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Unveiling the molecular dynamics of low temperature preservation in postharvest lotus seeds: a transcriptomic perspective

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Abstract

Background Postharvest quality deterioration poses a significant challenge to the commercial value of fresh lotus seeds. Low temperature storage is widely employed as the primary method for preserving postharvest lotus seeds during storage and transportation.

Results This approach effectively extends the storage life of lotus seeds, resulting in distinct physiological changes compared to room temperature storage, including a notable reduction in starch, protein, H_2O_2, and MDA content. Here, we conducted RNA-sequencing to generate global transcriptome profiles of postharvest lotus seeds stored under room or low temperature conditions. Principal component analysis (PCA) revealed that gene expression in postharvest lotus seeds demonstrated less variability during low temperature storage in comparison to room temperature storage. A total of 14,547 differentially expressed genes (DEGs) associated with various biological processes such as starch and sucrose metabolism, energy metabolism, and plant hormone signaling response were identified. Notably, the expression levels of DEGs involved in ABA signaling were significantly suppressed in contrast to room temperature storage. Additionally, nine weighted gene co-expression network analysis (WGCNA)-based gene molecular modules were identified, providing insights into the co-expression relationship of genes during postharvest storage.

Conclusion Our findings illuminate transcriptional differences in postharvest lotus seeds between room and low temperature storage, offering crucial insights into the molecular mechanisms of low temperature preservation in lotus seeds.

Keywords Fresh lotus seed, Low temperature storage, RNA-sequencing, Gene expression, Molecular mechanism

Introduction

Lotus (*Nelumbo nucifera* Gaertn.) is an ancient aquatic herbaceous plant with a rich cultural, ornamental, medicinal, and culinary heritage. Widely cultivated in China, Japan, India, and other Southeast Asian countries. Lotus is classified into seed-, rhizome-, and flower-varieties based on agricultural purposes [1]. Lotus seeds, primarily harvested from seed lotus varieties, are valued for their high content of starch, protein, vitamins, polyphenols, alkaloids, flavonoids, and other bioactive compounds, making them a valuable resource for both medicinal and culinary purposes [2, 3]. There are two edible forms of

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lotus seeds: fresh and dried. Fresh lotus seed-based products are increasingly popular among consumers for their nutritious, sweet, fresh, and crispy characteristics.

Fresh lotus seeds are seasonal fruits, primarily harvested during the high-temperature season from July to September. The milk-ripe stage, occurring 13–16 days after bloom, is considered the optimal period for consuming fresh lotus seeds. However, the high moisture content in postharvest lotus seeds often leads to elevated levels of respiration, resulting in swift nutrient metabolism within the cotyledons. This rapid deterioration adversely affects the flavor and quality of fresh lotus seeds [4]. Therefore, effectively reducing respiration is a crucial measure for delaying the aging process of fresh lotus seeds. Furthermore, postharvest fresh lotus seeds are highly susceptible to browning due to their high content of phenolic compounds and oxidases [5, 6]. Browning is a direct indication of reduced quality in postharvest fresh lotus seeds, potentially impacting their marketability.

It has been reported that approximately 22% of fruits and vegetables go to waste due to postharvest spoilage [7]. Optimal postharvest handling measures, such as storage temperature and chemical or physical treatments, play a crucial role in extending the shelf life of fruits and vegetables. For example, emerging novel technologies such as high hydrostatic pressure and pulsed electric fields have shown effectiveness in reducing nutrient losses, preserving fruit freshness, and prolonging shelf life [8]. Additionally, chemical treatments have been proven effective in fruit preservation. Treatments involving exogenous methyl jasmonate (MeJA) and 6-benzylaminopurine (6-BA) have been shown to enhance antioxidant activity in postharvest fruits [9, 10].

Nowadays, a variety of technologies, encompassing both physical and chemical methods, have been employed to maintain the quality, nutritional value, and freshness of postharvest fresh lotus seeds. Treatment with exogenous 1-methylcyclopropene (1-MCP) has been shown to reduce the respiration rate of fresh lotus seeds, significantly extending their shelf life to 8 days at 25℃ [11]. Additionally, 6-BA treatment has demonstrated the ability to delay the onset of browning and mitigate postharvest senescence in lotus seeds by preserving the levels of sucrose and starch [12]. Low temperature stands out as the primary physical measure for preserving postharvest lotus seeds and is widely used in their storage, transportation, and marketing. By effectively inhibiting the respiration of postharvest fresh lotus seeds and delaying the occurrence of browning, low temperature preservation greatly preserves their nutrients and extends shelf life.

In recent years, RNA-sequencing (RNA-Seq) has emerged as a widely used tool for investigating the postharvest regulatory mechanisms of various fruits, including banana [13], blueberry [14], and grape [15], particularly in the context of low temperature storage. Additionally, it has been employed to elucidate the molecular changes occurring in postharvest fresh lotus seeds under room temperature storage conditions [4]. However, to date, no regulatory mechanisms have been identified for postharvest fresh lotus seeds during low temperature storage. In this study, we conducted RNA-Seq analysis to elucidate the molecular changes occurring in postharvest fresh lotus seeds under different storage temperatures. The findings of this research are expected to provide new insights into the preservation of postharvest fresh lotus seeds at low temperatures, thereby establishing a theoretical foundation for their low temperature preservation.

Materials and methods

Plant materials and treatment

The seed-lotus variety 'Jianxuan17' (JX17), cultivated in the experimental fields at Shimen Village (Xianning, China), was used for this study. Lotus seeds were harvested in August, precisely at 13 days after pollination (DAP). A double-layered culture chamber (HP300GS-2, Ruihua, China) with a photoperiod cycle of 16 h (h) light and 8 h of dark was used to expose lotus seeds to different temperatures. The temperature in the room condition was set at 28 ± 0.5℃, while the low temperature was 4 ± 0.5℃. Cotyledons were collected at intervals of 0, 24, 48, 72, and 96 h post-storage. They were promptly frozen in liquid nitrogen and stored at -80 °C for subsequent total RNA isolation, as well as physiological and biochemical index measurements (denoted 'N' and 'C' for lotus seeds stored at room temperature and low temperature, respectively). For each time point, three biological replicates were set, each comprising 15 lotus seeds.

RNA extraction and sequencing

Total RNA extraction from lotus seed cotyledons was conducted using the Plant Total RNA Isolation Kit (Beijing Zoman Biotechnology Co., Ltd., Beijing, China). Subsequently, 1 μg of RNA per sample was used for sequencing library preparation with the NEBNext UltraTM RNA Library Prep Kit for Illumina (NEB, USA). Following the quality assessment of the prepared libraries, cDNAs were sequenced by Biomarker Technologies Corporation (Beijing, China) using the Illumina platform. The subsequent analyses were based on clean data of high quality, and all clean data generated in this study are accessible at the National Center for Biotechnology Information (NCBI) under the accession number PRJNA1111767.

Transcriptome sequencing data analysis

Clean reads were obtained from the raw data by eliminating adaptor sequences, low-quality reads, and poly-Ns. Subsequently, they were aligned onto the lotus reference genome sequence 'China Antique'. Gene expression levels were normalized as fragments per kilobase of transcript per million fragments mapped (FPKM). Differentially expressed genes (DEGs) were identified using the DESeq2 R package applying two criteria: (i) genes with Fold Change (FC) ≥ 2, and (ii) False Discovery Rate (FDR) ≤ 0.01 [16]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment pathways were analyzed using the KOBAS 3.0 [17]. Transcription factors (TFs) were identified using the PlantTFDB (v5.0) database (http://planttfdb.gao-lab.org/). The expression patterns of DEGs were visualized via Heatmap using TBtools software [18].

Weighted gene co-expression network analysis (WGCNA)

WGCNA was conducted using the programs available on the BMKCloud (http://www.biocloud.net) to generate clusters of highly correlated genes. All DEGs identified in this study were included in the WGCNA analysis. The power estimate parameter was set to 16. Hierarchical clustering and module division were conduct using the dynamic tree cut algorithm. The minimum module size was set to 50, and modules with a similarity threshold less than 0.25 were merged together.

Measurement of starch, protein, hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) content

The starch, protein, H_2O_2, and MDA content of the lotus cotyledons were assessed utilizing the Starch Content Assay Kit (BC0705, Solarbio, Beijing, China), Bradford Protein Assay Kit (PC0010, Solarbio, Beijing, China), Malondialdehyde Content Assay Kit (BC0025, Solarbio, Beijing, China), and Hydrogen Peroxide Quantitative Analysis Kit (water compatibility) (C500069, Sangon Biotech, Shanghai, China), respectively. The methods employed followed the manufacturer's instructions in detail.

