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Expression profile of *EXP, Succ-CoA* and *ALDH* genes in soursop (*Annona muricata* L.) fruits during ripening in response to refrigeration conditions

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Soursop (*Annona muricata* L.) is a climacteric fruit characterized by its rapid softening. Although many studies have improved the understanding of postharvest shelf life in soursop, the expression of genes involved in the loss of flesh firmness, organic acids and acetaldehyde metabolism are less well understood. We evaluated the expression profile of genes related to fruit softening, tricarboxylic acid cycle and acetaldehyde metabolism during ripening of soursop fruit stored at 28 ± 2ºC and 15 ± 2ºC. The fruit stored at 15 ± 2ºC prolonged the postharvest shelf life up to 9 days and showed higher firmness at 3 days compared to those stored at 28 ± 2ºC. Moreover, the postharvest storage at 15 ± 2ºC induced the expression of expansin (*EXP*), succinyl CoA ligase (*Succ-CoA*) and aldehyde dehydrogenases (*ALDH*) genes at 3 days. On the other hand, we recorded an increase in the gene expression of *EXP* and *Succ-CoA* at 6 days in fruit stored at 28 ± 2ºC. Based on the different gene expression patterns, we concluded that the postharvest storage at 15 ± 2ºC triggers the expression of *EXP, Succ-CoA* and *ALDH* genes at the early stages of soursop ripening. This suggests their role in cell disorganization, organic acids, acetaldehyde metabolism as well as in response to refrigeration during ripening.

Keywords: Gene expression profiles, postharvest refrigeration, softening, expansin, succinyl CoA ligase, aldehyde dehydrogenases, qRT-PCR.

Introduction

The soursop is a climacteric fruit that presents a high respiration rate and reaches the peak of ethylene production after 5 days of harvest[1-3]. Therefore, one of the main characteristics of soursop is the rapid decrease in flesh firmness during ripening. This fruit need storage temperatures between 15-18°C and other postharvest technologies to delay fruit softening and avoid chilling injury. In this regard, Espinosa *et al*[4] evaluated the effect of 1-methylcyclopropene (1-MCP) and refrigeration on soursop fruit. The results of that investigation showed that the fruit treated with 200 or 400 nLL^{-1} of 1-MCP and stored at 16ºC delayed ripening for 7 days without chilling injury. Further, these treatments were effective to decrease respiration rate, ethylene production, physiological weight loss and delay the physicochemical parameters such as titratable acidity, total soluble solids, pH and firmness. Likewise, in the same investigation authors found chilling injury in fruit stored at 16ºC. Jiménez-Zurita *et al*[5] evaluated the physicochemical parameters at 15 ± 2ºC and 22 ± 2ºC in two soursop selections (G1 and G2) at different days of storage. The authors found that the fruit stored at 15 ± 2ºC and then 4 days at 22 ± 2ºC increased their postharvest shelf life up to 8 days without chilling injury and conserving the organoleptic properties of mature fruit. Furthermore, fruit stored at 15ºC showed lower weight loss compared with fruits stored at 22ºC. Additionally, the authors showed the ethylene peak at six days after storage. Indeed, Balois-Morales *et al*[6] evaluated vitamin C content, soluble protein content and antioxidant activity using the same conditions previously mentioned. The authors found a decrease in soluble protein content during ripening at both temperatures. Furthermore, the fruit stored at 15ºC (refrigeration) presented a diminution in the vitamin C content.

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As can be seen no studies regarding the molecular mechanisms of soursop fruit response to abiotic stress can be found. Palma *et al*[7] and Thomashow *et al*[8] reported that fruit responses to abiotic stress are activated by cell membrane damage, activating signaling processes such as cellular structures, ethylene production, increased respiration, interference with energy production, reduced photosynthesis and accumulation of toxic compounds such as acetaldehyde.

