scientific reports

Check for updates

OPEN

Metabolic profiling and gene expression analyses shed light on the cold adaptation mechanisms of *Saposhnikovia divaricata* (Turcz.) Schischk

Ming Jiang, Yue Pan, Kanchao Yu, Yanshi Ma, Ying Cui, Yang Liu, Jicheng Liu, Keyong Zhang & Hui Li✉

The northeastern region of China experiences a distinctly cold climate influenced by the Siberian High during the winter months, thus resulting in severe cold weather conditions. Snow cover is prevalent and can persist for several months. This prolonged exposure to low temperatures necessitates specific adaptations in terms of agriculture and plant life, particularly for perennial herbs. *Saposhnikovia divaricate* (Turcz.) Schischk (SD) is a widely distributed perennial herb in the northeastern and northern provinces of China. However, there is limited documentation on the molecular mechanism through which this plant adapts to cold stress. Therefore, we elucidated the SD response to cold stress by transcriptome and metabolome analysis. Cold stress induced chlorosis and wilting in plants, thus leading to added function of antioxidant enzymes and higher levels of malondialdehyde, proline, soluble sugars. Notably, the differentially expressed genes (DEGs) were primarily related with sugar metabolism, ROS sweep, flavonoid and terpenoid biosynthesis, plant hormone signalling pathways, lipid metabolism, and transcription factors. Additionally, the differentially expressed metabolites (DEMs) mainly included lipids, flavonoids, terpenoid compounds, sugar-related metabolites, alkaloids and other metabolites. Furthermore, integrated analysis revealed coexpression patterns between carbohydrate metabolism-related genes and genes reference flavonoid and terpenoid biosynthesis, along with their corresponding metabolites. Finally, the qPCR results revealed notable over-expression levels of stress-related genes, including those participated in plant hormone signalling pathways (*PP2C* and *AUX*), flavonoid biosynthesis (*CH3*), antioxidant enzymes (*AOX* and *CAT*), and sugar-related metabolite metabolism (*TPS*, *SPS*, and *SS*). In conclusion, our findings suggest that cold stress strongly affects plant hormone signalling pathways, ROS scavenging mechanisms, unsaturated fatty acid synthesis and flavonoid and terpenoid biosynthesis in SD. These discoveries provide valuable insights into the impact of cold climates on herbaceous plants.

Keywords Cold stress, Medicinal plant, Metaboliltes

Northeast China encompasses a land area of 1.45 million square kilometres and is characterized by long winters, thus making it the coldest environment in China[1]. The markedly low temperatures, coupled with substantial snowfall and ice formation, present significant constraints on agricultural growing seasons[2]. The average annual air temperatures in the three observed provinces range from − 2 to + 3 °C, during winter daytime hours they can plummet as far as − 34 to − 30 °C. In most parts of Northeast China, the frost-free season does not exceed 160 days[3]. Multiple studies have demonstrated that cold stress poses diverse challenges to plants such as ion imbalances, osmotic stress, oxidative damage, compromised photosynthetic efficiency and cellular membrane rigidity along with disruptions in metabolic processes [4,5]. Therefore, developing resistance to low temperatures is a crucial mechanism for plants' ability to withstand cold conditions especially among perennial herbs [6,7].

Plants are particularly susceptible to significant temperature fluctuations due to large-scale atmospheric variations and the occurrence of extreme weather events[8]. For responding to negative abiotic-stress, sessile

Qiqihar Medical University, No.333, Bukui Street, Jianhua District, Qiqihar 161006, Heilongjiang Province, People's Republic of China. ✉email: lihui314220@126.com

plants need to perceive stress signals and respond to the generation of harmful reactive oxygen species (ROS). For counteracting detrimental results caused by excessive ROS, such as membrane rigidity damage, protein inactivation, and DNA breakage, plants have evolved various adaptive strategies that involve enzymatic scavengers (such as ascorbate peroxidase [APX], peroxidase [POD], superoxide dismutase [SOD], and catalase [CAT]), as same as nonenzymatic components, including proline, sugars, amino acids, and secondary metabolites[9]. The transcription factor *PtrbHLH* has been demonstrated to combine with the E-box motif belonging to the promoter region of *POD* gene sequence and activate POD activity for H_2O_2 scavenging. This activation enhances cold tolerance under freezing or chilling temperatures in tobacco and lemon plants[10]. The heterologous expression research of *StCBF1* and *StCBF4* enhanced cold tolerance in Arabidopsis to varying degrees. Furthermore, increased function of antioxidant enzymes was observed along with reduced ROS accumulation[11]. Soluble sugars (SSs) play dual roles as signalling molecules regulating various stress-related genes took part in sucrose metabolism, photosynthesis pathway and osmolyte biosynthesis under abiotic stress conditions[12]. Flavonoids and terpenoids are vital secondary metabolites that are known for their significant ROS scavenging activities. They have been directly associated with coping mechanisms against adverse climatic stresses[13,14]. Additionally, the abscisic acid (ABA)-dependent pathway has been shown to participate in plant regulation during plant responses. The transcript level analysis of the NAC transcription factor (*OsNAP*) showed that it was significantly reduced by ABA and abiotic stresses, and the enhanced expression resulted in notably increment resistance to drought, low temperature and high salinity based on an ABA-mediated network[15].

Saposhnikovia divaricate (Turcz.) Schischk (SD) is a perennial medicinal plant attached to the Apiaceae family that is extensively found in the northeastern and northern provinces of China due to its significant medicinal, nutritional, and economic value. It has a substantial domestic and international market and has served as a traditional Chinese medicine for several years[16]. SD is recognized as a top-grade medicinal plant by the Chinese Pharmacopoeia Commission and is commonly employed for treating rheumatism, stroke, fever, cold and arthralgia[17]. Extensive research on the roots and leaves of SD has identified numerous bioactive components, including polysaccharides, flavonoids, coumarins, volatile oils, lignins, and organic acids. These compounds have demonstrated antipyretic properties along with anti-inflammatory effects against hepatitis detoxification processes. These compounds also exhibit antiallergic activity while being effective against influenza-induced inflammation according to recent pharmacological investigations[18–24]. The literature suggests that genetic background and environmental factors significantly influence the presence of bioactive compounds. Therefore, it has become crucial (yet difficult) to elucidate the response mechanisms of key metabolites under different climatic conditions.

Currently, high-throughput omics application, for example genomics, metabolomics, transcriptomics, and proteomics, are effectively utilized to investigate the response mechanisms of plants under various abiotic stresses and elucidate crucial patterns of gene expression and metabolite accumulation[25,26]. Recently, RNA-seq has been utilized to dissect the mechanisms underlying low-temperature tolerance by examining gene expression and the regulation of target metabolites. Dynamic profiling of the transcriptome has identified hub-genes and pathways involved in low temperature stress in castor[27], faba bean[28], and Fragaria nilgerrensis[29]. Furthermore, studies have investigated complex mechanisms involving gene coexpression network analysis and the detection of stress tolerance genes linked to candidate metabolites involved in salt, water/drought, and heat responses[30–33]. Overall, integrated research combining transcriptomic and metabolomic approaches has made substantial progress in enhancing our understanding of intricate regulatory networks operating under stressful conditions. However, there is a need of comprehensive comparative studies on the molecular mechanisms involved in SD during cold stress. Therefore, an integrated assay encompassing transcriptome and metabolome analyses should be considered a highly effective approach for elucidating pivotal genes and metabolites involved in responsive molecular mechanisms under cold stress.

In this research, transcriptomic and metabolomic analyses of SD have been performed to determine the response mechanisms of gene expression and biosynthesis of key bioactive metabolites under different cold stress treatments. Integrated data analysis of the transcriptome and metabolome revealed significant upregulation or downregulation of numerous genes and metabolites. Notably, SS has emerged as one of the most significantly altered bioactive compounds. Intriguingly, compared with short-term stress, long-term cold stress treatment resulted in greater increases in the SS content. Furthermore, certain transcription factors, such as *MYB,* may play an important role in providing cold tolerance. These findings provide a comprehensive understanding of how genes and substances respond to cold stress in SD, thereby contributing valuable insights for plantation practices and breeding programs targeting highly commercial metabolite accessions.

Materials and methods

Plant sample gathering and cold stress treatment

Saposhnikovia divaricata (Turcz.) Schischk. seeds were collected from Tahe County, which is located in the Greater Khingan Mountains region of China, and were identified by Ma Dezhi, deputy director of Qiqihar Medical University. The plant collection and use was in accordance with all the relevant guidelines, the plant samples were deposited in Qiqihar Medical University Chinese medicinal herbarium (No. QMU172024935). This region employ a cold mild continental monsoon climate characterized by a typical yearly temperature of 0 °C, a mean rainfall of 460 mm, and approximately 2600 h of annual sunshine. Professor Ma Wei from Heilongjiang University of Chinese Medicine verified the authenticity of the plant material. The seeds were cultivated in a controlled environment in a controlled setting at Qiqihar Medical University. Following germination, seeds were planted for 48 days under light–dark alternating cycles of 14 h at 24 °C and 10 h at 20 °C while maintaining 55% humidity and a light intensity of 12,000 lx. To induce cold stress, 48 d-old seedlings were subjected to 4 °C for different durations: 6 h, 12 h, 24 h, 48 h, and a final temperature of up to 72 h. Afterwards, the leaf samples were promptly excised, frozen in liquid nitrogen, then stored at -80 °C for later analyses. These samples served

as both transcriptome sequencing and LC–MS/MS analysis materials. Additionally, a control group consisting of seedlings that did not undergo any cold stress treatment was established. For each treatment condition, the biological replicates comprised three uniformly growing plant groups.

Physiological and biochemical features of SD under cold stress

The relative conductivity of the SD seedlings during cold stress was assessed following methods described in the previous literature[34]. The biochemical characteristics of the seedlings were checked by reagent kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The malondialdehyde (MDA) content was analyzed through a thiobarbituric acid assay (product no. A003-3-1). Phenylalanine ammonia lyase (PAL), which is a key enzyme in phenylpropanoid metabolism, was quantified by using spectrophotometry (product no. A137-1-1). The soluble sugar content was measured through anthrone colorimetry (product no. A145-1-1). Peroxidase (POD) activity was assessed by monitoring absorbance changes at 420 nm (product no. A084-3-1). Catalase (CAT) function was measured by utilizing the colorimetric method with ammonium molybdate (product no. A007-1-1). Proline (PRO) levels were assessed through a response contacted with ninhydrin (product no. A107-1-1). Total antioxidant capacity (T-AOC) was gauged by through a colorimetric method (Product No. A105-1). Superoxide dismutase (SOD) activity was analyzed by using a xanthine-xanthine oxidase-nitro blue tetrazolium assay (product no. A001-1-2). All of the experiments were conducted in triplicate to ensure reliability.

Untargeted metabolic profiling

For metabolite analysis, samples from the cold stress groups (Cold6h and Cold48h) and the control group (CK) were prepared. Metabolite extraction, as well as qualitative and quantitative analyses, were conducted following previously established methods[35]. The plant samples were freeze-dried, pulverized into a fine powder, and then dissolved in aqueous methanol (70%) at a low temperature (4 °C) overnight. The resulting solutions were analysed by using ultrahigh-performance LC–MS/MS method with a Vanquish UHPLC system coupled to an Orbitrap Q Exactive™ HF mass spectrometer. The metabolites were subjected to orthographic projection of primary component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) for potential structure identification. Metabolites exhibiting a $|\log_2\text{fold change (FC)}| \geq 1$ and a variable importance in projection (VIP) ≥ 1 were identified as being differentially expressed metabolites (DEMs) between the groups (Cold6h vs. CK, Cold48h vs. CK). An enrichment analysis of these DEMs was subsequently performed by based on the KEGG database.

