The Relationship between CmADHs and the Diversity of Volatile Organic Compounds of Three Aroma Types of Melon (Cucumis melo)

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Alcohol dehydrogenase (ADH) plays an important role in aroma volatile compounds synthesis of plants. In this paper, we tried to explore the relationship between CmADHs and the volatile organic compounds (VOCs) in oriental melon. Three different aroma types of melon were used as materials. The principle component analysis of three types of melon fruit was conducted. We also measured the CmADHs expression level and enzymatic activities of ADH and alcohol acyl-transferase (AAT) on different stages of fruit ripening. An incubation experiment was carried out to investigate the effect of substrates and inhibitor (4-MP, 4-methylpyrazole) on CmADHs expression, ADH activity, and the main compounds of oriental melon. The results illustrated that ethyl acetate, hexyl acetate, (E,Z)-3,6-nonadien-1-ol and 2-ethyl-2-hexen-1-ol were the four principal volatile compounds of these three types of melon. AAT activity was increasing with fruit ripening, and the AAT activity in CH were the highest, whereas ADH activity peaked on 32 DAP, 2 days before maturation, and the ADH activity in CB and CG were higher than that in CH. The expression pattern of 11 CmADH genes from 24 to 36 day after pollination (DAP) was found to vary in three melon varieties. CmADH4 was only expressed in CG and the expression levels of CmADH3 and CmADH12 in CH and CB were much higher than that in CG, and they both peaked 2 days before fruit ripening. Ethanol and 4-MP decreased the reductase activity of ADH, the expression of most CmADHs and ethyl acetate or hexyl acetate contents of CB, except for 0.1 mM 4-MP, while aldehyde improved the two acetate ester contents. In addition, we found a positive correlation between the expression of CmADH3 and CmADH12 and the key volatile compound of CB. The relationship between CmADHs and VOCs synthesis of oriental melon was discussed.

Keywords: volatiles organic compounds, alcohol dehydrogenase, oriental melon, fruit ripening, gene expression

INTRODUCTION

Oriental melon (Cucumis melo var. makuwa makino) is a species of thin-pericarp melon, and it has extensive cultivated varieties and the largest plantation in China. The oriental melon has a sweet and crisp taste, juicy flesh and an edible rind, especially intense volatile aromas compound that is one of the most attractive qualities (Liu et al., 2012). Most volatile aroma compounds,
as a sign of fruit maturity, are produced and released during the maturation period (Visai and Vanoli, 1997; Goff and Klee, 2006). To date, more than 2000 types of volatile compounds have been detected in various plants, including melons, apples, strawberries, pears, tomatoes, and bananas (Dixon and Hewett, 2000; Maul et al., 2000; Urruty et al., 2002; Li et al., 2014, 2016). In different melon varieties, ~240 volatile compounds have been found, including volatile alcohols, aldehydes, terpenes, especially abundant esters (Kourkoutas et al., 2006; Khanom and Ueda, 2008; Obando-Ulloa et al., 2010). Specifically, the contents of aromatic compounds vary drastically according to the melon variety. In climacteric melon varieties, volatile esters are prominent, together with short-chain alcohols, aldehydes and terpenes, while non-aromatic varieties often have much lower levels of total volatiles, lacking the volatile esters (Gonda et al., 2010). Tang also found that, especially straight-chain esters were important VOCs in oriental melon (Tang et al., 2015). As the most abundant aroma in climacteric melon, esters are mainly produced from two ways, namely the amino acid way, producing the branched-chain esters and the lipoxygenase (LOX) way synthesizing the straight-chain esters (Zhang et al., 2014; Tang et al., 2015).

The lipoxygenase (LOX) pathway may be the most critical way for aroma foundation because of the high straight-chain esters content of oriental melon. The LOX way consist of four enzymes, including LOX, HPL (Hydroperoxide lyase), ADH (Alcohol dehydrogenase, EC1.1.1.1), and AAT (Alcohol acetyltransferase). As the last two steps in the foundation of volatile esters, some ADH and AAT have been extensively investigated, both in melons and in other plants. These steps involve alcohol dehydrogenase and alcoholacetyl transferase activities that convert volatile aldehydes to their respective alcohols and esters, and these activities are related to climactericity (Gonda et al., 2010).