Total RNA isolation and qRT-PCR analysis

Total RNA from lotus seed cotyledon was isolated using the RNAprep Pure Plant Kit (Tiangen Biotech, Beijing, China). For cDNA synthesis, the TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (Lot#M31212, Beijing TransGen Biotech Co., Ltd., Beijing, China) was employed. The qRT-PCR analysis was conducted on the StepOnePlus Real-time PCR System (Applied Biosystems, USA), following the previously described protocol [2]. The *NnACTIN* (Gene ID Chr01.g02496) gene was used as an internal reference gene. The gene-specific primers used in this study can be found in Table S1.

Statistical analysis

Statistical analysis in this study was conducted using the One-way ANOVA test and the least significant difference (LSD) test, utilizing Statistix 8 (Analytical Software, Version 8.0).

Results

Physiological changes in fresh lotus seeds during storage at different temperatures

To evaluate the impact of temperature on lotus seed storage, freshly harvested lotus seeds were placed in chambers set at room temperature and low temperature conditions, respectively. Over time, both sets of seeds exhibited browning (Fig. 1A). Browning began noticeably at 24 h in the room temperature condition, with subsequent shriveling and decay by 48 h. In contrast, seeds stored at low temperature showed delayed browning, shriveling, and decay. Physiological differences were also evident between seeds stored at room temperature condition and those at low temperature (Fig. 1B). Starch and protein content notably increased in cotyledons of seeds stored at room temperature over time, while showing slight increases at low temperature. Additionally, normal temperature condition led to greater accumulation of H_2O_2 and MDA. Conversely, H_2O_2 production in seeds stored at low temperature remained significantly lower throughout the storage period compared to those at room temperature. Furthermore, MDA content in cotyledons of seeds stored at room temperature condition from 24 to 96 h exceeded that of seeds stored at low temperature.

RNA-seq analysis of postharvest lotus seed

To delve into the molecular mechanisms underlying lotus seed storage at varying temperatures, RNA-seq analysis was conducted on cotyledons stored at room temperature and low temperature conditions for 0, 24, 48, 72, and 96 h. In total, 27 RNA libraries were generated, yielding 1.22 billion clean reads totaling 183.23G after sequencing data underwent quality control (Table S2). The average GC content and Q30 value was 46.37% and 93.44%, respectively, with 96.99% of clean reads successfully mapped to the reference genome. Moreover, these clean reads were assembled into 26,889 genes, with 2,967 identified as novel genes.

To ensure the reliability and consistency of the RNA-seq data, principal component analysis (PCA) was performed on all samples. PC1 and PC2 accounted for

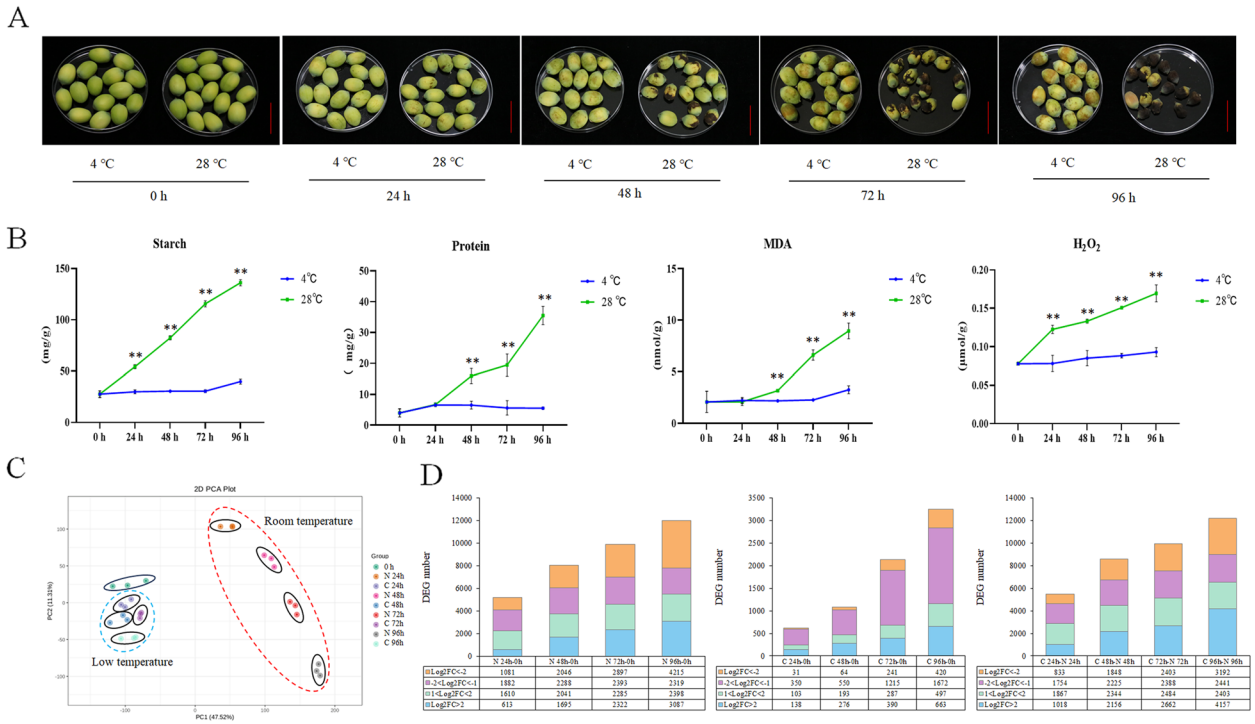

Fig.1 Physiological changes and DEGs identification in postharvest lotus seeds during low and room temperature storage. **A** Appearance of lotus seeds stored at low temperature and room condition after 0, 24, 48, 72, and 96 h. Bar = 3 cm. **B** Contents of starch, protein, MDA, and H_2O_2, and RWC in postharvest lotus seeds. Bars represent means ± SD ($n = 3$), $^*P < 0.05$, $^{**}P < 0.01$. **C** PCA analysis among different samples based on the transcriptomic profiles. **D** DEGs statistics in different comparison groups

47.52% and 11.31% of the total variance, respectively (Fig. 1C). Each treatment group at every time point exhibited close clustering among three biological replicates, displaying strong Pearson correlation coefficients ($r > 0.9$) (Fig. S1). Notably, a distinct segregation was observed between lotus seeds stored at room temperature and low temperature conditions. Furthermore, storage at the room temperature condition, samples displayed differentiation along PC2 across various time points.

Identification of DEGs

Two storage temperatures of lotus seeds induced differences in the number of DEGs. A total of 5,186 (2,223 up-regulated and 2,963 down-regulated), 8,070 (3,736 up-regulated and 4,334 down-regulated), 9,897 (4,607 up-regulated and 5,290 down-regulated), and 12,019 (5,485 up-regulated and 6,534 down-regulated) DEGs were identified, respectively, in the 24 h-0 h, 48 h-0 h, 72 h-0 h, and 96 h-0 h comparison groups in lotus seed cotyledons stored at room temperature condition (Fig. 1D; Fig. S2A). Meanwhile, 3,708 DEGs were shared among the four comparison groups (Fig. S3A). During storage at low temperature, the number of DEGs with respect to storage for 0 h reached 622 (241 up-regulated and 381 down-regulated), 1,083 (469 up-regulated and 614 down-regulated), 2,133 (677 up-regulated and 1,456 down-regulated), and 3,252 (1,160 up-regulated and 2,092 down-regulated) after storage for 24 h, 48 h, 72 h, and 96 h, respectively (Fig. 1D; Fig. S2B). A total of 439 common DEGs were detected among the four comparison groups (Fig. S3B). Additionally, a total of 14,547 DEGs were identified between room temperature and low temperature at each time point (Fig. 1D). Among these, 2,885, 4,500, 5,146, and 6,560 up-regulated DEGs, and 2,587, 4,073, 4,791, and 5,636 down-regulated DEGs were identified between room temperature and low temperature after storage for 24 h, 48 h, 72 h, and 96 h (Fig. S2C; Fig. S3C). Furthermore, 3,608 DEGs were identified in all four comparison groups, and the expression change of DEGs in these groups were mainly over four times. To evaluate the reliability of the RNA-seq results, 10 DEGs were randomly selected for qRT-PCR analysis. Consistent expression patterns were observed, with a high Pearson's correlation coefficient ($r = 0.79$) indicating strong linear regression (Fig. S4).