RNA extraction and Illumina sequencing

Transcriptome sequencing of SD seedling leaves was performed by Novogene Corporation, Inc. Total RNA was obtained based on the TRIzol procedure from samples collected at three time points, including 0 h (CK), 6 h (Cold6h), and 48 h (Cold48h), each under cold stress and with three biological replicates. The integrity, concentration, and purity of these RNA samples were rigorously verified to guarantee high quality. After data filtration, high-quality clean reads were obtained. Transcript assembly was conducted by using Trinity version 2.11.0. For differential expression analysis, DESeq2 software was used, and the screening criteria for DEGs were an absolute $|\log_2\text{fold change (FC)}| \geq 1$ and a false discovery rate (FDR) < 0.05.

WGCNA and gene network visualization

The weighted gene coexpression network analysis (WGCNA) method was utilized for constructing a coexpression network of the selected DEGs. The weighting coefficient β was determined based on the scale-free topology standard to maximize the correlation coefficient, which was then changed into a topological overlap matrix (TOM) according to β value. Subsequently, gene correlations were analysed, eigenvector genes for each module were calculated, and both total connectivity and intramodule connectivity were determined by using weighted correlation coefficients. Based on the clustering relationships among the different genes, DEGs were grouped into distinct modules, thus enabling investigation of the correlations between module eigengenes (MEs) and the physiological and biochemical features of SD at various time points under cold stress. The networks were visualized by using CYTOSCAPE software (v3.10.1, USA).

Quantitative real-time RT-PCR analysis

Quantitative real-time RT-PCR analysis (qRT-PCR) was utilized to verify the accuracy of the gene expression analysis results derived from the transcriptomic data. Total RNA was first isolated, followed by the preparation of cDNA. The qRT-PCR experiments were conducted through the BlazeTaq™ SYBR® Green qPCR Mix 2.0 Kit, utilizing GAPDH as the endogenous control gene. Relative expression degrees were estimated by using the $2^{-\Delta\Delta Ct}$ method, and every experiment included three technical replicates to ensure reliability.

Statistical analysis

The calculated informations are presented as the mean ± standard error of the mean (SEM). Statistical significance between different groups were assessed using Student's t test, with $^{*}P < 0.05$ and $^{**}P < 0.01$ demonstrating statistical significance. Correlation analyses were utilized by using the Pearson correlation coefficient, with screening criteria set at a correlation coefficient > 0.80 and a $P < 0.05$ for significance. DEGs and DEMs were annotated by using the KEGG database (www.kegg.jp/kegg/kegg1.html)[36]. Enrichment analysis was conducted with stringent filtering criteria, requiring a gene pathway $P < 0.01$ and a metabolic pathway $P < 0.05$ to decide significance.

Results

Morphological and physiological changes and enzyme activity of SD under cold stress

During cold stress, the leaves from the SD seedlings gradually wilt, and significant lodging of the plants is observed, as illustrated in Fig. 1A,B. To discover the physiological reactions of SD to cold stress, the biochemical experiments on samples subjected to both control and cold treatments at different time groups were conducted. Specifically, under stress, the MDA content increased significantly, showing a twofold increase at 12 h, a 2.33-fold increase at 48 h, and a 2.8-fold increase at 72 h, as indicated in Fig. 1C. Additionally, osmoregulatory substances such as proline, soluble sugar, and soluble protein also exhibited substantial increases. The proline levels rose markedly by 5.32-fold at 6 h and 3.66-fold at 48 h. The soluble sugar content reached its highest elevation at 72 h, with a 3.25-fold increase, as shown in Fig. 1D,E. Conversely, the soluble protein content initially dropped by 0.97-fold at 6 h but then rose significantly at 72 h, with a 1.8-fold increase (Fig. 1F).

Among the antioxidants, CAT, SOD, and APX all exhibited significant increases in activity during cold stress. Notably, CAT displayed the greatest increase, with a 1.59-fold change at 72 h (Fig. 1G). SOD showed the greatest increase at 6 and 48 h, thus demonstrating fold changes of 2.05 and 2.83, respectively (Fig. 1H). APX exhibited the most pronounced increase at 24 h, with a fold change of 2.67 (Fig. 1J). Conversely, PAL activity initially increased by a factor of 1.44 at six hours but subsequently decreased significantly to its maximum decrease of -0.53-fold at 72 h (Fig. 1I).

These findings collectively indicate a substantial accumulation in MDA content during cold environment, thus suggesting damage to the SD cell membrane structure along with severe lipid peroxidation within the membrane. Furthermore, there was a notable increase in the content of osmotic regulatory substances, as well as in the activity of antioxidant substances, which likely participated in the ability of SD to withstand cold stress.

Metabolomic response of SD under cold stress

To explore metabolic altering as a result of cold stress, we performed untargeted metabolomic profiling and compared differentially expressed metabolites (DEMs) between the Cold6h vs. CK, Cold48h vs. CK, and Cold48h vs. Cold6h groups. In total, 1396 metabolites were detected, containing fatty acids, amino acids and derivatives; lysolecithin (LPE, LPC, and LPI); alkaloids; organic acids; flavonoids; lignins and coumarins; terpenoids; phenolic acids; and several other compounds (Supplementary Table 1). PCA revealed significant changes among the different stress groups, with scores of 28.96% and 16.04%, respectively. Three distinct clusters were formed by three biological replicates within each group: CK, Cold6h, Cold48h, and QC (Fig. 2A). Venn diagrams illustrated the differences in DEMs among these groups. Esculetin and pimpinellin were shared among all three groups (Fig. 2B). By applying thresholds of $|\log_2$ Fold Change$| \geq 1$, VIP ≥ 1 and $(-\log_{10}$ P value$) \geq 1$, a total of 138 DEMs were identified in the comparison between Cold6h and CK, whereas 85 DEMs were identified in the comparison between Cold48h and CK (Fig. 2C,D). These metabolites were mainly classified into 10 categories: lipids, organic acids, amino acids and derivatives, lignin and coumarins, phenolic acids, alkaloids, nucleotides and derivatives, flavonoids, terpenoids and others. There were 86 DEMs upregulated and 52 DEMs downregulated in the Cold6h vs. CK group (Fig. 2C and Supplementary Table 2), whereas there were 59 DEMs

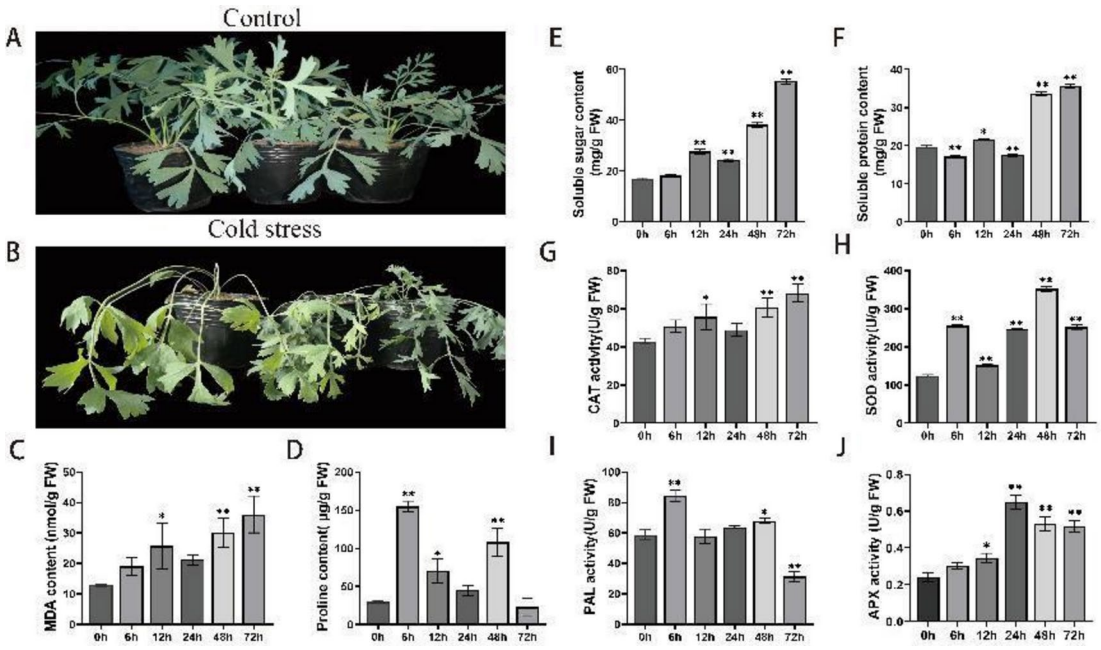

Fig. 1. *Saposhnikovia divaricata* (Turcz.) exhibits the potential for cold stress tolerance. (**A**) Phenotypes of SD cultured under normal conditions (control). (**B**) Phenotypes of SD cultured at 4 °C for 48 h to induce cold stress. (**C**–**J**) The physiological characteristics of SD under 4 °C treatment. The experiments were replicated thrice (*$P < 0.05$; **$P < 0.01$).

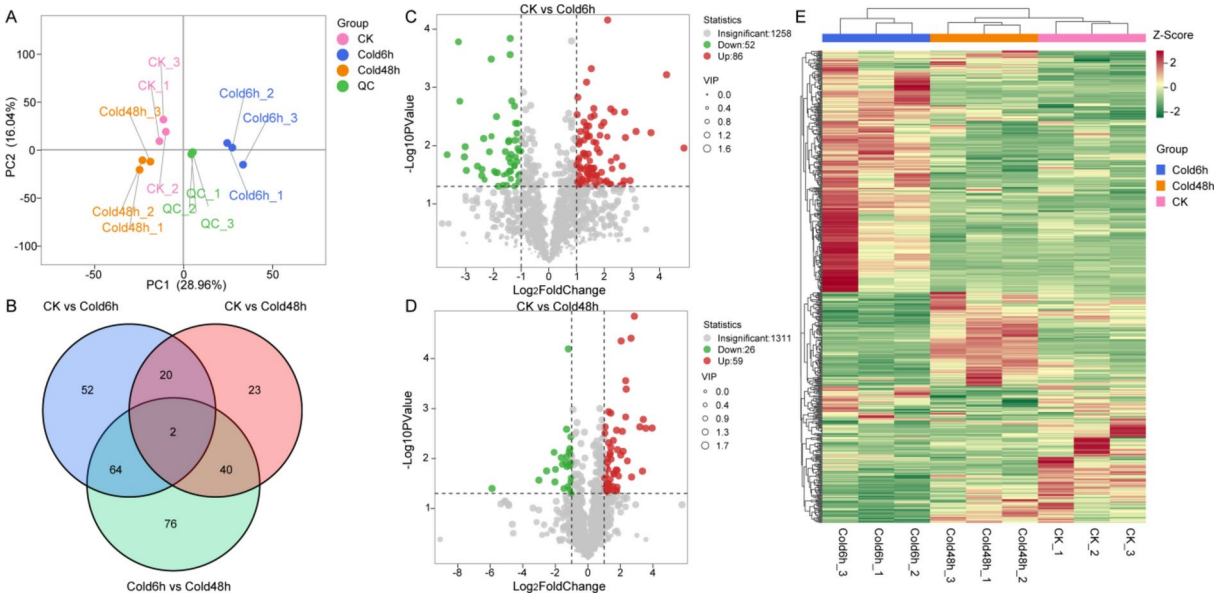

Fig. 2. Widely targeted metabolome result of SD exposed to cold stress. (**A**) Principal component analysis (PCA). CK is the control without cold treatment. QC indicates mixed samples. Cold6h and Cold48h are abbreviations for cold stress for 6 h and 48 h, respectively. (**B**) Venn diagram demonstrating crucial DEMs among various cohorts. (**C**) Volcano plot demonstrating the DEMs in the Cold6h vs. CK groups. (**D**) Volcano plot demonstrating the DEMs in the Cold48h vs. CK groups. (**E**) Hierarchical clustering analysis.

upregulated and 26 downregulated in the Cold48h vs. CK group (Fig. 2D and Supplementary Table 3). Notably, the upregulated metabolites were mainly saccharides, flavonoids and coumarins, thus implying that these compounds may facilitate the ability of SD to adapt to cold. Heatmap analysis revealed clear separation of these DEMs into three distinct groups, thus underscoring how cold stress significantly altered the metabolite profiles of SD (Fig. 2E).