The classic ADHs are Z-binding enzymes, relying on an NAD(P) co-factor to interconvert ethanol and acetaldehyde (and other short linear alcohol/aldehyde pairs). In petunia, PhADH2 and PhADH3 were involved in floral scent from the lipoxygenase pathway (Garabagi and Strommer, 2004). Previous reports also showed that ADHs were expressed in a developmentally-regulated manner, particularly during fruit ripening (Salas and Sánchez, 1998; Speirs et al., 2002; Lara et al., 2003; Manriquez et al., 2006). Over-expression of LeADH2 in tomato led to increasing the level of alcohols, particularly Z-3-hexenol of the fruit (Salas and Sánchez, 1998). The specific down-regulation of SlsADH1 in tomato fruit did not alter the aldehyde/alcohol balance of the volatiles compounds, but made higher content of C5 and C6 volatile compounds from the lipoxygenase pathway (Moummou et al., 2012). However, there were few reports on ADHs, participating in aroma synthesis, in oriental melon which has the extensive cultivated varieties and the largest plantation in China.

As our previous works, 12 CmADH genes (CmADH1-12) have been identified in the melon genome (http://melonomics.net/) and bioinformatics analyzed. We have also investigated the response of 12 CmADHs to ethylene in oriental melon (Jin et al., 2016), but the function of most members were far from clear, except for CmADH1 and CmADH2 in Countloup melon. The key CmADH gene participating in the accumulation of various volatile organic compounds (VOCs) in different aroma types of melon and the regulation of CmADHs family in the process of aroma foundation in oriental melon are still unknown. In this paper, to explore the potential CmADH genes participating in the key aroma compounds production, we analyzed the VOCs and investigated the activities and expression of ADH and AAT in ripening fruits of three different aroma types of melon. Simultaneously, a fruit disk incubation experiment was conducted to investigate the influence of substrates (ethanol and aldehyde) or inhibitor on ADH activity, CmADHs expression and VOCs productions in oriental melons.

MATERIALS AND METHODS

Plant Materials

Three different aromatic oriental melon varieties were used, including strong-aromatic melon (C. melo var. makuwa Makino) cultivar “Cai Hong” (CH), less-aromatic melon (C. melo var. makuwa Makino) cultivar “Cai Bao” (CB), and non-aromatic melon “Cai Gua” (CG) which is called as snake melon (C. melo L var. flexuosus Naud) in China. They were grown in pots (volume of 25 L and soil: peat: compost = 1: 1: 1) in a greenhouse under standard cultural practices for fertilization and pesticide treatments at Shenyang Agricultural University(Shenyang, China) from March to June in 2014. Female flowers were pollinated with “Fengchanji 2” to increase the rate of fruit set, and tagged on the day of bloom. Melons were harvested on 24, 26, 28, 30, 32, 34, 36 days after pollination (DAP).

Fruit Firmness, Soluble Solids Content (SSC) Evaluation

The firmness of melon fruit was measured with a hardness tester (FHM-1, Takemura, Japan) according to the method of Tijskens (Tijskens et al., 2009). The soluble solids content of melon fresh was determined by a digital refractometer (DBR45, Huixia, Fujian, China) described by Liu (Liu et al., 2012). A CR-400/410 spectrophotometer (Konica Minolta, Japan) was used to detect the rind color of melons. Six readings were taken from equatorial zone of each fruit (Liu et al., 2012). The firmness and SSC experiment was performed in triplicate.

ADH Enzyme Activity Assay

Reductase and dehydrogenase activities of ADH were evaluated by CARY 100 scan ultraviolet (UV)/visible spectrophotometer (Varian, USA). The method was optimized on the foundation of Longhurst et al. (1990) and Manriquez et al. (2006). Approximately 3 g fresh melon was ground into powder in liquid nitrogen using mortar and pestle, then mixed with 6 ml pre-cooling extract buffer [4°C, 100 mM MES-Tris (pH 6.5), 2 mM DTT (dithiothreitol), 1% PVP (polyvinyl pyrrolidone) (m/v)]. The ground slurry was centrifugated at 15,000g for 30 min at 4°C, and the supernatant was collected for ADH activity analyzing as crude enzyme. Reductase activity was measured in 1 ml total volume containing 200µl crude protein, 5 mM aldehyde, 0.25 mM NADH, or NADPH and 50 mM sodium
phosphate buffer (pH 5.8). Dehydrogenase activity was assayed in solution contained 5 mM ethanol, 0.25 mM NAD or NADP and glycine-NaOH buffer pH 9.4 in 1 ml. Reductase/dehydrogenase activity was measured by the increase/decrease in absorbance at 340 nm due to change of NAD(P). The reaction was initiated by the addition of ethanol or aldehyde and the rate of absorbance change without ethanol or aldehyde was subtracted to give the substrate dependent rate.

