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High throughput sequencing identifies chilling responsive genes in sweetpotato (*Ipomoea batatas Lam.*) during storage

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ABSTRACT

Sweetpotato (*Ipomoea batatas* L.) is a globally important economic food crop. It belongs to Convolvulaceae family and origins in the tropics; however, sweetpotato is sensitive to cold stress during storage. In this study, we performed transcriptome sequencing to investigate the sweetpotato response to chilling stress during storage. A total of 110,110 unigenes were generated via high-throughput sequencing. Differentially expressed genes (DEGs) analysis showed that 18,681 genes were up-regulated and 21,983 genes were down-regulated in low temperature condition. Many DEGs were related to the cell membrane system, antioxidant enzymes, carbohydrate metabolism, and hormone metabolism, which are potentially associated with sweetpotato resistance to low temperature. The existence of DEGs suggests a molecular basis for the biochemical and physiological consequences of sweetpotato in low temperature storage conditions. Our analysis will provide a new target for enhancement of sweetpotato cold stress tolerance in postharvest storage through genetic manipulation.

1. Introduction

Sweetpotato (*Ipomoea batatas* Lam.) is an important economic food crop that is globally cultivated due to its high nutrient content, stable and high yield, and adaptability under a variety of environmental conditions. In 2014, the annual global production of sweetpotato reached to 106,601,602 tons, with a plantation area of 8,352,323 ha (Food and Agriculture Organization of the United Nations; http://www.fao.org). Because sweetpotato is harvested within a relatively short time period but consumed over a long time, high postharvest loss becomes a major limiting factor for widening the range of applications of sweetpotato. Proper storage of sweetpotato to reduce postharvest losses is thus critical to solve this problem. Fresh tuberous roots of sweetpotato are large in size, have high moisture content, high respiration rate, and relatively thin and delicate skin. All these attributes make them highly sensitive to storage temperature [1]. As a tropical crop, sweetpotato is susceptible to chilling injury [2]. In temperate and subtropical regions, low temperature stress is a main restricting factor for the storage of sweetpotato. The optimal storage temperature varies among different sweetpotato cultivars, but it is generally accepted that 12 °C–15 °C is optimal. Storage at temperatures < 9 °C will cause chilling injury in sweetpotato. Chilling injury is characterized by a disruption in the metabolism of nutrient content as well as susceptibility to pathogen and pest stress. Formation of hard areas also occurs. Other signs of chilling injury in sweetpotato include soft rot disease caused by *Rhizopus stolonifer*, production of pectinases, destruction of tuberous root cell wall, and generalized softening. The present study aimed to compare the transcriptome profiles of sweetpotato subjected to different storage temperatures, which include 14 °C [appropriate temperature condition (ATC)] and 4 °C [low temperature condition (LTC)], while keeping other storage conditions the same.

Transcriptome analysis measures the mRNA expression of genes of an organism during a specific biological process or disease, which consequently reveals the molecular mechanisms underlying such processes. It is important to analyze the functions of stress-inducible genes at the transcriptome level not only to understand the molecular mechanisms of stress tolerance and plant responses to this stress but also to improve stress tolerance in crops by genetic manipulation. Recently

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advancements and applications in transcriptome analysis have allowed numerous investigations on the responses of plants to cold stress. Imbibition chilling injury causes delayed seed germination and poor seedling growth in maize. Using RNA sequencing technology, differentially expressed genes (DEGs) were identified in chilling-imbibed maize embryos, which provided new insights into early seed imbibition at the transcriptional level [3]. Low-temperature condition of peach fruit (pre-storage at 8 °C for 5 days) alleviates fruit chilling injury. Transcriptomic analysis has revealed that the regulation of ethylene response factors (ERFs) and lipid metabolism genes at the transcriptional level promotes softening, regulates lipid composition and desaturation, and modulates cell membrane stability, which eventually reduces browning and alleviates peach fruit CI [4]. Transcriptome sequencing of seedlings of *Dianthus spiculifolius* identified DEGs responsive to both cold and simulated drought stress. The DEGs and associated metabolic pathways may also play important roles in the responses of *D. spiculifolius* to combined stress. These genes and pathways may serve as new targets for enhancement of plant stress tolerance [5]. Longer rinsing combined with chilling of seeds affect the germination rate of the ornamental peach cultivar Yaguchi. Transcriptome analysis identified potential candidate genes responsible for dormancy progression in seeds of Yaguchi peach [6]. To date, transcriptome analysis has been used to investigate CI during plant storage. For example, in mango fruits, two days of chilling stress upregulated the expression of genes involved in the plant stress response, including those encoding transmembrane receptors, calcium-mediated signal transduction, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, mitogen-activated protein (MAP) kinases, and WRKYs, which can lead to cell death [7]. Cold storage can also induce CI in nectarine fruits. Postharvest treatment such as controlled atmosphere with specified CO_2 and O_2 content have been used under cold conditions to avoid CI. Transcriptomic analysis showed that when stored at a low temperature for 21 days, several transcripts related to ethylene production were down-regulated in nectarine fruits that had been stored under controlled conditions compared to those under uncontrolled settings [8].

Sweetpotato is hexaploid, with a genome size of about 4.4 Gb. Although several research groups have attempted to directly sequence the sweetpotato genome, details relating to its genome structure and organization remain unclear. However, recently two closed-related sweetpotato wild species (*I. trifida* and *I. triloba*) were sequenced (http://sweetpotato.plantbiology.msu.edu/index.shtml). Currently, high-throughput sequencing has been used in investigating various molecular regulatory mechanisms in sweetpotato. Transcriptome sequencing of the sweetpotato progenitor [*I. trifida* (H.B.K.) G. Don.] identified a potential drought tolerance protein, ItWRKY1 [9]. In another study, the transcriptomes of the salt-sensitive cultivar Lizixiang and the salt-tolerant cultivar ND98 were compared to identify genes and pathways involved in salt stress responses. The results showed that jasmonic acid biosynthesis and signaling pathways, as well as ion transport-related genes play important roles in the response of sweetpotato to salinity stress [10]. In addition, transcriptome sequencing has been employed to investigate sweetpotato genes related to carotenoid biosynthesis and anthocyanin biosynthesis, as well as flowering regulatory genes [11–13]. Phylogenetic analysis of homologous chromosome regions indicates that 30 out of 90 chromosomes of modern cultivated *I. batatas* may have originated from a diploid progenitor, whereas the other 60 chromosomes may have been derived from a tetraploid progenitor [14].

However, studies on the mechanisms underlying CI during sweetpotato storage are limited. In present study, we first measured the nutrient content in sweetpotato stored at ATC or LTC, and investigated the changes in nutrient content in chilling-injured sweetpotato. Meanwhile, transcriptomic analysis was performed to identify CI-related genes and to investigate the molecular mechanism underlying CI in sweetpotato. The aim of this study was to provide valuable information for the future genetic modification of sweetpotato.

2. Materials and methods

2.1. Plant materials and storage

Sweetpotato cultivar Xushu 18 (*I. batatas* L. cv. Xushu 18) was kindly provided by the Chinese Academy of Agricultural Science. Xushu 18 was developed in the 1970s and won the First Class Award of the National Technological Invention Award in China. Xushu 18 is resistant to root rot disease, easily adapts to a wide range of conditions, and has high yield. It is a widely grown cultivar in China. We selected Xushu 18 in this study because it well represents various sweetpotato cultivars and the results generated in this study have numerous practical applications.

Sweetpotato tuberous roots at same growing stage, with similar size and shape, and no pests or pathogenic infections were used in the present study. To further minimize the potential impact from microbial infections, the tuberous roots were cleaned, soaked in thiabendazole [500 mg/L; Shennong Chemicals Co., Ltd. (Xuzhou, Jiangsu, China)] for 3 min, and air-dried. The tuberous roots were then cured at 35 °C and a relative humidity of 85% for 3 days.

The sweetpotato were randomly divided into two groups, which were stored at 4 °C (LTC) or 14 °C (ATC) and with a relative humidity of 85%, respectively. Each group had the same number of samples. Sweetpotato not stored at the abovementioned specific conditions were considered members of the not stored condition group [15]. Sample tissues were collected every other week while in storage, immediately frozen in liquid nitrogen, and then stored at −80 °C prior to analysis. To minimize variations, seven sweetpotato tuberous roots were pooled into one biological replicate, and three biological replicates were assessed at each time point.

2.2. Changes in nutrient contents in sweetpotato stored at different temperatures

The moisture content in sweetpotato during storage was determined according to GB 5497-85 (Inspection of grain and oilseeds: Methods for determination of moisture content, Standards Administration of China). Three biological replicates were assessed.

The starch, sucrose, glucose, and fructose content in sweetpotato during storage was measured using near-infrared spectroscopy (NIRS). Three biological replicates were assessed.

Total protein content in sweetpotato during storage was measured using the BCA microplate method, according to manufacturer's instruction (Nanjing Jiancheng Bioengineering Institute). Three biological replicates were assessed.

2.3. RNA extraction, library preparation, and transcriptional sequencing

Total RNA was extracted from sweetpotato using TRIzol (Invitrogen). Each sample consisted of three biological replicates. RNA quality control and purification was conducted using a Bioanalyzer 2100 system and an RNA 6000 Nano LabChip Kit (Agilent, CA, USA), respectively. RNA samples with RNA integrity number (RIN) values ≥7 and rRNA 28S/18S ratios ≥0.7 were used in the subsequent experiments. Poly(A) mRNA was isolated and purified using 5 μg of total RNA and oligo(dT) magnetic beads (Invitrogen). After purification, the mRNA was randomly fragmented with RNA fragmentation reagents, dephosphorylated, 5′ phosphorylated, 3′ hydroxylated, and sequentially ligated to the adenylated 3' RNA adapter then to the 5' RNA adapter using T4 RNA ligase 2 (truncated). Reverse transcription [16] primers were mixed with the RNA ligation products. The adaptor-ligated fragments were then screened for 400-bp (± 50 bp) products, which were then PCR amplified for a total of 15 cycles. We sequenced the library using an Illumina Hiseq2500 platform (Illumina) as paired-end reads (2 × 150 bp) (LC Science, USA).

