research* 2020,
615 **9**:304.

- 616 26. Buchfink B, Reuter K, Drost HG: **Sensitive protein alignments at tree-of-life scale**
617 **using DIAMOND**. *Nature Methods* 2021, **18**:366-368.
- 618 27. Zheng Y, Jiao C, Sun H, Rosli HG, Pombo MA, Zhang P, Banf M, Dai X, Martin GB,
619 Giovannoni JJ *et al*: **iTAK: A Program for Genome-wide Prediction**
620 **and Classification of Plant Transcription Factors, Transcriptional Regulators,**
621 **and Protein Kinases**. *Molecular plant* 2016, **9**(12):1667-1670.
- 622 28. Diego Mauricio Ro-P, Ruzicic S, Dreyer I, Mueller-Roeber B: **PlnTFDB: an**
623 **integrative plant transcription factor database**. *Bmc Bioinformatics* 2007, **8**(1):42.
- 624 29. Jin J, Tian F, Yang D-C, Meng Y-Q, Kong L, Luo J, Gao G: **PlantTFDB 4.0: toward**
625 **a central hub for transcription factors and regulatory interactions in plants**.
626 *Nucleic acids research* 2017, **45**(D1):D1040-D1045.
- 627 30. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL,
628 Wold BJ, Pachter L: **Transcript assembly and quantification by RNA-Seq reveals**
629 **unannotated transcripts and isoform switching during cell differentiation**. *Nature*
630 *Biotechnology* 2010, **28**(5):511-515.
- 631 31. Love MI, Huber W, Anders S: **Moderated estimation of fold change and dispersion**
632 **for RNA-seq data with DESeq2**. *Genome Biology* 2014, **15**(12):550.
- 633 32. Ma X, Zhang Q, Zhu Q, Liu W, Chen Y, Qiu R, Wang B, Yang Z, Li H, Lin Y *et al*: **A**
634 **Robust CRISPR/Cas9 System for Convenient, High-Efficiency Multiplex Genome**
635 **Editing in Monocot and Dicot Plants**. *Molecular plant* 2015, **8**(8):1274-1284.
- 636 33. Li J-F, Norville JE, Aach J, McCormack M, Zhang D, Bush J, Church GM, Sheen J:
637 **Multiplex and homologous recombination-mediated genome editing in**
638 **Arabidopsis and Nicotiana benthamiana using guide RNA and Cas9**. *Nature*
639 *Biotechnology* 2013, **31**(8):688-691.
- 640 34. Mittler R, Vanderauwera S, Gollery M, Van Breusegem F: **Reactive oxygen gene**
641 **network of plants**. *Trends in Plant Science* 2004, **9**(10):490-498.
- 642 35. Gill SS, Tuteja N: **Reactive oxygen species and antioxidant machinery in abiotic**
643 **stress tolerance in crop plants**. *Plant Physiology and Biochemistry* 2010, **48**(12):909-
644 930.
- 645 36. Erpen L, Devi HS, Grosser JW, Dutt M: **Potential use of the DREB/ERF, MYB, NAC**
646 **and WRKY transcription factors to improve abiotic and biotic stress in transgenic**
647 **plants**. *Plant Cell, Tissue and Organ Culture (PCTOC)* 2018, **132**(1):1-25.
- 648 37. Zhao Q, Han R, Cai K, Yan H, Li Y, Qu G, Liu L, Zhao X: **Identification and Analysis**
649 **of the CBF Gene Family in Three Species of Acer under Cold Stress**. In:
650 *International Journal of Molecular Sciences*. vol. 24; 2023.
- 651 38. Krasensky J, Jonak C: **Drought, salt, and temperature stress-induced metabolic**
652 **rearrangements and regulatory networks**. *Journal of Experimental Botany* 2012,
653 **63**(4):1593-1608.
- 654 39. Monroe JD: **Involvement of five catalytically active Arabidopsis β -amylases in leaf**
655 **starch metabolism and plant growth**. *Plant Direct* 2020, **4**(2):e00199.
- 656 40. Ryoo N, Eom J-S, Kim H-B, Vo BT, Lee S-W, Hahn T-R, Jeon J-S: **Expression and**
657 **functional analysis of rice plastidic maltose transporter, OsMEX1**. *Journal of the*
658 *Korean Society for Applied Biological Chemistry* 2013, **56**(2):149-155.