To elucidate the functions of the differentially expressed metabolites, we enriched the KEGG pathways based on the enrichment factor, P value, and number of enriched genes. The results revealed that in the comparison between Cold6h and CK, DEMs were predominantly enriched in five pathways: starch and sucrose metabolism, galactose metabolism, phenylalanine metabolism, pyruvate metabolism, valine, leucine, and isoleucine biosynthesis (Fig. 3A). In the comparison between Cold48h and CK, DEMs were mainly accumulated in five pathways: galactose metabolism, glycolysis/gluconeogenesis, starch and sucrose metabolism, ABC transporters, and nicotinate and nicotinamide metabolism (Fig. 3B). In plants, the transcriptional coactivator MBF1c is closely related to heat-induced protein and trehalose biosynthesis (trehalose phosphate synthase 5) under heat stress[37]. Galactose metabolism is crucial for plant development, as are galactose biosynthesis, raffinose biosynthesis and lipid metabolism; moreover, it participates in salt, drought, osmotic, ABA and cold stress[38,39]. There is a significant correlation between amino acid metabolism and physiological reactions to abiotic stresses. Moreover, proline, glycine, leucine, and valine have been investigated in plants in reaction to abiotic stresses[40]. The top 20 DEMs, ordered by $|\log_2(\text{fold change})|$ in Cold6h vs. CK, revealed that 6-formyl-isoophiopogonanone A was the most markedly altered metabolite, with a $\log_2$(fold change) of 4.88 (Fig. 3C). In the Cold48h vs. CK comparison, ethyl-β-D-glucuronide was the most markedly altered metabolite, displaying a $\log_2$(fold change) of 3.95 (Fig. 3D). The results indicated that there was sufficient upregulation of saccharides, alkaloids, flavonoids and coumarins in SD under cold stress.

Transcriptomic response of SD under cold stress

The transcriptional pattern of SD plants exposed to low temperature was assessed by using a quantitative transcriptome sequencing strategy on the Illumina NovaSeq 6000 Platform, contained three independent replicates per group (CK, Cold6h, and Cold48h). After removing the raw data reads containing adapters, N bases, and low-quality sequences, we secured 20.37–24.72 million clean reads. Subsequent to quality assembly, we generated 6.1 GB of clean reads with an impressive Q30 percentage > 91.58%. Gene expression levels were normalized by using TMM technology, and differentially expressed genes were determined based on following testing parameters: |log2-fold change| > 1 and $P < 0.05$. A total of 6024 DEGs (4100 upregulated and 2104 downregulated) in Cold6h vs. CK and 13,155 DEGs (6932 upregulated and 6223 downregulated) in Cold48h vs. CK were identified (Fig. 4A,B). Notably, a Venn diagram depicted the presence of 1415 shared DEGs across the Cold6h vs. CK, Cold48h vs. CK, and Cold48h vs. Cold6h comparisons (Fig. 4C and Supplementary Table 4). These DEGs were verified to be correlated with sugar-related metabolite metabolism, ROS-scavenging and detoxification pathways, terpenoid biosynthesis and flavonoid biosynthesis pathways, plant hormone signal transduction, lipid metabolism, phenylpropanoid metabolism pathways (Supplementary Table 5).

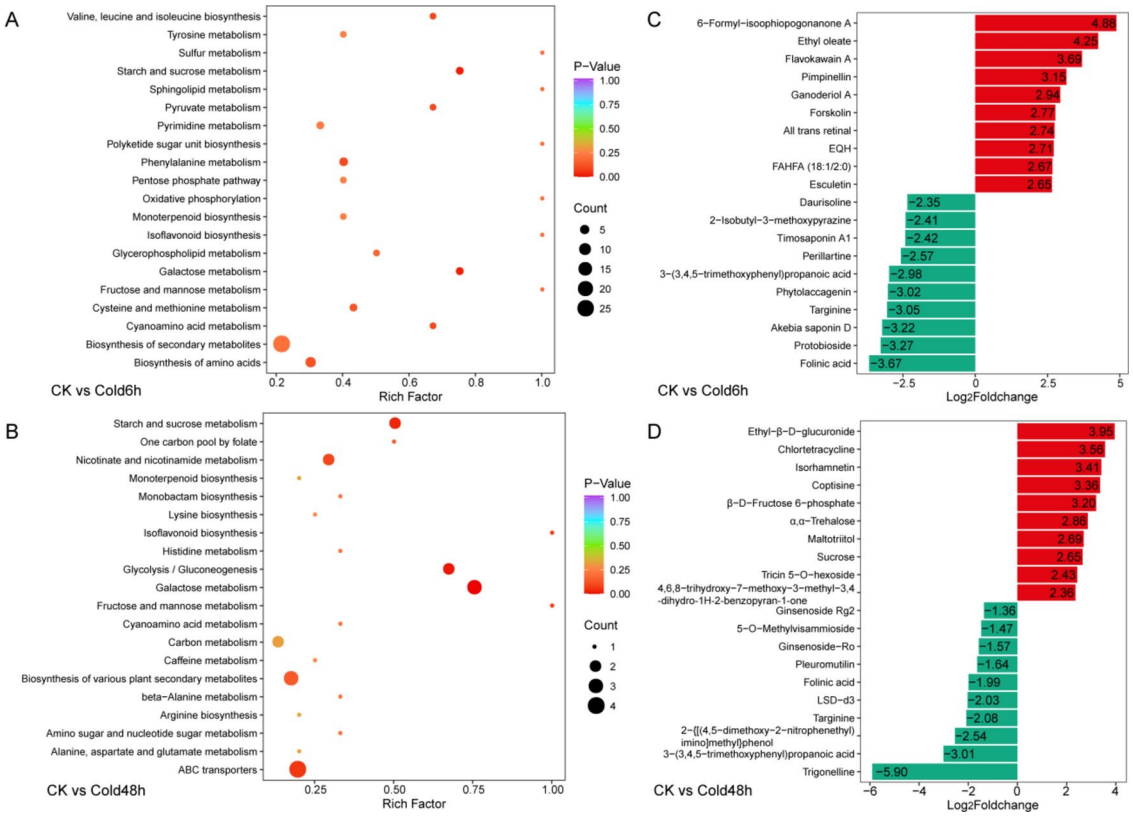

Fig. 3. Statistical examination of metabolites with differential changes. (**A**) KEGG enrichment analysis of the DEMs between Cold6h and CK. The enrichment factor represents the proportion of DEMs to the overall count of identified metabolites in the pathway. (**B**) KEGG enrichment analysis of the DEMs between Cold48h and CK. (**C**) The FC value of the top 20 markedly altered metabolites between Cold6h and CK. The numbers in the bars refer to $\log_2$(fold change). (**D**) The FC value of the top 20 markedly altered metabolites between Cold48h and CK.

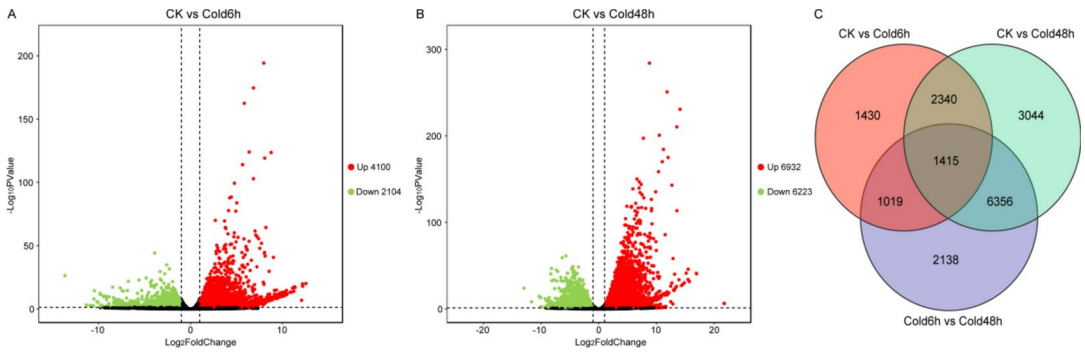

Fig. 4. Transcriptome sequencing information of SD exposed to cold stress. (**A**) Volcano plot of DEGs in the CK and Cold6h groups. In the illustration, every data point corresponds to a gene. The green dots signify genes that were downregulated, red dots indicate upregulated genes, and grey dots denote genes that were detected but did not exhibit significant variation. (**B**) Volcano plot of DEGs in the CK and Cold48h groups. (**C**) Venn diagram illustrating the common and distinct differentially expressed genes (DEGs) across various cohorts.