2.4. Data analysis, annotation, and differential expression analysis

De novo sweetpotato transcriptome assembly without a reference genome was conducted using the short reads assembling program, Trinity. The transcript sequences generated through Trinity were called unigenes. All assembled unigenes were first integrated with the published sweetpotato transcriptome data (ERP003402, SRP003171, SRP007758, SRP007846, SRP007847, and SRP007852), and then used in a basic local alignment search tool (BLAST) search and annotated against various databases, including the NCBI non-redundant protein (Nr) (http://www.ncbi.nlm.nih.gov), Cluster of Orthologous Groups (COG) (http://www.ncbi.nlm.nih.gov/COG), UniProtKB/Swiss-Prot (http://www.ebi.ac.uk/swissprot/), and Kyoto Encyclopedia of Genes and Genomes Ortholog (KEGG) (http://www.genome.jp/kegg). In addition, using Blast2GO and WEGO software [2,17], the unigenes were assigned into gene ontology [1] functional groups. Finally, the results of GO and KEGG pathway enrichment analysis were visualized as dot plot using ggplot2 (http://ggplot2.org). GENScan program (http://genes.mit.edu/GENSCAN.html) was then employed to predict the coding sequence (CDS) using the transcripts. RSEM software package [18] was used in the quantification of gene abundances using the RNA-Seq data. LTC and ATC samples were compared, an absolute value of log2-ratio > 1, $P < 0.05$, and FDR < 0.05 were used as filtering thresholds to identify differentially expressed genes (DEGs) in response to CI.

2.5. Quantitative reverse transcription PCR (qPCR) validation

Thirty representative DEGs identified by RNA-Seq were randomly selected for experimental validation by qRT-PCR. The gene-specific primers were designed and synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. The reaction was performed using a Takara PrimeScriptTM RT reagent Kit with a gDNA Eraser and the One Step SYBR® PrimeScriptTM RT-PCR Kit II. All reactions were performed in triplicate, and the results were expressed relative to the expression levels of beta-tubulin. To validate the RNA-Seq data, Pearson correlation analysis between the RNA Seq data and the qPCR data was performed using the linear regression function of Excel.

3. Results

3.1. Sweetpotato characteristics and nutrient content

The sweetpotato tuberous roots stored at 4 °C showed softening and shriveling (Fig. 1A). At day 28 of LTC storage, its internal flesh exhibited a darker colour (Fig. 1B). In contrast, such symptoms were not observed in the sweetpotato tuberous roots stored at ATC. These results indicate the CI occurred at day 28 under LTC. In a previous study, sweetpotato cultivar Yulmi did not exhibit CI symptoms when stored at 4 °C for 28 days [19], thereby suggesting that different sweetpotato cultivars may have different tolerance to chill stress.

Moisture content is an important quality indicator during sweetpotato storage. Fig. 1C shows that the moisture content in stored sweetpotato decreased over time. At LTC, the moisture content decreased to a stable state at day 28. In contrast, the moisture content in sweetpotato decreased faster at ATC. The moisture content in sweetpotato stored under LTC and ATC significantly differed at day 35. These results suggest that LTC facilitates moisture retention in sweetpotato.

Starch is the most abundant nutrient and an important parameter for quality control in sweetpotato. Fig. 1D shows that starch content fluctuates but shows an overall decline during storage. However, the decrease in starch content varied with storage temperature. At LTC, starch content decreased, whereas at ATC, the starch content fluctuated and then later stabilized. Furthermore, starch content decreased faster in LTC than in ATC. Starch content in sweetpotato stored under LTC and ATC significantly differed at day 14. Therefore, LTC accelerates the loss of starch in sweetpotato.

Protein content is an important nutritional indicator in sweetpotato. Fig. 1E shows that the total protein content in sweetpotato increased at both temperatures. Protein content of sweetpotato stored under ATC was significantly higher than that under LTC at day 35. These findings indicate that LTC inhibits the increase in total protein content in sweetpotato.

Sweetpotato contains soluble sugars such as sucrose, glucose, and fructose. Sucrose is a major type of sugar [20] and is also an important indicator in evaluating the taste and commercial quality of sweetpotato. Fig. 2 shows that the soluble sugar content in sweetpotato increased with storage. In LTC, the sugar content was relatively stable during the first 14 days. Then, the content of soluble sugar, particularly sucrose, rapidly increased. By day 21, glucose and sucrose content slightly decreased. In ATC, the sugar content in sweetpotato fluctuated but showed an overall increasing trend. At day 14, sucrose content peaked, which was then followed by a sharp drop and a gradual increase. Glucose content was lowest at day 14, followed by a rapid increase and a subsequent decrease. Fructose content was relatively stable but gradually increased.

3.2. Transcriptome sequencing

The transcriptome is the set of all RNA molecules in a given organism at a given developmental stage or functional status. Transcriptome analysis is the basis and starting point of investigations on gene function and structure. Understanding the transcriptome is thus essential to the interpretation of genomic functional components as well as in investigating the molecular composition of cells and tissues. Because the sweetpotato genome has not been sequenced to date, this study conducted transcriptome analysis, which does not require a reference genome, to investigate mRNA changes in response to cold exposure.

Sweetpotato that had been stored at NSC, LTC, or ATC for 28 days were evaluated. Biological triplicates were used for each treatment group. From nine libraries, a total of 874,967,752 raw reads were obtained. After a stringent-filtering process of raw sequence reads, approximately 110,110 clean transcripts reads were obtained. The mean size of the unigenes was 781 bp, with an N50 length of 1227 bp, and mean GC% of 42.09.

To determine the transcript abundance of the identified genes, the reads per kilobase of exon model per million mapped reads (RPKM) method was used, which was computed using the RNA-seq data with the expectation maximization (RSEM) tool (Table S1). The average value reflecting their expression levels for NSC, LTS or ATS was 10.37, 9.15, or 9.20, respectively.

Pearson correlation analysis was performed to validate the gene expression profiles of transcripts from nine different samples (Fig. 3A). The results showed that biological replicates from the same treatment group were highly correlated. In particular, ATC and NSC were highly correlated, whereas LTC and NSC were less correlated. The results of principle component analysis (PCA) (Fig. 3B) coincided with our correlation analysis findings. Additionally, we observed that ATC and NSC were highly correlated. These results show that the gene expression profile in sweetpotato stored at ATC was closes to that observed in NSC at day 28. However, the gene expression profile of sweetpotato in LTC and NSC showed more significant differences.

Out of 110,110 unigenes, 41,801 (37.96%) unigenes were annotated to known proteins in the Swiss-Prot database, 67,740 (61.52%) to the Nr database, 49,092 (44.58%) to the Pfam database, 23,716 (21.54%) to the KEGG database, 55,575 (50.47%) to the COG database, and 36,911 (33.52%) to the GO database. To evaluate the function of the assembled unigenes, we searched the annotated unigenes that were involved in COG. The cluster for “general function prediction” represented the largest group (7961 unigenes), followed by “signal transduction mechanisms” (5274 unigenes), and “post-translational

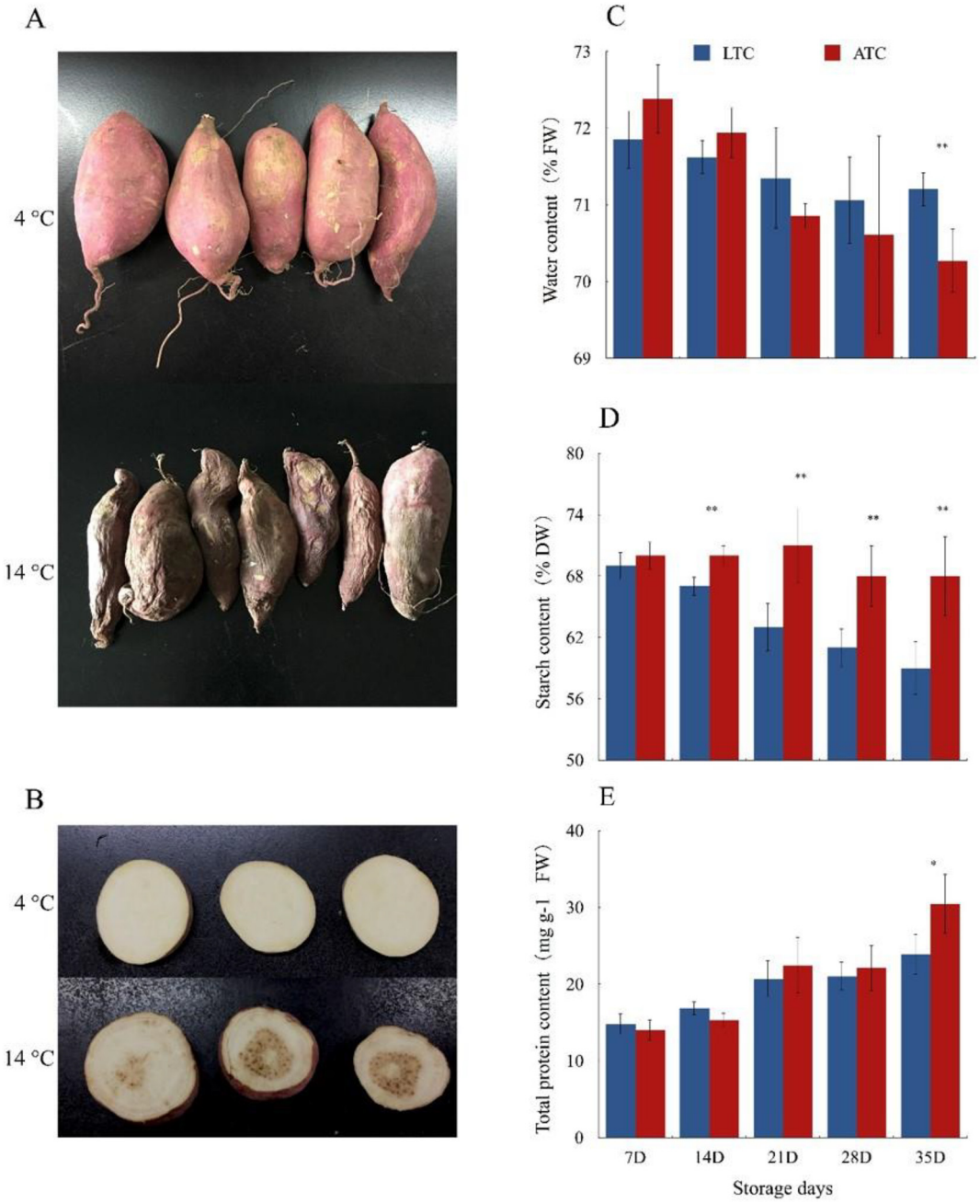

Fig. 1. Sweetpotato chilling injury symptoms and nutrient production at different storage temperatures. (A) Sweetpotato tuberous roots stored at 14 °C or 4 °C for 6 weeks. (B). Transverse section of sweetpotato tuberous roots stored at 14 °C or 4 °C for 28 days. (C) Water content of sweetpotato at different storage temperatures. (D) Starch content of sweetpotato at different storage temperatures. (E) Total protein content of sweetpotato at different storage temperatures. All data were assessed by ANOVA and the results are expressed as the mean ± SD. *$p < .05$, **$p < .01$.

modification, protein turnover, molecular chaperones" (4160 unigenes) (Fig. 4). For GO classification, genes were annotated to three categories, namely, molecular function, biological process, and cellular component. GO terms related to "ATP binding," "regulation of transcription, DNA dependent", and "integral to membrane" from each category was significantly enriched, with approximately 10,209, 2370, and 8648 genes, respectively (Fig. 5). For KEGG classification, genes were annotated to five categories. In the category of "metabolism," KEGG terms related to "carbohydrates metabolism," "amino acid metabolism," and "energy metabolism" was significantly enriched, with 2251, 1806, and 1233 genes, respectively (Fig. 6).

