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Relative expression of genes related to volatile organic compounds in non-climacteric and climacteric melons

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ABSTRACT. Melon (*Cucumis melo* L.) is an important species in the cucurbit family with a large economic importance in the world. Two melon cultivars commercially important in Brazil are 'Yellow' and 'Gaúcho'. In addition to their economic importance, these two cultivars display phenotypic differences in aroma, a major trait determining fruit quality. Volatile organic compounds (VOCs) impart the different aroma found in this fruit and their biosynthesis is associated with fatty acid and amino acid metabolism. Using RT-qPCR techniques, the expression of seven genes (*CmLOX9*, *CmLOX18*, *CmBCAT1*, *CmArAT1*, *CmPDC1*, *CmADH1*, and *CmAAT1*) was determined during ripening. The lipid pathway played a strong role in determining aroma composition in non-climacteric 'Yellow' melons. Most volatiles decreased during ripening, explaining the non-aromatic characteristic of this cultivar. In climacteric 'Gaúcho' melons, the amino acid pathway was the main one related to the biosynthesis of esters, which contribute to the aroma of this cultivar. Volatile products of the branched-chain amino acid pathway correlated with *CmADH1* and *CmAAT1* expression, demonstrating their role in volatile synthesis in this climacteric melon cultivar. In addition, *CmPDC1* contributes to the formation of aldehydes at the beginning of this pathway.

Keywords: aroma; amino acid; *Cucumis melo*; volatiles; fruit.

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Introduction

Melon (*Cucumis melo* L.) is an important species in the cucurbit family, with 28,467,920 tons of fruit produced globally in 2020 (FAO, 2018). Brazil ranks thirteenth as a melon producer, with 'Yellow' melons (non-climacteric) being the most important fresh fruit exported by the country (Instituto Brasileiro de Geografia e Estatística [IBGE], 2018). The 'Gaúcho' melon is a climacteric variety largely appreciated in the South of Brazil and has good adaptability for production in this region (Bissacotti & Londero, 2017).

Melon fruit exhibits a wide diversity of phenotypic traits, including its ripening physiology. Unlike most plant species, genotypes of melon can express either climacteric (ripening associated with an increase in respiration and ethylene synthesis) or non-climacteric (ripening without an increase in respiration or ethylene synthesis) ripening (Paris, Tadmor, & Schaffer, 2017; Pitrat, Hanelt, & Hammer, 2000). Ripening involves complex biochemical processes that determine fruit quality characteristics such as color, sweetness, acidity, firmness and aroma (White, 2002). Aroma is an important organoleptic trait characterized by a unique combination of volatile compounds determined by the genetics of the melon cultivar (Gonda et al., 2010; Schwab, Davidovich-Rikanati, & Lewinsohn, 2008).

Climacteric melon fruits are more aromatic than non-climacteric ones, producing high concentrations of total volatiles, with esters being the most abundant compounds (Gonda et al., 2016). The main pathways of aroma formation involve fatty acid and amino acid metabolism (see Figure 1). Ketones and terpenoids also play a minor role in melon aroma (Flores et al., 2002).

In the amino acid pathway, volatile organic compounds (VOCs) are derived mainly from five amino acids, two aromatics, L-phenylalanine and L-methionine, and three branched-chain amino acids, L-valine, L-leucine and L-isoleucine (Gonda et al., 2010). The initial stage of this pathway involves transamination of the

amino acid through the activity of branched-chain aminotransferases (BCATs) on branched-chain amino acids or aromatic aminotransferases (ArATs) on aromatic amino acids (Gonda et al., 2016). The volatiles formed in the branched-chain amino acid pathway are 2-methylbutanal, 3-methylbutanal, 2-methylpropanal and their related alcohols and esters (Gonda et al., 2016). As for the aromatic amino acid pathway, important volatiles formed are phenylacetaldehyde and its related esters (Gonda et al., 2016).

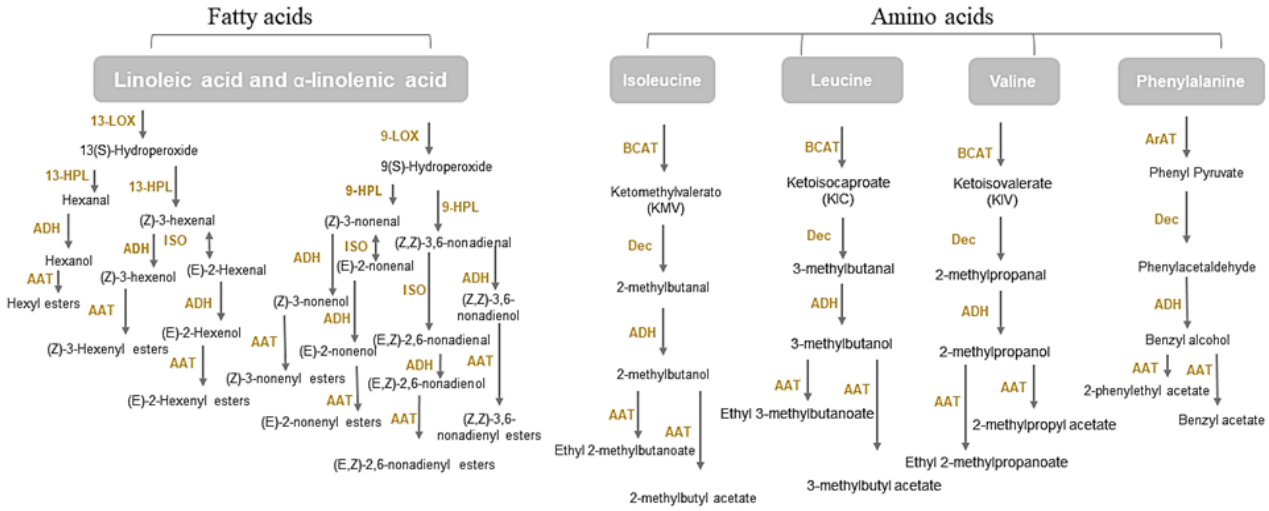

Figure 1. Biosynthetic routes for lipid and amino acid catabolism in plants. Enzymes known to act upon the precursors to form the following volatiles are highlighted in yellow beside the arrows. Adapted from Gonda et al. (2010) and Vincenti et al. (2019).