The GO enrichment examination revealed that DEGs were classified into different ontologies: BPs (biological processes), CCs (cellular components) and MFs (molecular functions). The significant DEGs were mainly clustered into BPs and MFs. In the Cold6h vs. CK comparison, the DEGs were mainly associated with oxidoreductase activity (MF) and embryo development (BP) (Fig. 5A). In the Cold48h vs. CK comparison, the DEGs included cellular protein modification process, transmembrane transport, lipid metabolic process and carbohydrate process in the BP category. In addition, in the MF category, the DEGs participated in transferase activity, kinase activity, oxidoreductase activity, transcription factor (TF) activity, ion binding and

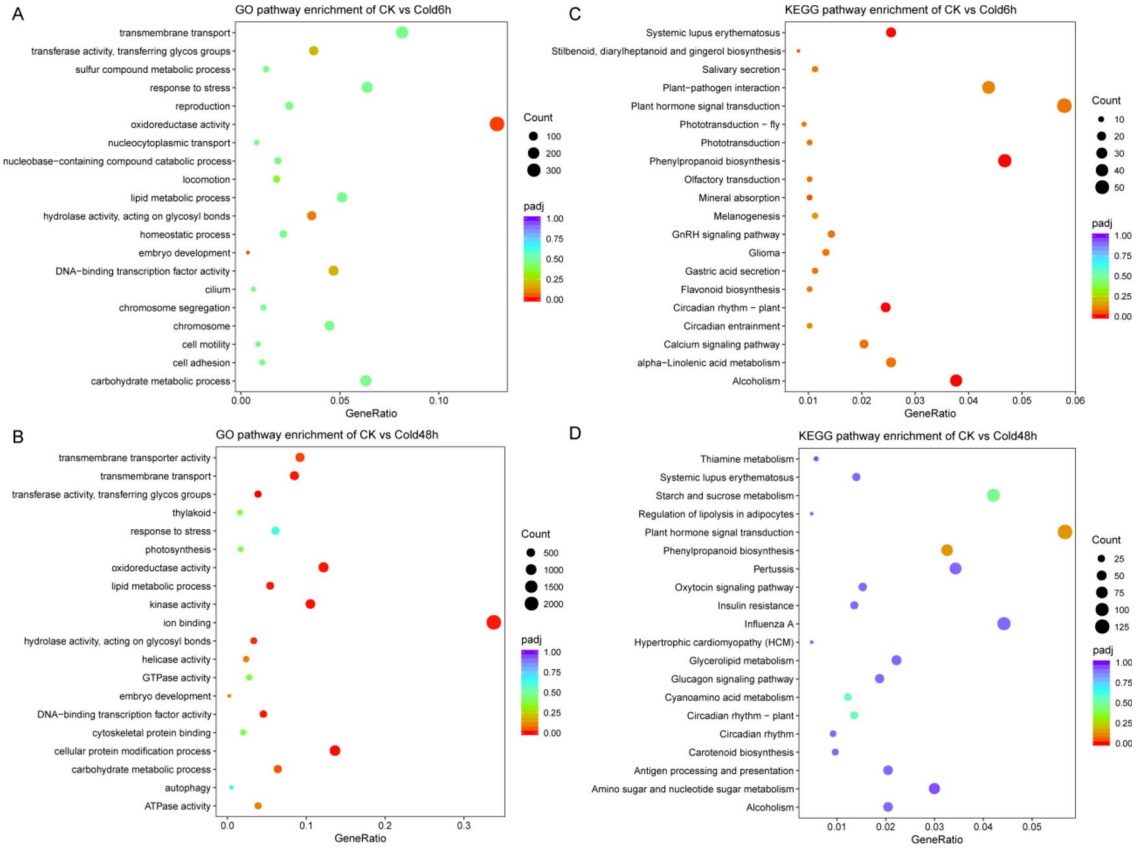

Fig. 5. GO and KEGG pathway enrichment. (**A**, **B**) GO pathway enrichment of the DEGs between the Cold6h vs. CK and the Cold48h vs. CK groups. (**C**, **D**) KEGG pathway enrichment of the DEGs between the Cold6h vs. CK and the Cold48h vs. CK groups.

transmembrane transporter activity (Fig. 5B). Afterwards, KEGG enrichment investigation was conducted based on DEGs information to examine influences of cold temperature on the enrichment pathways in SD. Several pathways, such as phenylpropanoid metabolism, plant hormone signal transduction, were significantly implicated under cold temperature. In addition, several other pathways were enriched, such as alpha-linolenic metabolism, flavonoid biosynthesis, the calcium signalling pathway, mineral absorption and alcoholism (Fig. 5C,D).

Transcription factors (TFs) and weighted gene coexpression network analysis (WGCNA)

The diversity in gene expression patterns ultimately impacts the response of SD to cold stress. We identified 2071 putative TFs from 90 distinct families, with the top ten TFs belonging to the AP2/ERF-ERF (122), C2H2 (106), MYB-related (100), NAC (92), others (88), C3H (88), bHLH (85), WRKY (80), bZIP (68), and MYB (59) families (Fig. 6A).

We employed WGCNA to explore the relationships between physiological indices and important DEGs. In WGCNA, gene clusters exhibiting high correlation were defined as modules, wherein genes within each module displayed strong correlations. WGCNA identified eight distinct modules, which were distinguished by different colours (Fig. 6B). The correlation coefficients between several physiological characteristics and these eight gene cluster modules were analysed (Fig. 6C). Notably, the MEyellow module exhibited significant correlations with SOD activity, MDA levels, and soluble sugar content ($P < 0.05$) (Fig. 6D). Based on the PPI analysis results, we identified twenty hub genes within this intriguing module (Fig. 6E). These hub genes included two *PP2Cs*, two *GH3s*, and two *HSPs*. Importantly, all twenty hub genes were upregulated in the Cold6h vs. CK and Cold48h vs. CK comparison groups, thus suggesting their potential association with SD resistance to cold environments.

Cold stress significantly affected the dynamic changes in plant hormones regarding SD. The 'plant hormone signal transduction' pathway was markedly enriched after Cold6h and Cold48h stress, and the DEGs enriched in this pathway included *PP2C-24*, *PP2C-50*, *GH3-6*, *SAUR50*, and *ETR2*, which are critical genes participating in the ABA, auxin and ET signal transduction pathways. The expression levels of those DEGs were significantly correlated with group Cold48h, indicating that they are key genes sensitive to cold treatment (Fig. 6F).

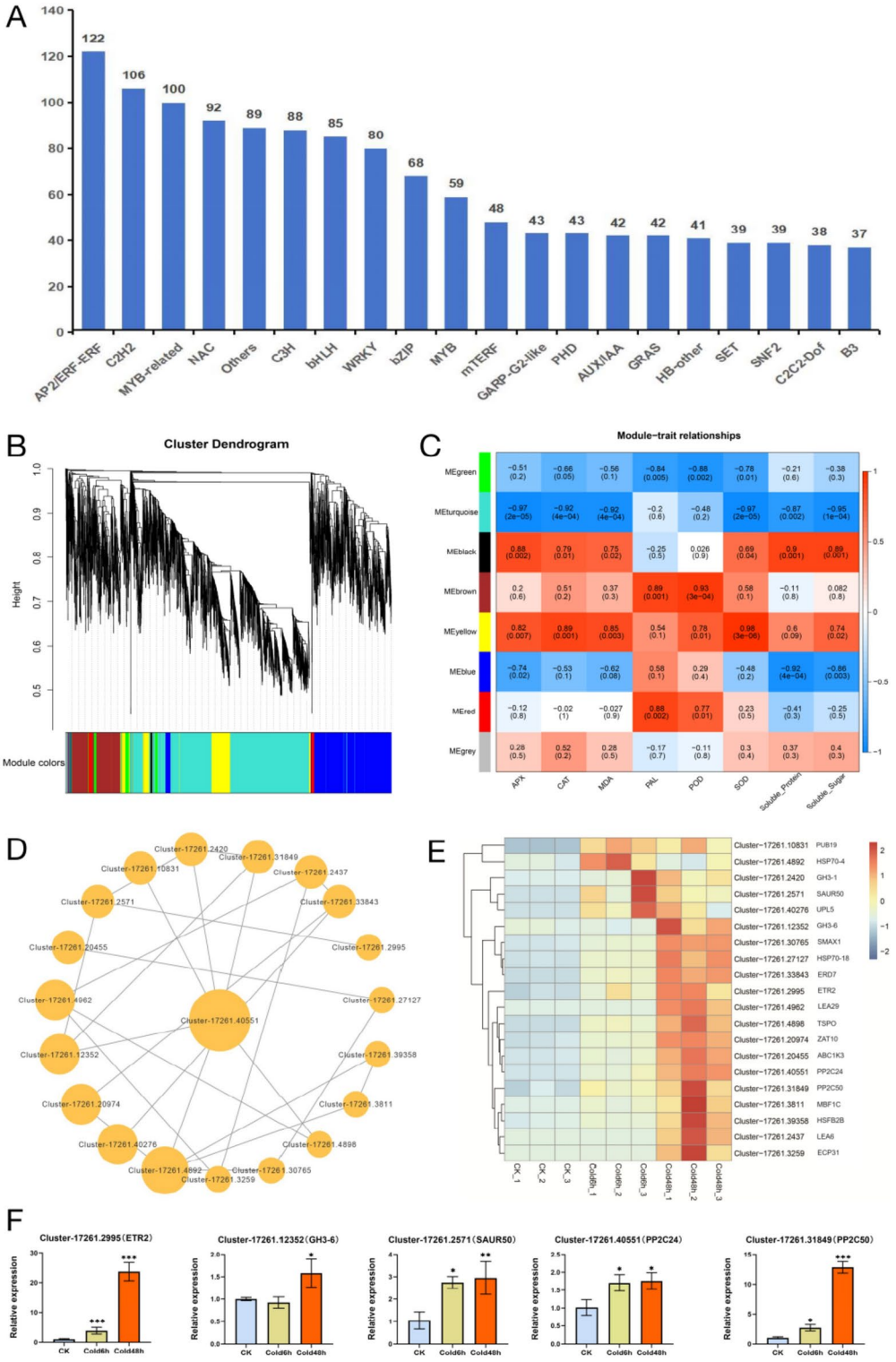

Fig. 6. Transcription factor (TF) and WGCNA results. (**A**) Analyzing of the top 20 TFs. (**B**) Hierarchical clustering diagram displaying coexpression modules identified based on WGCNA. (**C**) Module sample association correlations. (**D**) Correlation networks of the hub genes in the yellow module. (**E**) Heatmap of the hub genes in the yellow module. (**F**) The expression levels of key hub genes involved in ABA, auxin and ET signalling pathways.

Integrated metabolic and transcriptional assay exhibits key role of starch and sucrose metabolism pathways under cold stress

To further explore the correlation between DEGs and DEMs of SD under cold stress, we executed a overall conetwork evaluation of the transcriptome and metabolome information. A Pearson correlation coefficient (PCC > 0.8) was utilized to construct a histogram based on the information derived from the DEGs and DEMs. The correlation analysis was visualized by using a nine-quadrant plot, wherein each quadrant represented distinct correlation scenarios between genes and metabolites. Quadrants 3 and 7 indicated positive correlations between DEGs and DEMs; moreover, quadrant 5 showed no significant correlation, whereas the remaining quadrants suggested negative correlations between DEGs and DEMs (Fig. 7A,B). Furthermore, KEGG enrichment analysis of DEGs and DEMs revealed consistent enrichment of starch and sucrose metabolism, ABC transporter, amino sugar and nucleotide sugar metabolism in the Cold48h vs. CK group ($P < 0.05$), as well as enrichment of the phenylpropanoid biosynthesis pathway in Cold6h vs. CK group and enrichment of the plant hormone signal transduction pathway from Cold48h vs. Cold6h group. These findings highlighted the potential importance of starch/sucrose metabolism, as well as plant hormone signalling pathways, in conferring cold stress resistance to SD plants (Fig. 7C,D).