Several genes have been involved in the response to abiotic stress such genes encode enzymes related to several biochemical pathways in cell wall modification, ethylene biosynthesis and organic acids metabolism[9]. Endogenous ethylene is a phytohormone with a broad role in the regulation of metabolism at cellular, biochemical, molecular and physiological level[10-11]. In climacteric fruits, ethylene plays a leading role in the control of many ripening-related events, including fruit softening. However, the molecular mechanism of this process remains unclear. It has been suggested that the textural changes in mesocarp are due to cell wall disassembly that occurs through some members of the expansins (*EXP*) gene family[12]. The expansins are plant cell wall proteins that disrupt hydrogen bonds within the cell wall matrix, contributing to cell wall degradation, leading to a rapid decrease in flesh firmness[13]. This is one of the main characteristics of soursop fruits during ripening. Taking the above into account, soursop is a highly perishable fruit due to its metabolism during ripening and the use of refrigeration has demonstrated to increase the postharvest shelf life of soursop fruit[14]. In abiotic stress, Zhao[15] reported that cold stress induced ethylene concentrations in plants, promoting cold tolerance. In addition, Zhao *et al*[16] found that ethylene concentrations and cold tolerance are associated with tomato. However, no information related to soursop exists.

Aldehyde dehydrogenase (*ALDH*) conserved gene superfamily catalyzes the irreversible oxidation of an extensive range of aldehydes into corresponding carboxylic acids using NAD+ or NADP+ as a co-factor and play a key role in detoxifying aldehydes produced by various metabolic pathways and in abiotic stress tolerance[17]. In plants, the *ALDH2* gene family metabolizes acetaldehyde, producing acetate which is used for coenzyme A (CoA) synthesis by the acetyl-CoA synthase[18]. Further, these molecules can be oxidized at the mitochondria to give succinyl-CoA and pass into the tricarboxylic acid (TCA) cycle. Interestingly, succinyl-CoA synthetase (Succ-CoA) participates in the TCA cycle catalyzing the only step of substrate-level phosphorylation of GDP or ADP being one of the enzymes less studied in the TCA cycle[19-20]. Nevertheless, the molecular information of this fruit is still largely unknown due to the few sequences available are from a leaf soursop transcriptome which is not fully annotated[21].

Genes *EXP, ALDH* and *Succ-CoA* are involved in important biochemical processes during fruit ripening. Hence, it is important to perform an initial molecular approach to identify soursop fruit gene expression patterns during ripening in response to refrigeration conditions. This information will have a positive impact on the molecular understanding of this fruit in order to prolong the postharvest life of soursop.

Therefore, the objective of this study was to evaluate the effect of two postharvest storage temperatures on the expression profiles of genes related to fruit softening, organic acids and acetaldehyde metabolism during the ripening of soursop fruit.

Materials and Methods

Plant Material and Treatments

Soursop fruit 'GUANAY-1' was harvested at physiological maturity from 10 trees in an orchard located in Las Varas, Nayarit, Mexico. Five fruits per tree were collected at physiological maturity according to fruit shape, peel color and size. Fruit without mechanical and pathogenic damage was selected, disinfected with 2.0% sodium hyphochloride (NaOCl) and washed with distilled water. Then, 30 soursop fruits were stored at 28 ± 2°C and 15 ± 2°C until reaching senescence. In this regard, mesocarp from five fruit was removed at 0, 3 and 6 d after storage at 28 ± 2ºC. Also, fruit mesocarp at 3, 6 and 9 d after storage at 15 ± 2°C was taken. After, soursop mesocarp was frozen in liquid nitrogen, grounded with a mortar and pestle, and then stored at -80ºC for the subsequent analysis. In addition, firmness was measured in all the conditions mentioned with a digital penetrometer (SSEYL GY-4 Digital Fruit Penetrometer) in three different areas of fruit and reported as firmness N.

RNA Extraction and First cDNA Synthesis

Total RNA was isolated from 0.075 g of grounded tissue using the Spectrum Plant Total RNA kit (Sigma) following the manufacturer's instructions. Total RNA was quantified in a spectrophotometer Synergy HT/Take3 (BioTek Instrument Inc.). The integrity of the RNA was determined by electrophoresis on a 1.5% agarose gel stained with SYBR™ gold nucleic acid gel stain. RNA with a

260/280 ratio between 1.8 and 2.1 was used for the next experiments. First-strand cDNA was synthesized using the SuperScript III reverse transcriptase kit, according to the manufacturer's instructions.

