Study on the differences in aroma components and formation mechanisms of “Nasmi” melon from different production areas

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Abstract
Aroma is an important factor that guides consumers in purchasing and is thus very important in melon research. To our knowledge, the number of studies with a focus on the aroma differences of the same melon variety in different production areas is largely limited. In this study, the differences in aroma components of “Nasmi” melons from two different production regions were analyzed using gas-phase ion migration spectroscopy. Transcriptome sequencing was performed for analyzing fragrance-related genes. Results showed that there were significant differences in the aroma components between products from the two regions. The total amount of aroma compounds from the Turpan region (TT) was 1.7 times higher than that from the Altay region (AT). Through the analysis of transcriptome data, the key genes encoding melon aroma components in different regions were identified as ethanol dehydrogenase, 3-hydroxyl-coenzyme A (CoA) dehydrogenase, acyl-CoA oxidase, long-chain acyl-CoA synthetase, acetaldehyde dehydrogenase, and acetyl-CoA acyltransferase. Real-time quantitative polymerase chain reaction (RT-qPCR) showed that the verified genes were similar to the transcriptome. In this study, the main aroma components of the same variety of melon that differed in different production areas and the key genes causing these differences were identified. In addition, the aroma metabolic pathway of melon in different regions was preliminarily elucidated. These results could provide a theoretical basis for further study of the formation mechanism of melon aroma and breeding.

Keywords
aroma, gene, melon, transcriptomics
1 | INTRODUCTION

Melon (Cucumis melo L.) is a gourd family (Cucurbitaceae) cucumber (Cucumis) annual trailing herb and is grown on five continents worldwide. According to the FAOSTAT (Food and Agriculture Organization Statistical Database) published by the Food and Agriculture Organization (FAO) of the United Nations, the world’s total melon production in 2019 was 27.5013 million tons. China’s melon production was 13.5414 million tons, accounting for 49.24% of the world’s total production. Xinjiang is one of the main production areas in China, with the melon production ranking first in China year-round (Xiong et al., 2018). Melon fruit is favored by consumers because of its juicy pulp and unique aroma. Flavor (aroma and taste), color, texture, and nutrients are the main quality determining factors of melon, among which the flavor and color play a leading role in melon consumption (Obando-Ulloa, Jowkar, et al., 2020). Therefore, study on fruit aroma is very important in melon breeding and quality control. It has been receiving increasing attention from breeders and researchers. The main aroma compounds of melon and their metabolic pathways have been reported by other research groups (Shi et al., 2020).

It has been reported that the aroma of melon fruit is related to development period, variety, postharvest storage, and production area. Beaulieu et al. determined the volatile components of cantaloupe at different developmental stages using headspace solid-phase microextraction—gas chromatography–mass spectrometer (HS–SPME–GC–MS) technology (Beaulieu & Grimm, 2001). They found that the aroma of cantaloupe varied greatly during different developmental stages, and most ester compounds gradually increased with increasing maturity. Lamikanra et al. found that the most prominent volatile compounds in melon were methyl-butyl acetate and hexyl acetate when stored at 4°C. These two compounds, which contribute to the fruit aroma characteristics of many fruits, are usually present in relatively large proportions in Hami melon (Lamikanra & Richard, 2002). Elazar Falik et al. measured the aroma of two different varieties of melon in Israel and found that C8 fruit had higher levels of aroma volatiles than 5080 fruit (Falik et al., 2001). Xiao Z et al. determined and analyzed the volatile components of the melon varieties such as Jiashigua, Xizhou Mi 17, and Minqin from different production regions. They detected 45, 46, and 69 volatile compounds in the three cultivars, respectively. Among these volatile compounds, (Z)-6-nonenal-1-ol, 2-methyl-butyl acetate, 3-methyl-butyraldehyde, hexanal, and methyl thioacetate were particularly important in Minqin melon (Xiao et al., 2021). While in the study of Hasbullah et al., 3-pentene-2-alcohol, hexyl acetate, and 3-hydroxy-2-butanoate were determined to be the key aroma components of Gama Melon Parfum (GMP) melon (Hasbullah & Daryono, 2019).

