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

Zihui Huang[a], Lifang Guo[b], Hui Wang[a], Hongxia Qu[a,*], Sanmei Ma[b], Yifei Liu[a], Hongwen Huang[a], Yueming Jiang[a]

[a] Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China
[b] Department of Bioengineering, Jinan University, Guangzhou 510632, China

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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

* Corresponding author at: South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, PR China. Tel.: +86 20 37083042.
E-mail address: q-hxia@scbg.ac.cn (H. Qu).

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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

Table 1
Degenerate primers for cloning of energy related genes in kiwifruit.

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
AdAtpB	TGGAGTTGGAAAGACTGTTCTGathatggaryt	CATCCATTCCCAGAATAGCAatdatrtcytg
AdAAC1	TTAAGAAGGATAGAGATGGATATTGgaartggttyg	TTTCCAAACAAAATAATTTGCAGTtkrtcrtancc
AdAOX2	CAACTACTTTTCTGGATAAAATGGCTtwytggacngt	CAATAATCAATAGCAATAGCTGGAgcnggnacrtt
AdUCP	TGGATACTGCTAAGGTTAGACTGcarytncaraa	CATAAGAAGCCAGTTCAGCAgcrttdatdat
AdSnRK1	GGACTGTCTAATATTATGCGAGATggncayttyyt	CCAGCAGCAGATAATAAGCAACAgtnscytcrtt

AtpB, ATP synthase β subunit; *AAC1*, ADP/ATP carrier 1; *AOX2*, alternative oxidase 2; *UCP*, mitochondrial uncoupling protein; *SnRK1*, sucrose non-fermenting-1-related kinase 1.

Table 2
Homologies based on nucleotide sequences for energy related genes isolated from kiwifruit.

Gene	GenBank number	Top Arabidopsis BlAST match	Top BLAST match excluding Arabidopsis
AdAtpB	KJ466121	AtAtpB NM_120953.3 72%	VvAtpB XM_002283915.1 73%
AdAAC1	KJ466123	AtAAC1 NM_111692.3 71%	VvAAC1 XM_002279712.2 71%
AdAOX2	KJ466119	AtAOX2 NM_125817.2 52%	VvAOX2 NM_001281072.1 64%
AdUCP	KJ466120	AtPUMP AJ223983.1 69%	VvUCP3 XM_002277385.1 72%
AdSnRK1	KJ466122	AtSnRK NM_180157.1 75%	AaSnRK1 JX067541.1 97%

See legend of Table 1 for names of genes.

Table 3
Primers used for Real Time Quantitative PCR analysis.

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
AdAtpB	CAAGGAATCTGCTGCCTAGACAAGT	CCCCATCGGGATACAATCAAAACAG
AdAAC1	TTCCTTTGCCTTTTAGCAGTAGATG	ATAACCGAAACTCAATGACCAACAG
AdAOX2	GAGGCACCCGCTCCTGTTGGTTATC	TAACAGCCCCTGGAATGAGAAAACA
AdUCP	CTTTGGCTTTCTACAAGGGCTTCAT	CTTAGCCTGCTCCAAGGTCAGAAAC
AdSnRK1	ACGATGGTGTTGCTCGGTTAC	CCATTGGTCGCTTCAAGTTTC

See legend of Table 1 for names of genes.

neighbour-joining method using the MEGA programme and visualised with TreeView software.

Experiments were arranged in a completely randomised design. There were three replicates of each treatment, with 15 sample fruit per replicate. The experiment was repeated twice during two consecutive growing seasons. Significant differences were tested by one-way analysis of variance (ANOVA) using SPSS® version 13.0 (SPSS, Inc., Chicago, IL, USA). Significant differences were assessed with a significance level of 5%. Graphs were drawn with SigmaPlot 10.0.

3. Results

3.1. Firmness and TSS

Flesh firmness and TSS level reflect fruit maturity. Flesh firmness decreased whilst TSS increased during storage. Storage at a lower temperature delayed the decrease of flesh firmness and increase of TSS (Fig. 1A and B). Long-term O_2 treatment accelerated the decrease of flesh firmness, whereas long-term N_2 treatment blocked this process (Fig. 1C). In addition, TSS content in fruit treated with long-term O_2 showed a wavelike increase, but TSS was maintained at a relatively low level in fruit treated with long-term N_2 (Fig. 1D). This suggested that both 15 °C storage and long-term N_2 treatment delayed the ripening of kiwifruit, with the latter being more efficient, and long-term O_2 treatment clearly accelerated ripening.