Bioinformatics Analysis and Primer Design

Bioinformatics analysis was performed according to the methodology reported by Berumen-Varela *et al*[22]. Briefly, a basic local alignment search tool (BLAST) database was built from the soursop leaf transcriptome and then a BLASTn search against the database created was performed to identify the homologous genes in soursop. Based on the predicted coding sequences of these genes, primer sequences for *EXP*, *Succ-CoA* and *ALDH* genes were designed using primer quest tool (Integrated DNA Technologies, IA, USA) with the following parameters: primer length of 18-25 nucleotides, expected amplicon length between 100 - 220 bp; melting temperature (Tm) between 55 - 60ºC and GC% of 45-55. The sequence of the primers designed in this experiment is listed in Table 1. The specificity of the primers was tested by the Primer-BLAST tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/).

Polymerase Chain Reaction

Primer pairs were verified by conventional PCR using the RedTaq ready mix (Sigma) according to the manufacturer instructions. PCR was performed in a T-100 thermal cycler (Bio-Rad Laboratories, Inc.) with the following conditions: initial denaturation at 94ºC for 5 min, followed by 40 cycles of 94ºC for 45 s, 56ºC for 30 s and 72ºC for 30 s and a final extension of 72ºC for 10 min. Amplicons were analyzed by 1.5% agarose gel electrophoresis stained with SYBR™ gold nucleic acid gel stain and visualized in a photo doc-it imaging system (Ultra-Violet Products, Ltd.). PCR products were sequenced and compared with the National Center for Biotechnology Information (NCBI) database using BLAST. The *Succ-CoA* and *ALDH* sequences obtained were submitted to the NCBI database with the accession numbers shown in Table 1.

Quantitative Real Time-PCR (qRT-PCR)

Before the gene expression analysis, relative standard curves were generated using 3-fold serial dilutions of cDNA to determine amplification efficiency, specificity and the absence of primer dimers. The qRT-PCR reactions were performed in a Rotor-Gene Q-5 plex real-time cycler (Qiagen, Valencia, CA, USA) using Maxima SYBR Green/ROX qPCR master mix kit (Thermo Fisher Scientific, USA). The qRT-PCR reaction mixture contained 6 µL SYBR green master mix, 5 µL cDNA (20 ng), 1 µL (10 µM) of each forward and reverse primers and 7 µL PCR grade water in a total reaction volume of 20 µL. The expression analysis was normalized with the Ubiquitin (*UBC*) gene as an internal control as previously reported in soursop and cherimoya[22-23]. The qRT-PCR reactions were run in three technical replicates with two biological replicates of each condition tested. The qRT-PCR assay consisted of a two step protocol with an initial polymerase activation step of 94ºC for 5 min, followed by 35 cycles of 94ºC for 30 s and 58ºC for 60 s. The acquisition of fluorescece was performed at the melting temperature during each cycle. Melting curve analysis (55ºC to 95ºC) was performed at the end of each qRT-PCR analysis to verify primer specificity. Additionally, PCR products were analyzed in agarose electrophoresis to visualize the gene expression. The relative expression level for each gene was calculated by the formula $2^{-\Delta\Delta Ct}$ as reported by Livak and Schmittgen[24]. The final values included in the graphs were calculated using the gene expression of day zero as a control reference. Gene expression data were normalized to log 2 fold change and plotted in R studio using the heatmap 2 function of the gplots package.

Statistical Analysis

A completely randomized design was used to study the gene expression in each day of storage. The data

Table 1 — List and description of the genes and primers used in this study.

Genes	Accession no.	Sequence (5′–3′)	Amplicon size (bp)
EXP	FJ457025	F: CTCAGTACAGGGCTGGAATC R: CACTCCTACTTCAACCTGGTC	107
Succ-CoA	MN782306	F: GTTGATGCTGCATTTCCTCTTC R: TGTTATGGTTGGTGGGTTCC	119
ALDH	MN782307	F: TGACCATGAGGGTGGAAATG R: TTTAGCTGGTCCACTTCATGG	219
UBC	FJ664263	F: AACCTCTATCCAGTCTCTCCTC R: TGAGATAGTGGAGCAGAGCT	128

F and R means forward and reverse primers, respectively

**EXP*, expansin; *Succ-CoA*, Succinyl-CoA; *ALDH*, Aldehyde dehydrogenase; *UBC*, Ubiquitin carrier-like protein

were analyzed by analysis of variance (ANOVA) with P $\leq$ 0.05. LSD test was performed for the comparison of means. GraphPad Prism 6 software was used for all statistical analyses. Pearson correlation analysis was carried out using R studio to evaluate the association between gene expression of *EXP* and firmness.