The main identified metabolic pathways of melon aroma include the fatty acid pathway, amino acid pathway, secondary metabolic pathway, and conversion of alcohols and aldehydes into esters (Lewinsohn et al., 2001; Schwab et al., 2008; Tang et al., 2015). In the fatty acid pathway, straight-chain aliphatic alcohols, aldehydes, ketones, and esters can be synthesized. Saturated fatty acids are catalyzed by β-oxidation and acyl-CoA oxidase to produce lactones. In addition, some unsaturated fatty acids are directly oxidized to form C6 aldehydes and corresponding alcohols and esters by lipoxygenase (LOX). In the amino acid pathway, branched-chain alcohols and esters are formed mainly through the action of transaminase and dehydrogenase. Aldehydes produced in the above two pathways generate alcohols under the action of alcohol dehydrogenase (ADH). In combination with acyl-CoA, corresponding esters can be formed under the action of alcohol acyltransferase (AAT). In secondary metabolic pathways, synthesis of some melon volatile phenols and terpenoid substances via the shikimic acid pathway is one of the most important branches. With the combination of these various aroma metabolism pathways, melon forms a unique and strong aroma that appealing consumers love. Related studies have found that enzymes related to melon aroma metabolism mainly include lipoxygenase (LOX), alcohol dehydrogenase (ADH), alcohol acyltransferase (AAT) acyl-coenzyme A oxidase, aldehyde dehydrogenase, and others (Buchhaupt et al., 2012; Gur et al., 2016; Li et al., 2016a; Shalit et al., 2001).

With rich aroma compound content, “Nasmi” melon which was selected and bred by Academician of Mingzhu Wu was used as the model fruit in our study. Gas chromatography-ion mobility spectrometry (GC-IMS) technology was used to determine and analyze the aroma compounds of melons in different production areas. RNA-sequencing (RNA-seq) technology was used to carry out high-throughput sequencing on fruits of the same “Nasmi” melon from different production areas in Xinjiang. Based on the sequencing data, the genes related to melon fruit aroma metabolism were identified. The formation mechanisms of melon aroma were also subsequently analyzed. These studies may contribute to more fundamental research in melon aroma and industrial practices.

2 | MATERIALS AND METHODS

2.1 | Materials

The sampling area is shown in Table 1. The melons with same planting patterns from Turpan are provided by the Xinjiang and Altay experimental base. The same batches of seeds were provided by the Xinjiang Academy of Agricultural Sciences Research Center. Melons were sampled 43 days after pollination. Thirty melons with moderate maturity and no disease/insect pests were selected in the two test regions respectively. Samples were stored at ~80°C until use. Three biological replicates were obtained for each sample. The test sample “Nasmi” was planted in two experimental bases, with similar treating and environmental conditions, such as seed pretreatment, plant spacing, seedling stage management, fertilizer, and watering.

2.2 | Chemicals and instruments

Polysaccharide polyphenol total RNA extraction kit, FastKing RT Kit (with GDNase), and SuperReal PreMix Plus (SYBR Green) Kit
were purchased from Tiagen Biochemical Technology (Beijing) Co Ltd. Diethylpyrocarbonate (DEPC) water, 1,3-diethyltriazene (DET) buffer (5x), anhydrous ethanol, β-mercaptoethanol, and anhydrous ethanol were purchased from Shanghai Source Leaf Biotechnology. Primer synthesis is from Shanghai BioLeaf Biotech Co Ltd.

The following instruments and equipment were used in this study: medical cryopreservation box (Haier): DW-86 W100, Haier special electrical appliances, analytical balance (Model No. XSE204), Mettler Toledo (Switzerland), DNBSeq Sequencing Platform (BDA), FlavourSpec® Flavor analyzer (G.A.S. Company, Germany), Agilent 2100 Bioanalyzer (Agilent Technologies, USA), PCR Amplifier (SureCycler 8800, Agilent), and fluorescent PCR instrument (LightCycler 96 Roche).