3.2. Respiration rate and ethylene production rate

As a typical respiratory climactic fruit, kiwifruit showed obvious peak values for respiration rate and ethylene production rate on the 4th and 5th days, respectively, after commencing storage. Lower temperature storage significantly inhibited both respiration and ethylene production (Fig. 2A and B). The respiration and ethylene production rates were accelerated, and their peaks appeared earlier in long-term O_2-treated fruit, but they were maintained at significantly lower levels and postponed in long-term N_2-treated fruit (Fig. 2C and D). These results confirmed that lower temperature storage and long-term N_2 treatment significantly delayed the ripening of kiwifruit.

3.3. Energy status

ATP and ADP accumulation increased, with ATP increasing more rapidly, after 3 days of storage at 25 °C, whereas AMP accumulation decreased as storage progressed. They peaked from early to late in the order of AMP, ADP and ATP. Total adenylate (adenylate pool) followed a similar pattern to that of ATP (Fig. 3). Low temperature (15 °C) storage and long-term N_2 treatment decelerated the accumulation of ATP, ADP and AMP. EC and the ATP/AMP ratio were elevated quickly after peaks at 2 or 3 days of storage, with low temperature and long-term N_2 treatments accelerating these changes, whereas long-term O_2 treatment retarded these changes. Long-term O_2 treatment elevated the accumulation of ADP and AMP during storage and increased the accumulation of ATP and total adenylate in the first 3 days of storage but decreased ATP content thereafter. In addition, there was no significant difference in total adenylate after 3 days of storage between control and long-term O_2-treated fruit (Fig. 4).

3.4. Isolation and sequence analysis of energy-related genes

Partial sequence fragments of energy-related genes were isolated by reverse transcription PCR (RT-PCR) using degenerate primers (Table 1). Sequencing of several of these fragments revealed the existence of a single isoform for each gene. These genes were *AdAtpB*, *AdAAC1*, *AdAOX2*, *AdUCP* and *AdSnRK1*. Longer

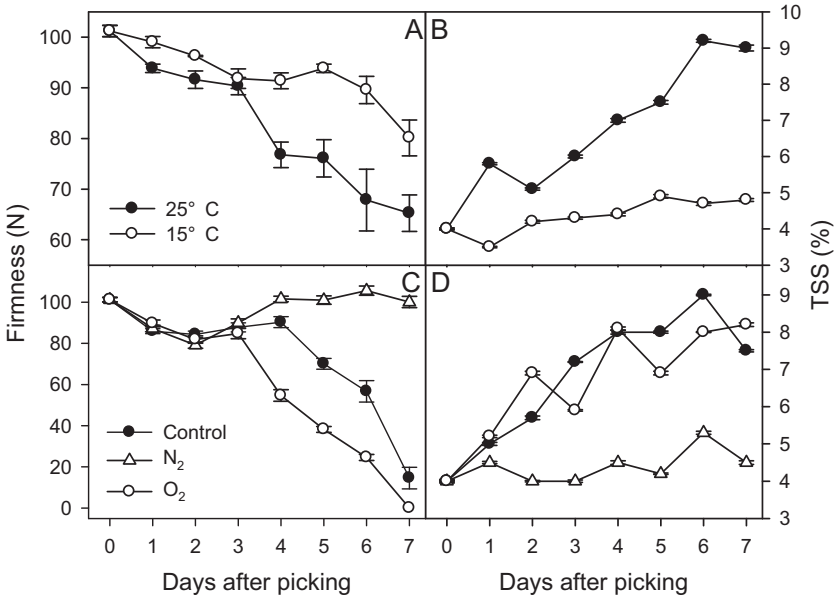

Fig. 1. Firmness and TSS in harvested kiwifruit stored under different temperatures or exposed to long-term N_2 or O_2. TSS, total soluble solids. Data are the mean ± standard deviation ($n = 3$). The vertical bars indicated deviation exceeded the symbol size.

sequences of these genes were obtained via 3′-RACE. These sequences were compared with known sequences from other species using NCBI BLAST. The GenBank accession codes of the sequences are listed in Table 2.