K-means clustering analysis was conducted to unveil the diversity in the expression profiles of DEGs identified between room condition and low temperature at the same storage time point. The temporal expression

patterns of a total of 14,547 DEGs were mainly clustered into nine modules, which contained 849, 2,453, 755, 4,519, 794, 855, 1,423, 740, and 2,159 DEGs from clusters I to IX, respectively (Fig. 2A, Table S3). Among them, the most DEGs (4,519) were displayed in cluster IV, showing significant expression changes with down-regulation during storage at room condition but up-regulation during storage at low temperature throughout the postharvest storage at each time point. To further investigate the molecular differences of lotus seeds under different storage conditions between room temperature and low temperature, KEGG enrichment analysis was performed on the DEGs in each of the clusters. As shown in Fig. 2B, DEGs enriched in metabolic pathways and biosynthesis of secondary metabolites pathways were widely identified in clusters I, IV, V, VII, and VIII. Moreover, numerous biological pathways were uniquely enriched in the DEGs from cluster IV, such as carbon metabolism, the TCA cycle, pyruvate metabolism, and other metabolism pathways. In addition, DEGs enriched in starch and sucrose metabolism pathways were detected in clusters IV and VIII.

Functional annotations and classifications

To elucidate the biological pathways associated with the DEGs between lotus seeds stored at room temperature and low temperature, we conducted KEGG pathway enrichment analysis. Within the KEGG category, ten pathways emerged as significantly enriched, encompassing metabolic pathway, biosynthesis of secondary metabolites, glycerolipid metabolism, carbon metabolism, amino sugar and nucleotide sugar metabolism, glycolysis/gluconeogenesis, flavonoid biosynthesis, starch and sucrose metabolism, biosynthesis of amino acids, and alanine, aspartate, and glutamate metabolism across all comparison groups (Fig. S5A). Notably, several additional pathways were enriched exclusively in comparison groups featuring up-regulated DEGs. Alongside the aforementioned pathways, fructose and mannose metabolism, and the citrate cycle (TCA cycle) were also significantly enriched (Fig. S5B). Furthermore, down-regulated DEGs were predominantly associated with metabolic pathway, MAPK signaling pathway, and plant hormone signal transduction terms (Fig. S5C).

Starch and sucrose metabolism

Significant differences in starch content were observed in lotus seeds during storage at room temperature and low temperature, with numerous DEGs enriched in the starch and sucrose metabolism pathway across all time points. To elucidate the underlying mechanism, the starch and sucrose metabolism pathways of lotus seeds stored at different temperatures were analyzed (Fig. 3). As a result, we identified five (Chr01.g01261, Chr02.g08022, Chr03.g13296, Chr05.g20750, and Chr08.g26173), one (Chr01.g00222), six (Chr02.g09801, Chr03.g14261, Chr04.g16687, Chr04.g16688, Chr04.g17322, and Chr07.g24289), three (Chr01.g05869, Chr02.g09480, and Chr08.g25546), five

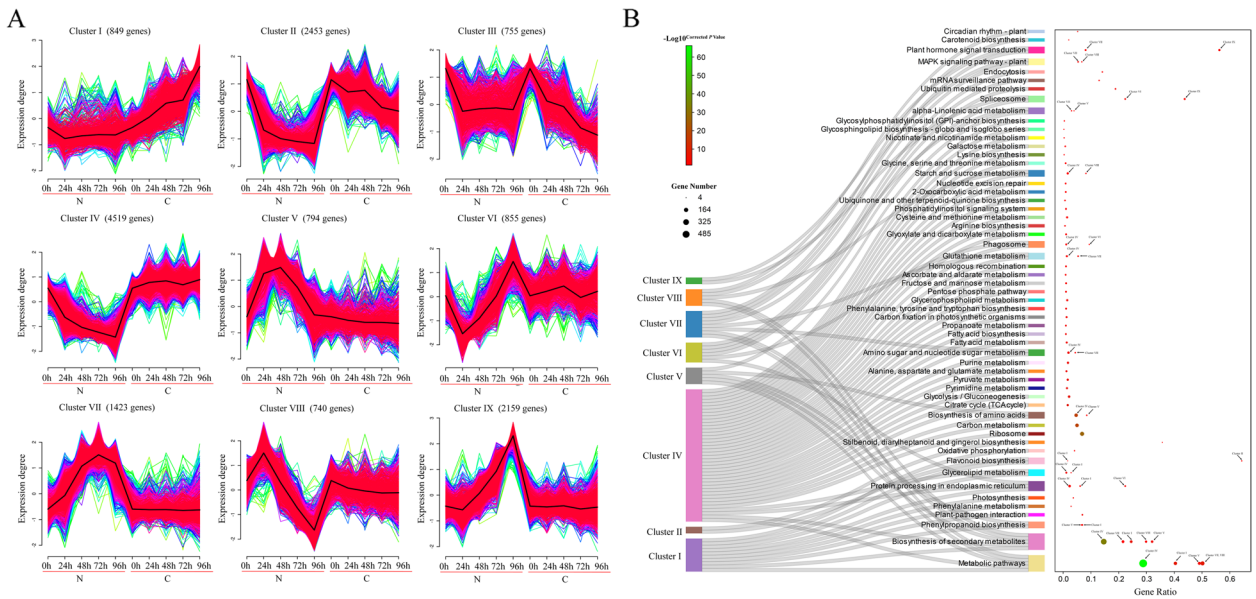

Fig.2 Cluster and functional enrichment analysis of DEGs between low temperature and room temperature storage. **A** Cluster analysis of 14,547 DEGs using the K-means method. In the panels, the black lines represent the consensus of all gene expressions in each subcluster. **B** KEGG analysis of DEGs in each cluster. The black arrow indicates the cluster where the term is located. 'N' and 'C' represent lotus seeds stored at room temperature and low temperature, respectively

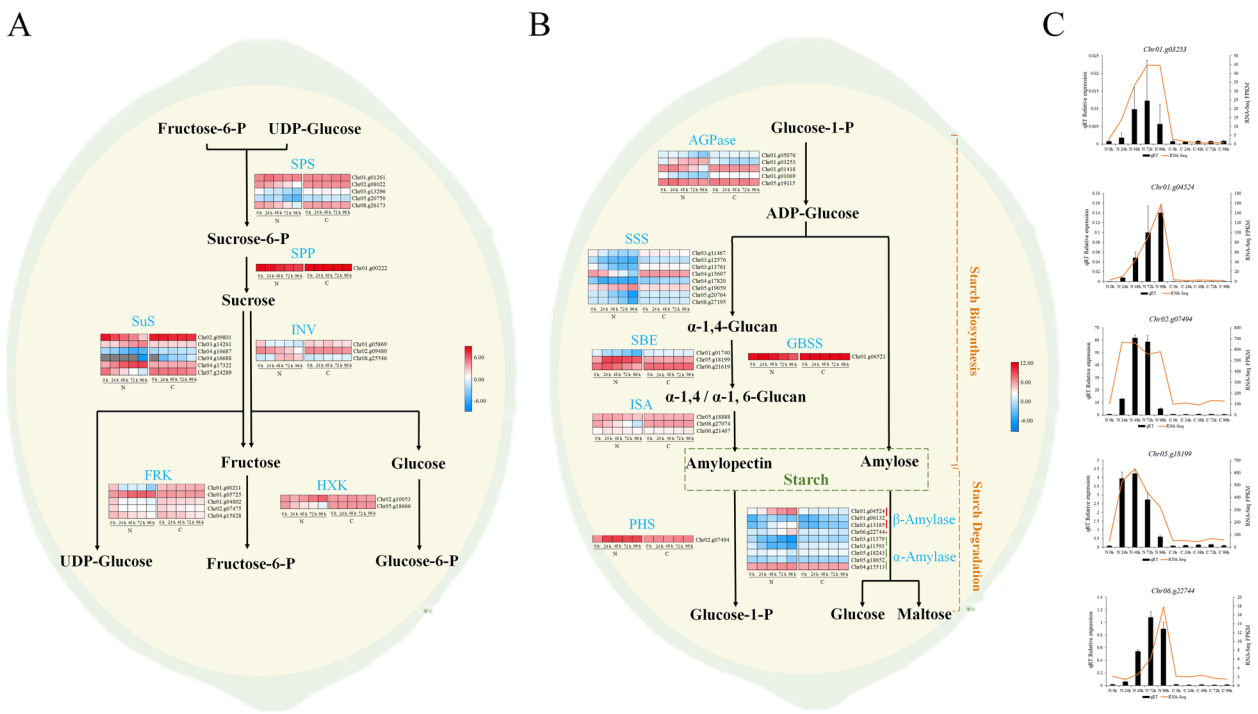

Fig.3 DEGs in the sucrose (**A**) and starch (**B**) metabolism pathways. **C** Expression analysis of five DEGs related to starch metabolism by qRT-PCR. SPS, sucrose phosphate synthase; SPP, sucrose phosphate phosphatase; SuSy, sucrose synthase; INV, invertase; FRK, fructokinase; HXK, hexokinase; AGPase, ADP-Glc pyrophosphorylase; SSS, soluble starch synthase; GBSS, granule bound starch synthase; SBE, starch branching enzyme; ISA, isoamylase; PHS, alpha-glucan phosphorylase. 'N' and 'C' represent lotus seeds stored at room temperature and low temperature, respectively