A prominent enzyme in melon fruit is pyruvate decarboxylase (PDC), which is associated with the decarboxylation of keto acids, generating pentanal and propanal and their derivatives (Vincenti et al., 2019; Wang et al., 2019). In the final steps of volatile biosynthesis, alcohol dehydrogenase (ADH) and alcohol acyltransferase (AAT) are key enzymes that convert aldehydes into alcohols and esters. In melon aroma, aldehydes and alcohols often display green, leafy, cucumber-like notes, while esters are related to the emission of fresh, floral, fruity notes (Gonda et al., 2016).

Considering the importance of aroma volatiles in the overall flavor of fruits, integrative studies relating gene expression data are crucial to better understand the organoleptic profile of the wide phenotypic diversity of melons, as well as the biochemical complexity involved in aroma production. Thus, the aim of this study was to understand the transcription of genes that encode enzymes of the fatty acid and amino acid metabolic pathways during climacteric and non-climacteric ripening in melons ('Gaúcho' and 'Yellow', respectively).

Material and methods

Plant material

Melon fruits (*C. melo* cv. 'Yellow' and 'Gaúcho') were greenhouse-cultivated in Ponta Grossa, Paraná, Brazil during the summer of 2018, and were collected at 15, 25, 35, and 45 days after pollination (DAP) for analysis of fruit quality. Specifically, only fruits at 35 and 45 DAP were used for quantitative reverse-transcription PCR (RT-qPCR) analysis, as at these stages mature fruits exhibit the highest levels of representative volatile compounds.

Post-harvest quality assessment

Three fresh fruits from each cultivar were measured for peel and pulp color at different ripening stages using a Minolta CR400 colorimeter. The CIE (Commission Internationale de l'Eclairage) L* (lightness), a* (green/red coordinate) and b* (blue/yellow coordinate) color scale was used. Hue angle was calculated using the equation $h° = \tan^{-1}(b^*/a^*)$, when $a^* > 0$ and $b^* > 0$ or $h° = 180 + \tan^{-1}(b^*/a^*)$, when $a^* < 0$ or $b^* > 0$. The soluble solids content (SSC – °Brix) and juice acidity (pH) were measured using a digital refractometer and automatic pH meter, respectively. Three measurements were made for each fruit, and a mean was calculated.

RT-qPCR analysis

Total RNA was isolated from fruit mesocarps in biological triplicate using the sodium perchlorate method as described by Campos et al. (2017). Once isolated, RNA samples were submitted to a TURBO™ DNase kit (Invitrogen) at 37°C for 30 min. to remove genomic DNA. Sample integrity was assessed by agarose gel electrophoresis and through the A260/A280 and A260/A230 absorbance ratios, with samples exhibiting values above 1.7 considered suitable. Then total RNA samples (2 µg) were converted to cDNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to manufacturer's instructions.

The primers used were designed using PrimerBlast and Beacon Design software, tested under gradient PCR and the oligonucleotide sequences are represented in Table 1. A 60 ng sample of cDNA was used for each RT-qPCR reaction. Thermocycling was initiated with a 15 min. incubation at 95°C, followed by 40 cycles (95°C, 15 s; primer-specific temperature (Supplementary Table 1), 72°C 20 s). The relative quantification of gene expression was achieved using the housekeeping genes ribosomal protein S15 (*CmRPS15*) and 60S ribosomal protein L (*CmRPL*) according to Kong et al. (2014) and the 35 DAP fruit of 'Gaúcho' as the calibration sample. The RT-qPCR reactions were run in duplicate for each primer combination (Supplementary File 1). The analyses were performed in an AriaMx Real-Time PCR System (Agilent) and expression values were calculated using the Livak and Schmittgen (2001) $2^{-\Delta\Delta Ct}$ equation.

Table 1. Genes tested and their Melonomics ID, oligonucleotide sequence, amplified fragment size and specific annealing temperature.

Gene	Melonomics ID	Oligonucleotide sequences (5'-3')	Amplified fragment size (bp)	Annealing temperature (°C)
CmRPS15 F	MELO3C006471	GAAGCTGCGTAAAGCGAAAC	132	61.8
CmRPS15 R		GGTCTTTCCATTGTAAACTCCAA		
CmRPL F	MELO3C023039	CGACAATACTGGAGCCAAGAA	100	63.1
CmRPL R		CATCACCATATCTCCCACACAA		
CmADH1 F	MELO3C023685	GGGGTGTTGCTGTTCTTGTG	129	60.4
CmADH1 R		GAATGTCGGTTCGGGGTTTG		
CmAAT1 F	MELO3C024771	CCACAGGGGCCAGAATTACA	104	61.8
CmAAT1 R		TGGAGGAGGCAAGCATAGACTT		
CmBCAT1 F	MELO3C010776	ATGATGAGAGCTGTGATTTTGAC	126	61.8
CmBCAT1 R		TCCCATAACGGCTCATTTG		
CmArAT1 F	MELO3C025613	AATGCTTGCTCGTCCTGGTG	121	60.4
CmArAT1 R		GCCTTGCTGAGGGAGTAGAT		
CmPDC1 F	MELO3C009145	CAGTGGAAGCAGCAGCACA	130	60.2
CmPDC1 R		GCCAAGGCATAGCCACAAG		
CmLOX9 F	MELO3C014482	CAGATCCATCTTGTGAAC	230	56.9
CmLOX9 R		AGTTGGTAGAGTCATTCC		
CmLOX18 F	MELO3C024348	TGGAGACTATCACAATCG	195	56.9
CmLOX18 R		CTTTCCCATCACCTCTAA		