AdAtpB was 73% homologous with the *AtpB*s from *Vitis vinifera*. The C-terminal domain of AdAtpB contained the highly conserved 'DELSEED' motif (Supplementary files S1 and S2) involved in mechanochemical coupling of ATP synthase (Mnatsakanyan et al., 2011). The *AdAAC1* gene isolated from kiwifruit exhibited 62% identity with *AAC1* from *A. thaliana* and 71% identity to *AAC1* from *V. vinifera*. AdAAC1 contained a highly conserved RRRMMM signature motif (Supplementary files S3 and S4). *AOX* belongs to a multigene family in many plants, including mango, tomato and other horticultural crops. In the present study, one isoform of *AdAOX2* was isolated and validated, and it showed 64% identity with that of *V. vinifera*. The highly conserved residues were identified in kiwifruit using multiple sequence alignments of AOX proteins from diverse organisms (Supplementary files S5 and S6). The major feature of UCPs from plants is the presence of three energy transfer protein signatures (ETPS) (Borecky et al., 2006) conserved in all UCP isoforms (Supplementary file S7). In the present study, three copies of the ETPS signature were identified in *AdUCP*, which shared 69% identity with *AtPUMP* (AJ223983.1) from *Arabidopsis thaliana* and 72% identity with *VvUCP3* (XM_002277385.1) from *V. vinifera* at the protein level (Supplementary files S7 and S8). The plant SnRK family can be divided into three subfamilies: SnRK1, SnRK2 and SnRK3. Among these subfamilies, SnRK1s play central roles in coordinating energy balance and nutrient metabolism in plants (Shen et al., 2009). In the present study, the deduced amino acid sequence of SnRK isolated from kiwifruit showed 97%

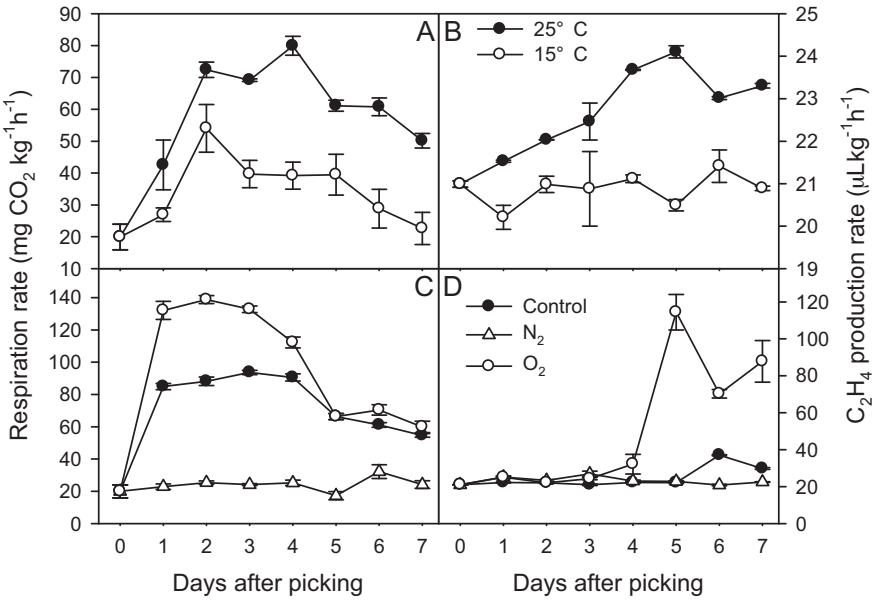

Fig. 2. Respiration rate and ethylene production rate in harvested kiwifruit stored under different temperatures or exposed to long-term N_2 or O_2. Data are the mean ± standard deviation ($n = 3$). The vertical bars indicated deviation exceeded the symbol size.