(Chr01.g00211, Chr01.g05725, Chr01.g04802, Chr02.g07475, and Chr04.g15828), and two DEGs (Chr02.g10053 and Chr05.g18666) encoding key enzymes in plant sucrose catabolism, including sucrose phosphate synthase (SPS), sucrose phosphate phosphatase (SPP), sucrose synthase (SuSy), invertase (INV), fructokinase (FRK), and hexokinase (HXK), respectively (Fig. 3A). Compared to lotus seeds stored at room temperature condition, 82% (18/22) of the DEGs identified in the sucrose metabolism pathway were up-regulated during storage at low temperature (Table S4). Notably, the expression of Chr02.g08022, Chr01.g00222, Chr01.g00211, and Chr05.g18666 showed continuous increases in lotus seeds throughout the entire postharvest storage period at low temperature.

Thirty DEGs involved in starch metabolic processes were identified, including ADP-Glc pyrophosphorylase (AGPase), soluble starch synthase (SSS), granule-bound starch synthase (GBSS), starch branching enzyme (SBE), isoamylase (ISA), alpha-glucan phosphorylase (PHS), alpha-amylase, and beta-amylase (Fig. 3B, Table S4). Compared to storage at room condition, most DEGs involved in starch biosynthesis exhibited a similar up-regulated expression pattern in postharvest lotus seeds stored at low temperature. Notably, Chr01.g03253, Chr05.g19059, and Chr05.g18199, encoding AGPase, SSS, and SBE, respectively, were continuously and significantly down-regulated throughout the postharvest storage period at low temperature. DEGs involved in starch degradation, such as PHS, alpha-amylase, and beta-amylase, showed varied expression patterns. Chr01.g04524, Chr03.g13185, and Chr06.g22744, encoding β-amylase, and Chr02.g07494, encoding PHS, exhibited up-regulated expression patterns during storage at room condition. Meanwhile, Chr03.g11591 and Chr05.g18243, encoding α-amylase, showed down-regulated expression patterns during storage at room temperature condition. Additionally, the expression of five DGEs involved in the starch metabolism process was verified by qRT-PCR, confirming a similar expression pattern between RNA-Seq and qRT-PCR (Fig. 3C).

Glycolysis and tricarboxylic acid cycle

Carbohydrate metabolism plays a pivotal role in providing energy for plant function, which is crucial for preserving the postharvest quality of fruits. Here, we identified fifty-five DEGs encoding key enzymes involved in the glycolysis (EMP) pathway (Fig. S6A, Table S5). Gene expression analysis revealed a consistent trend, with the majority of DEGs exhibiting up-regulated expression in lotus seeds stored at low temperature compared to

those at room temperature. In the tricarboxylic acid cycle (TCA) pathway, we identified forty-one DEGs encoding key enzymes (Fig. S6B, Table S5). All DEGs encoding aconitase (ACO), isocitrate dehydrogenase (IDH), oxoglutarate dehydrogenase (ODC), succinyl-CoA ligase (SCoAL), succinate dehydrogenase (SDH), and fumarase (FUM) showed continuous up-regulation in lotus seeds stored at low temperature compared to those stored at room temperature throughout the postharvest period. Additionally, Chr01.g06187 and Chr08.g25719, encoding malic enzyme (ME), Chr03.g13879 encoding citrate synthase (CSY), novel.1226 encoding IDH, and Chr04.g15011 and Chr05.g19479 encoding malate dehydrogenase (MDH), exhibited up-regulated expression patterns in lotus seeds during storage at room temperature condition.

Antioxidant system and MAPK cascade signaling pathway

As previously noted, the H_2O_2 content significantly increased in postharvest lotus seeds during storage at room temperature, leading to elevated levels of MDA. Conversely, these effects were effectively mitigated under low temperature storage condition. Respiratory burst oxidase homologs (RBOHs) are recognized as key ROS producers in plants. Here, we identified six DEGs encoding RBOHs, exhibiting diverse expression patterns (Fig. 4A, Table S6). Notably, Chr01.g04322 and novel.467 were consistently up-regulated in lotus seeds throughout the postharvest period at room condition, while Chr01.g05438, novel.848, and novel.856 exhibited up-regulation in lotus seeds stored at low temperature. Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) are pivotal antioxidant enzymes in the defense against ROS, as identified in this study (Fig. 4A, Table S6). In comparison to storage at room temperature condition, five DEGs (Chr02.g09208, Chr02.g10193, Chr04.g16705, Chr06.g22094, and Chr06.g22557) encoding SOD, and four DEGs (Chr01.g00063, Chr02.g06909, Chr02.g06998, and Chr05.g20026) encoding APX were identified, displaying up-regulated expression patterns throughout the postharvest period at low temperature. POD participates in the direct reduction of hydrogen peroxide to water and oxygen. Here, twenty-five DEGs encoding POD were identified, demonstrating varied expression patterns in postharvest lotus seeds under different temperatures. Seventeen DEGs were up-regulated during storage at low temperature, with ten DEGs (Chr01.g02556, Chr01.g04183, Chr03.g10875, Chr03.g11258, Chr04.g15393, Chr05.g18501, Chr05.g18865, Chr05.g18886, Chr05.g19352, and novel.292) continuously up-regulated throughout the low temperature storage period.

The MAPK cascade signaling pathway plays a crucial role in regulating plant growth, development, and stress responses, and it has also been found to be involved in ROS homeostasis [19, 20]. In this study, numerous DEGs associated with the MAPK signaling pathway

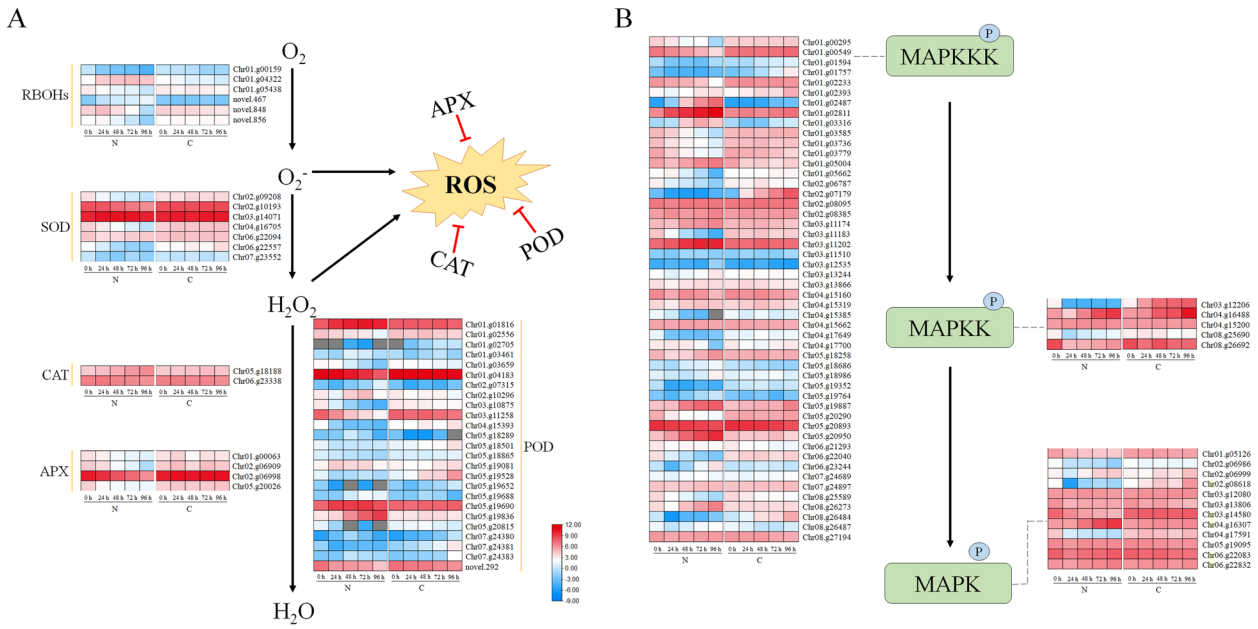

Fig. 4 Expression patterns of DEGs involved in ROS metabolism (**A**) and MAPK cascade signaling transduction (**B**) pathways. RBOH, respiratory burst oxidase homologue; SOD, superoxide dismutase; CAT, catalase; POD, peroxidase; APX, ascorbate peroxidase. 'N' and 'C' represent lotus seeds stored at room temperature and low temperature, respectively

were identified in postharvest lotus seeds. A total of fifty DEGs encoding MAPKKK, five encoding MAPKK, and twelve encoding MAPK proteins were identified (Fig. 4B, Table S7). Among these, twenty MAPKKK, four MAPKK, and five MAPK were significantly up-regulated in postharvest lotus seeds at low temperature compared to those stored at room condition.