*The specific primers were designed using the Primer Blast platform, and their quality was assessed by Beacon Designer software. The parameters used to design the primers were: 45-55% CG content, minimum Tm of 57°C and maximum of 63°C (with a maximum difference of 3°C between forward and reverse primers), primers up to 23 nucleotides, amplicons up to 250 bp, without formation of hairpins and inter- and intraprimer dimers.

Statistical analysis

To determine the significance of differences in gene expression among cultivars and stages, the Livak and Schmittgen (2001) equation was used to calculate the expression values, which were then subjected to ANOVA using the R statistical environment (R Core Team, 2010). Significance was defined as $p < 0.05$, and Tukey's test was performed at a 95% confidence level on the significant data.

Results

Phenotypic characterization

Melon maturity at different DAP was assessed by measuring the color, SSC and pH of two types of cultivated melon fruit. Colorimeters express color in numerical terms (see methods) along the L*, a* and b* axes (from white to black, green to red and blue to yellow, respectively). The L* value in the 'Yellow' fruit peel (Figure 2b) increased from 15 to 45 DAP, whereas in pulp, the pattern was the opposite, decreasing as fruit ripened. Coordinates on the a* axis for peel and pulp (Figure 2c) increased in 'Yellow' melons, and results for b* (Figure 2d) increased throughout ripening for the peel, decreased slightly for the pulp and the hue angle (H°) declined slightly as the fruits ripened (Figure 2a).

For 'Gaúcho' melons, L* peel values decreased from 15 to 35 DAP and then increased as the fruit reached 45 DAP (Figure 3b). The L* values of 'Gaúcho' pulp declined throughout ripening. The results showed that the coordinates on the a* axis increased for both pulp and peel in 'Gaúcho' melons (Figure 3c). Additionally, the coordinates on the b* axis (Figure 3d) increased throughout ripening for both peel and pulp. The hue angle exhibited a declining pattern for both peel and pulp, indicating a shift from greenish to reddish colors as the fruits ripened (Figure 3a). This is a phenotypic characteristic of fruits in this cultivar.

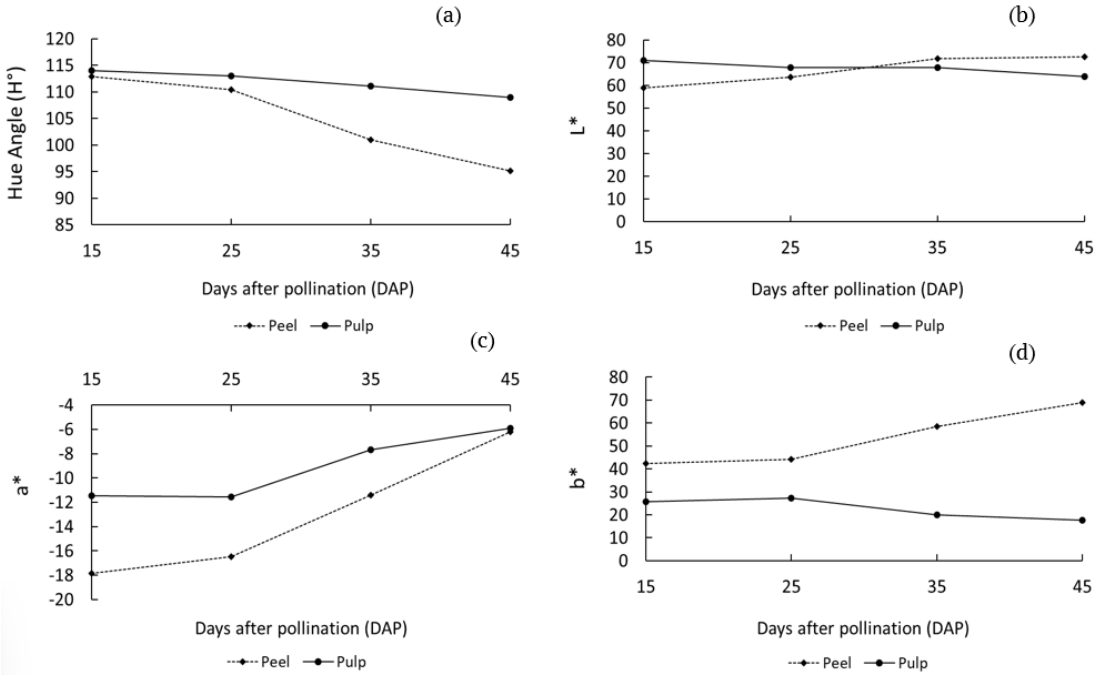

Figure 2. Means of peel and pulp colorimetry of 'Yellow' melon fruits at different stages of development (15 DAP, 25 DAP, 35 DAP, 45 DAP): (a) represents the hue angle (°), (b) represents luminosity, (c) indicates the colors that range from green (−a*) to red (+a*), and (d) indicates the color change from the blue scale (−b*) to yellow (+b*).