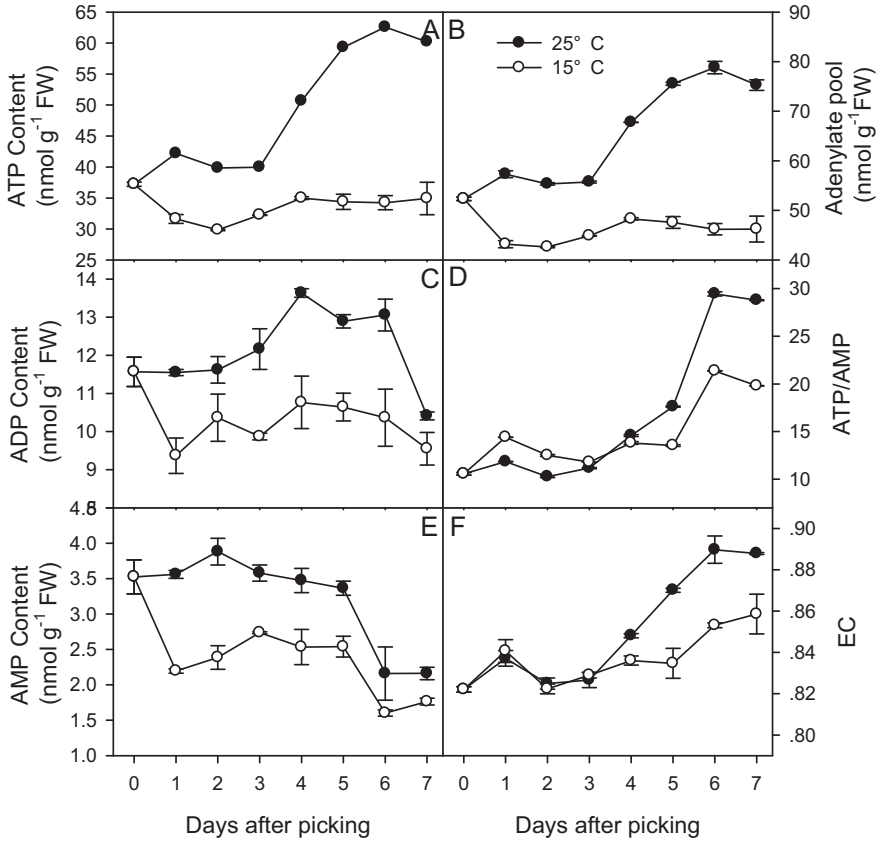

Fig. 3. Energy status in harvested kiwifruit stored under different temperatures. ATP, adenosine triphosphate (A); ADP, adenosine diphosphate (C); AMP, adenosine monophosphate (E); adenylate pool, total adenylate (B); ATP/AMP, ratio of ATP/AMP (D); and EC, energy charge (F). Contents of ATP, ADP, AMP and total adenylate are expressed as nmol g^{-1} FW. FW, fresh weight. Data are the mean ± standard deviation ($n = 3$). The vertical bars indicated deviation exceeded the symbol size.

homology with the SnRK from *Actinidia arguta*, a member of the same family of *Actinidia*. Similar to other *SnRK1* genes, the Thr protein kinase-specific subdomain (DFGLSNVMHDGHFLK-TSCGSPNYAAPE) was highly conserved (Supplementary file S9). In a neighbour-joining cladogram, *AdSnRK1* and its counterparts *AaSnRK*, *VvSnRK1* and others were clustered in subclass 1 (Supplementary file S10).

3.5. Transcript abundance of energy-related genes

Relative abundance of the five energy-related genes in fruit stored at 25 °C decreased after picking except for that of *AdUCP*, which increased during the first 2 days of storage, and that of *AdAOX2*, which decreased during the first half of storage and recovered thereafter. The levels of all of these genes were increased at the beginning of storage but decreased thereafter and were present at substantially higher levels in fruit stored at 15 °C than in fruit stored at 25 °C (Fig. 5).

The transcript abundances of *AdAAC1*, *AdAtpB* and *AdSnRK1* fluctuated during storage in fruit treated with long-term N_2 and O_2. Transcript abundance of *AdAAC1* was significantly reduced in long-term O_2-treated fruit but was upregulated in long-term N_2-treated fruit compared to fruit in control storage. Transcript abundance of *AdAOX2* and *AdUCP* was significantly elevated by long-term O_2 application during storage. Moreover, these transcripts were increased dramatically until their first peaks of 32.8 and 6.5, respectively, on the 1st day of storage and then their 2nd peaks of 7.4 and 1.8 on the 5th and 7th days of storage, respectively, in long-term O_2-treated fruit (Fig. 6). Transcript abundance of *AdAAC1* and *AdSnRK1* was upregulated by long-term N_2 application.