**AAT Enzyme Activity**

AAT activity was measured according to Shalit (Shalit et al., 2001). Total protein was extracted from 3 g melon fruit without peel and macerated with 6 ml 0.1 M sodium phosphate buffer (pH = 0.8) at 4°C. The supernatant was collected as the crude enzyme for AAT activity analyzing after the mixture was centrifuged at 16,000 g for 30 min at 4°C. The reaction system consisted of 2.5 ml 5 M Mgcl₂, 50 µl 0.5 mM acetyl CoA, 50 µl 200 mM butanol and 0.6 ml crude enzyme. 150 µl 5,5-disulfide double nitro benzoic acid (DTNB) was added into the mixture after 15 min. The AAT activity was determined by the changes of A412 measured by spectrophotometer and each measurement was repeated three times.

**Protein Content**

Total proteins were quantified with modifications (BioRad Protein Assay Kit, Bio-Rad, USA) according to the method of
coomassie brilliant blue G-250 described by Bradford (Bradford, 1976).

**Volatile Organic Compounds Analysis**

The VOCs of different melons were detected under the procedure of headspace (HP)-solid phase micro extraction (SPME)-gas chromatography-mass spectrometry (GC-MS), as Liu and Tang was used (Liu et al., 2012; Tang et al., 2015). About 100 g frozen melon flesh were thawed and squeezed into juice. 1-octanol (50 µl, 59.5 mg/l) were added into 10 ml juice samples as an internal standard. SPME needle was from Supelco (57347-U, Bellefonte, PA, USA), and GC-MS was from Thermo Scientific (Trace GC Ultra-ITQ 900, Waltham MA 02454). The GC system was equipped with a 30 m*0.25 mm*0.25 um thickness capillary column (Thermo TR-5 ms SQC, USA).

For incubation experiment, a 1 g aliquot of the melon powder was placed in a 10 ml glass vial containing 0.7 g of solid NaCl, 2 ml of a 20 % (w/v) NaCl solution (Gonda et al., 2010) and 10 µl of a 59.5 mg/l 1-octanol used as internal standard. Then, the sample was measured with the method mentioned above.

**Incubation Experiments**

Melon cubes (4 g) from CB mature fruit were put in sterile petri dish plates and 500 µl of a solution of 5 mM ethanol or 5 mM aldehyde and different concentrations of ADH inhibitor (4-methylpyrazole, 4-MP. 0.1, 1, and 5 mM) were applied on top of each cube, and distilled water was taken as control. The plate was covered and incubated overnight at room temperature. Then, each cube was frozen in liquid nitrogen and stored at −80°C (Gonda et al., 2010).

**Real-Time Quantitative (qPCR) Analysis**

The total RNA was isolated with TRIzol Reagent (Takara, Japan). DNase I (Promega, USA) was used to remove genomic DNA. cDNA template was obtained by reverse transcriptase (Invitrogen, Thermo fisher scientific, USA) with random primer. The PCR program parameters consisted of a preliminary step of 3 min at 95°C followed by 45 cycles at 95°C for 15 s and at 60°C for 30 s, finally, 68°C 30 s. The template cDNA was amplified in a 20

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**TABLE 1 | Total and different classes of volatile compounds and their concentrations in different aromatic melon types.**

| Volatile compounds | Different types of melon |
|--------------------|-------------------------|
|                    | CH          | CB          | CG          |
| Total esters       | 207.83 ± 17.21<sup>a</sup> | 127.16 ± 16.73<sup>b</sup> | 5.29 ± 1.82<sup>c</sup> |
| Total alcohols     | 30.24 ± 2.48<sup>b</sup> | 23.68 ± 1.87<sup>b</sup> | 136.85 ± 4.85<sup>a</sup> |
| Total acids        | 15.73 ± 5.28<sup>a</sup> | 8.14 ± 2.62<sup>b</sup> | 9.31 ± 3.97<sup>b</sup> |
| Others             | 54.15 ± 20.01<sup>a</sup> | 10.07 ± 1.32<sup>b</sup> | 6.14 ± 1.21<sup>b</sup> |

Duncan’s multiple range tests were performed, and different letters represent significant differences (P < 0.05) between different types of melon.