- 659 41. Pružinská A, Tanner G, Aubry S, Anders I, Moser S, Müller T, Ongania K-H, Kräutler
660 B, Youn J-Y, Liljegren SJ *et al*: **Chlorophyll Breakdown in Senescent Arabidopsis**
661 **Leaves. Characterization of Chlorophyll Catabolites and of Chlorophyll**
662 **Catabolic Enzymes Involved in the Degreening Reaction.** *Plant Physiology* 2005,
663 **139(1):52-63.**
- 664 42. Meng X, Zhang S: **MAPK Cascades in Plant Disease Resistance Signaling.** *Annual*
665 *Review of Phytopathology* 2013, **51(1):245-266.**
- 666 43. Loreto F, Schnitzler J-P: **Abiotic stresses and induced BVOCs.** *Trends in Plant*
667 *Science* 2010, **15(3):154-166.**
- 668 44. Nowicka B, Kruk J: **Occurrence, biosynthesis and function of isoprenoid quinones.**
669 *Biochimica et Biophysica Acta (BBA) - Bioenergetics* 2010, **1797(9):1587-1605.**
- 670 45. Davière J-M, Achard P: **Gibberellin signaling in plants.** *Development* 2013,
671 **140(6):1147-1151.**
- 672 46. Murase K, Hirano Y, Sun T-p, Hakoshima T: **Gibberellin-induced DELLA**
673 **recognition by the gibberellin receptor GID1.** *Nature* 2008, **456(7221):459-463.**
- 674 47. Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, Van Der Straeten
675 D, Peng J, Harberd NP: **Integration of Plant Responses to Environmentally**
676 **Activated Phytohormonal Signals.** *Science* 2006, **311(5757):91-94.**
- 677 48. Achard P, Genschik P: **Releasing the brakes of plant growth: how GAs shutdown**
678 **DELLA proteins.** *Journal of Experimental Botany* 2009, **60(4):1085-1092.**
- 679 49. Li H, Ye K, Shi Y, Cheng J, Zhang X, Yang S: **BZR1 Positively Regulates Freezing**
680 **Tolerance via CBF-Dependent and CBF-Independent Pathways in**
681 **Arabidopsis.** *Molecular plant* 2017, **10(4):545-559.**
- 682 50. Wang W, Wei X, Jiao G, Chen W, Wu Y, Sheng Z, Hu S, Xie L, Wang J, Tang S *et al*:
683 **GBSS-BINDING PROTEIN, encoding a CBM48 domain-containing protein,**
684 **affects rice quality and yield.** *Journal of Integrative Plant Biology* 2020, **62(7):948-**
685 **966.**
- 686 51. Colebrook EH, Thomas SG, Phillips AL, Hedden P: **The role of gibberellin signalling**
687 **in plant responses to abiotic stress.** *Journal of Experimental Biology* 2014, **217(1):67-**
688 **75.**
- 689 52. Yu S, Cao L, Zhou C-M, Zhang T-Q, Lian H, Sun Y, Wu J, Huang J, Wang G, Wang J-
690 W: **Sugar is an endogenous cue for juvenile-to-adult phase transition in plants.**
691 *eLife* 2013, **2:e00269.**
- 692 53. la Rosa NM-d, Sotillo B, Miskolczi P, Gibbs DJ, Vicente J, Carbonero P, Oñate-
693 Sánchez L, Holdsworth MJ, Bhalerao R, Alabadí D *et al*: **Large-Scale Identification**
694 **of Gibberellin-Related Transcription Factors Defines Group VII ETHYLENE**
695 **RESPONSE FACTORS as Functional DELLA Partners** *Plant Physiology*
696 2014, **166(2):1022-1032.**
- 697

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