4. Discussion

4.1. Energy metabolism in relation to respiration intensity

More and more studies have shown recently that ripening and senescence are close related to cellular energy status. Energy depletion was reported to be an early signal activating the programmed cell death of tulip petals (Azad et al., 2008). ATP is mainly produced by respiration. The results from Bennett et al. (1987) using ^{31}P nuclear magnetic resonance technology revealed that ATP content in avocado fruit was tightly connected with the increase of respiration rate. It was suggested that cellular ATP demand could be reduced by repressing the respiration rate. In addition, cellular energy metabolism could be inhibited if ATP biosynthesis or ATP utilisation was suppressed. Numerous reports have proved that low-temperature and low-O_2 conditions could effectively suppress respiration intensity. Temperature control and controlled atmosphere (CA) storage, as the most efficient preservation techniques, are widely used with horticultural products to delay senescence and to prolong shelf life based on their roles in inhibiting respiration intensity. The present study indicated that low temperature and long-term N_2 treatment repressed the respiration rate in harvested kiwifruit. Meanwhile, levels of ATP, ADP, and AMP and the adenylate pool declined significantly in fruit subjected to low temperature or long-term N_2 treatment. Obviously, low-temperature storage and long-term N_2 application delayed the senescence of harvested kiwifruit, which showed higher firmness and lower TSS through repression of the respiration rate. In contrast, long-term O_2 application stimulated the respiration rate, accelerated the release of soluble sugars from starch, and consequently promoted maturity and senescence, which was reflected in the rapid loss of

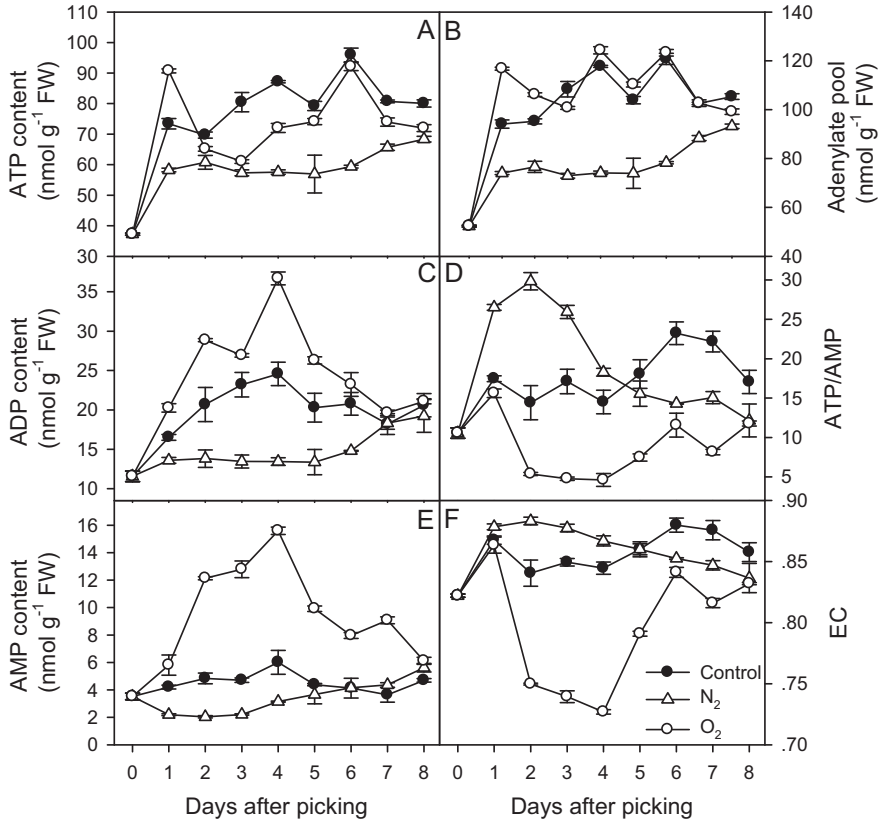

Fig. 4. Energy status in harvested kiwifruit exposed to long-term N_2 or O_2. Data are the mean ± standard deviation ($n = 3$). The vertical bars indicated deviation exceeded the symbol size. See Fig. 3 legend for names of abbreviations.

firmness and increase of TSS (Nardozza et al., 2013). This differed from our previous result in litchi, in which short-term treatment with pure oxygen delayed fruit senescence (Duan et al., 2004). However, short-term pure oxygen had no significant effect on ripening and senescence of kiwifruit in our preliminary experiment, showing their distinctive responses to different dosages of oxygen. Furthermore, peak values in ATP, ADP and AMP levels appeared to coincide with the respiration climacteric, indicating a positive correlation between energy status and respiration rate. Interestingly, the times of their peak values appeared to be in the order of AMP, ADP and ATP. This was different in the non-climacteric litchi fruit, which showed decreased ATP content while peak values of ADP and AMP appeared at the same time after harvest. This discrepancy was probably because climacteric fruits such as kiwifruit finish their ripening and senescence process after harvest, and ATP is synthesised robustly along with climacteric respiration. However, non-climacteric fruit such as litchi (*Litchi chinensis* Sonn.) finish their ripening process on the tree. Peak values in the order of AMP, ADP and ATP were also observed in ripening and senescent fruit of pre-harvest litchi in our previous study (Wang et al., 2013). Different characteristics of energy metabolism between climacteric fruit and non-climacteric fruit were probably based on their individual respiration styles.