3.3. Identification of DEGs responsive to CI in sweetpotato

Compare LTC and ATC transcriptome, DEGs related to CI were identified. The distribution of significant changes in expression is illustrated as a volcano plot (Fig. 7). Non-DEGs are shown with blue dots, and DEGs are indicated by red dots. Compared to ATS, there were 18,681 transcripts that were significantly upregulated and 21,983 transcripts that were significantly downregulated in the LTS samples.

3.4. GO enrichment and KEGG analyses of DEGs

Twenty genes with the smallest *p* values from the GO and KEGG analyses of DEGs were used in generating a scatterplot using ggplot2

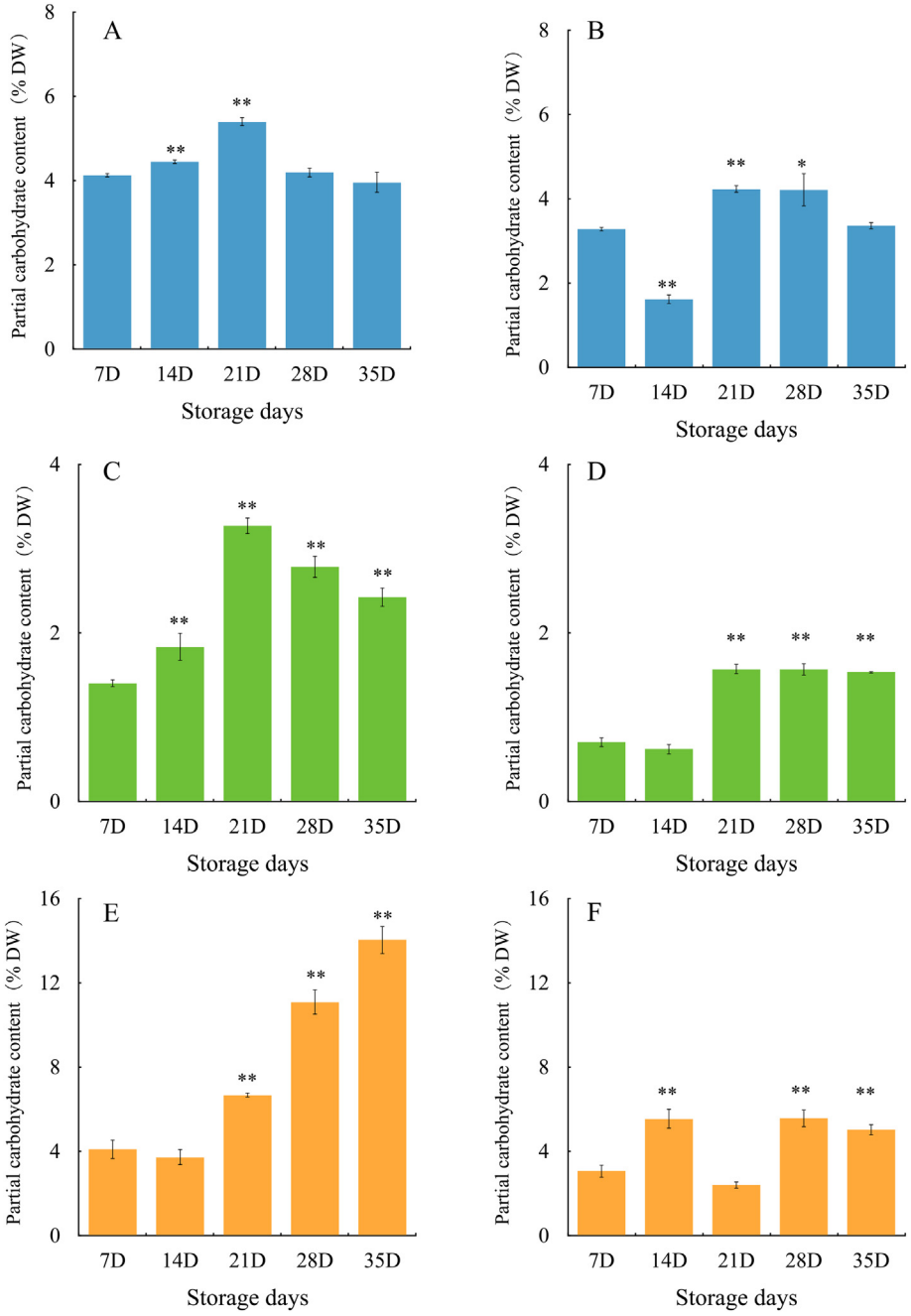

Fig. 2. Reducing sugar content of sweetpotato at different storage temperatures. (A) Glucose content of sweetpotato in LTC. (B) Fructose content of sweetpotato in LTC. (C) Sucrose content of sweetpotato in LTC. (D) Glucose content of sweetpotato in ATC. (E) Fructose content of sweetpotato in ATC. (F) Sucrose content of sweetpotato in ATC. All data were assessed by ANOVA and the results are expressed as the mean ± SD. *p < .05, **p < .01.

software (Fig. 8). Rich factor is defined as the ratio of the number of DEGs in a GO pathway to the total number of genes in the GO pathway. Based on the GO categories, 3916 DEGs were categorized into "membrane composition," and 2480 DEGs were categorized into "plasma membranee." Notably, "nutrient reservoir activity" and "starch biosynthesis process" were significantly enriched with DEGs. KEGG pathway analysis of DEGs showed significant enrichment in the categories relating to the synthesis, metabolism, and degradation of macromolecules. Top pathways that were significantly enriched with DEGs were "starch and sugar metabolism" (388 DEGs). In particular, among the 189 transcripts enriched in phenylalanine metabolism, 150 DEGs showed significant differential expression, which results in a high rich factor.

3.5. Validation of RNA-seq results

To validate the expression profile, qPCR was performed using 30 randomly selected genes. Fig. 9A shows that the qPCR expression profile was in agreement with the RNA-seq data for all genes evaluated. Linear regression analysis confirmed that the RNA-seq data was positively correlated with the qPCR results, with an r^2 value of 0.8122 (Fig. 9B). Overall, the miRNA and transcript expression profiles obtained from qPCR were in complete agreement with those from RNA-seq. Similar expression profiles and the observed high correlation confirmed the reliability of our RNA-seq results.