Results

Firmness and Gene Expression Profile of *EXP* in Soursop Fruit Stored at Two Temperatures

Fruit firmness at 28 ± 2ºC dramatically decreased by 83% at 3 d and almost completely lost at 6 d of storage (Fig. 1a). Conversely, after 3 d of storage, firmness was higher in fruit stored at 15 ± 2ºC than in those stored at 28 ± 2ºC. In fruit stored at 15 ± 2ºC, the loss of firmness was observed after 6 d, increasing the shelf life up to 9 d (Fig. 1b). To investigate the role of *EXP* in fruit ripening the gene expression profiles were associated with the loss of firmness during fruit ripening under two different temperatures (Fig. 1a & 1b).

Gene expression analysis of *EXP* at 28 ± 2ºC showed no significant differences between 0 and 3 d (p > 0.05). However, a 5-fold increase of the *EXP* expression levels was observed after 6 d of storage at 28 ± 2ºC compared to 0 d (Fig. 1a). Pearson's correlation coefficient showed a -0.47 value, indicating a negative association between the firmness and gene expression pattern at 28 ± 2ºC. On the other hand, when the fruit was stored at 15 ± 2ºC, a 2.8 fold increase in gene expression was observed at 3 d of storage compared to day 0 and then a notable decrease in the expression of *EXP* gene at 6 and 9 d was exhibited, reaching low values in comparison with 0 and the 3 d of storage (Fig. 1b). Pearson's correlation coefficient showed a 0.79 value, demonstrating a positive association between the firmness and gene expression pattern at 15 ± 2ºC.

Succ-CoA Gene Expression in Soursop Fruit Stored at Two Temperatures

Furthermore, to explore the function in the organic acid metabolism in soursop fruit the gene expression profile of *Succ-CoA* was analyzed during fruit ripening of soursop fruit stored at two temperatures (Fig. 2a & 2b). In fruit stored at 28 ± 2ºC, a 2 fold and 9 fold increase was found in the *Succ-CoA* gene

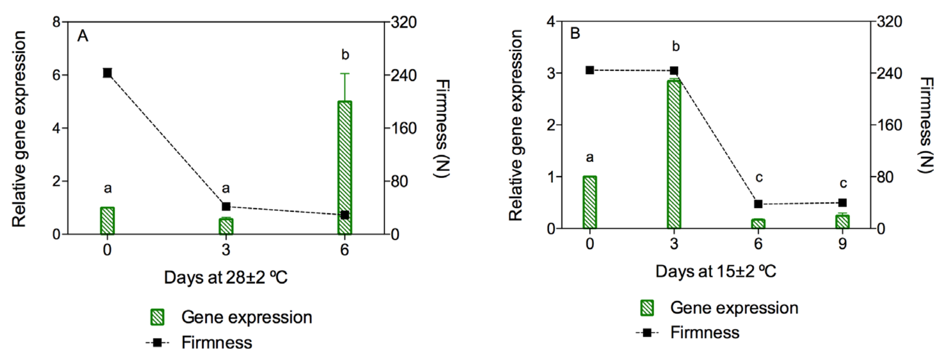

Fig. 1 — Gene expression analysis of *EXP* and firmness in soursop fruit. a) Fruit stored at 28 ± 2ºC. b) Fruit stored at 15 ± 2ºC. Each value represents the mean of three technical replicates and two biological replicates. Vertical lines represent the standard deviation. Means with different letters are significantly different according to the LSD test at p < 0.05.