4.2. Energy regulation in kiwifruit stored under low-temperature and long-term N_2 conditions

ATP is synthesised by ATP synthase (F_OF_1-ATPase). The mitochondrial FoF1 ATP synthase is a bottleneck in the provision of metabolic energy by oxidative phosphorylation, converting the energy of transmembrane proton flow into the high energy bond between ADP and phosphate, and it synthesises most of the ATP in living organisms. Subunit β (atpB) is the key catalytic component in the catalytic domain of F1 ATP synthase. Mutations in the DELSEED loops on the N-terminal edge of β subunits affect the mechanochemical coupling in ATP synthase (Mnatsakanyan et al., 2009). Knockdown of the beta subunit by RNA interference resulted in disrupted assembly of complex V, decreased respiratory rate, impaired coupling of ATP synthesis to the respiratory activity, and deformity of mitochondria, which were deprived of cristae in the green alga *Chlamydomonas reinhardtii* (Lapaille et al., 2010). Lower temperature increased the transcript abundance of *AtpB* in Japanese flounder (Itoi et al., 2007). Hypoxia caused an increase in the transcript abundance of *atpB* in shrimp and a subsequent decrease when shrimp were re-oxygenated (Kane et al., 2010). In the present study, low temperature upregulated the transcript abundance of *AdAtpB*, supporting the above results. Furthermore, atpB negatively regulated plant cell death as a pro-cell-death protein (Chivasa et al., 2011). In the present study, long-term N_2 treatment blocked the peak of *AdAtpB* transcript abundance and postponed its expression. However, long-term O_2 treatment significantly increased the transcript abundance of *AdAtpB* after 1 day of storage, with rapid loss of fruit firmness in kiwifruit. The dramatic increase in *AdAtpB* expression suggested that *AdAtpB* expression may mark the onset of senescence of kiwifruit. These results were consistent with our previous work in litchi and supported the hypothesis that a surge of *AtpB* expression marks the beginning of senescence in fruit (Wang et al., 2013). Meanwhile, low temperature increased the *AdAtpB* transcript abundance and was accompanied by postponed maturation and senescence, implying that moderate upregulation of *AdAtpB* transcript abundance was favourable for storage of kiwifruit, and this may also imply that transcription regulation in *AdAtpB* under different storage conditions is complex.