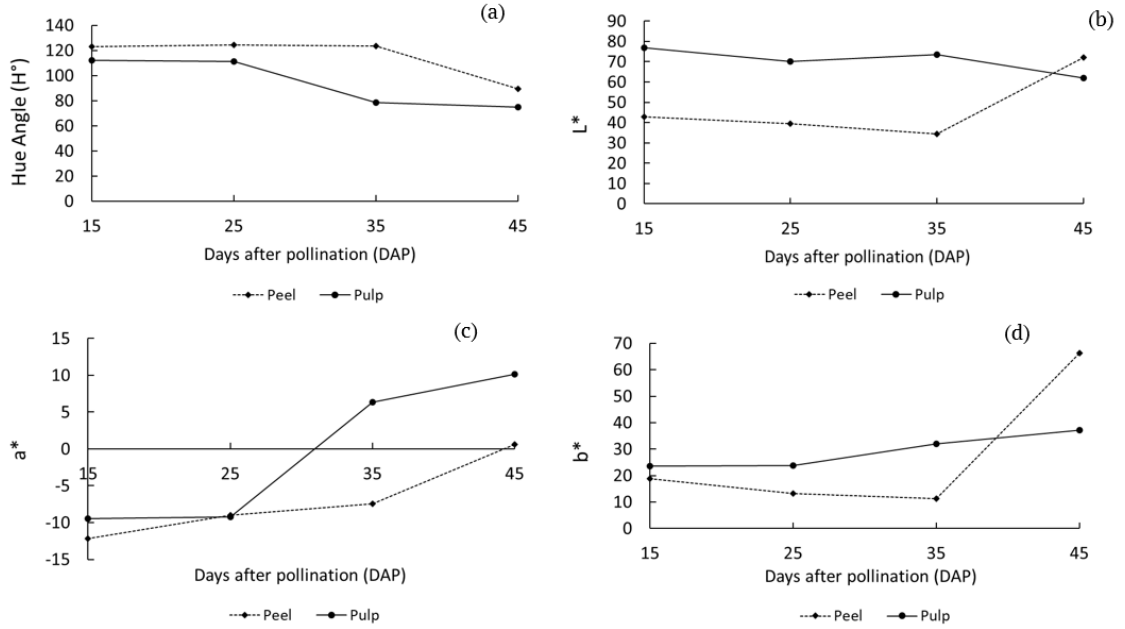

Figure 3. Means of peel and pulp colorimetry of 'Gaúcho' melon fruits at different stages of development (15 DAP, 25 DAP, 35 DAP, and 45 DAP): (a) represents the hue angle (°), (b) represents luminosity, (c) indicates the colors that range from green (−a*) to red (+a*), and (d) indicates the color change from the blue scale (−b*) to yellow (+b*).

The SSC in 'Gaúcho' melons presented a slight decrease from 15 to 25 DAP, and a marked increase from 35 to 45 DAP, reaching approximately 8 °Brix in fully ripe fruits (Figure 4a). When it comes to pH, 'Gaúcho'

fruit showed a slight decline from 15 to 35 DAP, increasing again at 45 DAP. In 'Yellow' melon, the SSC increased gradually throughout maturation, reaching its maximum value at full maturity of approximately 9 °Brix (Figure 4b).

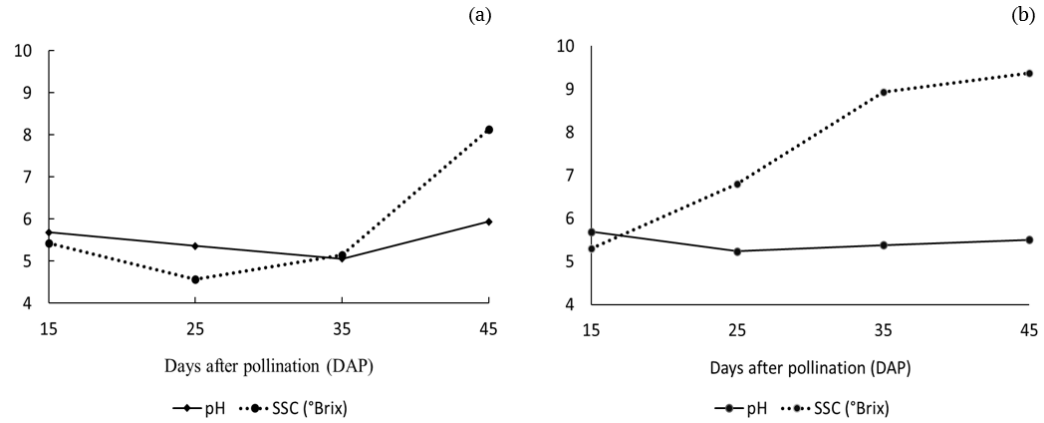

Figure 4. Soluble solids content (SSC, °Brix) and pH were measured in (a) 'Gaúcho' melons at different stages of development (15 DAP, 25 DAP, 35 DAP, and 45 DAP) and (b) 'Yellow' melons at different stages of development (15 DAP, 25 DAP, 35 DAP, and 45 DAP).