Sugars and starches, as the primary carbohydrates within plants, serve both as sources of energy and as crucial substances for plant stress resistance. Sugars play multiple roles in cold adaptation, including acting as osmoprotectants to shield cells from freeze damage, serving as signalling molecules in the signal transduction pathways of the cold reaction, and modulating the antioxidant enzyme system to mitigate oxidative stress induced by low temperatures. Metabolomic analysis revealed significant accumulation of raffinose, sucrose,

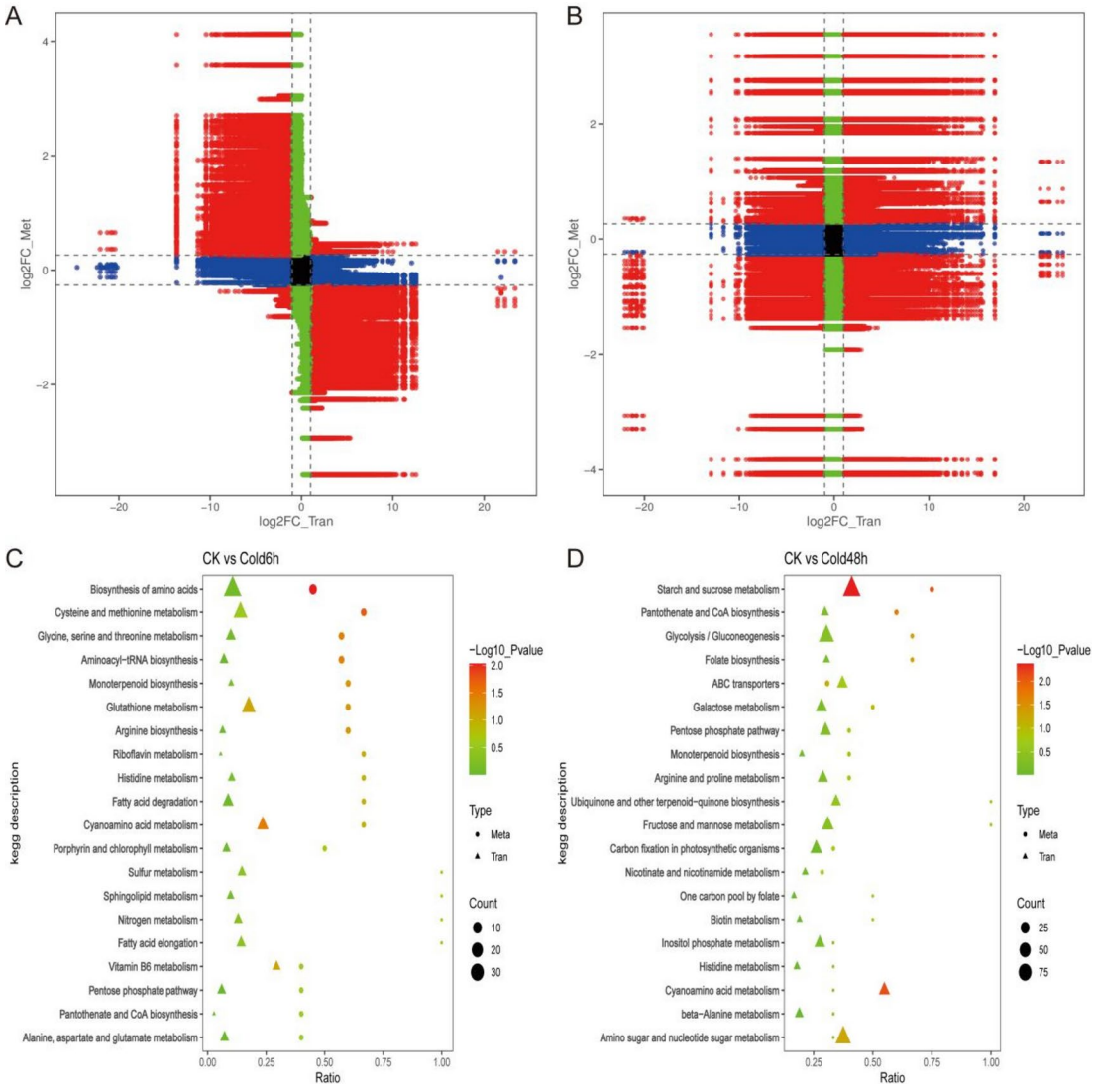

Fig. 7. Relationship examination of transcriptomic and metabolomic data from SD under cold stress. (**A**, **B**) The nine-quadrant diagram indicated the relationship of genes and metabolites between Cold6h vs. CK and Cold48h vs. CK groups. (**C**, **D**) KEGG enrichment examination of DEGs (circle) and DEMs (triangle) enriched within the same biological pathway.

D-glucose-6P, β-D-fructose-6P and trehalose under cold stress (Fig. 8A,C). Moreover, gene expression analysis indicated positive regulation of various DEGs encoding enzymes participating in pathway of sugar metabolism , such as α-galactosidase (*α-Gal*), sucrose synthase (*SS*), sucrose phosphate synthase (*SPS*), invertase (*INV*), sugar cotransporter kinase (*ScrK*), trehalose-6-phosphate synthase (*TPS*), trehalase (*TREH*) and oligosaccharyltransferase B (*otsB*). qPCR analysis demonstrated the substantial regulation of several genes associated with sugar biosynthesis under cold stress, such as SdINV3 in the Cold6h group and SdSPS1/SdSS5 in the Cold48h group (Fig. 8B). In summary, our transcriptome and metabolome data suggest that sugar-related metabolic pathways, along with flavonoid/terpenoid biosynthesis and plant hormone signalling pathways, are primarily responsible for the resistance of SD to cold stress (Fig. 9).

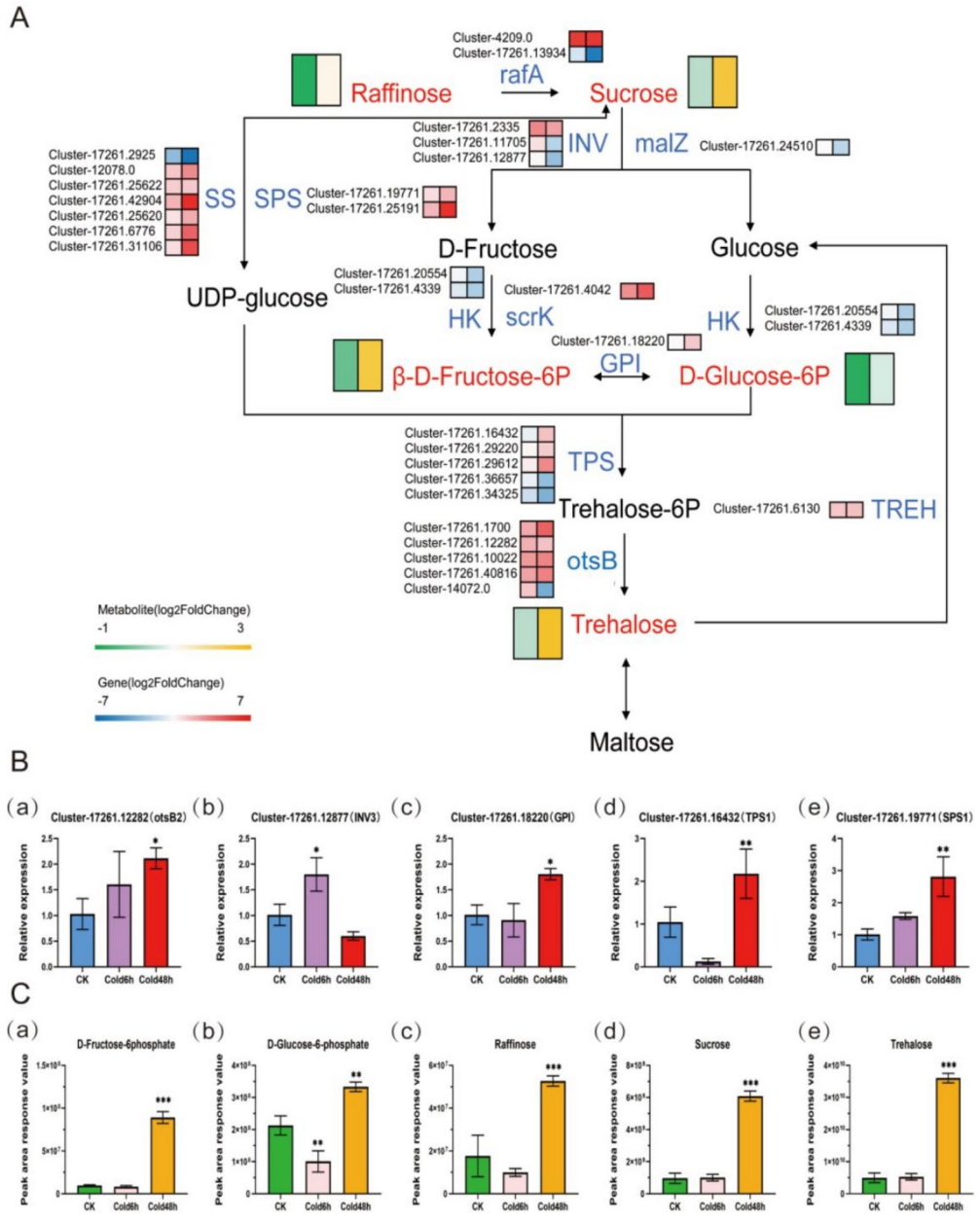

Fig. 8. Comprehensive study based on transcriptome and metabolome analysis emphasized the critical involvement of the sugar metabolism pathway in response to cold stress. (**A**) Integrated examination of KEGG enrichment pathways. (**B**) qPCR assay validated that sugar synthase genes showed upregulation under cold environment. otsB2, trehalose-6-phosphate phosphatase; INV3, glycoside hydrolase; GPI, glucose 6 phosphate isomerase; TPS1, trehalose-6-phosphate synthase; SPS1, sucrose phosphate synthase. The data were calculated from three biological replicates. Significant differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) (**C**) Contents of key sugar-related metabolites were overexpressed under cold stress.

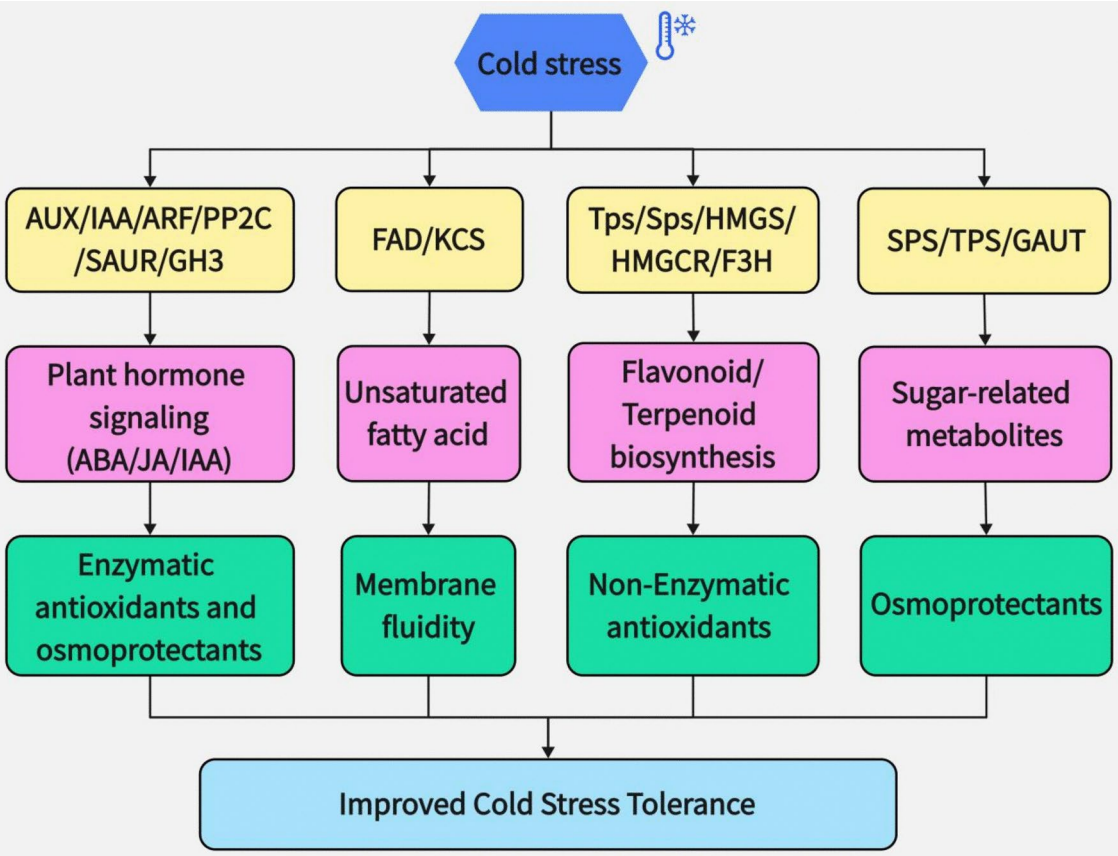

Fig. 9. An illustrative chart outlining the primary pathways affected by cold. AUX/IAA, auxin/indoleacetic acid protein; ARF, auxin response factor; PP2C, type 2C protein phosphatase; SAUR, small auxin-up RNA; GH3, indole-3-acetic acid-amido synthetase; FAD, fatty acid desaturase; KCS, 3-ketoacyl-CoA synthase; Tps, terpene synthase; Sps, solanesyl diphosphate synthase; HMGS, hydroxymethylglutaryl-CoA synthase; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; F3H, flavanone 3-dioxygenase; SPS, sucrose-phosphate synthase; TPS, trehalose-phosphate synthase; GAUT, galacturonosyltransferase.