2.3 Method

2.3.1 Melon aroma composition determination

Volatile components were determined by the GC-IMS technology (Wang, Chen, & Sun, 2019). An automatic headspace (HS) device was used to extract samples. Three-gram sample was placed in a 20 ml headempty bottle with a magnetic cap, followed by an incubation at 50°C for 15 min. The rotating speed was set to 500 rpm (revolutions per minute). Three hundred microliter sample was injected each time with the injection needle temperature of 55°C. The GC was equipped with a FS-SE-54-CB-1 capillary column (15 m x 0.53 mm ID, 1 μm). The column temperature stayed at 60°C during the process. The running time was set to 30 min. Nitrogen (99.99%) was used as a carrier gas and its flow rate was initially set at 2 ml/min for 2 min, then increased to 100 ml /min within 18 min and held for 10 min. The 9.8 cm drift tube was operated at constant temperature (45°C) and voltage (5 kV). The flow rate of the drift gas (nitrogen) was set to 150 ml/min. Volatile compounds were identified by comparing the retention index (RI) and the drift time (DT) (the time it takes for ions to reach the collector through drift tube, in milliseconds) of standard in the GC-IMS library.

2.3.2 Transcriptome sequencing

Total RNA extraction from melon fruit

Total RNA extraction was carried out according to the kit operation manual. After extraction, the RNA integrity was detected by an Agilent 2100 biological analyzer.

Library preparation and sequencing

Following magnetic bead enrichment of messenger RNA mRNA with Oligo(dT) poly (A), RNA was obtained in segmentation buffer using random N6 primers for reverse transcription, according to the instructions for the retrovirus kit. After that, second strand complementary DNA (cDNA) was synthesized to form double-stranded DNA. The double-stranded DNA underwent phosphorylation at the 5’ end and 3’ end to form the sticky end of an “A”. The 3’ end had a bulge in the “T” drum bubble joint, which facilitated connection of the product through PCR amplification with specific primers into a single PCR product following thermal denaturation. Then bridge-type primers facilitated cyclization of single-stranded circular DNA to create a single-strand DNA library. Finally, the DNBSEQ platform was used for sequencing.

2.3.3 qRT-PCR verification

DNA extraction and reverse transcription were performed according to the instructions of kit. The reaction system was prepared using cDNA as template according to the SuperReal PreMix Plus (SYBR Green) kit. As shown in Table 2, the system volume was 10 μl. Each gene was prepared and analyzed in triplicate. The target gene and internal reference gene were placed in the same plate and measured simultaneously. According to the PCR amplification reaction and melting temperature (Tm) values provided by internal reference genes and target genes, the real-time fluorescence PCR conditions were set as follows: predenaturation at 94°C for 5 min followed by a cycle with 10S denaturation at 94°C, 30S annealing at 72°C, 15S extensions, 40 cycles, and 57–95°C melting curve plotted with a heating rate of 1°C/min. The obtained detection results were processed by the 2−ΔΔCT method (Livak & Schmittgen, 2001). The relative contents of multiple differential genes in the same melon fruit from different production areas were calculated to analyze their differential expression. Primer information for 11 different genes designed using Primer 5.0 software is shown in Table 3.

| The serial number | Sample number | Sampling area | Sampling time | Longitude | Latitude | Instruction |
|-------------------|---------------|---------------|---------------|-----------|----------|-------------|
| 1                 | AT            | Burjin County, Altay, Xinjiang, China | August 26, 2019 | 89.198021 | 42.941948 | Same variety, different producing area |
| 2                 | TT            | Turpan, Xinjiang, China | May 20, 2019 | 86.86187 | 47.704739 |

| Reagent | Amount |
|---------|--------|
| 2×SuperReal PreMix Plus | 5 μl |
| Upstream and downstream primers | 0.3 μl each |
| cDNA | 1 μl |
| RNase-Free ddH2O | Fill up to 10 μl |
2.4 | Data processing

2.4.1 | Aroma components

The instrumental analysis software includes LAV (Laboratory Analytical Viewer), three plugins, and GC-IMS Library Search tools, which can be used for sample analysis from different angles.

2.4.2 | Transcriptome data analysis

Sequence quality control and cleaning

SOAPnuke V1.5.2 software (https://github.com/BGI-flexlab/SOAPnuke) was used for filtering and statistical processing of the raw data. The steps were as follows: (I) Removal of reads containing joints (joint contamination); (II) removal of reads with unknown base N content greater than 5%; (III) removal of low-quality reads. Clean reads were obtained and stored in FASTQ format for subsequent analysis.