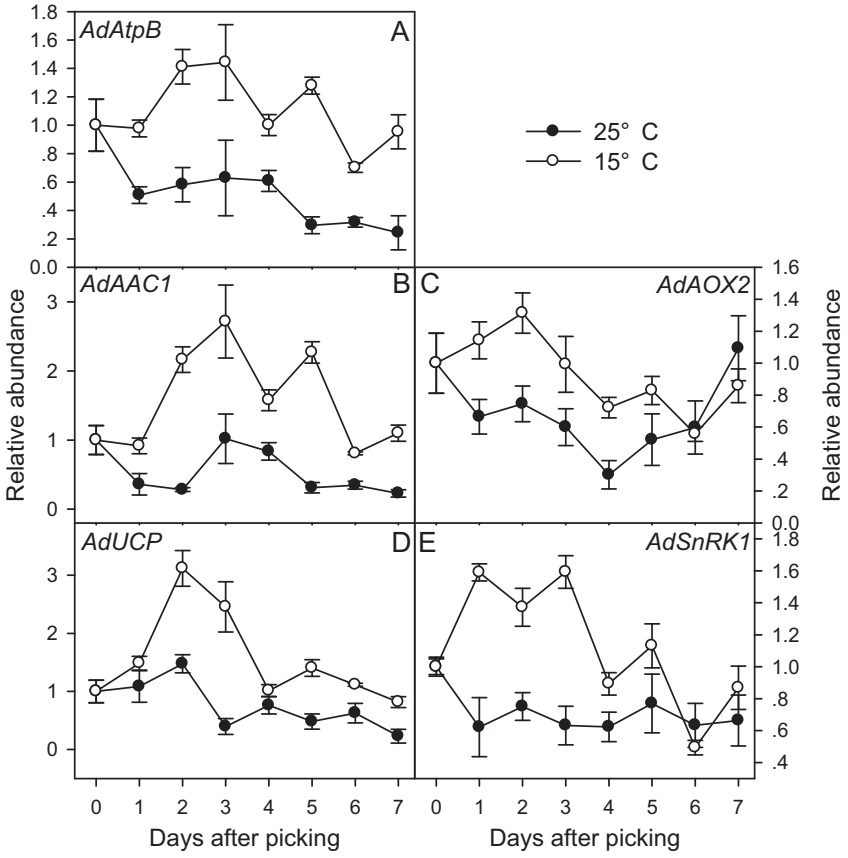

Fig. 5. Relative abundance of gene transcripts in harvested kiwifruit stored under different temperatures. Transcript abundance was determined using qRT-PCR and was normalised using *AdACTIN*. *AtpB*, ATP synthase β subunit; *AAC1*, ADP/ATP carrier 1; *AOX2*, alternative oxidase 2; *UCP*, mitochondrial uncoupling protein; *SnRK1*, sucrose non-fermenting-1-related kinase 1. Data are the mean ± standard deviation ($n = 3$). The vertical bars indicated deviation exceeded the symbol size.

Mitochondrial ADP/ATP carrier (AAC), described as a decisive player in programmed cell death, is a prominent actor in energetic regulation of the cell, importing ADP into mitochondria, where most cellular energy is produced, and exporting ATP towards cytosol, where cellular energy is mainly consumed. Any deficiency or dysfunction in this membrane protein leads to serious consequences for cell metabolism and can cause various diseases (Palmieri, 2013). ADP/ATP exchange in isolated *T. brucei* mitochondria was eliminated upon depletion of TbMCP5 (a type of AAC) (Pena-Diaz et al., 2012). Mutations of prolines in the signature sequences of AAC affected respiratory rates, cytochrome contents and mitochondrial biogenesis and morphology (Babot et al., 2012). Low temperature and long-term N_2 treatment may maintain cell viability by increasing the cellular ATP supply by upregulating *AdAAC1* transcript abundance, thus delaying the ripening and senescence of kiwifruit.

Two other important enzymes that play crucial roles in energy metabolism are alternative oxidase (AOX) and mitochondrial inner membrane uncoupling proteins (UCP). AOX is a terminal oxidase in the mitochondrial electron transport chain and is found in the majority of plants as well as many fungi and protists. It directly accepts electrons from the ubiquinone pool and transfers them to oxygen, which bypasses energy production associated with complexes III and IV, thereby dramatically reducing ATP formation (Gupta et al., 2012; Moore et al., 2013). UCP catalyses a proton conductance that dissipates the proton electrochemical gradient established by the respiratory chain, thus affecting the yield of ATP synthesis. AOXs and UCPs are two major energy dissipation systems. Both of them play key roles in cellular metabolism, thermogenesis and energy homeostasis. They are generally considered to be major stress-induced proteins. Reduced carotenoids, respiration and ethylene production and downregulation of ripening-associated genes with retarded ripening were observed in tomato with reduced *LeAOX* levels. In contrast, more lycopene was accumulated in tomato with overexpressed *LeAOX1a*, suggesting that AOX was involved in respiratory climacteric and ethylene-mediated fruit ripening (Xu et al., 2012). A transgenic tomato overexpressing *LeUCP* showed reduced ROS accumulation and enhanced heat stress tolerance compared with the control plants, and it exhibited significant increases in tolerance to *Botrytis cinerea* (Chen et al., 2013). Peaks of *AdAOX2* and *AdUCP* transcript abundance coincided with that of respiratory rate appearing after 2 days of storage, indicating that they played pivotal roles in fruit ripening and senescence. Low temperature storage significantly increased the transcript abundance of *AdAOX2* and *AdUCP*, whereas long-term N_2 treatment reduced the *AdAOX2* transcript abundance in the first two days and reduced the *AdUCP* transcript abundance during storage, although both low temperature and long-term N_2 treatment postponed the ripening and senescence of kiwifruit, suggesting that different regulation mechanisms existed between these two storage environments. However, AdAOX2 and *AdUCP* were abruptly induced in long-term O_2-treated fruit during the first two days of storage, indicating their roles as stress proteins, and this may also imply that they contribute to reducing the levels of reactive oxygen species (ROS).