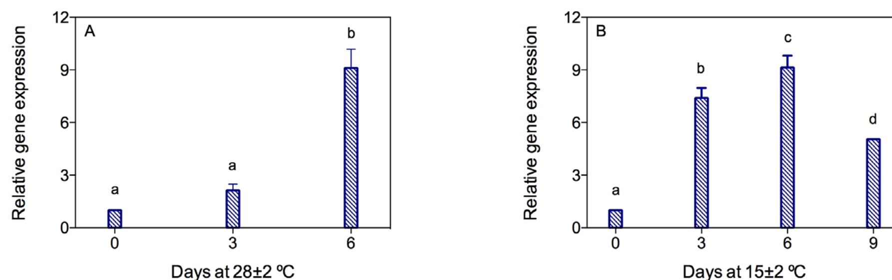

Fig. 2 — Gene expression analysis of *Succ-CoA* in soursop fruit. a) Fruit stored at 28 ± 2ºC. b) Fruit stored at 15 ± 2ºC. Each value represents the mean of three technical replicates and two biological replicates. Vertical lines represent the standard deviation. Means with different letters are significantly different according to the LSD test at p < 0.05.

expression at the 3 and 6 d of storage compared to 0 d respectively, (Fig. 2a). Indeed, significant differences between 0 and 6 d at 28 ± 2ºC were observed ($P < 0.05$). Otherwise, more than 7 fold, 9 fold and a 5 fold increase in the *Succ-CoA* gene expression was observed at the 3, 6 and 9 d in fruit stored at 15 ± 2ºC compared to 0 d respectively, (Fig. 2b). The highest expression levels of *Succ-CoA* were detected at the 6 d of storage. Significant differences between all days of storage at 15 ± 2ºC was observed ($p < 0.05$).

ALDH Gene Expression in Soursop Fruit Stored at Two Temperatures

ALDH gene expression during fruit ripening was analyzed in two storage temperatures as shown in (Fig. 3a & 3b). In fruit stored at 28 ± 2ºC, *ALDH* gene expression showed no differences in the 3 d of storage compared to the 0 d ($p > 0.05$). On the 6 d of storage, a decrease in *ALDH* gene expression was observed in comparison with 0 d ($p < 0.05$) as shown in Figure 3a. In fruit stored at 15 ± 2ºC a 1.7 fold increase in the *ALDH* gene expression on the 3 d of storage was observed, followed by a decrease in the 6 d of storage (Fig. 3b). No differences between the 6 and 9 d were recorded ($p > 0.05$). The highest *ALDH* expression levels were identified on the 3 d compared with the other days of storage ($p < 0.05$) as shown in Figure 3b.

Differential Gene Expression Analysis

Additionally, agarose gel electrophoresis and heatmap were made to visualize and group the *EXP, Succ-CoA* and *ALDH* genes based on their expression

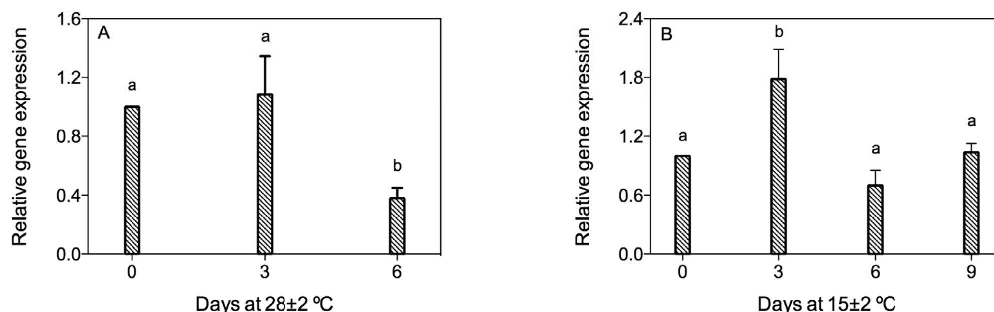

Fig. 3 — Gene expression analysis of *ALDH* in soursop fruit. a) Fruit stored at 28 ± 2ºC. b) Fruit stored at 15 ± 2ºC. Each value represents the mean of three technical replicates and two biological replicates. Vertical lines represent the standard deviation. Means with different letters are significantly different according to the LSD test at $p < 0.05$.