Quantitative analysis and screening of differential gene expression

Gene expression level, also known as expression abundance, is the first and most important factor in transcriptome data analysis (Sonali et al., 2020). FPKM (fragments per kilobase of transcript per million fragments mapped) was used as an indicator to measure the expression levels of transcripts or genes. The calculation formula is as follows:

\[
\text{FPKM} = \frac{\text{cDNA Fragments}}{\text{(Mapped Fragments (Millions) \times Transcript Length (kb))}}
\]

In this formula, cDNA fragments represent the number of double-ended reads. Mapped fragments (millions) represent the total number of fragments compared to the transcript. The RNA-Seq Expectation-Maximization (RSEM) software package was used to analyze the differentially expressed genes, with different multiples (fold change, FC) of gene expression variation: FC = FPKM (GA)/FPKM (CK). If Log2 FC > 0, it was considered to be upregulated. Otherwise, it was considered to be downregulated. The conditions for screening differential genes were that the differential multiple was more than 2 and the corrected p-value was less than or equal to .05 (Love et al., 2014).

Functional classification and annotation of differential genes

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (GO and KEGG) annotation databases are by far the most widely used public databases. The phyper function in R software was used for enrichment analysis of differential genes. The GO classification annotation of differential genes enables us to understand the classification of biological functions of differential genes. The GO database (http://www.geneontology.org/) notes mainly include biological processes, components of cells, and molecular functions. The KEGG database (https://www.kegg.jp/) system includes the functional systematic analysis of intracellular metabolic pathways and gene products, which is helpful for the study of complex gene biological behavior. Analyses on the obtained differential genes based on the KEGG database facilitate the understanding of major metabolic pathways which are demonstrating high gene expression.

3 | RESULTS

3.1 | Comparison of aroma components in different production areas

Figure 1 and Table 4 show the qualitative and quantitative results of volatile chemicals in melon samples from various production regions. Forty-seven signal peaks were detected by GC-IMS, from which 36 typical compounds were identified. The other 11 compounds got no qualitative results due to the limited data in the library database (Table 4). Based on the identified compounds, the volatile compounds in melon samples were esters (8), alcohols (7), aldehydes (10), ketones (2), pyrazines (1), terpenoids (1), and others.
As shown in Figure 1, one data point represents one volatile component. However, the same substance could detect monomers or dimers as a result of which one single compound might produce multiple signals. Each row in the figure represents all signal peaks selected from one sample. Each column represents signal peaks of the same volatile organic compounds in different samples. The depth of the color represents the content of the aroma component. The darker the color, the higher the content. The fingerprint contains all signals that can be detected by the instruments. Known substances were marked with existing names, while unknown substances were marked with numbers.