Gene expression of key genes of VOC metabolism

Seven key genes involved in VOC metabolism were selected for RT-qPCR analysis using *CmRPS15* and *CmRPL* as reference genes (Figure 5). The expression of these genes was evaluated in fruit at 35 and 45 DAP, the stages where the fruit has a desirable ripe aroma. Associated with lipid metabolism, the 9-lipoxygenase (*CmLOX9*) gene was downregulated in 'Yellow' melons from 35 to 45 DAP, *CmLOX9* expression at 35 DAP being 2.2-fold higher than at 45 DAP, although this difference was not significant. For 'Gaúcho' melons, this gene showed constant expression in the two ripening stages tested. When comparing non-climacteric with climacteric melons, the expression in 'Yellow' fruit was higher in both stages (4-fold at 35 DAP and 8.2-fold at 45 DAP) than that in 'Gaúcho' (Figure 5a). Expression of the gene responsible for encoding 18-lipoxygenase (*CmLOX18*) decreased between 35 and 45 DAP in both cultivars, being more pronounced in 'Gaúcho' melon (15.6-fold). For 'Yellow' melons at 35 DAP, the expression was 18.2-fold lower than in 'Gaúcho' melons at the same stage (Figure 5b).

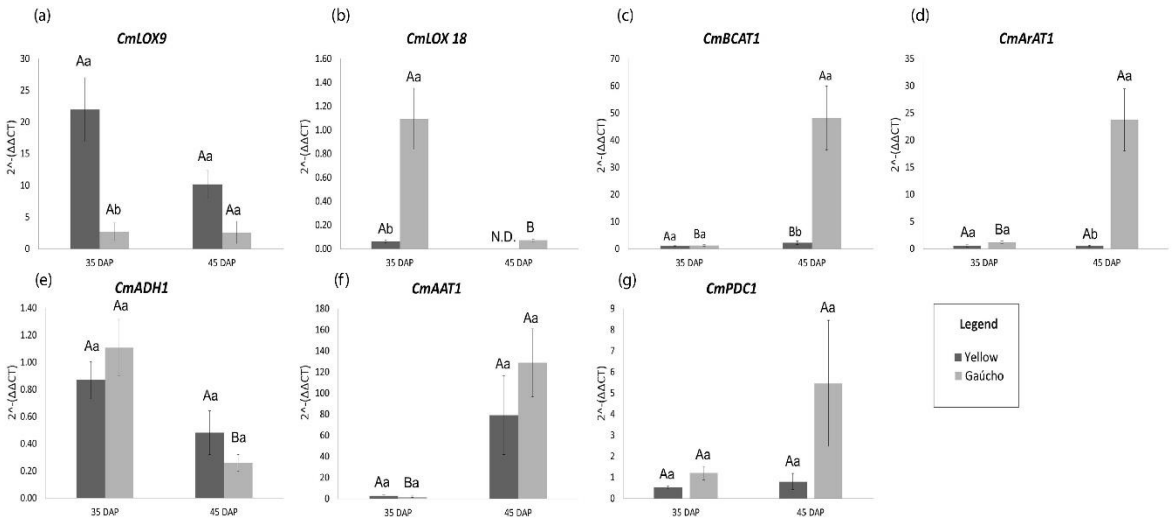

Figure 5. The relative mRNA expression of genes involved in VOC metabolism was determined by $2^{-\Delta\Delta Ct}$ (Livak & Schmittgen, 2001). The results are expressed as mean ± SEM and the significance of different developmental stages (35 DAP and 45 DAP) as defined by the Tukey test ($p \leq 0.05$) after data normalization by the Box-Cox method. Different capital letters indicate significant differences among stages within the same cultivar. Different lowercase letters indicate significant differences among cultivars within the same ripening stage. ND – not detectable.

Associated with amino acid metabolism, the expression of the branched-chain aminotransferase gene (*CmBCAT1*) increased 38.5-fold in 'Gaúcho' melons from 35 to 45 DAP (Figure 5c). In non-climacteric 'Yellow'

melons, the expression of this gene increased only 2-fold in 45 DAP fruit. When comparing these two cultivars at 35 DAP, the expression of *CmBCAT1* was similar, whereas at 45 DAP, the expression in 'Gaúcho' was notably higher than that in 'Yellow' melon (Figure 5c). The expression of the aromatic aminotransferase gene (*CmArAT1*) was considerably higher in fully ripe 'Gaúcho' melons, being 48.5-fold higher than in 'Yellow' melon fruit at the same stage. In 'Yellow' melons, the expression of this gene did not differ significantly from 35 to 45 DAP. For 'Gaúcho', the expression of *CmArAT1* at 45 DAP was 21-fold higher than at 35 DAP (Figure 5d).

Three genes coding enzymes at the end of VOC biosynthesis pathways were evaluated: alcohol dehydrogenase (*CmADH*), alcohol acyltransferase (*CmAAT*) and pyruvate decarboxylase (*CmPDC*). The expression of *CmADH1* decreased as fruit ripened in both cultivars, being 1.8- and 4.3-fold higher in 'Yellow' and 'Gaúcho' melons, respectively, at 35 DAP compared to fully ripe 45 DAP melons (Figure 5e). In contrast, the expression of *CmAAT1* was higher in 45 DAP fruit than in 35 DAP fruit for both cultivars (26-fold in 'Yellow'). However, only the increased expression for 'Gaúcho' melons (78-fold) was significant (Figure 5f). Considering both genotypes at the fully ripe stage, expression of *CmAAT1* was 1.6-fold higher in 'Gaúcho' melons than in 'Yellow' melons and the difference was not statistically significant. *CmPDC1* expression increased slightly as the fruit ripened. In 'Yellow' melons, the expression was 3.7-fold higher in 45 DAP ripe fruit than in 35 DAP fruit. In 'Gaúcho', the expression in ripe fruit was 13.3-fold higher than in fruit at 35 DAP (Figure 5g).