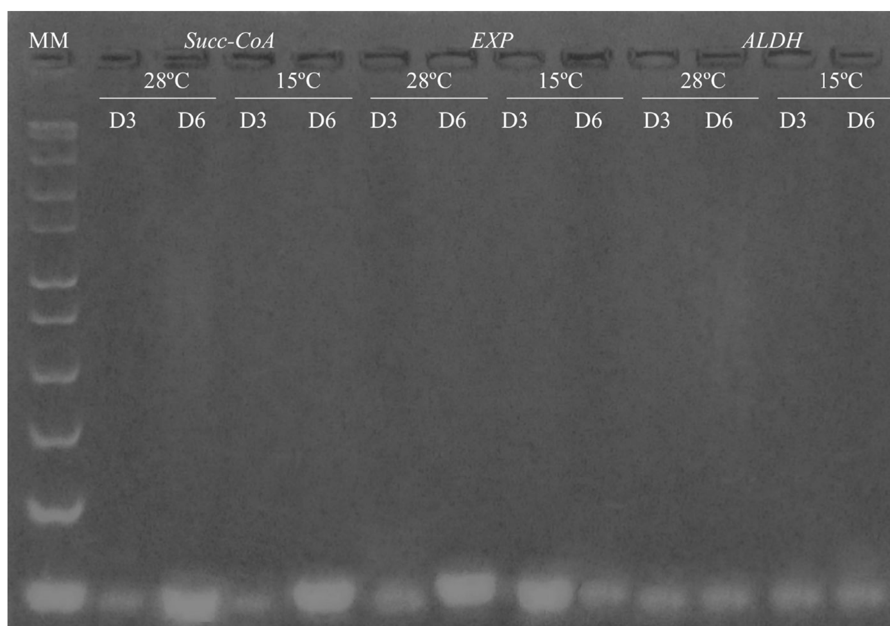

Fig. 4 — Agarose gel electrophoresis of qRT-PCR products of *EXP, Succ-CoA* and *ALDH* genes in soursop fruits stored at two temperatures. MM represents 1 kb molecular marker.

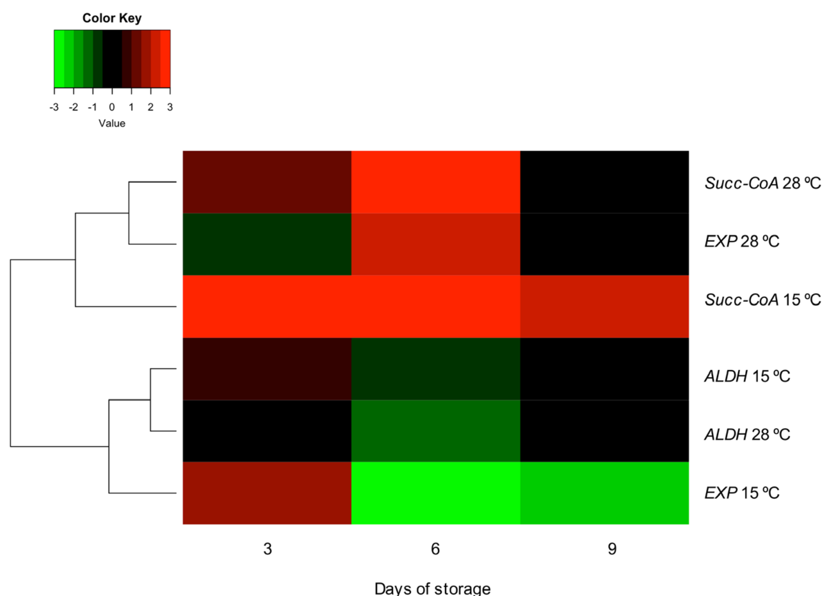

Fig. 5 — Heatmap of the changes in gene expression levels of *EXP, Succ-CoA* and *ALDH* in soursop fruit stored at 28 ± 2ºC and 15 ± 2ºC. Levels of down expression (green) or up expression (red) are shown on a log2 scale from the highest to the lowest expression of each gene.

pattern as shown in Figure 4 & 5. Interestingly, on the 3 days, all the evaluated genes in the soursop fruit stored at 15 ± 2ºC were up-regulated. Instead, *ALDH* and *EXP* genes at 28 ± 2ºC showed a down regulation after 3 days of storage. On the 6 d of storage, the *EXP* gene in fruit stored at 28 ± 2ºC and *Succ-CoA* gene in fruit stored at 28 ± 2ºC and 15 ± 2ºC showed an up-regulation of its gene expression. However, *ALDH* at both temperatures showed a down regulation of its gene expression at the 6 days. Finally, on the 9 days in fruit stored at 15 ± 2ºC a down-regulation of the *EXP* gene and up-regulation of the *Succ-CoA* gene was recorded.