As shown in Figure 1 and Table 4, the eight esters identified in different regions were isoamyl acetate, ethyl butyrate, isobutyral acetate, methyl 2-methylbutanoate, ethyl acetate, methyl 2-methylpropanoate, ethyl propanoate, and ethyl hexanoate. As shown in the purple box on the upper right of the map, the concentration of most ester substances in the AT sample was higher than that in the TT sample. Monomers and dimers are recognized in most ester products. According to the peak area, ethyl acetate was the most abundant ester compound in the samples from both production regions. There is no significant difference in ethyl acetate content between TT samples and AT samples. The contents of isobutyl acetate and ethyl propanoate in the AT sample were significantly higher than those in the TT sample. There are 7 alcohols, oct-1-en-3-ol, linalool, 3-methylbutanol, (E)-2-hexen-1-ol, 2-methylbutanol, n-hexanol, and 2-propanol, which can be detected in this study. The contents of n-hexanol, (E)-2-hexen-1-ol, and 2-propanol in the TT sample were significantly higher than those in the AT sample. There are 7 alcohols, oct-1-en-3-ol, linalool, 3-methylbutanol, (E)-2-hexen-1-ol, 2-methylbutanol, n-hexanol, and 2-propanol, which can be detected in this study. The contents of n-hexanol, (E)-2-hexen-1-ol, and 2-propanol in the TT sample were significantly higher than those in the AT sample. There are 7 alcohols, oct-1-en-3-ol, linalool, 3-methylbutanol, (E)-2-hexen-1-ol, 2-methylbutanol, n-hexanol, and 2-propanol, which can be detected in this study. The contents of n-hexanol, (E)-2-hexen-1-ol, and 2-propanol in the TT sample were significantly higher than those in the AT sample. There are 7 alcohols, oct-1-en-3-ol, linalool, 3-methylbutanol, (E)-2-hexen-1-ol, 2-methylbutanol, n-hexanol, and 2-propanol, which can be detected in this study. The contents of n-hexanol, (E)-2-hexen-1-ol, and 2-propanol in the TT sample were significantly higher than those in the AT sample. There are 7 alcohols, oct-1-en-3-ol, linalool, 3-methylbutanol, (E)-2-hexen-1-ol, 2-methylbutanol, n-hexanol, and 2-propanol, which can be detected in this study. The contents of n-hexanol, (E)-2-hexen-1-ol, and 2-propanol in the TT sample were significantly higher than those in the AT sample. There are 7 alcohols, oct-1-en-3-ol, linalool, 3-methylbutanol, (E)-2-hexen-1-ol, 2-methylbutanol, n-hexanol, and 2-propanol, which can be detected in this study. The contents of n-hexanol, (E)-2-hexen-1-ol, and 2-propanol in the TT sample were significantly higher than those in the AT sample. There are 7 alcohols, oct-1-en-3-ol, linalool, 3-methylbutanol, (E)-2-hexen-1-ol, 2-methylbutanol, n-hexanol, and 2-propanol, which can be detected in this study.
As shown in Figure 2b, the obtained differentially expressed genes...
were classified into KEGG biological pathways. A total of 4061 differentially expressed genes were classified into different groups involved in 21 pathways. These groups of genes are mainly associated with certain biological functions, such as cellular processes, environmental information processing, genetic information processing, metabolism, organizational systems, and human disease. "Metabolism"
was the major category for a single gene. Further division of each category showed the biggest differences in the "global and overview map" group (1690), followed by the "carbohydrate metabolism" group (751) and "translate" group (628). Some other groups, such as "fold, classification and degradation," "transportation and catalysis," and "signal transduction" gene annotation, are also showing substantial differences.

### 3.4 KEGG annotation pathway enrichment

As shown in Figure 3, Enrichment analysis showed that these differentially expressed genes were enriched in 136 metabolic pathways, including 10 pathways with a p-value ≤0.05 and only 1 pathway with a Q value ≤0.05. The top 20 pathways with low Q values are shown in Figure 3. The oxidative phosphorylation was significantly enriched (Q value ≤0.05). There are 107 genes that were enriched to medium level, of which 67 genes were upregulated. The pathways of the most enriched genes were RNA transport and endocytosis, with 248 (95 upregulated) and 244 (108 upregulated) genes, respectively.

### 3.5 Genes related to aroma metabolism of melon from different production areas

As shown in Figure 4, studies on aroma metabolic pathways have preliminarily demonstrated the metabolic pathway of melon fruit aroma (Beaulieu, 2006; Gonda et al., 2010; Lange et al., 2000; Mayobre et al., 2021; Song et al., 2021). The precursor substance pyruvate is synthesized from glycolysis or organic acid metabolism. Under the action of pyruvate decarboxylase (PDC), acetyl-CoA, which is crucial to aroma metabolism, is formed. Acetyl-CoA produces 3-oxohexadecanoyl-CoA through the action of acetyl-CoA acyltransferase 1, which is then transformed into 3-hydroxyhexadecanoyl-CoA through 3-hydroxy-CoA dehydrogenase. Transhexadec-2-enoyl-CoA is formed by enyl-CoA hydrase, which generates hexadecanoyl-CoA under the action of acyl-CoA oxidase, and fatty acids can be formed under the action of long-chain acyl-CoA synthetase. At the same time, fatty acids can be interconverted with aldehydes via aldehyde dehydrogenase (nicotinamide adenine dinucleotide (NAD+)). While aldehydes can be interconverted with alcohols under the action of alcohol dehydrogenase (ADH). Alcohol acyltransferase (AAT) plays an important role in ester synthesis by transferring the acyl group of acetyl-CoA to alcohols to produce ester compounds. A variety of amino acids are degraded into branched-chain fatty acids by transaminase and dehydrogenase, resulting in ester compounds.