Discussion

Fruit development and ripening are complex physiological processes that involve many biochemical pathways that determine the quality of the fruit, such as flavor (White, 2002). The chemical profiling of aroma is determined by specific combinations and proportions of volatile compounds, with the lipid and amino acid pathways having the greatest influence on this characteristic (Gonda et al., 2010; Pott, Osorio, & Vallarino, 2019; Schwab et al., 2008).

In addition, there are two types of fruit ripening, climacteric and non-climacteric, which have distinct regulatory mechanisms in determining organoleptic properties (McMurchie, McGlasson, & Eaks, 1972). Melon is an important species in which to study these differences because it presents both phenotypes, with climacteric cultivars generally being more aromatic and having esters as the most prominent volatiles, while non-climacteric cultivars are mostly non-aromatic, demonstrating that VOC content is an ethylene-dependent trait (Burger et al., 2006; Ezura & Owino, 2008; Pech, Bouzayen, & Latché, 2008; Portnoy et al., 2008).

Previous studies have reported on the chemical characteristics of aroma in climacteric 'Dulce' and 'Védrantais' and non-climacteric 'Piel de Sapo' cultivars, as well as many others (Obando-Ulloa et al., 2008; Spadafora et al., 2019). However, this is one of the first studies that presents an integrative analysis of gene expression of biochemical pathways related to volatile compound synthesis in non-climacteric ('Yellow') and climacteric ('Gaúcho') melons. Furthermore, the results of this study provide knowledge about the first steps in the synthesis of volatile, which have been little explored; previous studies have mainly focused on the last steps involving ADH and alcohol *acyltransferase* (Chen, Cao, Jin, Tang, & Qi, 2016; El-Sharkawy et al., 2005; Flores et al., 2002; Manríquez et al., 2006; Peng et al., 2020).

Factors such as fruit coloration and taste-related aspects, such as sucrose accumulation and acidity, are important factors in determining fruit maturity and quality. In the quality evaluation of climacteric and non-climacteric cultivars, the colorimetric results showed a similar behavior of hue angle for peel and pulp in 'Yellow' melon, where color changes are subtle and gradual throughout the ripening process, as well as those found by Schemberger et al. (2020). In contrast, in 'Gaúcho' there is a clear transition of pulp color change from 25 to 35 DAP and a marked change in peel color from 35 to 45 DAP, which coincides with the climacteric peak of this cultivar, since rind pigmentation is an ethylene-dependent factor in melon fruits (Ayub et al., 1996).

The color results demonstrate that 'Gaúcho' melons tend to be orange, while 'Yellow' melons have a yellowish tone (McLellan et al., 1995). The SSC increased during maturation in both cultivars, while slight changes in pH occurred. The SSC was slightly higher in 45 DAP 'Yellow' melons compared to 'Gaúcho' melons, and there was a minor variation in pH, which corresponds with previous studies of non-climacteric and climacteric melons (Saladié et al., 2015). However, it was observed that, similar to fruit coloration, the increase in SSC in 'Yellow' melon is gradual throughout maturation, while in 'Gaúcho' melon, this trait undergoes a sharp change from 35 to 45 DAP, possibly triggered by ethylene production at this developmental stage, although this characteristic is not ethylene-dependent.

Several comparative studies between climacteric and non-climacteric melons have reported a greater amount of total volatiles in climacteric fruit, as well as a greater amount of esters, which are generally responsible for the typical sweet and fruity aroma of climacteric melons. In contrast, non-climacteric melons have greater amounts of alcohols and aldehydes; therefore, this is an attribute that undergoes modifications in the presence of ethylene, just like skin coloration and the pattern of soluble solids accumulation (El-Sharkawy et al., 2005; Gonda et al., 2016; Shalit et al., 2001).

Lipid metabolism is an important route in determining the aroma composition of fruits. The main enzymes involved include the lipoxygenases (LOXs), which transform polyunsaturated fatty acids such as linoleic acid and linolenic acid into hydroperoxides (Andreou & Feussner, 2009). In melons, there are 18 genes encoding these enzymes (*CmLOX01–18*), but only a few play a role in determining aroma characteristics (Tang, Zhang, Cao, Wang, & Qi, 2015; Zhang et al., 2010). In our study, we evaluated two genes, *CmLOX9* and *CmLOX18*.

Vincenti et al. (2019) presented the role of 9-LOXs in the formation in plants of volatiles such as (Z)-3-nonenal, (E)-2-nonenal and (E, Z)-2,6-nonadienal and, consequently, their respective alcohols and esters. Expression of *CmLOX9* decreased from 35 to 45 DAP in 'Yellow' melons as the fruit ripened. Similar results have been found previously in RNA-Seq analysis of 'Yellow' melon, where expression of the *CmLOX9* gene was higher in 10 DAP than 40 DAP melons (Schemberger et al., 2020). In 'Gaúcho' melons, the expression of this gene remained relatively constant at 35 and 45 DAP and was lower than expression in 'Yellow' melons at both stages. These results indicate that *CmLOX9* has more influence on aroma determination in non-climacteric melons. However, contrary results were found by Tang et al. (2015), who worked with 18 *LOX* genes in four distinct melon cultivars, two aromatic (*C. melo* var. *makuwa* Makino group cultivars 'Yu Meiren' and 'Cui Bao') and two non-aromatic (*C. melo* var. *conomon* group, cultivar 'Shao Gua', and *C. melo* var. *flexuosus* Naud. group, cultivar 'Cai Gua'). They observed that the expression of this gene was higher in aromatic cultivars. It is possible that another isoform may be acting on the synthesis of these metabolites in 'Gaúcho' melons, as will be observed below.