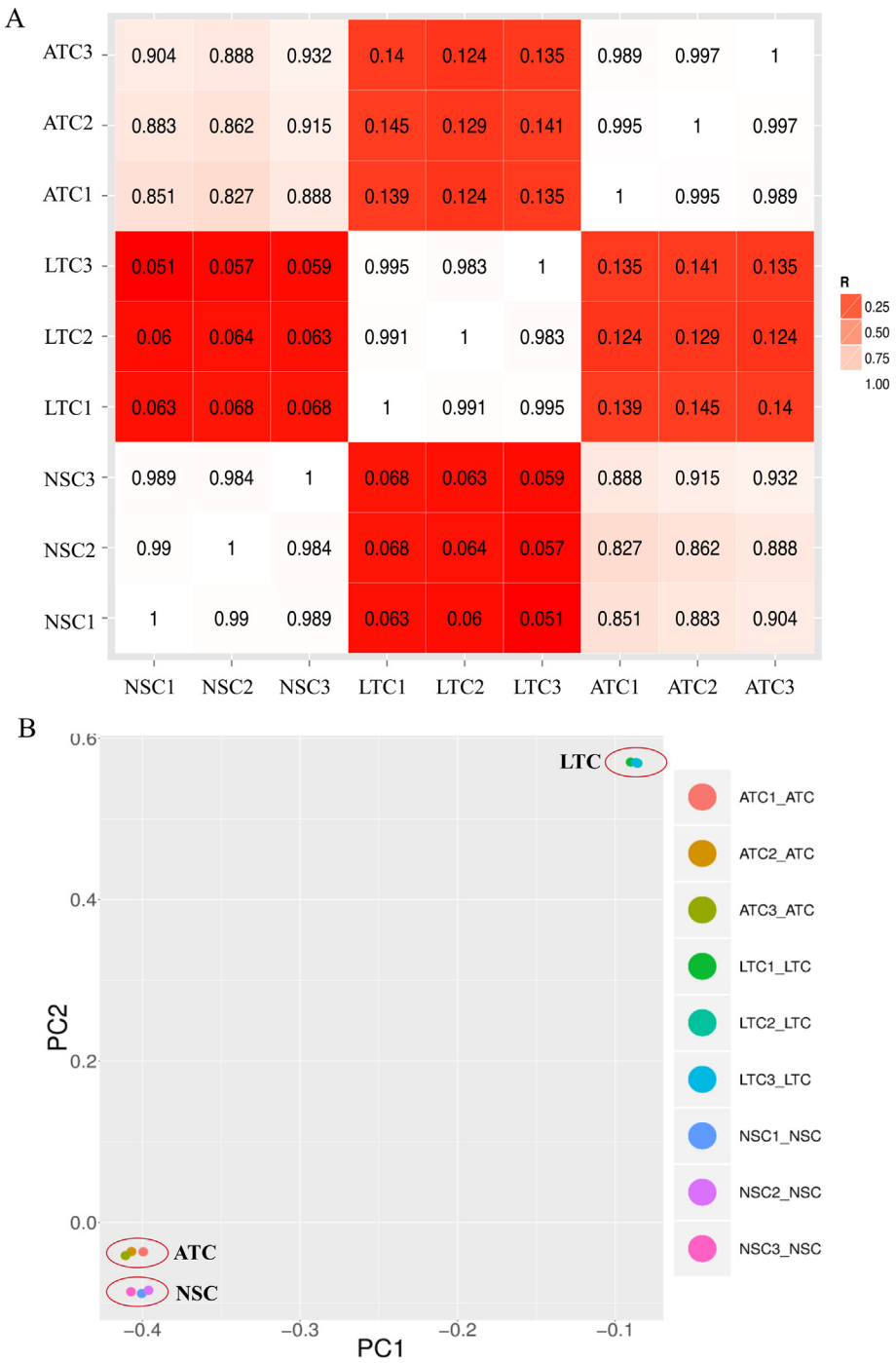

Fig. 3. Sweetpotato global transcriptomic response to cold storage (4 °C and 14 °C) at harvest (0 t) and after 21 days of cold storage. (A) 2D hierarchical clustering, white indicates high correlation, red represents low correlation. (B) Principal component analysis (PCA) at different time points and storage temperatures.

4. Discussion

4.1. Membrane-associated genes

Biological membranes participate in transport, signal transduction, metabolic regulation, energy conversion, and other physiological activities. Membranes are thought to be the primary sites for the development of CI. Membrane integrity is first affected by CI [21]; meanwhile, rapid cooling activates calcium-permeable channels in plant plasma membranes [22]. The membranes of chilled tissues undergo a phase transition from a flexible liquid-crystalline to a solid-gel structure, which significantly increases the risk of loss of cell membrane semi-permeability [23]. In the present study, GO analysis determined that 42.4% of the DEGs are associated with the categories of “integral to membrane” and “plasma membrane.” Mahajan et al. believed that the major malicious effect of low temperature is mechanical constraint, as well as consequent damage to cell wall and lipid membrane. In addition, membrane lipid peroxidation and membrane protein destruction also contribute to membrane damage [24].

When plant membranes are exposed to CI, lipid metabolism undergoes a series of changes to improve their resistance to cold stress by increasing unsaturated fatty acid content [25,26]. Lipid metabolism in sweetpotato may also change at low temperature in response to cold stress. Among the COG annotation categories, a total of 616 DEGs were

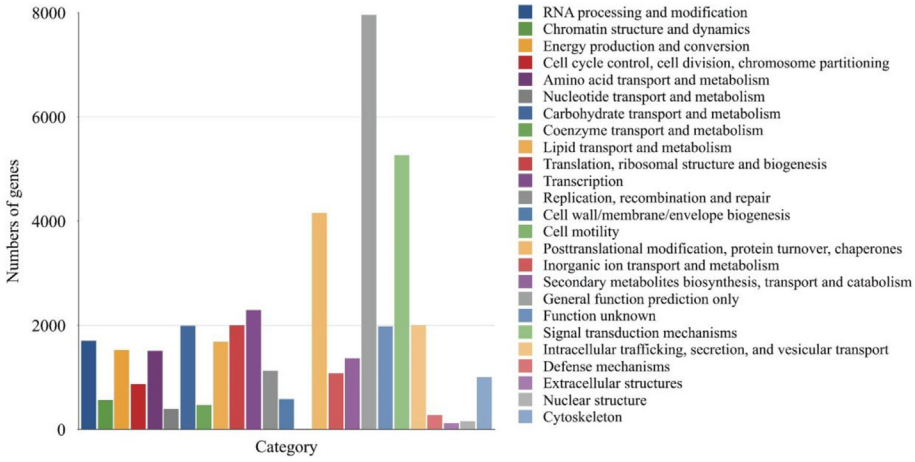

Fig. 4. COG functional categories.

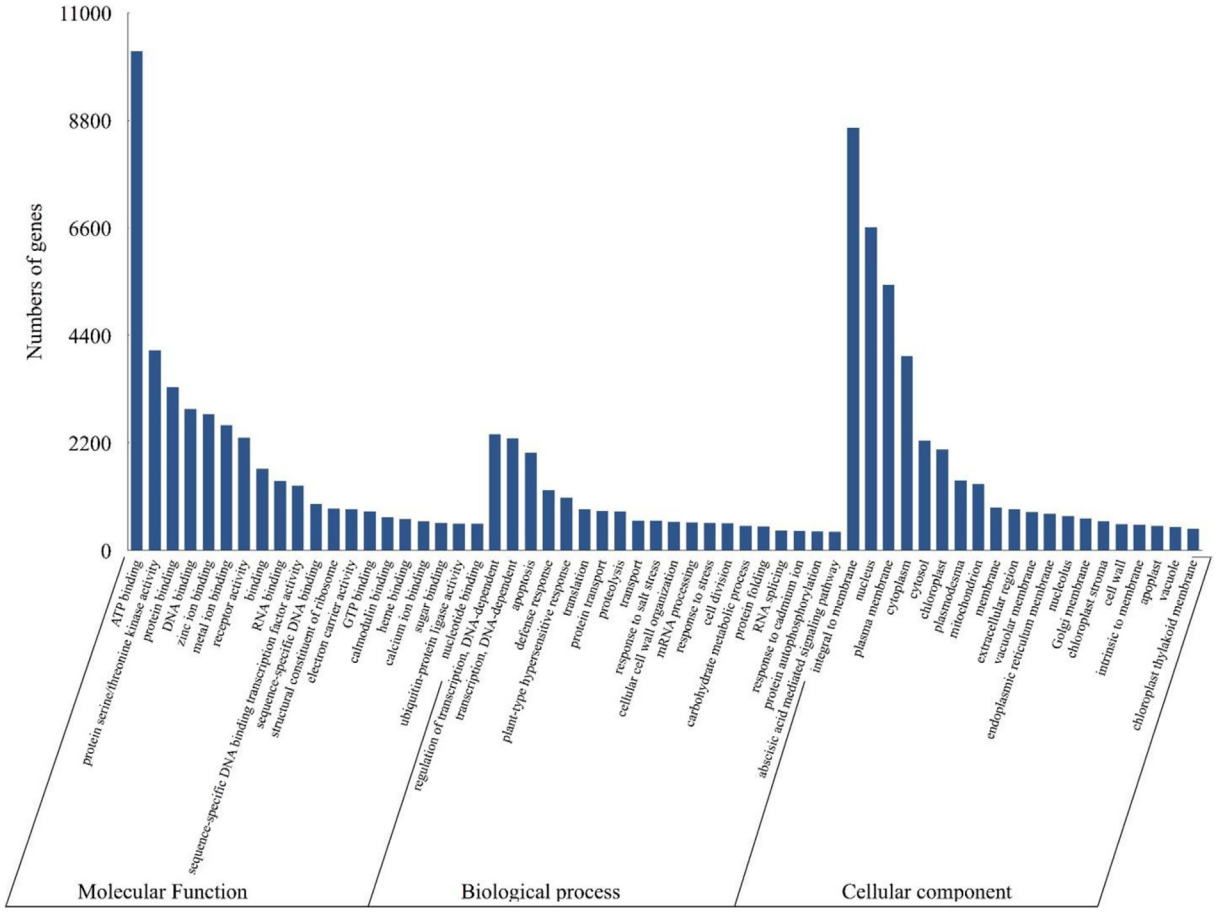

Fig. 5. GO functional categories.

associated with lipid metabolism, and an increase in unsaturated fatty acids could improve resistance to cold stress in plants. In the present study, the transcript level of fatty acid desaturase (*FAD7)* was significantly upregulated in the LTC. Moreover, multiple transcripts of four genes encoding phospholipase D significantly differed between two groups. These results were consistent with the findings of previous reports [19]. Fatty acid synthesis in plants requires acyl carrier protein [27], which is a key component of fatty acid synthetase (FAS) [28]. A candidate gene encoding acyl carrier protein 1 (mitochondrial), *MTACP1* (comp101566_c2), was upregulated 186.11-fold. The candidate gene encoding probable acyl-activating enzyme 1, *AAE1* (comp91405_c2), was upregulated 537.45-fold. Plant plasma membranes have proteins involved in transportation channels, signal transduction, membrane trafficking, and stress responses [29]. Among these membrane proteins, aquaporin (AQP) enhances the permeability of biological membranes to water and plays important roles in responses to osmotic stress [16]. Plasma membrane intrinsic protein (PIP) is one of the AQPs on the plasma membrane. In the present study, genes *PIP2-1* (comp98752_c1) and *PIP2-4* (comp82443_c1) were upregulated 694.58- and 604.67-fold, respectively. In addition, the *PIP1-1, PIP1-2,* and *PIP2-2* genes were downregulated. Differences in the effects of cold stress on mitochondrial or plasma membranes during sweetpotato