Discussion

Within the scope of traditional Chinese medicine, SD has been established for millennia, not only for its therapeutic efficacy but also for the complex interplay between its phytochemical constituents and environmental conditions. The phytochemical profile of SD is significantly influenced by various environmental factors, including temperature, light, soil composition, and water availability. These components, which primarily consist of coumarins, polysaccharides, and volatile oils, are pivotal for its pharmacological properties. Research indicates that environmental stresses, such as temperature fluctuations, can induce secondary metabolite biosynthesis in plants. For instance, chromones levels in SD have been observed to increase due to abiotic stress[41]. Under low-temperature stress, SD exhibits a fascinating adaptive mechanism that alters its active component profile. This physiological response is crucial for survival and the maintenance of medicinal properties under adverse conditions. A former literature confirmed that low-temperature stress substantially enhances the concentration of polysaccharides, which are thought to play protective roles against abiotic damage[42]. Furthermore, the literature has explored gene expression changes in SD during drought stress, thus demonstrating the positive regulated expression of DEGs related to secondary metabolite pathways, including those responsible for coumarin and flavonoid biosynthesis[43]. These findingsindicate that low-temperature conditions not only hinder plant survival but also trigger complex biochemical responses, thus enhancing plant medicinal and nutritional value. However, limited information is available on the key genes, enzymes and metabolites affiliated with the regulatory molecular biology mechanisms of low temperature resistance of SD. Herein, to elucidate the cold tolerance mechanism of SD, we conducted biochemical assays, along with transcriptome and metabolome analyses, under cold treatment at 4 °C. The results showed that the SD's responses to cold stress were chiefly linked to plant hormone signalling pathways, starch and sucrose metabolism, secondary metabolite biosynthesis, and the activities of ROS-scavenging pathways.

Under Cold6h and Cold48h stress, the downregulation of the chlorophyll-binding protein-encoding gene *CP24* significantly suggested a decrease in the plants' capacity to capture and utilize light effectively, thus potentially leading to reduced photosynthetic capabilities and overall energy acquisition in the SD treatment (Supplementary Table 4). Chlorophyll-binding proteins, including CP24, are integral components of light-harvesting complex (LHC) related to photosystem II, which is essential for capturing and transferring light

energy during photosynthesis[44]. The buildup of MDA during cold stress reflects the extent of oxidative damage and can trigger a cascade of antioxidative responses (Fig. 1C). It can modify proteins and nucleic acids, thereby altering their function and potentially activating stress response pathways. An increase in MDA can also act as signal for the induction of antioxidant enzymes for instance SOD and CAT, which aid in mitigating oxidative damage[45]. An elevation in MDA levels can also act as a signal for the induction of antioxidant enzymes such as SOD and CAT, which play a crucial role in ameliorating oxidative damage.

Upon reviewing the transcriptomic data, we identified *P5CS* (pyrroline-5-carboxylate synthetase) genes associated with MDA biosynthesis in both the Cold6h and Cold12h groups in comparison to control. CAT plays the crucial role within detoxifying hydrogen peroxide (H_2O_2), and an increase in CAT levels during cold treatment indicates an enhanced antioxidative defence mechanism. SOD is essential in the conversion of superoxide radicals into hydrogen peroxide and oxygen, thereby acting as a primary defence mechanism against ROS[46]. The enzyme PAL is crucial in the biosynthesis of phenylpropanoids, which are vital for plant adjustment to environmental stresses. The upregulation of PAL indicates a highly accumulation in the content of phenolic ingredients, which participate in structural reinforcement, antioxidative responses, and signalling (Fig. 1I). In reaction to cold stress, the cooperative action of SOD, CAT, and APX enzymes involves their spatial and functional complementation within cellular compartments. Specifically, SOD promotes the conversion of superoxide radicals into oxygen and hydrogen peroxide. Afterward, CAT as well as APX detoxify hydrogen peroxide through distinct mechanisms, whereas CAT decomposes it into water and oxygen (primarily in peroxisomes); furthermore, APX not only detoxifies hydrogen peroxide but also regenerates ascorbate as part of the ascorbate–glutathione cycle for further ROS scavenging (Fig. 1J). We hypothesized that SOD and CAT would exhibit greater sensitivity to cold stress than PAL, whereas APX would play a predominant role in scavenging ROS in SD.

We employed widely targeted metabolome analysis to investigate alterations in the metabolites of SD under low-temperature stress. A notable finding from our study is the significant accumulation of sugar-related metabolites, including sucrose, trehalose, and fructose (Fig. 3). Sugars play pivotal roles ranging from serving as primary sources of energy to acting as signalling molecules that regulate various physiological processes in plant development and abiotic-stress tolerance. Sugars are not only metabolic fuels but also crucial regulators of gene expression, thus influencing plant survival and development[47]. Under abiotic stresses, sugars act as osmoprotectants, thus playing a significant role in osmoregulation. This function is crucial for maintaining cellular integrity under stress by balancing the osmotic pressure within cells, thereby protecting them from damage caused by dehydration and ionic toxicity[48]. For example, trehalose is a nonreducing sugar that is known for its role in stabilizing proteins and lipid membranes under stress conditions (including low temperatures), thus enhancing plant stress tolerance[49]. Other sugar-related metabolites were also overaccumulated in our analysis (such as α,α-trehalose, sucrose, β-D-fructose 6-phosphate, stachyose, and α-lactose) (Supplementary Tables 2, 3). Stachyose, which is a tetrasaccharide contains two α-D-galactose units, one α-D-glucose, and one β-D-fructose, has been identified as being a determinant component in plant reaction to different stress. It acts as an osmoprotectant, thus contributing to the maintenance of cell membranes and proteins during stress conditions. Peters and Keller demonstrated that stachyose levels increase in plants exposed to drought, thus suggesting its involvement in osmotic adjustment and protection against dehydration[50]. Additionally, stachyose has been implicated in alleviating oxidative influence by neutralizing ROS, thus protecting cellular components from oxidative damage[51]. Integrating the metabolome and transcriptome results, we deduced that increased levels of sucrose-phosphate synthase (*SPS*) and trehalose-phosphate synthase (*TPS*) may promote high level accumulation of sugars (Supplementary Table 5). The *SPS* and *TPS* genes are crucial for the synthesis of glucose and trehalose, respectively, thus impacting the accumulation of sugar metabolites and playing vital roles in plant metabolism and stress response. The overexpression of *SPS* can significantly boost resistance to heat stress by improving various physiological and biochemical parameters (including increasing sucrose and chlorophyll contents), improving photosynthetic efficiency, and reducing cellular damage[52]. At the molecular level, the induction of *TPS* and the coordination of downstream gene expression under low temperatures are regulated by a complex network of CBF/DREB1 and ICE1[53]. The results inferred that *TPS* and *SPS* may play key roles in the cold tolerance of SD. Nevertheless, the molecular regulatory mechanism of these two genes remains unclarify.

In general, plant hormones such as auxins (IAA), gibberellins (GAs), ethylene (ETH), abscisic acid (ABA), salicylic acid (SA), and jasmonic acid (JA) coordinate a wide range of physiological and molecular responses that enable herbs to endure and adapt to unfavorable stress conditions. These hormones regulate diverse stress-responsive pathways, thereby contributing to the maintenance of cellular homeostasis, also the reinforcement of herb defense mechanisms[54]. Under normal growth conditions, SnRK2-PP2C-SnRK1 forms a complex that contributes to the suppression of SnRK1 function. This facilitates the effective functioning of TOR (a growth-promoting factor), thereby promoting plant growth. Conversely, under adverse conditions, accumulated ABA enhances the interaction between PYR/PYL receptors and PP2C, thus resulting in the disassembly of the SnRK2-PP2C-SnRK1 complex[55]. Cold treatment caused *SnRK1* and *PP2C* to be upregulated and inhibited TOR activity, thereby suppressing plant growth and enhancing cold resistance (Supplementary Table 5). In the auxin signalling pathway, the increased expression of *ARF* (auxin response factor) in the Cold6h and Cold48h environments not only controlled the increased expression of downstream *SAUR* and *GH3* (which are hub genes) to cope with abiotic stress but also influenced the orientation of plant growth factors by binding to Aux/IAA proteins (Fig. 6). In the JA signal transduction pathway, upregulated MYB TFs can bind to the promoters of genes involved in proline synthesis, such as *P5CS* (pyrroline-5-carboxylate synthetase), thereby enhancing their transcription and consequently promoting proline accumulation to improve resistance to abiotic stress (Fig. 1). The R2R3-MYB type TF *MtMYBS1* exhibits enhanced salinity resistance when organically expressed in *Arabidopsis thaliana*[56]. We also found that *OPR* and *LOX*, illustrated as two crucial enzymes in the biosynthetic pathway of jasmonic acid, were upregulated. *OPR* controls the final step of JA synthesis, thus reducing OPDA to the precursor of

JA, whereas *LOX* controls the initial step of JA synthesis, which involves the oxidization of unsaturated fatty acids to hydroperoxide (Supplementary Table 5). In this study, the simultaneous action of ABA, IAA, and JA is hypothesized to contribute to the maintenance of SD growth under cold exposure.