Plant hormone biosynthesis and signal transduction

Plant hormones play crucial roles in the process of plant growth and development. Gene Ontology results from this study revealed that many DEGs identified in postharvest lotus seeds were grouped into categories related to abscisic acid (ABA), ethylene, auxin, jasmonic acid (JA), gibberellic acid (GA), and brassinosteroid signaling pathways, respectively (Fig. 5A, Fig. S7). Additionally, a majority of the DEGs were associated with pathways related to the ABA signaling pathway in each comparison group of lotus seeds stored at low temperature vs. room temperature conditions. Specifically, one, three, two, and five DEGs encoding zeaxanthin epoxidase (ZEP), 9-cis epoxycarotenoid dioxygenase (NCED), ABA deficient 2 (ABA2), and abscisic aldehyde oxidase (AAO), respectively, were identified (Fig. 5B, Table S8). More DEGs involved in the ABA biosynthesis process exhibited up-regulated expression in postharvest lotus seeds stored at room temperature condition. Notably, DEGs such as Chr03.g13637 and Chr02.g10466, encoding NCED and AAO, respectively, displayed continuous and significant up-regulation throughout the storage period at room temperature. Furthermore, the ABA signaling transduction pathway was activated in postharvest lotus seeds stored at room temperature (Fig. 5C, Table S8). Seven DEGs encoding PYR/PYL were identified, with six of them showing up-regulation at room condition. Additionally, five DEGs encoding phosphatase 2C (PP2C) were significantly up-regulated at room condition. Chr01.g06110, encoding ABA-activated SNF1-related protein kinases 2 (SnRK2), was significantly up-regulated in postharvest lotus seeds at room condition after 48 h. Moreover, seven DEGs encoding downstream ABA-responsive TFs of AREBs/ABFs were identified, with three (Chr02.g09431, Chr05.g18672, and Chr05.g19723) of them significantly up-regulated at room condition.

Identification of differentially expressed transcription factors

TFs play pivotal roles in gene expression regulation, exerting significant influence on fruit physiology during postharvest storage. In this study, a total of 345, 1,046, and 1,080 differentially expressed TFs were identified in the low temperature, room temperature, and low temperature vs. room temperature comparison groups, respectively (Fig. 6A). These TFs were categorized into various families, with a notable abundance observed in the ERF, bHLH, and MYB families (Fig. 6B, Fig. S8). To elucidate the transcriptional regulatory mechanisms in lotus seeds during storage at low temperature, we scrutinized the expression patterns and putative functions of the differentially expressed TFs.

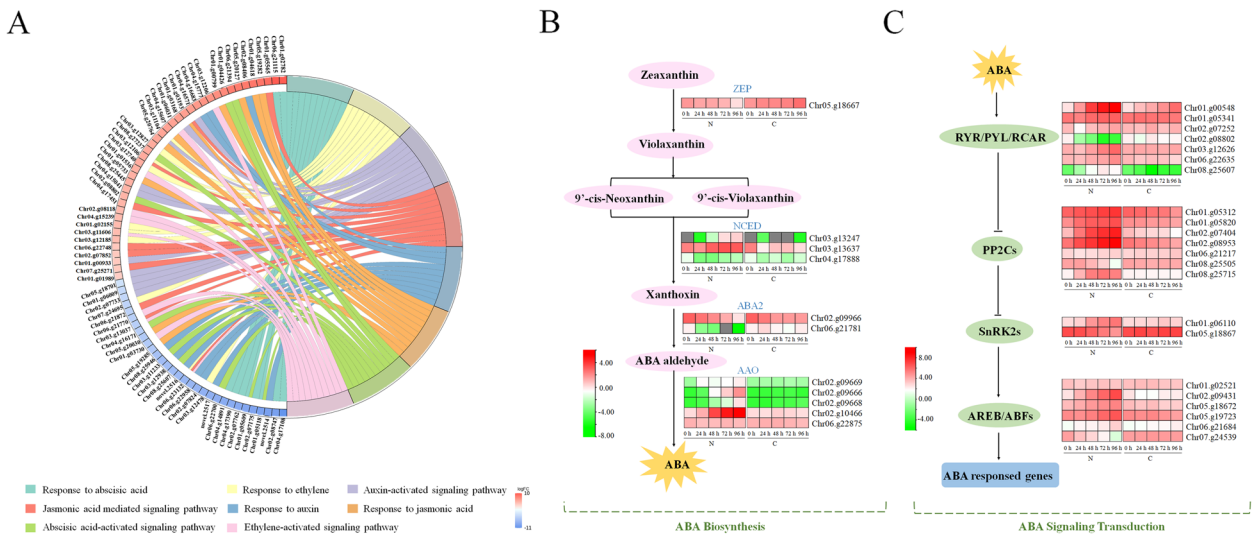

Fig. 5 DEGs related to plant hormone signaling pathways. **A** Chord diagrams display plant hormone response DEGs in lotus seeds at postharvest 96 h under storage temperatures between low temperature and room temperature condition. **B**-**C** Represent the DEGs involved in ABA biosynthesis and signaling transduction pathways, respectively. 'N' and 'C' represent lotus seeds stored at room temperature and low temperature, respectively

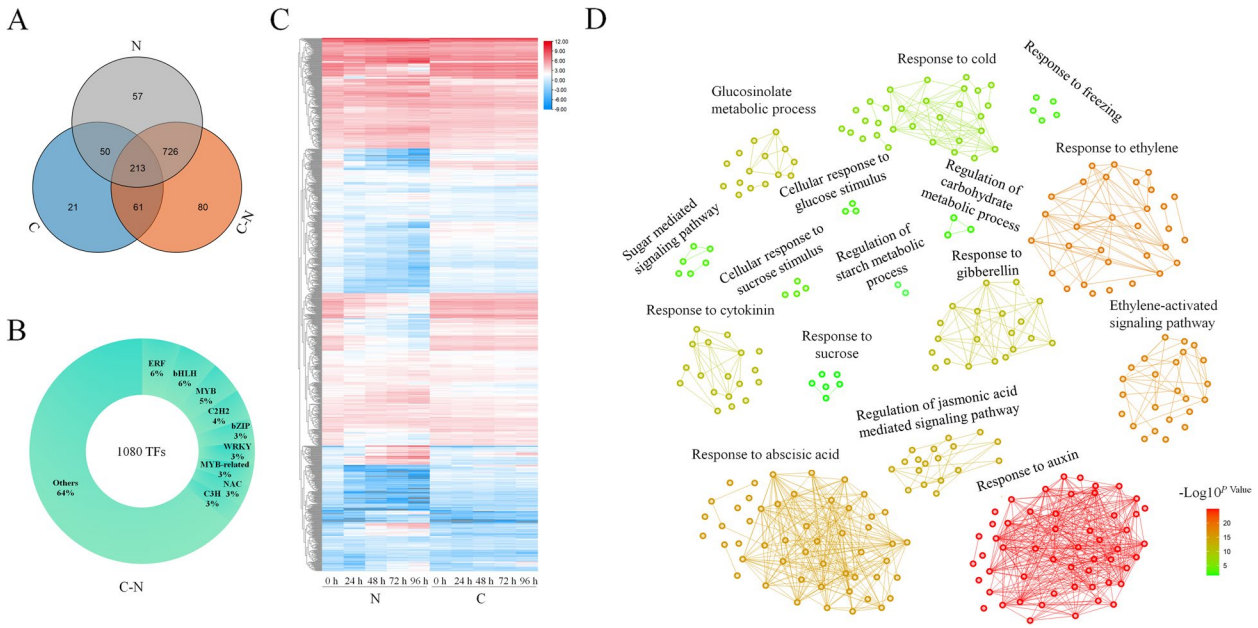

Fig. 6 Analysis of differentially expressed transcription factors (TFs) under different storage temperatures. **A** Venn diagram illustrating differentially expressed TFs in lotus seeds at different storage temperatures. **B** Proportions of differentially expressed TFs in lotus seeds under storage temperatures between low temperature and room temperature condition. **C** Expression patterns of differentially expressed TFs under storage temperatures between low temperature and room temperature. **D** Functional enrichment analysis of differentially expressed TFs. Solid lines represent correlation relationships between two TFs. 'N' and 'C' represent lotus seeds stored at room temperature and low temperature, respectively

