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Energy status of kiwifruit stored under different temperatures or exposed to long-term anaerobic conditions or pure oxygen

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ABSTRACT

Energy status is a key factor switching on ripening and senescence of fruit. In this study, kiwifruit was stored at 15 °C or 25 °C or exposed to long-term N_2 and O_2. Energy characteristics and transcript abundance of energy-related genes cloned from kiwifruit in relation to fruit quality, respiration rate and ethylene production rate were investigated. The concentrations of adenylate triphosphate (ATP), adenylate diphosphate (ADP) and adenylate monophosphate (AMP) peaked during storage in the following order: AMP, ADP and ATP. The transcript abundances of ADP/ATP carrier 1 (*AdAAC1*), ATP synthase β subunit (*AdAtpB*) and sucrose non-fermenting-1-related kinase 1 (*AdSnRK1*) fluctuated during storage. Transcript abundance peaks of alternative oxidase 2 (*AdAOX2*) and uncoupling protein (*AdUCP*) appeared after 2 days of storage, consistent with the peak in respiratory rate. Low temperature (15 °C) and long-term N_2 treatment maintained higher firmness, blocked respiration and energy production, and lowered the total soluble solids (TSS) content, ATP level, and ATP/AMP ratio, whilst these treatments increased the transcript abundance of *AdAAC1* and *AdSnRK1*. Furthermore, low temperature storage increased the transcript abundance of *AdAtpB*, *AdAOX2* and *AdAUCP*. Long-term O_2 application dramatically elevated the transcript abundance of *AdAOX2* and *AdUCP*, especially at the beginning of storage. It was suggested that ripening and senescence of kiwifruit was closely related to the energy level, which in turn was positively correlated with respiration activity and regulated in coordination with *AdAAC1*, *AdAtpB*, *AdAOX2*, *AdAUCP* and *AdSnRK1*.

1. Introduction

Adenylate triphosphate (ATP) is the common energy currency for cellular metabolism in living organisms. Energy metabolism plays crucial roles in postharvest physiology of horticultural crops. Studies on apple (Saquet et al., 2000), pear (Saquet et al., 2001, 2003; Veltman et al., 2003), longan (Su et al., 2005), litchi (Wang et al., 2013), lettuce (Braidot et al., 2014) and cut carnation flowers (Song et al., 2006) have shown that the senescence process was negatively correlated with the cellular energy status. Furthermore, exogenous ATP application could delay pericarp browning of litchi (Yi et al., 2008) and prolong the bottle life of cut carnation flowers (Song et al., 2006). High oxygen treatment maintained the ATP level and inhibited litchi fruit pericarp browning (Duan et al., 2004). This was also tested in "Conference" pears with controlled atmosphere storage by Xuan et al. (2005). Therefore, postharvest browning and senescence of horticultural products may be due to a lower cellular energy level or a restricted energy supply. Cellular energy status is a key factor in maintaining basic cellular metabolism, which is indispensable for the maintenance of quality in fruits and vegetables during storage or transportation (Jiang et al., 2007). However, the physiological and molecular mechanisms of energy regulation in horticultural products remain unclear.

ATP is generated mainly by mitochondrial respiration. Its level is tightly controlled by the energy regulation network, which is responsible for the synthesis, transportation and consumption of ATP. Within this network, ATP synthase is a key enzyme in the biosynthesis of ATP. Subunit β is located in the centre of ATP synthase and plays a pivotal role in ATP degradation and synthesis (Brandt et al., 2013). ADP/ATP carrier (AAC) is the core of the mitochondrial adenosine transportation system in higher

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organisms, where it is located in the mitochondrial inner membrane and transports ATP from the site of synthesis to the site of utilisation (Spetea et al., 2011). In addition, plants have two energy dissipation systems, including mitochondria uncoupling protein (UCP) and alternative oxidase (AOX) to reduce the ATP synthesis rate and the reactive oxygen species (ROS) accumulation, respectively, while increasing heat dissipation, thereby maintaining cell energy homeostasis (Borecky et al., 2006). Sucrose non-fermenting-1-related protein kinase (SnRK), as an energy sensor, responds to the sucrose signalling pathway and globally regulates the metabolism of energy resources such as carbohydrates and other alternative substances that provide energy for cells under stress (Baena-Gonzalez et al., 2007; Guerinier et al., 2013).

Kiwifruit is a climacteric fruit that softens rapidly and is perishable after respiration and ethylene peak. Cold storage is the most efficient technique for suppressing respiration and consequently preventing qualitative deterioration of horticultural products, and it is widely used for transportation and storage of fruit and vegetables. It was reported that anaerobic treatment can prolong the storage life of harvested litchi (Liu et al., 2007) and kiwifruit (Song et al., 2009). High oxygen treatment can also extend shelf-life and maintain the quality of fruit and vegetables (Kader and Ben-Yehoshua, 2000). Furthermore, both of these treatments improved the energy level of harvested litchi fruit (Duan et al., 2004; Liu et al., 2007). Although cold storage, anaerobic and high oxygen treatments have been proved to be effective in reducing respiration rate and ethylene output, they generally decrease enzyme activity levels and thus delay ripening and extend the storage life of fruit and vegetables. The physiological and molecular mechanisms related to energy metabolism underlying these responses are far from being understood. In this study, two experiments were designed. In the first experiment, kiwifruit were packed individually and stored under different temperature conditions. In the second experiment, kiwifruit were exposed to long-term O_2 or long-term N_2. The energy-related genes described above were cloned, and energy status and the transcript abundance of those genes in relation to ripening and senescence of harvested kiwifruit were analysed. Meanwhile, correlations between respiration, ethylene production and energy status as well as gene expression were analysed.