The 13-LOX enzymes, including LOX18, are involved in the formation of volatiles such as hexanal, (Z)-3-hexenal and (E)-2-hexenal, as well as their respective alcohols and esters in plants (Vincenti et al., 2019). Tang et al. (2015) reported that *CmLOX18* is a gene responsible for the formation of volatiles in melons. The *CmLOX18* gene showed much lower expression in 'Yellow' compared to 'Gaúcho' melons at 35 DAP and no expression could be detected in this cultivar at 45 DAP. These results differ from those found by Tang et al. (2015): in their study, expression in two aromatic cultivars, 'Yu Meiren' and 'Cui Bao' (*makuwa* Makino group), and the non-aromatic cultivar 'Cai Gua' (*flexuosus* Naud. group) was constant and significantly higher than in the aromatic cultivar 'Shao Gua' (*conomon* group). In 'Gaúcho' melons, it was possible to detect expression in ripe fruit, even though it was significantly lower than that obtained at 35 DAP. Thus, it is possible to attest that *CmLOX18* has a similar expression profile to *CmLOX9* studied by Tang et al. (2015), and the expression of this gene is dependent on the cultivar.

In the case of 'Yellow' melon fruit, the expression of *CmLOX9* may be directly related to the formation of alcoholic compounds, while the *CmLOX18* isoform in 'Gaúcho' may participate in the formation of esters present in the ripe fruit. Zhang et al. (2010) observed expression of the *CmLOX9* gene in treated fruits to verify its dependence on ethylene and maturation and found that *CmLOX9* expression is decreased in the presence of ethylene and is not affected under ethylene inhibitors such as 1-methylcyclopropene (1-MCP), ethanol vapor or low temperatures, results resembling those found here. As for the *CmLOX18* isoform, Zhang et al. (2010) found that the expression of this gene is upregulated when ethylene is present, and it is downregulated in treatments that promote ripening delay, results resembling those presented here. Moreover, Tang et al. (2015) suggested that there is a decrease in LOX activity as aldehyde levels increase, which could imply the downregulation we have noted for both *CmLOX9* and *CmLOX18* from 35 to 45 DAP.

VOCs derived from the catabolism of amino acids are present in both non-climacteric and climacteric cultivars, and the biochemical reaction consists of a transamination followed by a decarboxylation. In climacteric melons, most compounds are esterified and are more abundant than in non-climacteric melons, which have aldehydes and alcohols as the most prominent products (Gonda et al., 2016). The main amino acids involved in VOC synthesis are L-valine, L-leucine and L-isoleucine, which have a branched chain; and L-phenylalanine with an aromatic chain (Gonda et al., 2010). The first three have the transamination process mediated by BCATs, while for the latter it is mediated by ArATs (Gonda et al., 2010).

In this study, the expression of *CmBCAT1* increased as the fruit ripened in both 'Yellow' and 'Gaúcho' melons, and expression was highest in ripe 'Gaúcho' melons. These results are similar to those of Gonda et al. (2010), who found higher expression in ripe climacteric melons ('Dulce', 'Védrantais' and 'Noy Yizre'el') than

in non-climacteric ones ('Tam Dew', 'Rochet' and 'Piel De Sapo'). A correlation between the downregulation of *CmBCAT1*, caused by 1-MCP treatment, and a concomitant reduction in the activity of aminotransferase enzymes related to the gene has previously been described in melons. This leads to a subsequent decrease in fruit aroma content, indicating a close relationship between this gene and volatile synthesis in an ethylene-dependent ripening process (Li et al., 2016). This expression pattern in different melon fruits is reflected in the metabolites formed (directly or indirectly) by the enzyme encoded by this gene. Several studies have also indicated that climacteric melons tend to produce more total volatiles, including esters resulting from the degradation of branched-chain amino acids (Gonda et al., 2010; Schwab et al., 2008).

ArATs degrade tyrosine and phenylalanine, forming several different volatile compounds (Gonda et al., 2010; Lee & Facchini, 2011). Gonda et al. (2010) demonstrated that the expression of *CmArAT1* in melons is directly involved in the biosynthesis of acetaldehyde and its respective alcohol and esters, through the degradation of the amino acid phenylalanine. In this study, we noted upregulation of the expression of *CmArAT1* during ripening of climacteric 'Gaúcho' fruit, while in 'Yellow' fruit this expression remained constant. The expression in ripe fruit was considerably higher in climacteric melons. Gonda et al. (2010) presented a similar pattern; in climacteric 'Dulce' melons, the expression of this gene was higher in ripe fruit compared to 25 DAP fruit. Additionally, higher expression of this gene in ripe climatic fruit than in non-climacteric fruit was observed in the cultivars 'Tam Dew', 'Rochet' and 'Piel De Sapo'. Just as for *CmBCAT1*, Li et al. (2016) observed that the expression of *CmArAT1* is ethylene-dependent, supporting our results.

The final steps of lipid and amino acid metabolism involve two enzymes that are crucially important: ADH and alcohol *acyltransferase* (AAT). ADH is a zinc-binding enzyme that converts aldehydes into alcohols in a reversible manner, while AAT promotes the esterification of alcohols (Chen et al., 2016; El-Sharkawy et al., 2005; Manríquez et al., 2006). At least 12 isoforms of *CmADH* have been reported, with *CmADH1* and *CmADH2* being the most well-characterized for the formation of volatiles in fruits (Jin et al., 2016).