In contrast to storage at room temperature, TFs from diverse families exhibited varying expression profiles in postharvest lotus seeds preserved at low temperature (Fig. 6C), with a majority displaying down-regulation during the storage period at low temperature. For instance, expression levels of 26 MYB genes, 34 ERF genes, and 30 bHLH genes decreased during storage at low temperature, while expression of 21 MYB genes, 23 ERF genes, and 27 bHLH genes increased (Fig. S8). Gene Ontology analysis revealed that a significant portion of the differentially expressed TFs were enriched in metabolic processes, cold response, and phytohormone signal transduction pathways (Fig. 6D), with strong co-expression relationships observed among TFs within each cluster. Notably, TFs associated with metabolic processes include Chr03.g12925 and Chr04.g16569, encoding members of the bZIP and C2H2 TF families, respectively. Correlation analysis unveiled 814 and 3,312 co-expressed genes associated with Chr03.g12925 and Chr04.g16569, respectively. Additionally, 23 and 62 co-expressed genes were implicated in starch and sucrose metabolism, as well as carbon metabolism (Fig. S9). Furthermore, homologous genes to Chr03.g12925 and Chr04.g16569 in *Arabidopsis*, *AtBZIP63* (AT5G28770) [21] and *AtIDD14* (AT1G68130) had been found to be involved in sugar and starch metabolism process [22].

Gene co-expression network of postharvest lotus seed during storage

Co-expression network analysis is used to predict functional modules and gene functions, aiding in the investigation of the regulation mechanisms of lotus seeds during postharvest storage at different temperature conditions. Through hierarchical clustering dendrograms and cluster heatmaps of DEGs, nine co-expression gene modules were identified (Fig. 7A, B). For instance, 454 DEGs clustered into the "Green" module were down-regulated during postharvest storage at low temperature from 24 to 72 h. The "Blue" module, containing 2,306 DEGs, represented genes down-regulated during postharvest storage at low temperature from 48 to 96 h. Conversely, the "Black" module, comprising 302 DEGs, represented genes up-regulated during postharvest storage at low temperature from 24 to 72 h (Fig. S10). Additionally, the Brown module (containing 1,571 DEGs) and "Turquoise" module (containing 12,736 DEGs) represented genes continuously down- and up-regulated throughout the postharvest storage period at low temperature, respectively (Fig. 7C). Furthermore, most DEGs from these two modules were enriched in pyruvate metabolism, starch and sucrose metabolism, and glyoxylate and dicarboxylate metabolism processes (Fig. 7D). For instance, DEGs such as Chr01.g03253 (encoding large AGPase subunit) and Chr04.g17322 (encoding sucrose

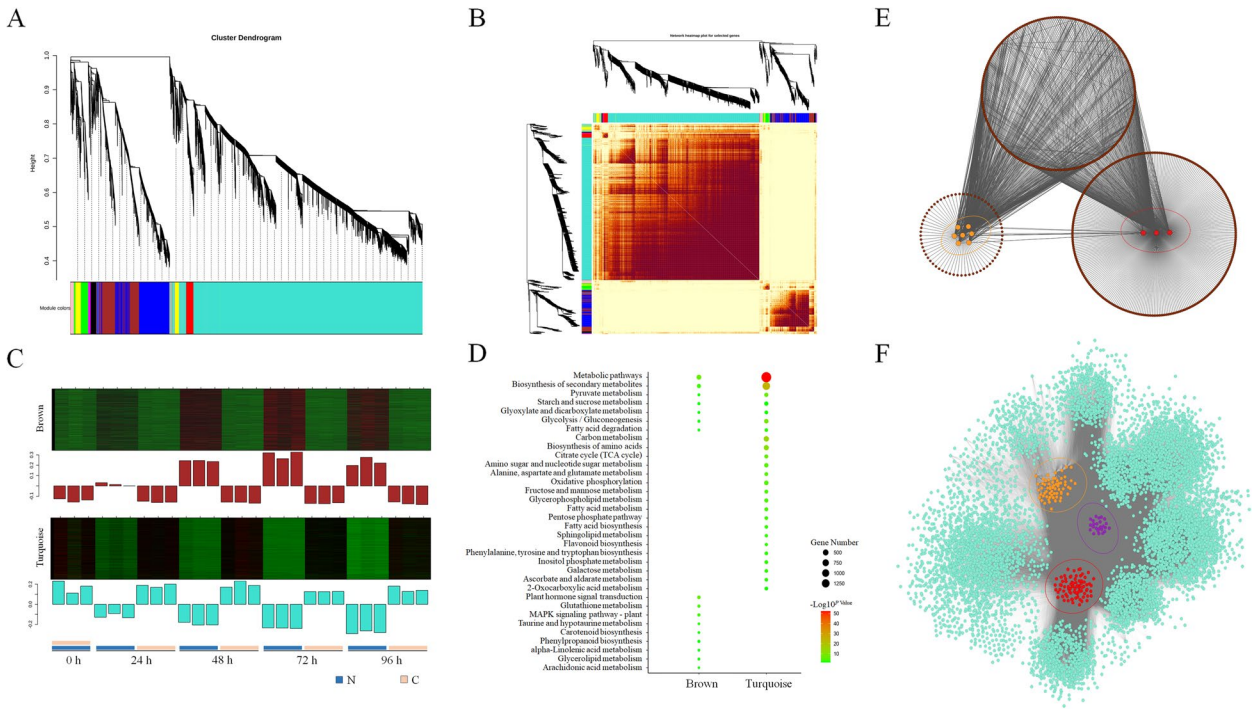

Fig. 7 WGCNA for the DEGs identified in lotus seeds during storage at low temperature compared to room temperature. **A** Identification of co-expression modules using a hierarchical clustering tree. Nine co-expression gene modules are indicated with different colors. **B** Heatmap displaying the topological overlap matrix of 1,000 randomly selected DEGs from each co-expression module. **C** Expression patterns of DEGs in the "Brown" and "Turquoise" co-expression modules. **D** KEGG enrichment analysis of DEGs in the "Brown" and "Turquoise" co-expression modules. **E**–**F** Represent the co-expression networks of DEGs associated with material and energy metabolism processes in the "Brown" and "Turquoise" modules, respectively. DEGs enriched in sucrose and starch metabolism processes, TCA cycle, and those involved in both processes are colored in yellow, red, and purple, respectively. Brown and turquoise nodes represent other DEGs in the "Brown" and "Turquoise" modules, respectively. 'N' and 'C' represent lotus seeds stored at room temperature and low temperature, respectively

synthase) involved in sucrose and starch metabolism processes were identified in the "Brown" module. Similarly, DEGs like Chr01.g05409 (encoding pyruvate kinase), Chr04.g17294 (encoding fumarate hydratase), Chr01.g05770, and Chr02.g09311 (encoding enolase) related to glyoxylate and dicarboxylate metabolism processes clustered into the "Turquoise" module. Moreover, a series of DEGs in the Turquoise and Brown modules, associated with starch and sucrose metabolism processes, and the citrate cycle, exhibited high correlation with other DEGs (Fig. 7E, F), suggesting their involvement in the postharvest preservation process of lotus seeds at low temperature.