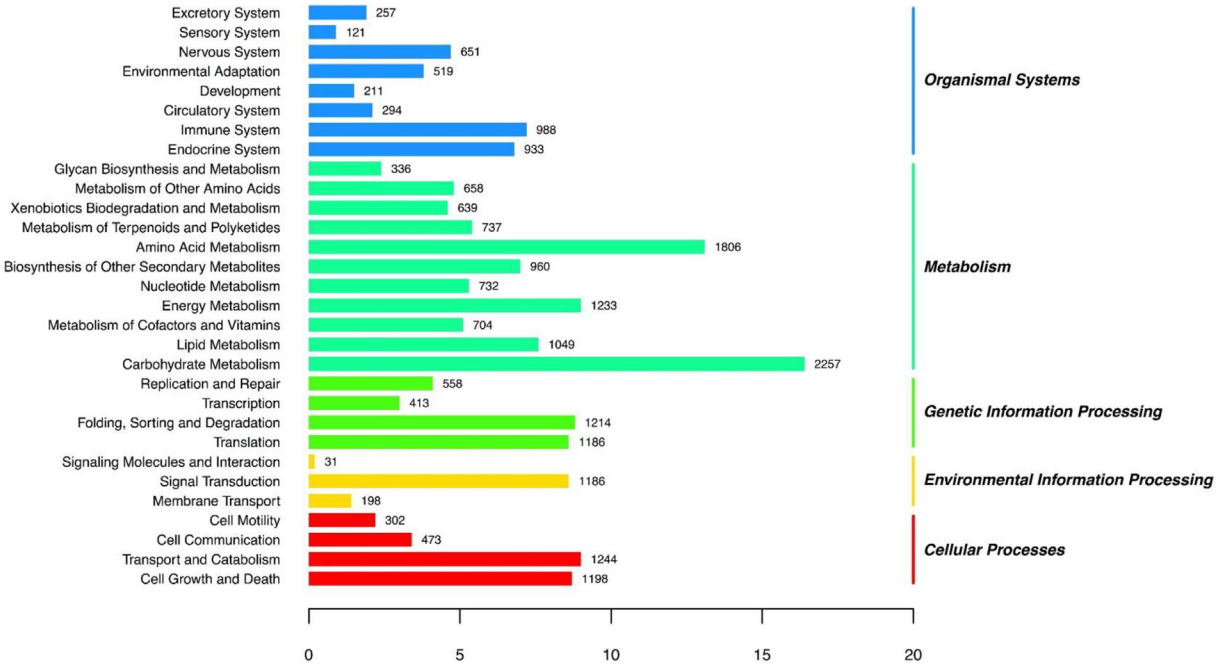

Fig. 6. KEGG functional categories.

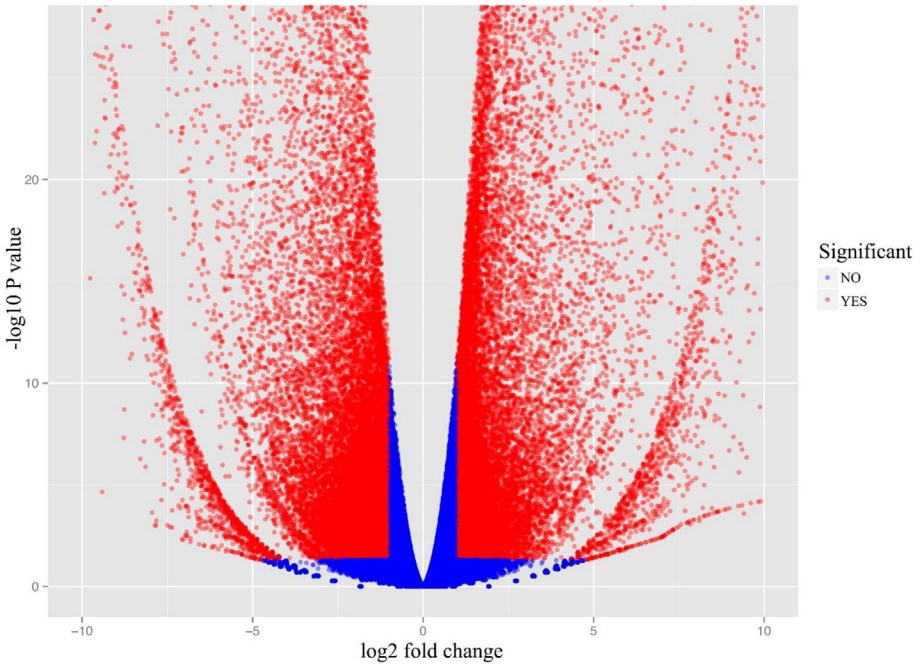

Fig. 7. The volcano diagram depicting the differential expression of genes. Blue represents not significant, red indicates significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

storage may be attributable to the differential expression of these enzyme-encoding genes.

Mitochondrial oxidative phosphorylation and electron transport chain, also known as the mitochondrial respiratory chain, occurs in the inner membrane of mitochondria. It consists of a multiprotein structure containing four high-molecular weight proteins that span the intermembrane space, ubiquinone, and cytochrome C. DEG analysis showed that respiratory chain electron transport-related genes and oxidative phosphorylation-related genes were affected by CI. Among these DEGs, the expression of the potential transcript of *NAD7* (comp4524_c1) encoding NADH dehydrogenase (ubiquinone) Fe-S protein 2 was downregulated. The expression of six transcripts (comp78486_c1, comp85541_c1, comp99446_c1, comp95837_c1, comp79676_c14, and comp84262_c9) related to succinate dehydrogenase was all upregulated. Moreover, the expression levels of *Mtco1, Mtco2* (which encodes the subunit of cytochrome C oxidase), and the potential transcript of *Mtco3* (comp107647_c1, comp107877_c1, and comp106823_c1) were upregulated 5220.60-, 2304.12-, and 205.07-fold, respectively.

These results indicate that the genes associated with lipid metabolism that are closely linked to plasma membrane phospholipid metabolism, as well as the genes associated with protein structures of plasma membrane are affected by cold stress during sweetpotato storage. Changes in the expression of these genes consequently affect cellular respiration and eventually lead to CI in sweetpotato.

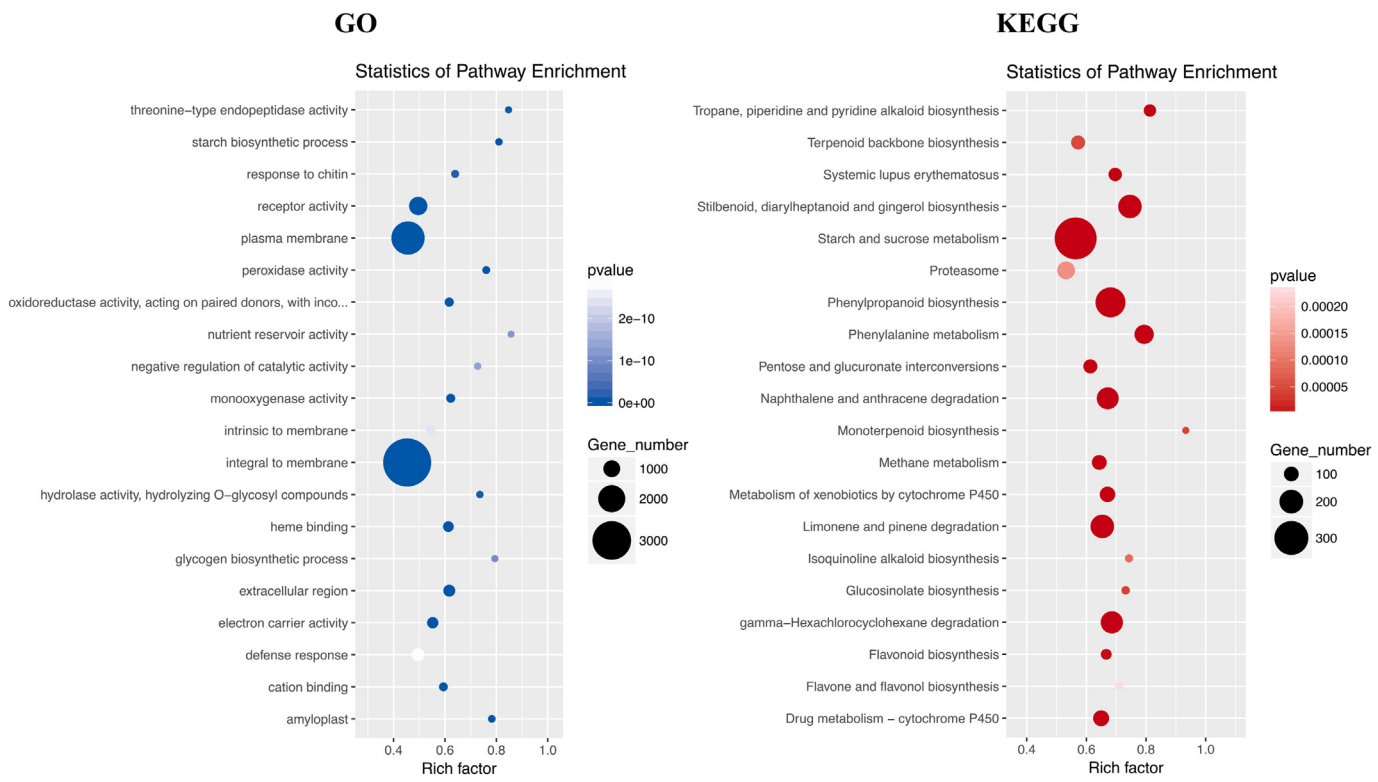

Fig. 8. The enrichment scatter diagram depicting the differential expression of genes.