In melon, studies have reported an affinity of the isoform *CmADH1* for acetaldehyde (also known as ethanal), catalyzing its conversion into ethanol. The role of this enzyme in the conversion of butanal to butanol and hexanal to hexanol is also known, although it also acts in a less significant way with other aldehydes (Chen et al., 2016; El-Sharkawy et al., 2005). In this study, the gene expression of *CmADH1* decreased as both 'Yellow' and 'Gaúcho' melons ripened from 35 to 45 DAP. Manríquez et al. (2006) observed in wild 'Védrantais' melon (*cantalupensis* group) that there is a significant increase in the expression of *CmADH1* and *CmADH2* during the peak of ethylene production (around 39 DAP), with a decrease in expression after the climatic peak, agreeing with the present study. However, in climacteric and strongly aromatic melons (*makuwa* Makino group, cultivar 'Cai Hong'), this gene presented the opposite behavior, with increased expression during maturation (El-Sharkawy et al., 2005); as a result, it is possible to attest that the expression of this gene is cultivar-dependent.

CmAAT1 is involved in the esterification of several alcohols, including 1-butanol, hexanol, heptanol, octanol, (Z)-2-hexen-1-ol, (E)-2-hexen-1-ol, (Z)-3-hexen-1-ol, (E)-3-hexen-1-ol, 2-methylpropanol, 2-methylbutanol, 3-methylbutanol and benzyl alcohol (El-Sharkawy et al., 2005). In this study, the *CmAAT1* gene was upregulated in both 'Yellow' and 'Gaúcho' melons during ripening. Considering these two melon genotypes at full ripeness, the expression of *CmAAT1* in non-climacteric fruit was significantly lower than that in climacteric fruit.

The results obtained for 'Gaúcho' were similar to those found by Obando-Ulloa et al. (2008): the expression of this gene was upregulated throughout ripening in climacteric 'Védrantais' melons (*cantalupensis* group). Chen et al. (2016) confirmed these results by analyzing the enzymatic activity of AAT, which was found to be high and increasing with fruit ripening in aromatic melon fruits (var. *makuwa* Makino) and to a lesser extent in non-aromatic melon fruits (var. *flexuosus* Naud.). Other authors have also reported that the accumulation of volatile esters occurs in ripe fruit and that they are strongly correlated with ethylene production (Flores et al., 2002; Schemberger et al., 2020). The expression of *CmAAT1* is negatively regulated in the presence of the ethylene antagonist 1-MCP and in antisense ACC oxidase fruits, as reported by El-Sharkawy et al. (2005), thus explaining why non-climacteric melons have much lower formation of esters compared to climacteric fruits, resulting in their classification as low aromatic or non-aromatic fruits (Chen et al., 2016).

PDC contributes to the formation of volatile compounds by catalyzing the transformation of a keto acid into an aldehyde (Sugimoto, Daniel Jones, & Beaudry, 2011; Wang et al., 2019). As this enzyme acts in the early stages of volatile formation, it has the potential to influence the formation of various volatiles derived

from its substrates. Wang et al. (2019) identified seven substrates of the PDC1 enzyme: pyruvate, 2-oxobutanoate, 2-oxopentanoate, 2-oxohexanoate, ketoisocaproate, ketomethylvalerate and ketoisovalerate. Moyano et al. (2004) described the involvement of the *PDC1* gene in aroma formation in strawberries, and Zhu et al. (2020) also suggested the involvement of a *PDC* gene in aroma biosynthesis in bananas.

The results of the present study show that *CmPDC1* expression remains constant throughout ripening of 'Yellow' melons and is upregulated during ripening of 'Gaúcho' melons. Wang et al. (2019) identified the gene function of *CmPDC1* in melon fruits by silencing this gene and measuring enzymatic activity. They found that the activity of PDC1 was significantly reduced in fruit where the gene had been silenced. Furthermore, with the gene silenced and enzymatic activity lowered, there was a significant decrease in the levels of the aromatic compound related to it. Thus, based on our results, it is possible to attest that the expression of *CmPDC1*, accompanied by the expression of *CmAAT1*, plays an important role in the formation of esters in ripe 'Gaúcho' melon fruits, and also explains, in part, the smaller amount of volatile compounds present in non-climacteric melon fruits, such as the 'Amarelo' cultivar.

Conclusion

Aroma plays an important role in determining the organoleptic properties of fruit, which can influence consumption and demand. In our study, we conducted gene expression analysis during fruit development of 'Yellow' and 'Gaúcho' melons. In the lipid pathway, there is an opposite profile of expression between the isoforms of LOX: in non-climacteric 'Yellow' fruit aroma formation, there is higher expression of *CmLOX9*, possibly participating in the formation of alcoholic compounds, while *CmLOX18* is more active in climacteric melon fruit ('Gaúcho'), possibly to form esters. The amino acid pathway was the primary one related to the biosynthesis of esters. The branched amino acid pathway correlated with expression of *CmADH1*, demonstrating that this gene is more strongly linked to the production of volatiles at the 35 DAP stage, decreasing at the end of maturation, for both cultivars. As for *CmAAT1*, its expression is significantly higher in climacteric fruits, as is that of *CmPDC1*, which contributes to the formation of aldehydes at the beginning of the pathway, partly explaining the lower content of volatile compounds in mature 'Yellow' melon fruits. Thus, these results open new perspectives to further explore the mechanisms of VOC formation in melons.

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