2. Materials and methods

2.1. Materials and treatments

Kiwifruit (*Actinidia deliciosa* cv. Miliang) were harvested from a commercial orchard in Heyuan, Guangdong, PR China. Fruit were selected for uniformity of shape and size and for absence of visible diseases and blemishes. The selected fruit were surface-sterilised in 0.5% sodium hypochlorite solution for 5 s, air-dried and divided into two groups. One group was divided into two subgroups. Fruit was packaged individually in low-density polyethylene bags (0.015 mm thick) before storage at 15 °C (subgroup I) or 25 °C (subgroup II) for 7 days. The other group was divided into three subgroups. Seventy-five fruit were placed in 35-L dry boxes. There were three boxes per treatment in a flow-through gas system. Pure oxygen and nitrogen gases (Guangzhou Gas Factory, China) were applied. Fruit samples were continuously kept in humidified air (control), pure O_2 at 0% CO_2 or nitrogen gas for up to 8 days at 25 °C and 80–90% RH according to Song et al. (2009). Respiration rate, ethylene production rate, fruit firmness and TSS were determined using 15 fresh fruit randomly sampled from each group every day after the beginning of storage. The remaining pulp tissue was collected, frozen in liquid nitrogen and stored at −80 °C for RNA extraction and for determination of energy level.

2.2. Fruit firmness, TSS, respiration rate and ethylene production rate

Fruit firmness was determined with a GY-1 sclerometer (Zhejiang Tuopu Instrument Co., Ltd., Zhengjiang, China). TSS was tested with a hand-held refractometer (J1-3A, Guangzhou Scientific Instruments, Guangdong, China). Respiration rate was measured with a Li-6262 CO_2/H_2O analyser (LI-COR, Inc., Lincoln, NE, USA) using the infrared carbon analysis method. Ethylene production rate was measured by gas chromatograph GC-2010 (Shimadzu Corporation, Kyoto, Japan) with an HP-PLOT/Q column (30 m × 0.32 mm × 0.20 μm). The conditions were as follows: column temperature: 80 °C; FID temperature: 180 °C; injection port temperature: 120 °C; flow rate: 1.62 mL min^{-1}; diversion ratio: 1:3.

2.3. HPLC analysis of ATP, ADP and AMP

Extraction of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) was performed as described by Liu et al. (2006). ATP, ADP and AMP levels were determined by HPLC (Waters, Inc., Milford, MA, USA) with a Pinnacle II-C18 column (4.6 mm × 250 mm) and a UV detector at 254 nm. Mobile phase: 100% phosphate buffer (0.06 mol L^{-1} KH_2PO_4 and 0.04 mol L^{-1} K_2HPO_4 dissolved in deionised water, adjusted to pH 6.8 with 0.1 mol L^{-1} KOH, and filtrated with 0.45-μm filter membrane); flow rate: 1.0 mL min^{-1}; injection volume: 10 μL; Elution time: 20 min. ATP, ADP and AMP concentrations were calculated according to the external standard programme and normalised to fresh weight (FW). Energy charge (EC) was calculated as

$$([ATP] + 0.5 \times [ADP])/([ATP] + [ADP] + [AMP])$$

2.4. RNA extraction, gene cloning, sequence and determination of gene transcript levels

Total RNA was extracted from kiwifruit pulp using the hot borate method (Wan and Wilkins, 1994). Extracted RNA was purified and reverse transcribed with Prime-Script™ RT-PCR Kit (TaKaRa, Dalian, China) according to the manufacturer's protocol. The resulting cDNA was subjected to degenerate PCR using primers (Table 1) designed based on conserved nucleotide sequences of *AtpB*, *AAC*, *AOX*, *UCP*, and *SnRK* from other species. The longer fragments of these genes were obtained after 3′ rapid amplification of cDNA ends (3′-RACE). The sequences obtained were then compared with known sequences from other species using NCBI BLAST. The resulting sequences were designated as *AdAtpB*, *AdAAC1*, *AdAOX2*, *AdUCP* and *AdSnRK1* and were deposited in GenBank (http://www.ncbi.nlm.nih.gov) with the accession numbers KJ466121 (*AdAtpB*), KJ466123 (*AdAAC1*), KJ466119 (*AdAOX2*), KJ466119 (*AdUCP*), and KJ466122 (*AdSnRK1*) (Table 2).

Quantitative real-time PCR (qPCR) was performed on an ABI 7500 Real-time PCR System (Applied Biosystems, Carlsbad, CA, USA) with the LightCycler 480 SYBR Green I Master Mix (Roche Applied Science, www.roche-applied-science.com) to detect the relative transcript abundance of genes. The programme was as follows: 30 s at 95 °C, 40 cycles of 5 s at 95 °C and 34 s at 60 °C. Primers are listed in Table 3. The *AdACTIN* gene was used for quantitative normalisation.

2.5. Bioinformatics and statistical analysis

Identification of nucleotide sequences from RT-PCR clones was performed with NCBI BLAST (http://www.ncbi.nlm.nih.gov/BLAST). Alignments were made using Clustal X and Jalview software, and cladograms were constructed by the