The sucrose non-fermenting 1 protein kinase (SnRK), a homologue of sucrose non-fermenting 1 (SNF1) in yeast and AMP-activated protein kinase (AMPK) in animals, is widely present in plants. As a cellular energy sensor, SnRK is an integrator of the balance between energy supply and demand, and it plays

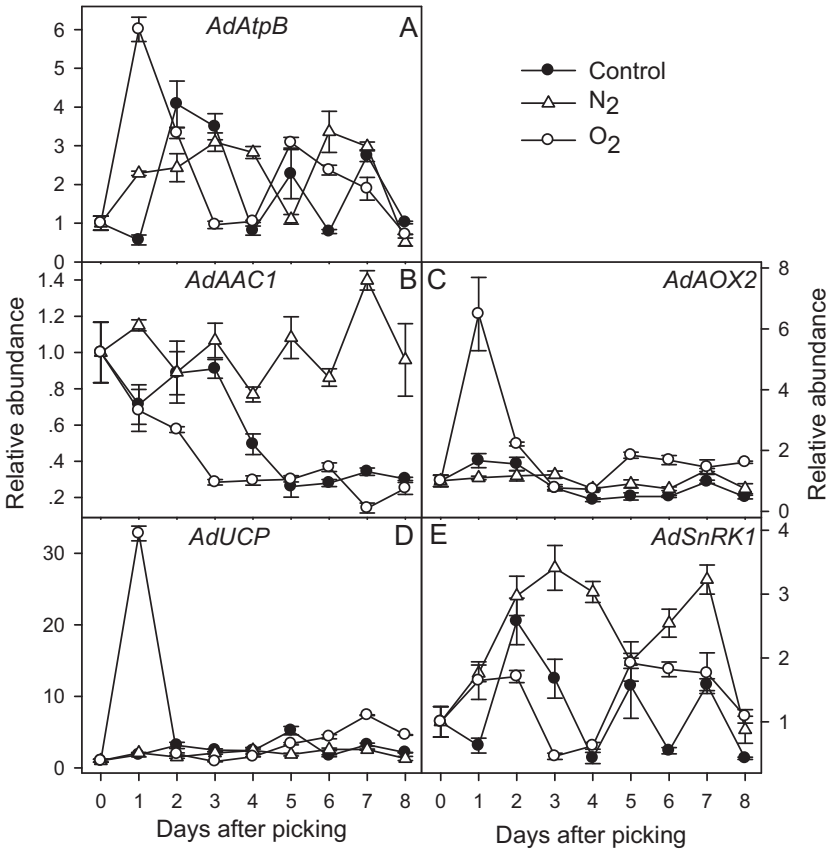

Fig. 6. Relative abundance of gene transcripts in harvested kiwifruit exposed to long-term N_2 or O_2. Transcript abundance was determined using qRT-PCR and was normalised using *AdACTIN*. Data are the mean ± standard deviation ($n = 3$). The vertical bars indicated deviation exceeded the symbol size. See Fig 5 legend for names of genes.

critical roles in the regulation of cellular energy homeostasis and in the stress response by downregulating ATP-consuming biosynthetic processes while stimulating energy-generating catabolic reactions through gene expression and post-transcriptional regulation (Nietzsche et al., 2014). *AdSnRK1* transcript abundance was induced by low temperature and long-term N_2 treatments in this study, which favoured ATP conservation and might account for the delayed ripening and senescence of kiwifruit stored under these two environments. Furthermore, *AdSnRK1* may affect ATP status by controlling the expression and phosphorylation of key metabolic enzymes, and this process might involve ATP synthase, AAC, AOX and UCP (Liu et al., 2013; Wang et al., 2013).

5. Conclusion

The observed ATP level in this research was the difference calculated as the total ATP synthesised minus the ATP dissipated and the ATP consumed in the tissue. Therefore, the mechanism by which low temperature and long-term N_2 treatment postponed the ripening and senescence of kiwifruit may be as follows: first, downregulating the adenylate pool to efficiently economise energy; second, increasing ATP synthesis and transportation in tissues; third, accelerating SnRK signal transduction to further promote ATP synthesis. In addition, the tissue might accelerate ATP dissipation to adapt to low-temperature conditions. However, the detailed molecular mechanisms by which the low-temperature and long-term N_2 treatments affect the energy metabolism and the relationship between the relative enzymes remain unclear. This requires further investigation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.postharvbio.2014.07.008.

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