Discussion

Soursop fruit suffers a rapid softening and ripening due to its high respiration rate and ethylene production. The right storage conditions and postharvest technologies need to be applied in soursop fruit to control fruit ripening. In this study, the postharvest storage at 15 ± 2ºC prolongs the shelf life of soursop fruit up to 9 days and enhanced the gene expression at the 3 days of storage.

Expansins are cell wall proteins in all plants that intermediate the cell wall loosening. The *EXP* gene at 28 ± 2ºC showed an increase in the 6 days which coincides with the loss of firmness (Fig. 1a). The α-expansin mRNA has been reported to be expressed in the late stage of fruit ripening such as peach, strawberry and kiwifruit[25-28]. The previous statement agrees with the reported in this investigation due to the highest expression values of the *EXP* gene at 28 ± 2ºC were observed in the last stage of fruit ripening. Rose and Bennett[29] indicated that expansins contribute to cell wall polymer disassembly, in turn, causes fruit softening, which was observed in the fruit stored at 28 ± 2ºC. On the Annonaceae family, one of the most studied species is cherimoya. In this regard, Shen *et al* isolated and characterized the mRNA expression patterns of *EXP* genes from ripe cherimoya at different temperatures. The results of that investigation showed that *AcEXP2* and *AcEXP3* mRNA increased after 5 d of storage at 15ºC. An increase in the gene expression of *EXP* was found after 3 d of storage at 15 ± 2ºC and a decrease of gene expression after 6 and 9 d, whereas the firmness value decreased to similar levels to those found in fruit at 28 ± 2ºC after 6 d of storage (Figs. 1a & 1b). Probably, the results found in this investigation differ from the previously mentioned because the *EXP* gene family possesses multiple members with different biological roles, which causes differential expression patterns.

Furthermore, these results are similar to those reported by Carvajal *et al*[30] who analyzed the gene expression of an *EXP* gene (*CpEXP1*) by qRT-PCR in zucchini fruit stored for 48 h at 15ºC and then stored at 4ºC (preconditioned fruit). The authors of that investigation found that on preconditioned fruit the

CpEXP1 gene increased the expression levels on the seventh day and then decreased by 14th day, suggesting that might have a role in the appearance of the damage. Taken together, these data suggest that the *EXP* gene is associated with cell disorganization caused by either low temperature or senescence.

Some authors have mentioned that cherimoya fruit softening is related to the increase of the polygalacturonase (PG), pectin methylesterase (PME), endoglucanase enzymatic activity and genes related to cell wall disassemblies such as endotransglycosylases and expansins[31-32]. Franco-Mora *et al*[33] applied resveratrol (1.6 mM) and benzylaminopurine (1.0 mM) 8 - 15 days before harvest and then analyzed the PME and PG enzymatic activities in 'Ruth' and 'Fino de Jete' cherimoya fruits during postharvest storage (14 - 18ºC). The authors found that the highest enzymatic activity of both enzymes was at the five day after postharvest storage in all treatments. On the other hand, the highest PG activity was recorded two days earlier compared with the fruits treated with resveratrol and benzylaminopurine. Moreover, Salomon-Castaño *et al*[34] used the same concentration of the previous solution on soursop fruits. The authors reported higher firmness in the treated fruits at the five day of storage and prolonged the soursop shelf life for three days compared with the control. Jiménez-Zurita *et al* evaluated the PME and polyphenol oxidase (PPO) enzymes at 15 ± 2ºC and 22 ± 2ºC in two soursop selections (G1 and G2) at different days of storage founding that PPO and PME activity increased at the end of storage (consumption maturity) at both temperatures could be involved in the loss of firmness.

In this study the expression of genes involved in organic acid and acetaldehyde metabolism was analyzed. Succinyl-CoA ligase is an enzyme involved in the Krebs cycle that hydrolyzes succinyl-CoA to succinate and free coenzyme A and converts ADP or guanosine diphosphate (GDP) to ATP or guanosine triphosphate (GTP), respectively[35]. *Succ-CoA* gene expression showed a gradual increase during fruit ripening at the 3 and 6 days of storage at both temperatures (Figs. 2a & 2b). Furthermore, the highest *Succ-CoA* gene expression was recorded at six day of storage in both temperatures, which coincides with the ethylene peak and respiration rate reported by Marquez-Cardozo *et al* and Jimenez-Zurita *et al* in soursop fruits. This result suggests that the *Succ-CoA* gene is involved in ethylene biosynthesis and therefore in fruit ripening. Nonetheless, a decrease of *Succ-CoA* gene expression on the 9 days at 15 ± 2ºC was observed. This decrease in the 9 days could be related to the senescence of the fruit due to cellular energy controls ripening and senescence events[36]. In this regard, the decline of ATP levels is related to the delayed senescence in which the *Succ-CoA* gene is involved. The conversion of Succ-CoA to succinate is highly variable where most organisms use ADP-forming Succ-CoA ligase[37].