### 3.6 Real-time quantitative RT-PCR verification

To verify the transcriptome data, we randomly selected 11 genes for real-time fluorescence quantitative verification. As shown in Table 8, annotated information, expression levels obtained from transcriptome data, and relative contents obtained using real-time fluorescence quantitative technology for 11 verified genes are presented. The results showed that the 11 verified genes were consistent with the transcriptome data, indicating the high reproducibility and reliability of transcriptome analysis.

### 4 DISCUSSION

#### 4.1 Influence of region on melon aroma components

Aroma can be classified into different types based on different proportions of aroma components, such as flower flavor, green flavor, fruit flavor, aldehyde flavor, and others. Flower fragrance refers to substances with flower fragrance. Green flavor refers to the aroma of fresh grass, mainly containing C6 aldehydes, C9 aldehydes, and alcohols. Fruity flavor is mainly represented by ripe apples, strawberries, and other odors, including phenols, ethers, and esters (Selli et al., 2012). Aldehyde flavor mainly manifests as melon odor substances, which are represented by C7 to C12 aliphatic aldehyde substances. In melon, the main characteristic aromas are fruit flavor and green flavor. The results showed that the fruit aroma in different regions was significantly different as a result of different altitudes,
temperatures, light conditions, and soil environments. For example, Xiao Z et al. compared and analyzed the volatile components of melon from Jiashigua, Xizhou Mi 17, and Minqin in Xinjiang. They found that there were great differences in the types and contents of aroma components among different producing areas and varieties (Fallik et al., 2001). Zehra Guler measured volatile components of 3 groups of melon samples from different regions of Turkey. The results showed that the volatile components of melon in the three groups were significantly different in both quality and quantity (Güler et al., 2013). Melon is a photophilous and thermophilic species. Different temperatures and light have notable effects on melon production and quality. The optimal temperatures for melon growth and maturation are 27–30°C in the daytime and approximately 18°C at night. The light compensation point of melon is approximately 4000 lx, while the light saturation point is approximately 55,000 lx. Under sufficient light conditions, the melon plant grows robustly. There were significant differences in temperature, illumination, altitude, and other conditions between the products from two regions in this study. This is the main cause of the significant differences in aroma components of the same variety of “Nasmi” melon. Not all aroma ingredients are related to fragrance. Some characteristic aroma contents are very high, but it is difficult for us to smell because of the high flavor threshold of the odor. Those with a high aroma value (the ratio of aroma components to aroma threshold) are called "characteristic effector compounds." The characteristic aroma compounds of melon have been identified as hexyl acetate, ethyl caproate, ethyl butyrate, ethyl 2-methyl butyrate, ethyl 2-methyl propionate, ethyl 3-methyl butyrate, (Z, Z)3,6-noneny lactate, (Z)-6-nonenal, (E, Z)-2,6-nonenal, (E)-2-nonenal, 2-(methylthyl) ethyl acetate, 3-(methylenehy) propionate ethyl ester, and (Z)-1,5-octadiene-3-ketone (Obando-Ulloa et al., 2008; Obando-Ulloa, Nicolai, et al., 2009). Some of these characteristic effector compounds were identified in the melons used in this study.

4.2 Aroma metabolic main way

In recent years, research on aroma has attracted great attention from many research groups. The main pathways of fruit aroma synthesis can be divided into the fatty acid pathway, secondary metabolic pathway, amino acid pathway, and conversion of alcohol and aldehyde compounds to esters. In this study, all four pathways were identified. More emphases were put on the fatty acid pathway in our analysis. Amino acid pathways have been found to play an important role in the synthesis of branched-chain esters, in which valine, leucine, and isoleucine are important precursors. Yan Li et al. analyzed the amino acid pathway during the synthesis of melon aroma and found that the amino acid pathway mainly depended on the decomposition of leucine, isoleucine, valine, phenylalanine, and cysteine into aroma volatile substances through transamination and dehydrogenation (Li et al., 2016b). In the fatty acid pathway, pyruvate is an important precursor...
TABLE 7  Selected genes related to melon fruit aroma climate in different regions