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**FIGURE 2 | Principal component analysis (PCA) of aroma volatiles identified in three types of melon at mature period.** Loading plots of the two main PCA of the aroma volatiles identified in three types of melon at mature period. One hundred percent of the variability in the volatile compounds in the melon cultivars could be explained by two principal PCs. PC1 explained 57.57% of the variability, while PC2 explained 42.43% of the variability. Each sample consisted of three replicates. Codes were corresponding to the volatile compounds number in Table S1.
FIGURE 3 | Four principal volatile compounds of three types of melon at mature period. All of the data for volatile compounds are means ± SE value of three replicates.

FIGURE 4 | ADH and AAT activities in three aroma types of melon at different DAP. (A) ADH activities in three types of melon. (B) AAT activities in three types of melon. Each experiment was performed in triplicate and the means ± SE value of their activities were shown in the line chart.
μl reaction (2xSYBR Green PCR Master Mix, Tiangen Biotech Co. Ltd. Beijing, China) on an ABI 7500 sequence detection system. All qPCR experiments were performed in triplicate with different cDNA template. The ADH/18s rRNA ration for samples were related to the ratio for CH in Figure 5 and for CB in Figure 8 which were set to 1, respectively. The $2^{-\Delta\Delta C_{\text{t}}}$ method was used to calculate relative genes expression of the *CmADH* genes produced by real time PCR.

**Statistical Analysis**

A principal component analysis (PCA) was employed to identify the key aroma compounds of the three aromatic melon varieties according to their VOCs by the SPSS 20.0. And significant analysis was conducted by a one-way ANOVA following Duncan’s multiple range tests for experiment at a $p < 0.05$ level. The figures were produced by Origin 9.0.
RESULTS

Firmness, Soluble Solids Content (SSC), and Rind Color

In order to determine the maturation period, SSCs of the three types of melon was chosen for the signal of fruit maturation (Tang et al., 2015). We chose DAP34 as the maturation period when nearly all of the SSC of three types of melon reached the highest concentration at the same time (Figure S1). The SSC of oriental melon was significantly different between the three types of melon (Figure 1B). Moreover, CH, CB, and CG fruits also exhibited various morphological and physical characteristics, implying the ripening of different type melons.

Volatile Organic Compounds of Three Types of Melon

We had detected 49 VOCs, including esters, alcohols, acids, and other aroma in three types melons (Table S1). Esters were the most abundant volatile compounds in CH and CB (207.83 μg.g⁻¹FW and 127.16 μg.g⁻¹FW, respectively). On the other hand, alcohol contributed the aroma of CG. We also found that the content of esters or total aroma accumulated in CH was nearly twice of those in CB, although esters was the main compounds in both of them (Table 1).

To further distinguish the variety of aroma in three types of melon, we conducted PCA of aroma volatiles identified in three types of melon at mature period of different DAP (Figure 2). It was clearly that CG was separated from the others in account of V29 [(E,Z)-3,6-nonadien-1-ol], V24 (z-6-nonenal), V23 (3-carene), V34 (2-octyn-1-ol), V44 [Stearic acid, 3-(octadecyloxy) propyl ester], and V45 (10,12-Octadecadiynoic acid) (Figure 2), and (E,Z)-3,6-Nonadien-1-ol was the representative volatile compound of CG considering the content (Table S1). We regarded ethyl acetate, hexyl acetate, (E, Z)-3, 6-nonadien-1-ol and 2-ethyl-2hexen-1-ol as the main aroma compounds of three types of melon. In Figure 3, it was obvious that esters were most abundant in melon of “CH” or “CB” and were separated from “CG.”

Reductase Activity of ADH and AAT Activity in Three Types of Melon at Different DAP

During fruit development from 24 to 36 DAP, reductase activity of ADH in three types of melon showed a trend of increasing at first and decreasing subsequently, which reached a peak at 32 DAP. ADH activity was significantly different between different SSCs and fruit development of melons. The SSC of three types of melon nearly reached the highest concentration at the same time at 34 DAP (Figure S1). The firmness of CH and CG were similar and lower than that of “CB” (Figure 1A). Both CH and CB fruit had higher SSCs than CG (Figure 1B). In terms of rind color, CH and CG were brighter or yellow, while CB was dark green (Figures 1C–E; Figure S2). Moreover, CH, CB, and CG fruits also exhibited various morphological and physical characteristics, implying the ripening of different type melons.