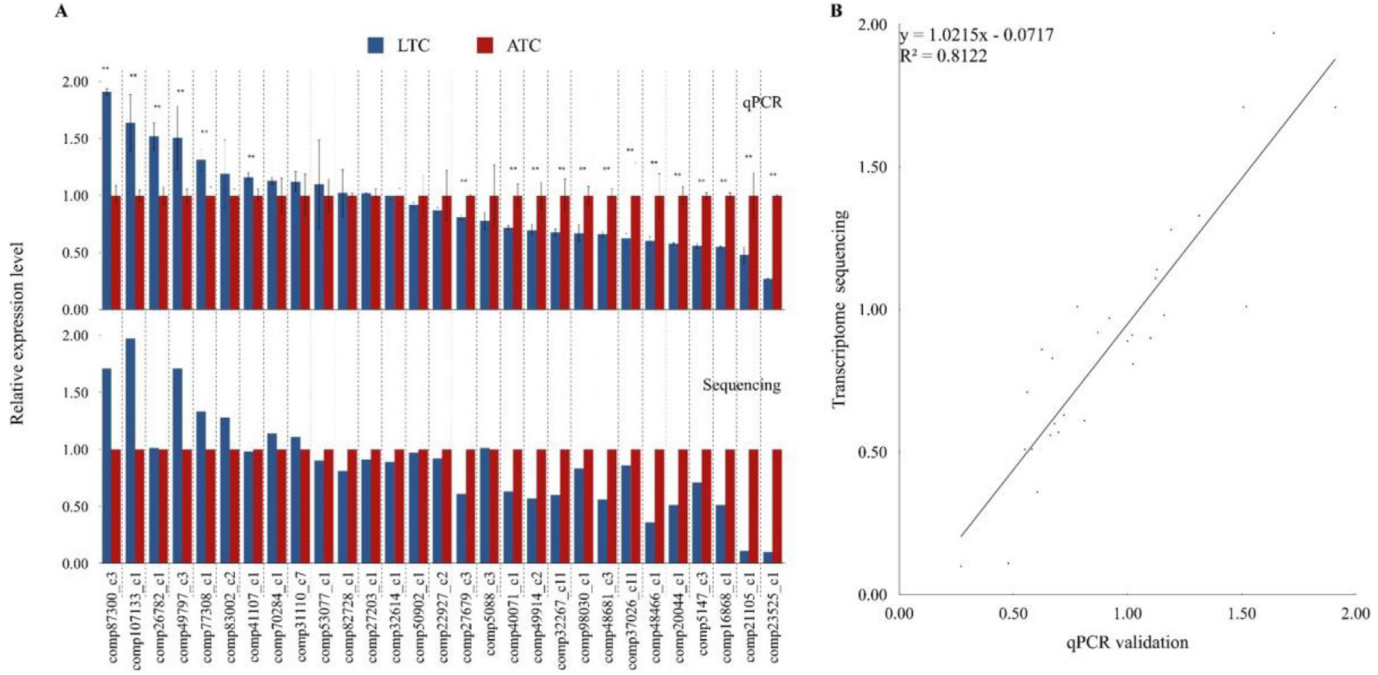

Fig. 9. Validation of transcriptome sequencing results. (A) Expression patterns of selected mRNAs obtained by qRT-PCR and sequencing. (B) Coefficient analysis between mRNA expression ratios obtained from sequencing and qRT-PCR analysis. mRNA expression levels during qRT-PCR analysis were normalized to the level of *β-tubulin*. All qRT-PCRs were repeated three times for each sample and subjected to ANOVA, and the results expressed as the mean ± SD. *P < .05 or **P < .01 were considered statistically significant.

4.2. Antioxidase-related genes

When exposed to stress, the accumulation of reactive oxygen species [30] leads to metabolic disorders in plants. Plants have developed very efficient defense mechanisms that include the expression and activation of genes related to intracellular and extracellular ROS scavengers to cope with oxidative stress to protect themselves against toxic ROS. For example, superoxide dismutase [31] converts super-oxygen ions to H_2O_2, which can then be cleared by catalase (CAT). In addition, peroxidase (POD) can also remove the accumulated ROS, prevent membrane lipid peroxidation, and therefore protect cells from injury. Several peroxidase genes, including *CuZnSOD, swAPX1, GST, APX,* and

swPAs, have been isolated and identified in sweetpotato [32–35]. The expression of almost all the antioxidant genes can be induced under abiotic stresses, including cold exposure. In addition, the overexpression of these genes by transgenic techniques enhances the tolerance of sweetpotato to stress. These genes respond to cold-induced oxidative stress via various signal transduction pathways. The results of the present study were consistent with the findings of previous reports. The gene encoding superoxide dismutase (belonging to the Fe-Mn family) *SOD* (comp89000_c2) was upregulated, whereas that encoding Cu/Zn superoxide dismutase, *SODCC* (comp60799_c2), was downregulated under cold stress. These variations in responses of SODs under cold stress may be explained by the type, cellular location, and mechanisms of action of these SODs. A previous study showed that catalase (*CAT*)-related genes were downregulated during cold stress [19]. However, we observed that transcripts encoding CAT were upregulated [e.g., *CAT3* (comp89371_c1)], downregulated [*CAT4* (comp47313_c3)] or remained unchanged (comp104765_c1, comp49627_c2, and comp22026_c2). This discrepancy may be attributable to the use of different sweetpotato cultivars or distinct regulatory mechanisms in different organs of sweetpotato.

4.3. Carbohydrate metabolism-related genes

Plants enhance freezing tolerance by breaking down starch and accumulating soluble sugars. Soluble sugars play an important role in cold acclimation [36]. DEG analysis showed that the expression of carbohydrate metabolism-related genes was modulated during cold storage, which led to the breakdown of starch and the accumulation of sucrose in response to cold stress (Fig. 10).

Sucrose is one of the main products of photosynthesis in higher plants, which is the most common form of carbon that is transported from the source to sink organs [37]. After storage at LTC for 14 days, the sucrose content in the sweetpotato significantly increased compared to those stored at ATC. During DEGs analysis, we investigated the expression levels of genes closely related to sucrose metabolism such as sucrose synthase-, sucrose phosphate synthase-, and sucrose-converting enzyme-encoding genes.

Sucrose synthase is a key enzyme in sucrose metabolism that catalyzes the reversible reactions involved in the synthesis and breakdown of sucrose [38]. In wheat, sucrose synthase activity increases at low temperatures [39]. In the present study, 24 DEGs were related to sucrose synthase, of which 16 DEGs were regulated and 8 DEGs were downregulated in LTC. The upregulated DEGs included comp85494_c1 and a potential *SUS6* transcript comp72027_c1, which showed 6.905- and 3.16-fold increase in expression levels, respectively. The downregulated genes included the *SUS3* potential transcripts comp21832_c1 and comp21833_c2, which exhibited a decrease in expression levels to 0.097 and 0.195, respectively.

Sucrose-phosphate synthase catalyzes the transfer of a hexosyl group from uridine diphosphate glucose (UDPG) to D-fructose 6-phosphate to form D-sucrose-6-phosphate. It is a rate-limiting enzyme in the synthesis of sucrose [40]. In tomato, chilling delays the circadian pattern of sucrose phosphate synthase [41]. The activity of sucrose phosphate synthase in the leaves of chilling-tolerant and chilling-sensitive maize seedlings increased by about 3.3-fold when temperature was lowered from 30 °C to 10 °C [42]. Our results showed that the *SPS5* transcript (comp81588_c3) was upregulated by 2.522-fold. However, the expression levels of *SPS4* transcript (comp11694_c1) and *SPS1* transcript (comp22724_c2) decreased to 0.001 and 0.223, respectively.

Invertase, also known as β-fructosidase (EC 3.2.1.26), catalyzes the hydrolysis of sucrose, yielding glucose and fructose. It was first cloned in tomato [43]. Transgenic tomato plants expressing a constitutive antisense invertase (*TIV1*) transgene have higher sucrose content [44]. Our study has shown that the expression levels of two potential transcripts for *TIV1*, comp41186_c1 and comp9832_c1, decreased to 0.105 and 0.047, respectively.

The present study also showed that the starch content in LTC was significantly lower than that in ATC after 14 days of storage. We then investigated the expression levels of the enzymes closely related to starch metabolism. Glucose-1-phosphate adenylyltransferase is an important enzyme that participates in starch metabolism [45]. Our results showed that the potential genes encoding the large subunit of *AGPS1,* comp11306_c1, comp26958_c1, and comp75210_c1, showed a decrease in mRNA expression by 2702.35-, 652.57-, and 308.68-fold, respectively. The expression levels of potential genes encoding starch synthase *SS1* (comp4181_c4) and *SS4 (*comp62228_c1) exhibited a 556-, and 10-fold decrease, respectively. The expression levels of potential genes encoding 1,4-alpha-glucan branching enzyme (*SBE1*), comp23075_c1, comp107159_c1, and comp67391_c5 showed a 98.36-, 49.52-, and 32.67-fold decrease, respectively. The expression level of potential gene encoding glycogen (starch) synthase (*WAXY*), comp18538_c4, was downregulated by 40.50-fold.

4.4. Hormone-related genes

Plant hormones are vital to the generation of adaptive responses to

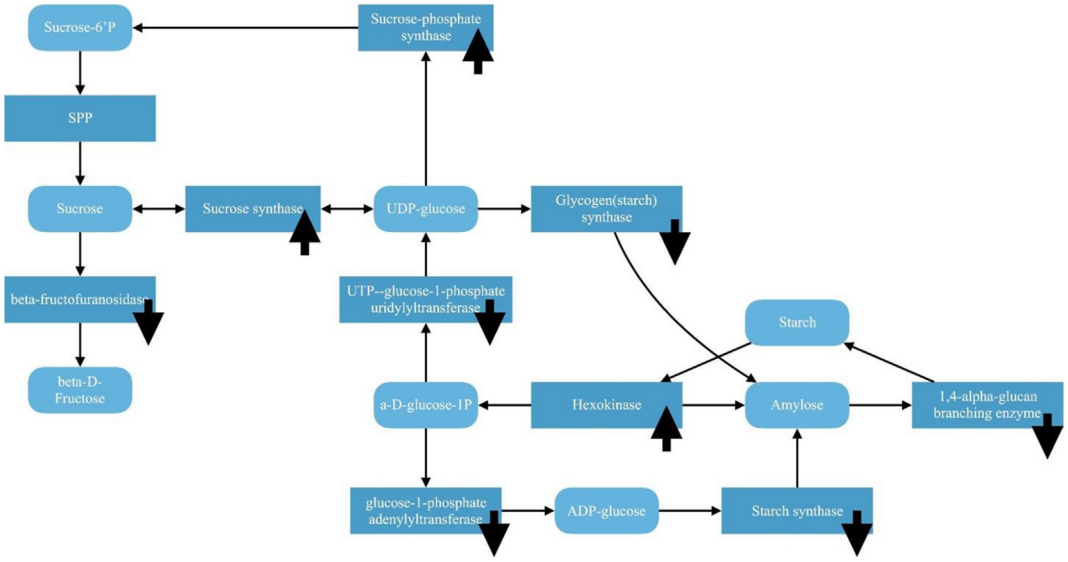

Fig. 10. Starch and sucrose metabolism pathways are induce in response to cold storage.

environmental changes. Plant tolerance to abiotic stress can be enhanced by regulating the synthesis, signal transduction, and action mechanisms of plant hormones using transgenic technologies [46].

Gibberellins are endogenous phytohormones that are essential for the entire life cycle of higher plants. GA biosynthesis and response pathways are activated during *Arabidopsis thaliana* seed inhibition at low temperature [47]. GA-2 oxidase is a key multifunctional enzyme during the third stage of GA synthesis that involves GA deactivation [48]. The genes encoding GA-2 oxidase, which included comp89137_c1, comp95250_c, comp45362_c1, comp73874_c1, comp55658_c2, comp62353_c1, comp49007_c1, and comp69442_c3, were upregulated in the present study. Some GA-responsive genes also showed differential expression between groups. For example, the expression of *LBD40* (comp107496_c1), *BHLH137* (comp95431_c1, mp83029_c1), and *WRKY27* (comp46605_c1) were upregulated, whereas *MYB59* (comp100_c2, comp24798_c1, comp32006_c2), *ABI5* (comp13734_c2, comp33984_c1, comp24706_c1), and *EXPA1* (comp65787_c1) were downregulated.

Abscisic acid [49] is one of the most important signal transduction molecules. Previous studies have shown that ABA has a significant impact on cold resistance in wheat, maize, and other plants [50,51]. DEG analysis showed that the expression of ABA synthesis-related genes significantly differed when sweetpotato was stored in LTC. For example, the expression of *NCED3* (comp53246_c2, comp53247_c3) and *NCED1* (comp56932_c1) were downregulated. 9-*cis*-epoxycarotenoid dioxygenase participates in the early steps of ABA biosynthesis and catalyzes the conversion from carotenoids to ABA. The expression of the gene encoding 9-*cis*-epoxycarotenoid dioxygenase, *NCED5* (comp85733_c1), was upregulated. In addition, xanthoxin dehydrogenase is an enzyme that catalyzes the conversion of xanthoxin to ABA. The expression levels of genes encoding xanthoxin dehydrogenase (*ABA2* transcripts comp106384_c1, comp80130_c1, and comp22404_c1) were upregulated.

Ethylene plays an important role in the storage and ripening of fruits and vegetables. During cold storage, exogenous ethylene causes discoloration of the flesh of avocado fruits and different plum cultivars, prior to other symptoms of CI [30,52]. In subtropical non-climacteric loquat fruits, modulation of the ethylene signaling pathway leads to different responses in two cultivars during post-harvest storage [53]. In the present study, the expression of several ethylene biosynthesis-related genes was upregulated. These genes included genes encoding aminocyclopropanecarboxylate oxidase, *ACO* (comp82943_c2 and comp83275_c4), *ACO3* (comp83277_c29), and *DK-ACO1* (comp83014_c1), as well as those encoding 1-aminocyclopropane-1-carboxylate synthase, *ACS1* (comp90554_c1, comp34255_c5, comp86312_c16, and comp77565_c1). The ERF (ethylene-responsive transcription factor), which is involved in ethylene-mediated signal pathways, has been extensively studied. The CBF/DREB endogenous pathway is involved in various structural, biochemical, and physiological alterations that are associated with cold acclimation [54]. In the present study, several genes encoding ERF were upregulated, e.g., *ERF098* (comp96679_c3, comp94456_c2), *ERF091* (comp77173_c1), *ERF1B* (comp106614_c6, comp82217_c1). Similar to other plants, cold exposure affects the biosynthesis of ethylene and ethylene-related signaling pathways. However, further studies elucidating how ethylene regulates the responses of sweetpotato against cold exposure are warranted.

Salicylic acid is an important endogenous signal molecule that is involved in the activation of plant responses against certain stresses. Salicylic acid solution treatment of young maize plants under normal growth conditions provides protection against subsequent low-temperature stress [55]. Jasmonic acid is ubiquitous in higher plants and has been proven to improve chilling tolerance various plant species [56]. The treatment of tomato fruit with low concentrations of methyl salicylic acid (MeSA) or methyl jasmonate (MeJA) induces the transcription of pathogenesis-related genes, which in turn increases chilling tolerance and pathogen resistance [57]. Similarly, postharvest treatment with MeSA or MeJA significantly reduces CI symptoms and increases antioxidant activities in pomegranates [49]. Salicylates and jasmonates alleviate CI in fruits and vegetables via several mechanisms, which include enhancement of membrane fluidity, increase in the expression and accumulation of heat shock proteins (HSPs), improvement in antioxidant system activity, enhancement of arginine pathway, activation of the CBP pathway, and alterations in PPO and PAL activities [58]. The present study observed an upregulation of genes that were related to the biosynthesis of SA biosynthesis [*BAH1* gene encoding E3 ubiquitin protein ligase, (comp98381_c1)] and JA [*4CLL7* gene encoding 4-coumaroyl-CoA synthetase (comp85751_c3, comp105927_c1), as well as the *PED1* gene that encodes acetyl-CoA acyltransferase (comp823_c10, comp822_c5, comp77386_c12, and comp44686_c1). In addition, putative carboxylesterase is associated with the activity of methyl salicylate esterase and methyl jasmonate-specific methyl esterase. We observed the upregulation of multiple genes encoding putative carboxylesterases (CXEs) (comp82032_c1, comp102441_c4, comp93850_c22, comp93848_c12, and comp93849_c15). Among these genes, one transcript of C*XE2* (comp82032_c1) exhibited a 180-fold upregulation.

5. Conclusions

Refrigeration is considered one of the most effective ways of extending the postharvest life of a variety of crops. However, sweetpotato originated from tropical areas and is thus susceptible to CI. The present study employed transcriptome sequencing to comprehensively investigate the molecular mechanism underlying the response of sweetpotato to CI during postharvest low temperature storage. The characteristic features and nutrient content in sweetpotato changed after storage at 4 °C for 28 days. RNA-sequencing analysis also showed significant changes at the transcriptomic level. Changes in the expressions levels of genes related to the cell membrane system, antioxidant enzymes, carbohydrate metabolism, and hormone metabolism were observed, which in turn lead to the occurrence of CIs and decrease in sweetpotato quality. Our study has revealed the molecular mechanism underlying CI during low temperature storage, which in turn facilitates improvements in postharvest storage of sweetpotato.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygeno.2018.05.014.

References

[1] V. Ravi, J. Aked, C. Balagopalan, Review on tropical root and tuber crops. I. Storage methods and quality changes, Crit. Rev. Food Sci. Nutr. 36 (1996) 661–709.
[2] A. Conesa, S. Gotz, J.M. Garcia-Gomez, J. Terol, M. Talon, M. Robles, Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research, Bioinformatics (Oxford, England) 21 (2005) 3674–3676.
[3] F. He, H. Shen, C. Lin, H. Fu, M.S. Sheteiwy, Y. Guan, Y. Huang, J. Hu, Transcriptome analysis of chilling-imbibed embryo revealed membrane recovery related genes in maize, Front. Plant Sci. 7 (2016) 1978.
[4] K. Wang, X.R. Yin, B. Zhang, D. Grierson, C.J. Xu, K.S. Chen, Transcriptomic and metabolic analyses provide new insights into chilling injury in peach fruit, Plant Cell Environ. 40 (2017) 1531–1551.
[5] A. Zhou, H. Ma, E. Liu, T. Jiang, S. Feng, S. Gong, J. Wang, Transcriptome sequencing of dianthus spiculifolius and analysis of the genes involved in responses to combined cold and drought stress, Int. J. Mol. Sci. 18 (2017).