| Gene ID            | FPKM(TT) | FPKM(at) | Description [EC:NO]                                      |
|--------------------|----------|----------|-------------------------------------------------------|
| LOC103482592       | 24.576   | 51.336   | Acyl-CoA oxidase [EC:1.3.3.6]                          |
| LOC103482821       | 8.193    | 2.593    | Long-chain acyl-CoA synthetase [EC:6.2.1.3]            |
| LOC103486958       | 44.22    | 52.156   | Long-chain acyl-CoA synthetase [EC:6.2.1.3]            |
| LOC103487766       | 867.386  | 5647.706 | Acyl-[acyl-carrier-protein] desaturase [EC:1.14.19.2 1.14.19.11 1.14.19.26] |
| LOC103483284       | 146.956  | 66.893   | Acetyl-CoA carboxylase carboxyl transferase subunit alpha [EC:6.4.1.2 2.1.3.15] |
| LOC103500505       | 111.556  | 553.813  | Acetyl-CoA C-acetyltransferase [EC:2.3.1.9]            |
| LOC103497788       | 62.923   | 187.83   | Enoyl-CoA hydratase [EC:4.2.1.17]                      |
| LOC103501532       | 73.04    | 5.46     | Fatty acyl-ACP thioesterase B [EC:3.1.2.14 3.1.2.21]   |
| LOC103500074       | 174.696  | 2261.113 | Alcohol dehydrogenase [EC:1.1.1.1]                     |
| LOC103483849       | 1639.03  | 5491.8   | Aldehyde dehydrogenase (NAD+) [EC:1.2.1.3]             |
| LOC103483275       | 26.67    | 41.816   | Acyl-coenzyme A thioesterase 1/2/4 [EC:3.1.2.2]        |
| LOC103496981       | 61.103   | 205.602  | Acetyl-CoA acyltransferase 1 [EC:2.3.1.16]             |
| LOC103497860       | 114.62   | 206.155  | 3-hydroxyacyl-CoA dehydrogenase [EC:1.1.1.35 1.1.211] |
| LOC103501981       | 14.82    | 66.556   | Acetate-CoA ligase [EC:6.2.1.1]                         |
| LOC103498837       | 45.006   | 11.47    | Pyruvate dehydrogenase E1 [EC:1.2.4.1]                 |
| LOC103488170       | 64.613   | 624.273  | Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) [EC:1.1.1.40] |
| LOC103499065       | 26.333   | 44.133   | Malate dehydrogenase (decarboxylating) [EC:1.1.1.39]   |
| LOC103487805       | 39.116   | 977.476  | Branched-chain amino acid aminotransferase [EC:2.6.1.42] |

Substance. Under the action of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH), pyruvate degrades to synthesize acetyl-coenzyme A, which provides a precursor for the production of fatty acids. Min Min Wang et al. studied PDC1 (pyruvate decarboxylase 1) in melons and found that maturation-induced PDC1 encodes a pyruvate/α-ketoate decarboxylase, which is involved in the biosynthesis of acetaldehyde, propionate, and valeraldehyde in melon fruits and plays an important role in the decarboxylation of pyruvate and 2-oxy-caproate (Wang, Zhang, et al., 2019). Liu et al. (Liu et al., 2020) resequenced 297 melon materials to reveal the genome improvement history of melon and the loci related to fruit traits. This study found that the Cm AAT gene was a special gene in melon fruits and was closely related to aroma formation. CM AATS plays an important role in the last step of ester biosynthesis, leading to the synthesis of various ester aromas (Chen et al., 2016).