CmADHs Expression in Three Types of Melon during Fruit Ripening

A total of 11 CmADHs were expressed during ripening of oriental melon (Figure 5), as CmADH11 was not detected during our experiment. Transcript analysis indicate that these 11 CmADH genes were specifically expressed in ripening fruit of three aroma types of melon. CmADH2 and CmADH6 were specifically expressed in strong-aromatic melon CH and less-aromatic melon CB and CmADH4 was only expressed in non-aromatic melon CG. The expression of CmADH3, CmADH7, and CmADH12 in CH and CB were higher than that in CG, and most of the genes were consistently expressed with an increase in transcript abundance and reached the peak at 34 DAP or 36 DAP in CH and CB. In addition, the expression level of CmADH5, CmADH8, CmADH9, CmADH10 were either not expressed or maintained a low level during fruit ripening.

Volatile Aroma Compounds of CB in Incubation Experiment

Production of ethyl acetate and hexyl acetate in CB were significantly affected by substrates or inhibitor (Figure 6). Both ethyl acetate and hexyl acetate abundance reduced after ethanol addition.
FIGURE 7 | ADH activities depended on four co-factors (0.25 mM NADPH/NADP and NADH/NAD) of CB flesh melon incubated with multiple solutions, including 5 mM ethanol (Ethanol), 5 mM aldehyde (Aldehyde), 0.1 mM 4-methylpyrazole (4MP0.1), 1 mM 4-methylpyrazole (4MP1), and 5 mM 4-methylpyrazole (4MP5) (A–D). (A) ADH activity depended on 0.25 mM NADPH. (B) ADH activity depended on 0.25 mM NADH. (C) ADH activity depended on 0.25 mM NADP. (D) ADH activity depended on 0.25 mM NAD. Flesh melon incubated with distilled water were used as control. Duncan’s multiple range tests have been performed with different letters above the columns represent significant differences (P < 0.05) between different treatments.

ADH Activity in Incubation Experiment

In incubation experiment, the ADH reductase activity was suppressed by ethanol, a production of ADH in melon, regardless of NADH or NADPH was used and ethanol showed a stronger suppression than 4-MP (Figures 7A,B). The dehydrogenase activity were increased by ethanol treatment, though activity change was more significant when the co-factor was NADP. 4-MP also worked as an inhibitor, but it depended on co-factor and its concentration (Figures 7C,D). Aldehyde did not promoted the ADH reductase activity, but it significantly inhibited the dehydrogenase activity when the co-factor was NAD (Figure 7D).

CmADHs Expression in Incubation Experiment

Based on the incubation experiment, 11 CmADH genes were expressed in oriental melon “CB” (Figure 8). CmADH1, CmADH4, CmADH9, and CmADH12 were up-regulated following the addition of aldehyde, while CmADH2, CmADH3, and CmADH7 seemed to not response to aldehyde. Most of CmADHs genes were down-regulated under ethanol treatment except CmADH4, CmADH7, and CmADH9. Different dose of 4-MP (0.1, 1, and 5 mM) reduced the levels of most CmADHs except CmADH4, CmADH7, CmADH9, and CmADH12 (Figure 8).

DISCUSSION

Aroma was an important quality of ripe fruit, and it differed between varieties of the same species, which was found in many plants (Poll, 1981; Visai and Vanoli, 1997; Kourkoutas et al., 2006; Goulet et al., 2012). For example, esters and alcohols were the main aroma volatiles of Cantalope melon, sulfur esters and straight-chain compounds of six-carbon or nine-carbon were
most abundant volatile in CG. These results illustrated that there were differences on the primary VOCs among different aroma types of melon and esters, especially ethyl esters were important aromatic compounds in oriental melons (Li et al., 2011; Liu et al., 2012).