[6] W. Kanjana, T. Suzuki, K. Ishii, T. Kozaki, M. Iigo, K. Yamane, Transcriptome analysis of seed dormancy after rinsing and chilling in ornamental peaches (Prunus persica (L.) Batsch), BMC Genomics 17 (2016) 575.
[7] V. Sivankalyani, N. Sela, O. Feygenberg, H. Zemach, D. Maurer, N. Alkan, Transcriptome dynamics in mango fruit peel reveals mechanisms of chilling stress, Front. Plant Sci. 7 (2016) 1579.
[8] D. Sanhueza, P. Vizoso, I. Balic, R. Campos-Vargas, C. Meneses, Transcriptomic analysis of fruit stored under cold conditions using controlled atmosphere in Prunus persica cv. "Red Pearl", Front. Plant Sci. 6 (2015) 788.
[9] Q. Cao, A. Li, J. Chen, Y. Sun, J. Tang, A. Zhang, Z. Zhou, D. Zhao, D. Ma, S. Gao, Transcriptome sequencing of the sweet potato progenitor (ipomoea Trifida (H.B.K.) G. Don.) and discovery of drought tolerance genes, Trop. Plant Biol. 9 (2016) 63–72.
[10] H. Zhang, Q. Zhang, H. Zhai, Y. Li, X. Wang, Q. Liu, S. He, Transcript profile analysis reveals important roles of jasmonic acid signalling pathway in the response of sweet potato to salt stress, Sci. Rep. 7 (2017) 40819.
[11] R. Li, H. Zhai, C. Kang, D. Liu, S. He, Q. Liu, De novo transcriptome sequencing of the orange-fleshed sweet potato and analysis of differentially expressed genes related to carotenoid biosynthesis, Int. J. Genom. 2015 (2015) 843802.
[12] P. Ma, X. Bian, Z. Jia, X. Guo, Y. Xie, De novo sequencing and comprehensive analysis of the mutant transcriptome from purple sweet potato (Ipomoea batatas L.), Gene 575 (2016) 641–649.
[13] X. Tao, Y.H. Gu, Y.S. Jiang, Y.Z. Zhang, H.Y. Wang, Transcriptome analysis to identify putative floral-specific genes and flowering regulatory-related genes of sweet potato, Biosci. Biotechnol. Biochem. 77 (2013) 2169–2174.
[14] J. Yang, M.H. Moeinzadeh, H. Kuhl, J. Helmuth, P. Xiao, S. Haas, G. Liu, J. Zheng, Z. Sun, W. Fan, G. Deng, H. Wang, F. Hu, S. Zhao, A.R. Fernie, S. Boerno, B. Timmermann, P. Zhang, M. Vingron, Haplotype-resolved sweet potato genome traces back its hexaploidization history, Nat. Plants 3 (9) (Sep 2017) 696–703.
[15] S. Saraf, N. Sanan-Mishra, N.R. Gursanscky, B.J. Carroll, D. Gupta, S.K. Mukherjee, 3 ' and 5 ' microRNA-end post-biogenesis modifications in plant transcriptomes: evidences from small RNA next generation sequencing data analysis, Biochem. Biophys. Res. Commun. 467 (2015) 892–899.
[16] A. Weig, C. Deswarte, M.J. Chrispeels, The major intrinsic protein family of Arabidopsis has 23 members that form three distinct groups with functional aquaporins in each group, Plant Physiol. 114 (1997) 1347–1357.
[17] J. Ye, L. Fang, H. Zheng, Y. Zhang, J. Chen, Z. Zhang, J. Wang, S. Li, R. Li, L. Bolund, J. Wang, WEGO: a web tool for plotting GO annotations, Nucleic Acids Res. 34 (2006) W293–W297.
[18] Q. Zhao, V. Hautamaki, P. Franti, RSEM: An Accelerated Algorithm on Repeated EM, 2011 Sixth International Conference on Image and Graphics, 12–15 Aug. 2011, 2011, (2011), pp. 135–140.
[19] C.Y. Ji, W.H. Chung, H.S. Kim, W.Y. Jung, L. Kang, J.C. Jeong, S.S. Kwak, Transcriptome profiling of sweetpotato tuberous roots during low temperature storage, Plant Physiol. Biochem. 112 (2017) 97–108.
[20] D. Truong Van, C.J. Biermann, J.A. Marlett, Simple sugars, oligosaccharides and starch concentrations in raw and cooked sweet potato, J. Agric. Food Chem. 34 (1986) 421–425.
[21] H. Rui, S. Cao, H. Shang, P. Jin, K. Wang, Y. Zheng, Effects of heat treatment on internal browning and membrane fatty acid in loquat fruit in response to chilling stress, J. Sci. Food Agric. 90 (2010) 1557–1561.
[22] A. Carpaneto, N. Ivashikina, V. Levchenko, E. Krol, E. Jeworutzki, J.K. Zhu, R. Hedrich, Cold transiently activates calcium-permeable channels in Arabidopsis mesophyll cells, Plant Physiol. 143 (2007) 487–494.
[23] J.M. LYONS, Chilling injury in plants, Annu. Rev. Plant Physiol. 24 (1973) 445–466.
[24] S. Mahajan, N. Tuteja, Cold, salinity and drought stresses: an overview, Arch. Biochem. Biophys. 444 (2005) 139–158.
[25] J.R. Hazel, Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? Annu. Rev. Physiol. 57 (1995) 19–42.
[26] T. Wongsheree, S. Ketsa, W.G.V. Doorn, The relationship between chilling injury and membrane damage in lemon basil (Ocimum × citriodourum) leaves, Postharvest Biol. Technol. 51 (2009) 91–96.
[27] N. Kinoshita, H. Wang, H. Kasahara, J. Liu, C. MacPherson, Y. Machida, Y. Kamiya, M.A. Hannah, N.-H. Chua, IAA-ala Resistant3, an evolutionarily conserved target of miR167, mediates Arabidopsis root architecture changes during high osmotic stress, Plant Cell 24 (2012) 3590–3602.
[28] D.J. Guerra, J.B. Ohlrogge, M. Frentzen, Activity of acyl carrier protein isoforms in reactions of plant fatty acid metabolism, Plant Physiol. 82 (1986) 448–453.
[29] E. Alexandersson, G. Saalbach, C. Larsson, P. Kjellbom, Arabidopsis plasma membrane proteomics identifies components of transport, signal transduction and membrane trafficking, Plant Cell Physiol. 45 (2004) 1543–1556.
[30] E. Pesis, M. Ackerman, R. Ben-Arie, O. Feygenberg, X. Feng, A. Apelbaum, R. Goren, D. Prusky, Ethylene involvement in chilling injury symptoms of avocado during cold storage, Postharvest Biol. Technol. 24 (2002) 171–181.
[31] C. Wang, A. Isoda, P. Wang, Growth and yield performance of some cotton cultivars in Xinjiang, China, an arid area with short growing period, J. Agron. Crop Sci. 190 (2004) 177–183.
[32] Y.H. Kim, J.C. Jeong, H.S. Lee, S.S. Kwak, Comparative characterization of sweetpotato antioxidant genes from expressed sequence tags of dehydration-treated fibrous roots under different abiotic stress conditions, Mol. Biol. Rep. 40 (2013) 2887–2896.
[33] S.Y. Park, S.H. Ryu, I.C. Jang, S.Y. Kwon, J.G. Kim, S.S. Kwak, Molecular cloning of a cytosolic ascorbate peroxidase cDNA from cell cultures of sweet potato and its expression in response to stress, Molecular Genet. Genom. 271 (2004) 339–346.
[34] S.Y. Park, S.H. Ryu, S.Y. Kwon, H.S. Lee, J.G. Kim, S.S. Kwak, Differential expression of six novel peroxidase cDNAs from cell cultures of sweetpotato in response to stress, Molecular Genet. Genom. 269 (2003) 542–552.
[35] H. Yan, Q. Li, S.C. Park, X. Wang, Y.J. Liu, Y.G. Zhang, W. Tang, M. Kou, D.F. Ma, Overexpression of CuZnSOD and APX enhance salt stress tolerance in sweet potato, PPB 109 (2016) 20–27.
[36] Y.Y. MA, Y. ZHANG, J.A. LU, Roles of plant soluble sugars and their responses to plant cold stress, Afr. J. Biotechnol. 8 (2009) 2004–2010.
[37] J. Farrar, C. Pollock, J. Gallagher, Sucrose and the integration of metabolism in vascular plants, Plant Sci. 154 (2000) 1–11.
[38] L. Elling, Effect of metal ions on sucrose synthase from rice grains—a study on enzyme inhibition and enzyme topography, Glycobiology 5 (1995) 201–206.
[39] C.S. Santoiani, J.A. Tognetti, H.G. Pontis, G.L. Salerno, Sucrose and fructan metabolism in wheat roots at chilling temperatures, Physiol. Plant. 87 (1993) 84–88.
[40] S.C. Huber, J.L. Huber, Role and regulation of sucrose-phosphate synthase in higher plants, Annu. Rev. Plant Physiol. Plant Mol. Biol. 47 (1996) 431–444.
[41] T.L. Jones, D.E. Tucker, D.R. Ort, Chilling delays circadian pattern of sucrose phosphate synthase and nitrate reductase activity in tomato, Plant Physiol. 118 (1998) 149–158.
[42] P. Sowiński, A. Dalbiak, J. Tadeusiak, P. Ochodzki, Relations between carbohydrate accumulation in leaves, sucrose phosphate synthase activity and photoassimilate transport in chilling treated maize seedlings, Acta Physiol. Plant. 21 (1999) 375–381.
[43] E. Klann, S. Yelle, A.B. Bennett, Tomato fruit acid invertase complementary DNA : nucleotide and deduced amino acid sequences, Plant Physiol. 99 (1992) 351–353.
[44] E.M. Klann, B. Hall, A.B. Bennett, Antisense acid invertase (TIV1) gene alters soluble sugar composition and size in transgenic tomato fruit, Plant Physiol. 112 (1996) 1321–1330.
[45] B. Muller-Rober, G. Nast, L. Willmitzer, Isolation and expression analysis of cDNA clones encoding a small and a large subunit of ADP-glucose pyrophosphorylase from sugar beet, Plant Mol. Biol. 27 (1995) 191–197.
[46] Z. Peleg, E. Blumwald, Hormone balance and abiotic stress tolerance in crop plants, Curr. Opin. Plant Biol. 14 (2011) 290–295.
[47] Y. Yamauchi, M. Ogawa, A. Kuwahara, A. Hanada, Y. Kamiya, S. Yamaguchi, Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of Arabidopsis thaliana seeds, Plant Cell 16 (2004) 367–378.
[48] S.G. Thomas, A.L. Phillips, P. Hedden, Molecular cloning and functional expression of gibberellin 2- oxidases, multifunctional enzymes involved in gibberellin deactivation, Proceedings of the National Academy of Sciences of the United States of America, vol. 96, 1999, pp. 4698–4703.
[49] M. Sayyari, M. Babalar, S. Kalantari, D. Martínez-Romero, F. Guillén, M. Serrano, D. Valero, Vapour treatments with methyl salicylate or methyl jasmonate alleviated chilling injury and enhanced antioxidant potential during postharvest storage of pomegranates, Food Chem. 124 (2011) 964–970.
[50] Y.D. HOU, Z.F. GUO, Y. YI, Effects of cold acclimation and exogenous pytohormone abscisic acid treatment on physiological indicators of winterness wheat, J. Plant Sci. 5 (2010) 125–136.
[51] F. JANOWIAK, E. LUCK, K. DÖRFFLING, Chilling tolerance of maize seedlings in the field during cold periods in spring is related to chilling-induced increase in abscisic acid level, J. Agron. Crop Sci. 189 (2003) 156–161.
[52] A.P. Candan, J. Graell, C. Larrigaudière, Roles of climacteric ethylene in the development of chilling injury in plums, Postharvest Biol. Technol. 47 (2008) 107–112.
[53] P. Wang, B. Zhang, X. Li, C. Xu, X. Yin, L. Shan, I. Ferguson, K. Chen, Ethylene signal transduction elements involved in chilling injury in non-climacteric loquat fruit, J. Exp. Bot. 61 (2010) 179–190.
[54] M.T. Pino, J.S. Skinner, Z. Jeknic, P.M. Hayes, A.H. Soeldner, M.F. Thomashow, T.H. Chen, Ectopic AtCBF1 over-expression enhances freezing tolerance and induces cold acclimation-associated physiological modifications in potato, Plant Cell Environ. 31 (2008) 393–406.
[55] T. Janda, G. Szalai, I. Tari, E. Páldi, Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize (Zea mays L.) plants, Planta 208 (1999) 175–180.
[56] S. Meir, S. Philosoph-Hadas, S. Lurie, S. Droby, M. Akerman, G. Zauberman, B. Shapiro, E. Cohen, Y. Fuchs, Reduction of chilling injury in stored avocado, grapefruit, and bell pepper by methyl jasmonate, Can. J. Bot. 74 (1996) 870–874.
[57] C.K. Ding, C.Y. Wang, K.C. Gross, D.L. Smith, Jasmonate and salicylate induce the expression of pathogenesis-related-protein genes and increase resistance to chilling injury in tomato fruit, Planta 214 (2002) 895–901.
[58] M.S. Aghdam, S. Bodbodak, Physiological and biochemical mechanisms regulating chilling tolerance in fruits and vegetables under postharvest salicylates and jasmonates treatments, Sci. Hortic. 156 (2013) 73–85.