The high content of alcohols and volatile esters in melon is related to the activity of CMAAT (Melo3C024771), which determines the high content of alcohols and volatile esters in melon volatiles (Galaz et al., 2013). This study showed that the action of AAT produces alcohols and acetyl in combination with acetyl coenzyme A, resulting in esters. Additionally, this study found that alcohol and aldehyde compounds result in the transformation of esters. In this way, there is mutual transformation between material, alcohols, and aldehydes, which are subsequently proceeded to be involved in the formation of esters, or fatty acids proceed through aldehyde dehydrogenase and participate in the fatty acid pathway to form esters. The secondary metabolic pathways of aroma metabolism can be divided into terpene pathways and the synthesis of volatile phenols. In terpene pathways, the main enzyme is terpene synthase, under which semiterpenes, polyterpenes, and other terpenes are synthesized (Portnoy et al., 2008). In addition, shikimic acid can synthesize many aromatic compounds, such as coumarin and flavonoids, through the shikimic acid pathway. Song and Forney et al. found that benzyl acetates are one of the major ester groups in melon, and this ester is usually synthesized through the shikimic acid pathway (Song & Forney, 2008). Additionally, many sulfur compounds contribute to melon flavor, but they have not been identified in “Nasim” melon in this study due to the fact that the specific melon varieties were used, or the incomplete records in the IMS database.

4.3  | Melon aroma content and amount of gene expression in different regions

Aroma content is regulated by a variety of different genes. In this study, transcripome technology was used to identify the key genes responsible for the difference in aroma metabolism in different production areas. Aroma components and relative contents between production areas were determined by the GC-IMS technology. Combined analysis showed that aroma content was inextricably related to these key genes. In aroma metabolism, pyruvate dehydrogenase is an important enzyme in the process of pyruvate conversion to acetyl-CoA. The expression levels of several differential genes encoding pyruvate in melon from different production areas changed significantly between the two regions. The expression levels of most of them in the TT samples were significantly higher than those in the AT samples. The same aroma metabolic pathways encoding aldehyde dehydrogenase in eight different genes express seven up-regulated genes in TT samples compared to AT samples. The total
aldehyde material in AT samples is relatively lower than that in TT samples, which may indicate a specific correlation between these observations. Results in alcohol dehydrogenase (ADH) (multiple genes are upregulated in TT vs AT) are in agreement with the observation that the content of aroma ingredients found in quantitative AT relative content is lower than that in TT samples. For the acetyl-CoA - LOC103496981 acyltransferase gene, the expression in TT and AT samples was 61.103 and 205.602, respectively. This gene is closely related to ester synthesis, which may be the reason why the content of most ester compounds in AT is higher than that in TT. Through gene screening, comparison, and analysis of aroma quantities, the relevant contents of aroma demonstrated a positive correlation between the expression levels of key genes and their effect, thus facilitating preliminarily evaluation. Because of the roles
of these key genes, the aroma composition between the two regions shows large differences.

This study focused on the fatty acid and amino acid pathways of melon aroma metabolism. We discovered that the genes primarily involved in aroma metabolism were acetyl-CoA acyltransferase, 3-hydroxy-CoA dehydrogenase, acyl-CoA oxidase, long-chain acyl-CoA synthetase, aldehyde dehydrogenase, and alcohol dehydrogenase in transcriptome analysis, which is consistent with previous research (Li et al., 2011; Zhang et al., 2017). At the same time, based on several aroma studies and the current study, we preliminarily obtained the synthesis pathways of aroma components, which provides strong support for melon breeding and other related studies.

5 | CONCLUSION

By studying the volatile components of the same melon from different production areas, it was observed that there was no difference in the types of volatile components of the melon from the two regions. However, there was a notable difference in the aroma contents. The main components that differed between the two regions were esters, alcohols, and aldehydes. The total aroma of the TT sample was 1.7 times higher than that of the AT sample. In the two regions, with the same variety of melon, transcriptome sequencing analysis produced 9658 different genes, and through the classification based on GO annotations, the three major categories all demonstrated notable amounts of enrichment. Through the analysis of aroma-related pathways and KEGG analysis, a large number of fruit-related metabolic pathways were obtained. Investigation of aroma and analysis of multiple genes related to regional differences identified the acetyl-CoA acyltransferase, 3-hydroxy acyl-coenzyme A, dehydrogenase, acyl-coenzyme A oxidase long-chain acyl-coenzyme A synthetase, acetaldehyde dehydrogenase, and alcohol dehydrogenase genes. Some differentially expressed genes were verified by real-time fluorescence PCR. The results showed that the transcriptome sequencing results were reliable.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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