The synthesis of straight-chain ethyl ester, such as hexyl acetate and butyl acetate, was directly correlated with the main enzymes activity in LOX pathway (Senesi et al., 2002; Echeverría et al., 2004; Altisent et al., 2009; Paige and Sheryl, 2012). ADH, as one of the key enzymes in LOX pathway, plays an important role in diverse volatile compounds synthesis in many plants. In olive, ADH activity may account for the diversity in aldehydes and alcohols of two cultivars, Carolea, and Coratina (Iaria et al., 2012). The high expression of PuADH3 in pear during fruit ripening also indicated the relationship between PuADHs and aroma (Li et al., 2014). CmADH1 and CmADH2 were involved in fruit development due to their highly expression in Cantaloupe melon and ethylene-induced regulation. Particular substances preferences of two ADHs indicated their particular functions in the formation of various flavor of melon (Manríquez et al., 2006). But ADH is not the final step of LOX pathway, some alcohols produced by ADH would convert into esters under the function of AAT. So that there may be a complex relationship between ADH, AAT and volatiles: During the development of apricot fruit, the expression levels of PaADH and PaLOX stayed constant at all stages, however PaAAT levels showed a sharp increase in the late harvest stages, with the changes observed in ester levels (González-Agüero et al., 2009); Silencing SlsCADH1, a specifically expressing gene in tomato fruit, resulted in the accumulation of C5 and C6 compounds rather than the alternation of alcohols/aldehydes balance (Moummou et al., 2012). In our study, ADH activity of all cultivars increased slightly first and raised up to several fold 2 days before the fruits ripened. There was no obvious difference between Less-aromatic melon CB with high esters content and non-aromatic CG with low esters content on ADH activity during fruit development, indicating that ADH activity might not be a key regulator of esters abundance in oriental melon. Increase of AAT activity was detected during ripening of fruit in CH and CB, but there was no significant change about AAT activity in non-aromatic melon CG. It seems there was no direct correlation between the total ADH activity and the total content of VOCs or the alcohols, and the AAT activity was positively correlated with the content of esters in oriental melons. The gene expression pattern of CmADHs also varied in three cultivars during fruit ripening (Figure 5). The specific CmADH genes expression might be an important reason for the diversity of alcohols and follow-up ester components in oriental melon considering that different ADH had particular preferences for various substrates (Manríquez et al., 2006; Moummou et al., 2012) and further more studies are needed to prove the speculation.

We cannot analysis the specific substrate preference of each CmADH using crude enzyme, but the change of expression of every CmADH caused by some substrate could be detected in incubation experiment. Ethanol was immediate precursor of ethyl acetate, the most abundant characteristic aroma compound in oriental melon. Ethanol and aldehyde could be converted

![Figure 8](image_url)
FIGURE 9 | Relative expression of two CmADH genes (CmADH12 and CmADH3) and volatile content of two principle esters (ethyl acetate and hexyl acetate) in incubation experiment. (A) CmADH12 and CmADH3 relative expressions in CB melon incubated with multiple treatments. (B) Volatile content of ethyl acetate and hexyl acetate in CB melon incubated with multiple treatments. The treatments were ethanol (Ethanol), aldehyde (Aldehyde), 0.1mM 4-MP (4MP0.1), 1mM 4-MP (4MP1), and 5mM 4-MP (4MP5). All of the data for ADH gene expression and volatile contents are means of three replicates.
reduce activity when NADPH acted as the co-factor, and the accumulation of hexyl acetate or ethyl acetate in incubation experiment (Figure 9), although the CmADHs gene expression and the changes of enzyme activity would not directly affect the synthesis of esters in theory. It hinted CmADH3 and CmADH12 might involve in synthesis of aroma compounds of oriental melon. Considering that their expression levels were up-regulated by ethylene in our previous study (Jin et al., 2016). The recombinant protein or the transgenic plants were needed to obtain more information about the role of certain CmADHs in oriental melon aroma formation.

CONCLUSIONS

In this paper, volatile esters, especially ethyl acetate, and hexyl acetate, as the primary aroma were identified in strong and less aromatic oriental melons, and alcohols, (E, Z)-3, 6-nonadien-1-ol, as the principle volatile, were also identified in non-aromatic melon. We found that the specific CmADH genes expression might be an important reason for the diversity of alcohols and follow-up ester components in three types of melon. ADH activity, CmADH genes expression and the content of two principle esters were significantly inhibited by ethanol, and the 4-MP, a kind of competitive inhibitor of ADH enzyme. While affection of aldehyde on CmADH activity or CmADH expression depended on co-factors or genes. We also found the relationship between CmADH3, CmADH12 and the characteristic volatile, namely ethyl acetate or hexyl acetate. In conclusion, our study provide some evidences for the relationship between CmADHs and volatile compounds of oriental melon, and more studies are needed to make it clear.

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AUTHOR CONTRIBUTIONS

HC and HQ designed research; HC performed research; HC analyzed data; HC and HQ wrote the paper; SC, YJ, and YT helped to revise the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fphys.2016.00254

Table S1 | Volatile compounds and their concentrations (µg.g-1FW) in different aroma types of the melon ripen fruit. Include “Cai Hong” (CH), “Cui Bao” (CB) and “Cai Gua” (CG). Each experiment was performed in triplicate and the mean value of their concentrations were shown in this table.

Figure S1 | The SSC of three types of melon at different days after pollination (DAP). The three aroma types melon are CH (short for “Cai Hong”), CB (short for “Cai Bao”), and CG (short for “Cai Gua”). