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Research article

Transcriptome profiling of sweetpotato tuberous roots during low temperature storage

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ABSTRACT

Sweetpotato [*Ipomoea batatas* (L.) Lam] is a globally important root crop with high industrial value. However, because sweetpotato tuberous roots undergo chilling injuries that negatively affect their quality at temperatures below 10 °C, postharvest damage during the winter season is a major constraint for industrialization. To understand chilling injury response during postharvest low temperature storage, we used next-generation sequencing technology to comprehensive analyze the transcriptome of tuberous roots stored at optimal (13 °C) or low temperature (4 °C) for 6 weeks. From nine cDNA libraries, we produced 298,765,564 clean reads, which were *de novo* assembled into 58,392 unigenes with an average length of 1100 bp. A total of 3216 differentially expressed genes (DEGs) were detected and categorized into six clusters, of which clusters 2, 4, and 5 (1464 DEGs) were up-regulated under low temperature. The genes in these three clusters are involved in biosynthesis of unsaturated fatty acids, pathogen defense, and phenylalanine metabolism. By contrast, genes in clusters 1, 3, and 6 (1752 DEGs), which were generally down-regulated at low temperature, encode antioxidant enzymes or are involved in glycerophospholipid, carbohydrate, or energy metabolism. We confirmed the results of the transcriptome analysis by quantitative RT-PCR. Our transcriptome analysis will advance our understanding of the comprehensive mechanisms of chilling injury during low temperature storage and facilitate improvements in postharvest storage of sweetpotato tuberous roots.

1. Introduction

Sweetpotato [*Ipomoea batatas* (L.) Lam] is an important root crop, with an annual production of more than 100 million tons worldwide (FAO, 2013). It is a major source of human food, animal feed, and industrial raw materials for biofuel production, as well as an abundant source of nutrients and natural antioxidant compounds (Yoshinaga et al., 1999; Teow et al., 2007). In addition, sweetpotato is an abiotic and biotic stress-tolerant crop that can be grown on marginal land, making it a globally important food source (Ziska et al., 2009). In light of these properties, sweetpotato is industrially valuable. However, the postharvest storage of sweetpotato is a major bottleneck for industrial applications of sweetpotato. In East Asia and China, which are responsible for more than 80% of world production, sweetpotato is usually harvested in late autumn and early winter, and the tuberous root is prone to a

Abbreviations: ATPase B, ATP synthase B chain; APL, ADP-glucose pyrophosphorylase large subunit; APS, ADP-glucose pyrophosphorylase small subunit; CAT, catalase; CCoAOMT, caffeoyl-COA O methyltransferase; CML, calmodulin like; CuZnSOD, copper-zinc superoxide dismutase; DEGs, differentially expressed genes; FAD, fatty acid desaturase; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genome; KOG, eukaryotic Ortholog Groups database; MCP, mitochondrial acyl carrier protein; MDA, malondialdehyde; PAL, phenylalanine ammonia lyase; PLD, phospholipase D; PPO, polyphenol oxidase; PR, pathogenesis-related; RIN, RNA integrity number; ROS, reactive oxygen species; SS, starch synthase; STP, sucrose-transporter protein; SUS, sucrose synthase; TAIR, The Arabidopsis Information Resource; V-ATPase A, vacuolar ATP synthase subunit A.

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physiological disorder known as chilling injury during low temperature storage. Chilling injury can arise in farm storage facilities, during long-distance transport, in wholesale or retail storage facilities, in markets, and in consumer refrigerators. Previous studies aimed at extending the storage period identified that the optimal storage condition is a controlled environment at 13–16 °C with 80–85% (high) relative humidity (Woolfe, 1992). However, temperature-controlled storage of sweetpotato may not be economically feasible in some developing countries, and prolonged storage requires expensive and sophisticated facilities as well as significant maintenance costs.

Low temperature storage is widely used to extend the postharvest life of a variety of crops. However, some tropical and subtropical plants are sensitive to temperatures below 10 °C, which induce the development of chilling injury in these crops. The physiological responses to chilling injury have been comprehensively elucidated in some horticultural species (Sevillano et al., 2009). The primary response involves modification of cell membrane conformation and structure via changes in the lipid composition of the membrane, resulting in reduced permeability and fluidity; these changes affect the functions of both mitochondria and chloroplasts. These changes cause an increase in reactive oxygen species (ROS) production, resulting in elevation of oxidative stress, which is considered to be a secondary response to chilling injury.

Several previous studies investigated the physiological and biochemical changes that occur at low temperature in sweetpotato tuberous roots. Although limited periods of low temperature exposure (5 °C for 4 weeks) increased the nutraceutical value (i.e., the concentrations of phenolic compounds) of the roots, longer periods resulted in chilling injury, which decreased their market value (Padda and Picha, 2008). Long-term exposure to low temperature results in vacuolar membrane degradation, mitochondrial membrane swelling (Yamaki and Uritani, 1972), internal tissue darkening (Lieberman et al., 1958; Minamikawa et al., 1961), elevated sucrose and total soluble sugar content (Picha, 1987), reduced starch content, surface pitting, and susceptibility to decay (Edmunds et al., 2008). The damaged roots undergo irreversible deterioration and are more easily infected by nonpathogenic fungi (Uritani, 1999). However, the mechanism of damage during postharvest storage at low temperature has not been elucidated at the molecular level. The lack of genomic information of sweetpotato was an obstacle to this type of studies.

Introduction of Next-generation RNA sequencing (RNA-seq) enabled the gene expression study at whole transcriptome level without prepared genome information, because RNA-seq allows transcript identification and discovery of genes involved in specific biological processes in non-model crops (Strickler et al., 2012; Varshney et al., 2009). RNA-seq has successfully applied for Tiger lily and turf grass to identify genes involved in low temperature stress (Wang et al., 2014; Wei et al., 2015). The transcriptomic studies, about crops such as peach, Ponkan mandarin, tomato, and grapefruit flavedo investigated changes in gene expression levels while the crops were exposed to low temperature storage (Puig et al., 2015; Zhu et al., 2011; Cruz-Mendívil et al., 2015; Maul et al., 2008). Those works resulted in dramatic improvements in our understanding of the plant response to cold including the molecular mechanism of chilling injury during low temperature storage.

In this study, we performed the large-scale transcriptome analysis of sweetpotato tuberous roots under low temperature stress. Specifically, we investigated the transcriptional profile of sweetpotato tuberous roots during low temperature storage by performing RNA-seq on the Illumina HiSeq 2000 platform. To the best our knowledge, this study is the first work that shows the transcriptome profile of sweetpotato tuberous roots under low temperature stress with the high-throughput sequencing technology. The data revealed a mass of gene resources of sweetpotato and elucidate the biological processes that occur under low temperature storage. These new findings shed light on the molecular mechanisms of chilling injury in tuberous roots, and will also facilitate improvements in prolonged cold storage of sweetpotato.

2. Materials and methods

2.1. Plant materials and low temperature treatment

Sweetpotatoes (*Ipomoea batatas* Lam., cv. Yulmi, one of the most widely grown varieties in Korea) were planted in the experimental station of the National Institute of Crop Science, Muan, Korea. Tuberous roots were harvested in early November, followed by curing. Afterwards, the storage temperature was shifted to 13 °C at relative humidity >80%. Samples were taken from controls (0 week) and at specified internals during 6 week exposure to storage at 13 °C or 4 °C. Sampled tissues were immediately frozen in liquid nitrogen and stored at −70 °C prior to analysis.

2.2. Physiological response assays

Malondialdehyde (MDA) content was measured in sweetpotato tuberous root using a modified thiobarbituric acid method (Horie et al., 2005). The specific absorbance of extracts was recorded at 532 nm. Non-specific absorbance was measured at 600 nm, and these values were subtracted from the 532 nm readings. The MDA concentration was taken as a measure of lipid peroxidation. Relative H_2O_2 content was assessed using xylenol orange: H_2O_2 is reduced by ferrous ions in an acidic solution, yielding a ferric product–xylenol orange complex that can be detected at 560 nm (Bindschedler et al., 2001). Total phenolic compound contents were analyzed using gallic acid to generate a standard curve (Ainsworth and Gillespie, 2007). Soluble sugars were quantified by the 3,5-dinitrosalicylic acid method at 540 nm as proposed by Miller (1959), using glucose to generate the standard curve. Experiments were conducted in three biological replicates, and results are expressed as means ± standard error (SE).

2.3. Total RNA extraction, RNA-seq library construction, and sequencing

Total RNA was extracted using a Trizol-based protocol and further purified using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA), with three biological replicates per condition. The RNA was quality-controlled and quantified on a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). A total of 10 μg of total RNA was subjected to Illumina RNA-seq. RNA integrity was confirmed on a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). All nine samples had 28S:18S ratios in the range of 1.8–2.0 with intact 28S, 18S and 5S RNA bands, high RNA purity, and mean RNA integrity number (RIN) > 8.0, which satisfied the requirements for library construction and sequencing. The established cDNA libraries were sequenced on an Illumina HiSeq 2000 system to generate 100 bp paired-end reads. The raw sequence reads were deposited in the National Center for Biotechnology Information (NCBI) SRA database.

2.4. Preprocessing and de novo transcriptome assembly

Raw sequencing data consisting of 100 bp paired-end reads were filtered by Phred quality score ($Q \geq 20$) and read length (≥25 bp) using SolexaQA (Cox et al., 2010). Trimming resulted in reads