The metabolome analysis indicated that the metabolites with notable alterations participated in lipid metabolism and flavonoid and terpenoid biosynthesis (Supplementary Tables 2, 3). It was observed that the majority of the top 20 DEMs were associated with flavonoids, terpenoids and lipids (Fig. 3). The accumulation of flavonoids and terpenoids, which are important secondary metabolites found in medicinal herbs, primarily occurs in resistance to adverse environmental conditions and the mitigation of abiotic stresses. The biosynthetic pathway and regulatory mechanism of flavonoids and terpenoids in SD have not been extensively characterized, unlike those in other well-studied model plants. Based on the analysis of the top 20 DEMs, we found that 6-formylisoophiopogonanone A, flavokawain B and irsorhamnetin were the main flavonoids that accumulated. The compound 6-formyl-isoophiopogonanone A was identified as a homoisoflavonoid, and the majority of homoisoflavonoids exhibited certain scavenging abilities towards ·OH and H_2O_2 in vitro[57]. Flavokawain B was demonstrated to induce ER stress in glioma cells, subsequently leading to the activation of autophagy pathways[58]. Isorhamnetin exhibited significant protective effects on heart muscle cells against oxidative stress-induced damage through two primary mechanisms: scavenging ROS and inhibiting the extracellular signal-regulated kinase (ERK) pathway[59]. These flavonoids may synergistically upregulate key enzymes and detoxification proteins related to ROS cleaning. Additionally, under cold stress conditions, *F3H*, which is an essential enzyme in flavonoid biosynthesis, was observed to be overexpressed. (Supplementary Table 5). The overproduction of the terpenoids ganoderiol A and forskolin enhanced resistance to environmental stress, as determined via the use of SIMX1[60]. In this research, several enzymes associated with terpenoid biosynthesis were identified: *Tps* (terpene synthase), S*ps* (solanesyl diphosphate synthase), *FPS* (farnesene synthase), *HMGS* (hydroxymethylglutaryl-CoA synthase), and *HMGCR* (3-hydroxy-3-methylglutaryl coenzyme A reductase).

Among the identified DEMs, lipids accounted for a significant portion, especially unsaturated fatty acids (Supplementary Tables 2, 3). Chilling-resistant plants exhibit elevated levels of unsaturated fatty acids in their membranes as a result of enhanced desaturase enzyme activity during cold acclimation, which enhances membrane fluidity and provides protection against low temperatures[61]. Through transcriptome analysis, we that upregulated temperature-sensitive sn-2 acyl-lipid omega-3 desaturase (FAD) may promote the synthesis of unsaturated fatty acids. The FAD-catalysed condensation process is the crucial rate-limiting step in the biosynthesis of unsaturated fatty acids, thus employing substrate, product and location selectivity[62]. Mutant *Arabidopsis thaliana* lacking *Fad2* grows slowly in low-temperature environments and exhibits significantly lower cold resistance than wild-type plants[63]. Our study indicated that *FAD* plays an important role in cold resistance in SD; nevertheless, the mechanism by which *FAD* regulates cold resistance remains unclear. To summarize, our findings indicate that sugar-related metabolism, flavonoid and terpenoid biosynthesis, unsaturated fatty acid biosynthesis, and plant hormone signalling are the main protective mechanisms employed by SD against cold stress. This information will support us better estimate the significant effects of low-temperature climates on SD.

Conclusion

Our findings clearly illustrate a comprehensive analysis integrating physiological parameters with metabolomic and transcriptomic data to elucidate crucial metabolites, genes, and pathways associated with cold stress in SD plants. This approach identified a total of 1396 differentially expressed metabolites (DEMs), including 138 DEMs for the comparison between Cold6h versus CK as well as 85 DEMs for Cold48h versus CK. Integration of transcriptomic data led to annotation of 1415 common differentially expressed genes (DEGs) across these comparisons. Furthermore, the combined insights from transcriptomics and metabolomics unveiled mechanisms governing SD's response to low temperatures. Exposure to 4 °C was found to modulate sugar-related metabolic pathways, flavonoid/terpenoid biosynthesis, and plant hormone metabolism through regulation of gene expression involving *PP2C*, *AUX*, *CH3*, *TSP*, *GPI*, *INV3*, *otsB2*, *SPS* and *SS* while elevating levels of antioxidant compounds and plant hormones in order to sustain normal growth. In conclusion, our results suggest that protective responses mounted by SD against cold stress primarily involve sugar-related metabolism, secondary metabolite synthesis, unsaturated fatty acid production,and plant hormone signaling. These findings will facilitate further assessment of the profound impacts exerted by low-temperature climates on SD.

Data availability

Sequence data that support the findings of this study have been deposited in NCBI with the primary accession code GSE276339.

Received: 20 August 2024; Accepted: 18 February 2025
Published online: 27 February 2025

References

1. Wu, B. Y., Su, J. Z. & Zhang, R. H. Effects of autumn-winter Arctic sea ice on winter Siberian High. *Chin. Sci. Bull.* **56**, 3220–3228. https://doi.org/10.1007/s11434-011-4696-4 (2011).
2. Luo, Y., Li, C., Shi, J., An, X. & Sun, Y. Wintertime cold extremes in northeast china and their linkage with sea ice in Barents–Kara seas. *Atmosphere* **12**, 386. https://doi.org/10.3390/ATMOS12030386 (2021).
3. Wang, Y. J., Li, Y. S., Wang, X. Q., Fang, Y.L., & Yang, J. M. The effect of climate change on the climatic regionalization of wine grapes in Northeast of China. In *IOP Conf. Series: Earth and Environmental Science*, vol 559 012008 (2020). https://doi.org/10.1088/1755-1315/559/1/012008.
4. Ruelland, E., Vaultier, M. N. & Zachowski, A. Cold signalling and cold acclimation in plants. *Adv. Bot. Res.* **49**, 35. https://doi.org/10.1016/S0065-2296(08)00602-2 (2009).

5. Yang, Q. S. et al. Comparative transcriptomics analysis reveals difference of key gene expression between banana and plantain in response to cold stress. *BMC Genomics* **16**, 446. https://doi.org/10.1186/s12864-015-1551-z (2015).
6. Huang, H. et al. Study on the mechanism of exogenous serotonin improving cold tolerance of rapeseed (*Brassica napus* L.) seedlings. *Plant Growth Regulat.* **94**, 161–170. https://doi.org/10.1007/s10725-021-00700-0 (2021).
7. Liu, S. H. et al. Metabolic profiling and gene expression analyses provide insights into cold adaptation of an Antarctic moss *Pohlia nutans*. *Front. Plant Sci.* **13**, 1006991. https://doi.org/10.3389/fpls.2022.1006991 (2022).
8. Ding, Y. L. & Yang, S. H. Surviving and thriving: How plants perceived and respond to temperature stress. *Dev. Cell.* **8**, 947–958. https://doi.org/10.1016/j.devcel.2022.03.010 (2022).
9. Xiong, L. M., Schumaker, K. S. & Zhu, J. K. Cell signaling during cold, drought, and salt stress. *Plant Cell.* **14**, S165–S183. https://doi.org/10.1105/tpc.000596 (2002).
10. Huang, X. S., Wang, W., Zhang, Q. & Liu, J. H. A basic Helix-Loop-Helix transcription factor PtrbHLH of *Poncirus trifoliata* confers cold tolerance and modulates POD-mediated scavenging of H_2O_2. *Plant Physiol.* **162**, 1178–1194. https://doi.org/10.1104/pp.112.210740 (2013).
11. Song, Q. P. et al. CBF1 and CBF4 in *Solanum tuberosum* L. differ in their effect on low-temperature tolerance and development. *Environ. Exp. Bot.* **185**, 104416. https://doi.org/10.1016/j.envexpbot.2021.104416 (2021).
12. Rosa, M., Prado, C. & Podazza, G. Soluble sugars–metabolism, sensing and abiotic stress: A complex network in the life of plants. *Plant Signal Behav.* **4**, 388–393. https://doi.org/10.4161/psb.4.5.8294 (2009).
13. Wang, J. Z., Wang, F. B., Jin, C., Tong, Y. & Wang, T. A R2R3-MYB transcription factor VvMYBF1 from grapevine (*Vitis vinifera* L.) regulates flavonoids accumulation and abiotic stress tolerance in transgenic Arabidopsis. *Taylor Francis.* **95**, 147–161. https://doi.org/10.1080/14620316.2019.1665480 (2020).
14. Bodede, O., More, G. & Prinsloo, G. Antimicrobial cytotoxic and oxidative stress inhibitory activities of Terpenoids and Flavonols from *Senegalia nigrescens*. *Iran J. Pharm. Res.* **20**, 329–338. https://doi.org/10.22037/ijpr.2021.115653.15463 (2021).
15. Chen, X. et al. The NAC family transcription factor OsNAP confers abiotic stress response through the ABA pathway. *Plant Cell Physiol.* **55**, 604–619. https://doi.org/10.1093/pcp/pct204 (2014).
16. Kreiner, J., Pang, E., Lenon, G. B. & Yang, A. W. H. *Saposhnikoviae divaricata*: A phytochemical, pharmacological, and pharmacokinetic review. *Chin J. Nat. Med.* **15**, 255–264. https://doi.org/10.1016/S1875-5364(17)30042-0 (2017).
17. Pharmacopoeia of People's Republic of China. 2020. 1, 214–215.
18. Kang, J. et al. Three new compounds from the roots of *Saposhnikoviae divaricata*. *J. Asian Nat. Prod. Res.* **10**, 971–976. https://doi.org/10.1080/10286020802217556 (2008).
19. Kim, M. K. et al. Simultaneous determination of chromones and coumarins in Radix Saposhnikoviae by high performance liquid chromatography with diode array and tandem mass detectors. *J. Chromatogr.* **1218**, 6319–6330. https://doi.org/10.1016/j.chroma.2011.06.103 (2011).
20. Yu, L. F., Li, X. R., Liu, S. Y., Xu, G. W. & Liang, Y. Z. Comparative analysis of essential components between the herbal pair Radix Saposhnikoviae–Rhizoma seu Radix Notopterygii and its single herbs by GC-MS combined with a chemometric resolution method. *Anal. Methods* **1**, 45–51. https://doi.org/10.1039/b9ay00044e (2009).
21. Yang, J. L. et al. Three new coumarins from *Saposhnikoviae divaricata* and their porcine epidemic diarrhea virus (PEDV) inhibitory activity. *Tetrahedron.* **71**, 4651–4658. https://doi.org/10.1016/j.tet.2015.04.092 (2015).
22. Dong, C. X. et al. Structural characterization of polysaccharides from *Saposhnikovia divaricata* and their antagonistic effects against the immunosuppression by the culture supernatants of melanoma cells on RAW264.7 macrophages. *Int. J. Biol. Macromol.* **113**, 748–756. https://doi.org/10.1016/j.ijbiomac.2018.03.022 (2018).
23. Chen, L. X., Chen, X. Y., Su, L., Jiang, Y. Y. & Liu, B. Rapid characterisation and identification of compounds in Saposhnikoviae Radix by high-performance liquid chromatography coupled with electrospray ionisation quadrupole time-off-light mass spectrometry. *Nat. Prod. Res.* **32**, 898–901. https://doi.org/10.1080/14786419.2017.1366482 (2017).
24. Urbagarova, B. M. et al. Chromones and coumarins from *Saposhnikovia divaricata* (Turcz) Schischk Growing in Buryatia and Mongolia and their cytotoxicity. *J. Ethnopharmacol.* **261**, 112517. https://doi.org/10.1016/j.jep.2019.112517 (2020).
25. Hultman, J. et al. Multi-omics of permafrost, active layer and thermokarst bog soil microbiomes. *Nature* **521**, 208. https://doi.org/10.1038/nature14238 (2015).
26. Raza, A., Tabassum, J., Kudapa, H. & Varshney, R. K. Can omics deliver temperature resilient ready-to-grow crops?. *Crit. Rev. Biotechnol.* **41**, 1209–1232. https://doi.org/10.1080/07388551.2021.1898332 (2021).
27. Wang, X. Y. et al. Dynamic transcriptome profiling revealed key genes and pathways associated with cold stress in castor (*Ricinus communis* L.). *Ind. Crop Prod.* **178**, 114610. https://doi.org/10.1016/j.indcrop.2022.114610 (2022).
28. Lyu, J. I. et al. Unraveling the complexity of faba bean (*Vicia faba* L.) transcriptome to reveal cold-stress-responsive genes using long-read isoform sequencing technology. *NPG.* https://doi.org/10.1038/S41598-021-00506-0 (2021).
29. Liu, J., Li, J. M. & Fu, C. X. Comparative physiology and transcriptome analysis reveals the regulatory mechanism of genome duplication enhancing cold resistance in *Fragaria nilgerrensis*. *Environ. Exp. Bot.* **188**, 104509. https://doi.org/10.1016/j.envexpbot.2021.104509 (2021).
30. Chen, P. F. et al. Gene coexpression network analysis indicates that hub genes related to photosynthesis and starch synthesis modulate salt stress tolerance in *Ulmus pumila*. *Int. J. Mol. Sci.* **22**, 4410. https://doi.org/10.3390/ijms22094410 (2021).
31. Palma, M. D., Docimo, T., Guida, G., Salzano, M. & Tucci, M. Transcriptome modulation by the beneficial fungus *Trichoderma longibrachiatum* drives water stress response and recovery in tomato. *Environ. Exp. Bot.* **190**, 104588. https://doi.org/10.1016/j.envexpbot.2021.104588 (2021).
32. Gong, L. et al. Transcriptome profiling of the potato (*Solanum tuberosum* L.) plant under drought stress and water-stimulus conditions. *PLoS ONE.* **10**, e0128041. https://doi.org/10.1371/journal.pone.0128041 (2015).
33. Gonzalez-Schain, N. et al. Genome-wide transcriptome analysis during anthesis reveals new insights into the molecular basis of heat stress responses in tolerant and sensitive rice varieties. *Plant Cell Physiol.* **57**, 57–68. https://doi.org/10.1093/pcp/pcv174 (2016).
34. Luo, L. et al. Preliminary studies on cold resistance evaluation of modern roses. *Acta Hortic.* **937**, 889–900. https://doi.org/10.17660/ActaHortic.2012.937.109 (2021).
35. Jiang, M. et al. Identification of the bZIP gene family and regulation of metabolites under salt stress in *Isatis indigotica*. *Front. Plant Sci.* **13**, 1011616. https://doi.org/10.3389/fpls.2022.1011616 (2022).
36. Kanehisa, M. & Goto, S. KEGG: Encyclopedia of genes and genomes. *Nucl. Acids Res.* **28**, 27–30. https://doi.org/10.1093/nar/28.1.27 (2000).
37. Suzuki, N., Bajad, S., Shuman, J., Shulaev, L. & Mittler, R. The transcriptional co-activator MBF1c is a key regulator of thermotolerance in *Arabidopsis thaliana*. *J. Biol. Chem.* **283**, 9269. https://doi.org/10.1074/jbc.M709187200 (2008).
38. Broad, R. C. et al. Effect of Rice GDP-L-galactose phosphorylase constitutive overexpression on ascorbate concentration, stress tolerance, and iron bioavailability in rice. *Front. Plant Sci.* https://doi.org/10.3389/fpls.2020.595439 (2020).
39. Gu, L. et al. Maize HSFA2 and HSBP2 antagonistically modulate raffinose biosynthesis and heat tolerance in Arabidopsis. *Plant J.* **100**, 128–142. https://doi.org/10.1111/tpj.14434 (2019).
40. Naidu, B. P., Paleg, L. G., Aspinall, D., Jennings, A. C. & Jones, G. P. Amino acid and glycine betaine accumulation in cold-stressed wheat seedlings. *Phytochemistry.* **30**, 407–409. https://doi.org/10.1016/0031-9422(91)83693-F (1991).
41. Han, Z. M. et al. Effect of drought stress on leaf protective enzyme activities, contents of osmoregulation substances and quality of *Saposhnikovia divaricata*. *J. South China Agric. Univ.* **37**, 91–97 (2016).