Discussion

Storage temperature affect the quality of postharvest fresh lotus seeds

Fresh lotus seeds are seasonal fruits cherished by consumers for their crisp, tender, and sweet taste. High-quality lotus seeds boast elevated moisture and soluble sugar levels, coupled with reduced starch, alkaloid, and hardness [23]. However, postharvest lotus seeds are prone to quality deterioration, marked by increasing starch, protein, and H_2O_2 contents, and decreasing soluble sugar content, posing significant challenges to their storage and transportation [4]. Low-temperature storage stands out as a simple yet highly effective method for fruit preservation, effectively curtailing enzymatic activities across various metabolic pathways, particularly post-harvest respiration [24, 25]. In this study, we observed markedly lower starch and protein accumulation in postharvest lotus seeds stored at low temperatures compared to room temperature throughout the storage period (Fig. 1B). Consequently, reduced protein levels suggest subdued metabolic activity in fresh lotus seeds stored at low temperatures. Furthermore, the negative correlation between starch and sucrose content implies that low-temperature storage preserves the superior flavor and taste of postharvest fresh lotus seeds. In addition, the accumulation of Reactive Oxygen Species (ROS) can cause severe damage to the membrane system, leading to rapid senescence of postharvest fruit [26]. The MDA content serves as an indicator to assess the extent of cell damage [14]. Here, we found that low-temperature storage effectively delays

the browning of postharvest fresh lotus seeds (Fig. 1A), with significantly decreased H_2O_2 and MDA contents (Fig. 1B), indicating minimal damage to the membrane structure of postharvest fresh lotus seeds during low temperature storage. Overall, our findings further underscore that low temperature reduces the metabolic activity of fresh lotus seeds, leading to diminished starch, protein, H_2O_2, and MDA contents, effectively preserving the quality of fresh lotus seeds post-harvest.

To delve into the postharvest preservation mechanism of fresh lotus seeds during low-temperature storage, we conducted deep transcriptome sequencing of postharvest fresh lotus seeds stored at different temperatures. A total of 14,547 DEGs were identified in lotus seeds between low-temperature and room-temperature storage, with 4,519 DEGs up-regulated during low-temperature storage (Fig. 1D). Numerous DEGs were implicated in metabolic pathways, notably starch and sucrose metabolism, glutathione metabolism, and carbon metabolism (Fig. 2B). Additionally, DEGs associated with plant hormone signal transduction, phenylpropanoid biosynthesis, and flavonoid biosynthesis were identified based on enrichment results. Altogether, our study sheds light on key mechanisms underlying the low-temperature preservation of postharvest fresh lotus seeds at the transcriptional level.

Effect of storage temperature on material and energy metabolism of postharvest fresh lotus seeds

Sugars and starch serve as crucial indicators for assessing the quality and flavor of fresh lotus seeds. Sucrose, a key component, is synthesized through the sequential actions of two enzymes. Sucrose-phosphate synthase (SPS) acts as the rate-limiting enzyme in sucrose synthesis in plants, catalyzing the formation of sucrose-6-phosphate [27]. In this study, five DEGs encoding SPS (Chr01.g01261, Chr02.g08022, Chr03.g13296, Chr05.g20750, Chr08.g26173) were identified, all of which were up-regulated in fresh lotus seeds during low-temperature storage (Fig. 3A). Additionally, Chr01.g00222, encoding sucrose-6-phosphate phosphatase, was up-regulated during low-temperature storage, potentially contributing to the regulation of sucrose biosynthesis flux [28]. Sucrose breakdown in plants is facilitated by invertases (INV) and sucrose synthase (SuSy) [29], which exhibited diverse expression profiles in postharvest fresh lotus seeds under different temperature storage conditions. For instance, Chr02.g09801 and Chr07.g24289, encoding SuSy, were up-regulated during low-temperature storage, whereas the expression of Chr03.g14261 and Chr04.g17322 was suppressed. Three DEGs encoding INV were also identified, with Chr02.g09480 and Chr01.g05869 up-regulated during low-temperature storage, while Chr08.g25546 exhibited down-regulated expression (Fig. 3A), potentially priming subsequent metabolic activities during postharvest storage at different temperatures.

AGPase, SSS, SBE, GBSS, SBE, and ISA are key enzymes involved in starch biosynthesis, with their transcript abundances increasing during the starch biosynthesis process in lotus seeds [30, 31]. In this study, genes related to starch biosynthesis exhibited diverse expression patterns during postharvest storage at low and room temperatures (Fig. 3B). Interestingly, a series of starch biosynthesis-related genes were induced during the early postharvest storage period at room temperature. For example, Chr01.g05076 and Chr01.g01418, encoding AGPase, Chr01.g06521, encoding GBSS, and Chr06.g21619, encoding SBE, were up-regulated at 24 h post-harvest during room temperature storage. Moreover, Chr05.g19115, encoding AGPase, Chr05.g18888, and Chr06.g21467, encoding ISA, were up-regulated after 48 h of room temperature storage. Additionally, enzyme activities of AGPase, SuSy, SSS, and SBE were increased at room temperature in postharvest lotus seeds [4], partially explaining the increased starch content in lotus seeds during postharvest storage at room temperature.

Membrane damage occurs in fruits and vegetables during postharvest low-temperature storage, often associated with depleted energy status [32, 33]. Previous studies have indicated that high levels of ATP content and energy charge could mitigate damage in postharvest fruits caused by low-temperature storage [34]. The Embden-Meyerhof-Parnas (EMP) and tricarboxylic acid (TCA) pathways are two major metabolic pathways that provide sufficient energy for plant life activities. Our study revealed significant induction of the EMP and TCA pathways in lotus seeds during postharvest low-temperature storage (Fig. S6), potentially generating more energy to alleviate damage induced by low temperatures. Similarly, intermediates produced by the TCA pathway may accumulate significantly and be used as substrates for gluconeogenesis [24, 35], effectively preserving the flavor of postharvest lotus seeds during low-temperature storage by promoting glucose biosynthesis.

Effect of storage temperature on the homeostasis of antioxidant system of postharvest fresh lotus seeds

H_2O_2 represents a significant type of ROS, and its excessive accumulation can induce oxidative damage in postharvest fruits and vegetables, consequently leading to quality deterioration [36, 37]. Therefore, maintaining the balance of intracellular ROS homeostasis is paramount for preserving the quality of postharvest fruits and vegetables. Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) are key ROS-scavenging enzymes in plants. SOD catalyzes the conversion

of superoxide radicals ($O_2^{\cdot-}$) into H2O2, which is then promptly decomposed by POD and CAT, thus averting oxidative damage to cells [38]. Ascorbate peroxidases (APXs) represent crucial enzymes involved in the Ascorbate-Glutathione (AsA-GSH) cycle, playing a vital role in ROS detoxification [39]. In this study, a higher expression of SODs, PODs, and APXs was observed in lotus seeds during low-temperature storage compared to storage at room temperature (Fig. 4A). Furthermore, the activities of SOD and POD in postharvest fruits and vegetables were enhanced during low-temperature storage [40, 41]. Additionally, the increased energy production and elevated ATP levels generated by the EMP and TCA pathways in fresh lotus seeds during low-temperature storage could potentially augment the activities of antioxidant enzymes, including SOD and APX [42]. Consequently, the preservation and prolonged shelf life of postharvest fresh lotus seeds during low-temperature storage may be attributed, at least in part, to heighten enzymatic antioxidant defense mechanisms.

Hormone signals regulation involved in low temperature preservation of postharvest fresh lotus seeds

Plant hormones play pivotal roles in modulating the ripening process of fruits and vegetables, with exogenously applied plant hormones significantly impacting postharvest fruit quality [43, 44]. In lotus seeds, hormones such as ABA, SA, IAA, and JA-Ile exhibited notable increases within 24 h of room temperature storage, accompanied by induced expression of genes associated with their transduction pathways [4]. Furthermore, several DEGs identified in our study were linked to phytohormone signaling responses (Fig. 5A), suggesting the involvement of plant hormones in regulating the preservation process of postharvest fresh lotus seeds under low temperatures. ABA, a key hormone in regulating postharvest ripening and senescence of non-climacteric fruits, has been shown to accelerate senescence processes with its increased presence in postharvest fruits [45]. Additionally, fruit ripening and senescence entail intricate crosstalk among various phytohormones, with ABA documented to interact with other hormones such as IAA, GA, and ethylene during this process [44, 46, 47]. In our study, an upregulation of genes involved in the ABA biosynthesis pathway was observed in lotus seeds during room temperature storage (Fig. 5B). For instance, four genes (Chr02.g09669, Chr02.g09666, Chr02.g09668, and Chr02.g10466) encoding AAO were significantly induced in lotus seeds between 72 and 96 h postharvest under room temperature conditions. Concurrently, signaling transduction pathways were activated (Fig. 5C), partially explaining the rapid senescence observed in postharvest fresh lotus seeds during room temperature storage.