Studart-Guimarães *et al*[38] developed transgenic tomato plants expressing a fragment of the β-subunit of Succ-CoA ligase by an RNA interference construct showing that the enzyme does not catalyze a key function in mitochondrial respiration. Nevertheless, the same authors evaluated the metabolite and transcript profile of the transgenic lines, concluding that the succinate production is important for the mitochondrial metabolism by several pathways of its production. These results support the evidence that *Succ-CoA* plays a role in organic acid metabolism during fruit ripening and therefore, might be an important control point of the TCA cycle. However, further analysis should be performed to prove this statement.

Few reports can be found regarding the function of *Succ-CoA* genes in plants. Studart-Guimaraes *et al*[39] analyzed the mRNA levels by northern blot and semi-quantitative RT-PCR of the two genes of *Succ-CoA* ligase (subunit α & subunit β). The authors found that the genes were strongly similar expressed in flower, root and stem in tomato. Both genes were expressed in leaves and during fruit development, reaching a peak at 55 days after flowering in tomato plants. These findings agree with the reported in this investigation, where the *Succ-CoA* was expressed mainly at the late stages of fruit ripening at 28 ± 2ºC.

ALDH genes play key roles in numerous metabolic pathways such as synthesis and catabolism of several biomolecules as well as in response to stress in plants[40]. However, no previous studies regarding the *ALDH* gene expression in climacteric fruit such as soursop can be found. ALDH enzymes can generate NADPH and NADH in its enzymatic reaction contributes to redox equivalents balance and catalyzes the oxidation of several aldehydes into carboxylic acids[40]. The results obtained in this investigation showed that the *ALDH* gene expression remained stable and down regulated at both temperatures in most of the days after postharvest storage (Figs. 3a, 3b & 4). Jimenez-Lopez *et al*[41] identified and classified *ALDH* genes in the tomato genome, founding that the

ALDH2 gene family is located in the mitochondria and cytosol and their specific functions have not been determined. The have mentioned that *ALDH2* genes could be involved in different oxidative stresses as detoxifying molecules. Based on the results obtained, plant cells seem to maintain stable in the metabolism of acetaldehyde during fruit ripening. However, exactly how this metabolic regulation function remains unknown. On the other hand, the highest *ALDH* gene expression values were found on the 3 days of storage at 15 ± 2ºC (Figs. 3b & 4). In plants, *ALDH* genes have been highly expressed in the response of several abiotic stress[41]. Xia *et al*[42] demonstrated that the *ZmALDH7B6* gene (maize *ALDH7* subfamily member) was expressed in roots, leaves, immature ears, tassels and developing seeds by using qRT-PCR analysis. Likewise, when plants were subject to NaCl, the *ZmALDH7B6* mRNA levels increased after 3 h and peaked at 6 h. According to the data obtained the expression of the *ALDH* gene in soursop fruit was up-regulated in response to 15 ± 2ºC at the 3 days of storage by the refrigeration temperature and fruit ripening. Given the above, the *ALDH* gene could play an important role in abiotic stress such as low temperature suggesting a critical function to environmental adaptation.

The expression levels of *EXP* and *Succ-CoA* genes increased after 6 days of postharvest storage at 28 ± 2ºC. Moreover, the *EXP Succ-CoA* and *ALDH* genes were up-regulated at 15 ± 2ºC after 3 days of postharvest storage. Based on these findings, it can be concluded that postharvest storage at 15 ± 2ºC induced the gene expression at the early stages of soursop ripening, suggesting that these genes might play a function in cell disorganization, organic acids and acetaldehyde metabolism as well as in response to refrigeration during fruit ripening. These results provide the first approach for future analyses to understand the function of these genes.

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