42. Comas-Serra, F. et al. Role of acemannan and pectic polysaccharides in saline-water stress tolerance of Aloe vera (*Aloe barbadensis* Miller) plant. *Int. J. Biol. Macromol.* **268**, 131601. https://doi.org/10.1016/j.ijbiomac.2024.131601 (2024).
43. Meng, Y. Q., Wang, D. H., Li, B. Z., Su, Y. L. & Chen, G. L. Effects of drought stress on the antioxidant system, osmolytes and secondary metabolites of *Saposhnikovia divaricata* seedlings. *Acta Physiol. Plant.* **40**, 191. https://doi.org/10.1007/s11738-018-2762-0 (2018).
44. Wei, Y. C. et al. Identification and expression analysis of chlorophyll a/b binding protein gene family in grape (*Vitis vinifera*). *Physiol. Mol. Biol. Plants* **28**, 1147–1158. https://doi.org/10.1007/s12298-022-01204-5 (2022).
45. Sharma, P., Jha, A. B., Dubey, R. S. & Pessarakli, M. Reactive oxygen species oxidative damage, and Antioxidative defense mechanism in plants under stressful conditions. *AJB* **1–26**, 217037. https://doi.org/10.1155/2012/217037 (2021).
46. Alscher, R. G., Erturk, N. & Heath, L. S. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J. Exp. Bot.* **53**, 1331–1341. https://doi.org/10.1093/jexbot/53.372.1331 (2002).
47. Rolland, F., Baena-Gonzalez, E. & Sheen, J. Sugar sensing and signaling in plants: Conserved and novel mechanisms, Annual. *Review.* **57**, 675–709 (2006).
48. Kaplan, F. & Guy, C. L. β-Amylase induction and the protective role of maltose during temperature shock. *Plant Physiol.* **135**, 1674–1684. https://doi.org/10.1104/pp.104.040808 (2004).
49. Lunn, J. E., Delorge, I., Figueroa, C. M., Van, D. P. & Stitt, M. Trehalose metabolism in plants. *Plant J.* **79**, 544–567. https://doi.org/10.1111/tpj.12509 (2014).
50. Winaya, A., Maftuchah, M. & Zainudin, A. The identification of osmoprotectant compounds from *Jatropha curcas* Linn. plant for natural drought stress tolerance. *Energy Rep.* **6**, 626–630. https://doi.org/10.1016/j.egyr.2019.09.037 (2020).
51. Nishizawa, A., Yabuta, Y. & Shigeoka, S. Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. *Plant Physiol.* **147**, 1251–1263. https://doi.org/10.1104/pp.108.122465 (2008).
52. Zhang, Y. Y., Zeng, D. & Zhu, W. M. SlSPS, a sucrose phosphate synthase gene mediates plant growth and thermotolerance in tomato. *Horticulturae.* **8**, 491. https://doi.org/10.3390/horticulturae8060491 (2022).
53. Hu, Y., Jiang, L., Wang, F. & Yua, D. Jasmonate regulates the inducer of CBF expression–c-repeat binding factor/DRE binding factor1 cascade and freezing tolerance in Arabidopsis. *Plant Cell.* **25**, 2907–2924. https://doi.org/10.1105/tpc.113.112631 (2013).
54. Liu, S. L. et al. Response of phytohormones and correlation of SAR signal pathway genes to the different resistance levels of grapevine against *Plasmopara viticola* infection. *Plant Physiol. Bioch.* **107**, 56–66. https://doi.org/10.1016/j.plaphy.2016.05.020 (2016).
55. Borja, B. P. et al. A dual function of SnRK2 kinases in the regulation of SnRK1 and plant growth. *Nat. Plants.* **6**, 1345–1353. https://doi.org/10.1038/s41477-020-00778-w (2024).
56. Dong, W. et al. The *Medicago truncatula* R2R3-MYB transcription factor gene MtMYBS1 enhances salinity tolerance when constitutively expressed in Arabidopsis thaliana. *Biochem. Biophys. Res. Commun.* **490**, 225–230. https://doi.org/10.1016/j.bbrc.2017.06.025 (2017).
57. Zhou, Y. F., Qi, J., Zhu, D. N. & Yu, B. Y. Homoisoflavonoids from *Ophiopogon japonicus* and Its oxygen free radicals (OFRs) scavenging effects. *Chin J. Nat. Med.* **6**, 201–204. https://doi.org/10.3724/SP.J.1009.2008.00201 (2008).
58. Wang, J. W. et al. Inhibition of glioma growth by flavokawain B is mediated through endoplasmic reticulum stress induced autophagy. *Autophagy.* **14**, 2007–2022. https://doi.org/10.1080/15548627.2018.1501133 (2018).
59. Sun, B. et al. Isorhamnetin inhibits HO-induced activation of the intrinsic apoptotic pathway in H9c2 cardiomyocytes through scavenging reactive oxygen species and ERK inactivation. *J. Cell Biochem.* **113**, 473–485. https://doi.org/10.1002/jcb.23371 (2012).
60. Ewas, M. et al. RNA-seq reveals mechanisms of SlMX1 for enhanced carotenoids and terpenoids accumulation along with stress resistance in tomato. *Sci Bull.* **67**, 476–485. https://doi.org/10.1016/j.scib.2017.03.018 (2017).
61. Khodakovskaya, M., Mcavoy, R., Peters, J., Wu, H. & Li, Y. Enhanced cold tolerance in transgenic tobacco expressing a chloroplast ω-3 fatty acid desaturase gene under the control of a cold-inducible promoter. *Planta.* **223**, 1090–1100. https://doi.org/10.1007/s00425-005-0161-4 (2006).
62. Mian, X., Zhang, L., Hu, X., Nan, S. & Fu, H. Cloning and functional analysis of the FAD2 gene family from desert shrub *Artemisia sphaerocephala*. *BMC Plant Biol.* **19**, 481. https://doi.org/10.1186/s12870-019-2083-5 (2019).
63. Matos, A. R. et al. Alternative oxidase involvement in cold stress response of *Arabidopsis thaliana* fad2 and FAD3+ cell suspensions altered in membrane lipid composition. *Plant Cell Physiol.* **48**, 856–865. https://doi.org/10.1093/pcp/pcm061 (2007).

Acknowledgements

We would like to thank Springer Nature for providing linguistic assistance for manuscript preparation.

Author contributions

All authors contributed to the conception and design of the study. Ming Jiang: Writing-original draft, Software, Investigation, Formal analysis & Funding acquisition. Yue Pan: Writing-review &editing. Kanchao Yu: Visualization, Software, Formal analysis. Yanshi Ma: Data collection. Ying Cui: Software, Formal analysis. Yang Liu: Formal analysis. Jicheng Liu: Formal analysis. Keyong Zhang: Resources. Hui Li: Supervision, Project administration, Methodology.

Funding

All the authors have reviewed and approved the manuscript and agree with its submission to Scitific Reports. Permission was granted for the collection of seeds from *Saposhnikovia divaricata (Turcz.)* Schischk. Sequence data that support the findings of this study have been deposited in NCBI with the primary accession code GSE276339. This work was supported by Academy Project of Qiqihar Medical University (no. QMSI2021M-13).

Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

This publication does not report any research involving human participants or animals conducted by the authors.

Informed consent
This article does not involve any informed consent.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-91094-w.

Correspondence and requests for materials should be addressed to H.L.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.