Conclusion

This study used RNA-Seq analysis to investigate the transcriptional dynamics underlying quality deterioration in postharvest fresh lotus seeds stored at room and low temperature conditions. In comparison to room temperature storage, low temperature storage significantly suppressed the accumulation of H_2O_2, MDA, protein, and starch in postharvest lotus seeds, effectively preserving their quality and flavor. During low temperature storage, key enzyme genes associated with sucrose metabolism were up-regulated, while those involved in starch biosynthesis, such as AGPase, GBSS, SBE, and ISA, were induced early in the storage period under room temperature condition. This resulted in

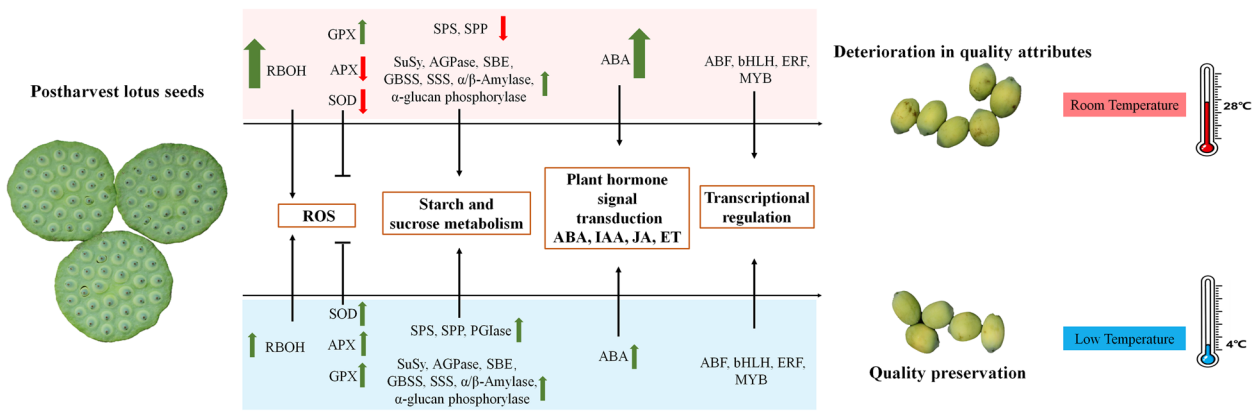

Fig. 8 A hypothetical molecular model of changes in postharvest fresh lotus seeds during storage at low temperature and room temperature conditions

enhanced sucrose accumulation and delayed starch biosynthesis, ultimately preserving the flavor of postharvest lotus seeds in the short term. The EMP-TCA pathways were notably activated during low temperature storage, providing sufficient energy to mitigate cold damage and serve as substrates for sucrose biosynthesis, thus promoting a balance between cold damage and freshness preservation. Additionally, the antioxidant system was significantly activated in lotus seeds during low temperature storage, effectively delaying postharvest senescence. Moreover, the interplay between different plant hormones played a crucial role in lotus seed preservation, particularly the inhibition of gene expression involved in ABA biosynthesis and signaling transduction processes under low temperature condition, which, to some extent, benefited quality maintenance by delaying senescence in postharvest lotus seeds (Fig. 8). Low temperature storage emerges as the simplest and most effective preservation technology for postharvest lotus seeds. These findings enhance our understanding of the transcriptional molecular mechanisms underlying low-temperature preservation in postharvest lotus seeds and provide insights to optimize low temperature storage protocols, thereby extending the shelf life of postharvest fresh lotus seeds.

Abbreviations

SPS	Sucrose phosphate synthase
SPP	Sucrose phosphate phosphatase
SuSy	Sucrose synthase
INV	Invertase
FRK	Fructokinase
HXK	Hexokinase
AGPase	ADP-Glc pyrophosphorylase
SSS	Soluble starch synthase
GBSS	Granule bound starch synthase
SBE	Starch branching enzyme
ISA	Isoamylase
PHS	Alpha-glucan phosphorylase
RBOH	Respiratory burst oxidase homologue
SOD	Superoxide dismutase
CAT	Catalase
POD	Peroxidase
APX	Ascorbate peroxidase
PGI	Glucose-6-phosphate isomerase
PFK	6-Phosphofructokinase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
TPI	Triose-phosphate isomerase
PGK	Phosphoglycerate kinase
PGAM	Phosphoglyceratemutase
ENO	Enolase
PK	Pyruvate kinase
PDC	Pyruvate dehydrogenase complex
CSY	Citrate synthase
ACO	Aconitate hydratase
IDH	Isocitrate dehydrogenase
ODC	Oxoglutarate dehydrogenase complex
SCoAL	Succinyl-CoA ligase
SDH	Succinate dehydrogenase
FUM	Fumarate hydratase
ME	Malic enzyme
MDH	Malate dehydrogenase

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05468-9.

Supplementary Material 1: Fig. S1. Correlation results for samples. 'N' and 'C' represent lotus seeds stored at room temperature and low temperature, respectively.

Supplementary Material 2: Fig. S2. Volcano map depicting the significance level of DEGs in different comparison groups during storage at room temperature (A), low temperature (B) and low temperature compared to room temperature (C). 'N' and 'C' represent lotus seeds stored at room temperature and low temperature, respectively.

Supplementary Material 3: Fig. S3. Upset diagram representing the number of DEGs during postharvest storage period at room temperature (A), low temperature (B), and low temperature compared to room temperature (C). 'N' and 'C' represent lotus seeds stored at room temperature and low temperature, respectively.

Supplementary Material 4: Fig. S4. Correlation analysis between RNA-Seq and qRT-PCR results based on 10 randomly selected genes.

Supplementary Material 5: Fig. S5. KEGG analysis of DEGs in different comparison groups during storage at room temperature (A), low temperature (B), and low temperature compared to room temperature (C). 'N' and 'C' represent lotus seeds stored at room temperature and low temperature, respectively.

Supplementary Material 6: Fig. S6. DEGs involved in glycolysis (A) and the citrate cycle (B). PGI, glucose-6-phosphate isomerase; PFK, 6-phosphofructokinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TPI, Triose-phosphate isomerase; PGK, Phosphoglycerate kinase; PGAM, phosphoglyceratemutase; ENO, enolase; PK, pyruvate kinase; PDC, pyruvate dehydrogenase complex; CSY, citrate synthase; ACO, aconitate hydratase; IDH, isocitrate dehydrogenase; ODC, oxoglutarate dehydrogenase complex; SCoAL, succinyl-CoA ligase; SDH, succinate dehydrogenase; FUM, fumarate hydratase; ME, malic enzyme; MDH, malate dehydrogenase. 'N' and 'C' represent lotus seeds stored at room temperature and low temperature, respectively.

Supplementary Material 7: Fig. S7. Plant hormone response DEGs in lotus seeds during storage at low temperature compared to room temperature at postharvest 24 h (A), 48 h (B) and 72 h (C), displayed in chord diagrams. 'N' and 'C' represent lotus seeds stored at room temperature and low temperature, respectively.

Supplementary Material 8: Fig. S8. Proportions of differentially expressed TFs in lotus seeds under postharvest storage at low temperature (A) and room temperature (B). 'N' and 'C' represent lotus seeds stored at room temperature and low temperature, respectively.

Supplementary Material 9: Fig. S9. Co-expression networks of Chr03.g12925 and Chr04.g16569 with material and energy metabolism-related DEGs. Green and yellow colors represent positive and negative correlation relationships, respectively.

Supplementary Material 10: Fig. S10. Expression patterns of DEGs in different co-expression modules. 'N' and 'C' represent lotus seeds stored at room temperature and low temperature, respectively.

Supplementary Material 11: Table S1. Primers used in this study.

Supplementary Material 12: Table S2. Summary of sequencing data.

Supplementary Material 13: Table S3 Statistical analysis of DEGs in each cluster.

Supplementary Material 14: Table S4. FPKM values of DEGs in the sucrose and starch metabolism pathways.

Supplementary Material 15: Table S5. FPKM values of DEGs in the glycolysis and the citrate cycle pathways.

Supplementary Material 16: Table S6. FPKM values of DEGs in ROS metabolism pathway.

Supplementary Material 17: Table S7. FPKM values of DEGs in MAPK cascade signaling pathway.

Supplementary Material 18: Table S8. FPKM values of DEGs in ABA biosynthesis and signaling transduction pathways.

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Authors' contributions
L.C. Data curation, Formal analysis, Writing - original draft. G.Q.D. Project administration, Writing- Reviewing and Editing. H.Y.S. and J.X. Data curation, Methodology. Y.Y.S. and W.C. Writing- Reviewing and Editing. M.Y. Supervision, Writing - review & editing. H.S. Supervision, Project administration, Writing - review & editing.

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Availability of data and materials
The subsequent analyses were based on clean data of high quality, and all clean data generated in this study are accessible at the National Center for Biotechnology Information (NCBI) under the accession number PRJNA1111767.

Declarations

Ethics approval and consent to participate
The authors declare that the experimental research on plants were comply with the Ethical Rules applicable to BMC Plant Biology.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests or personal relationships that could have appeared to influence the work reported in this paper.

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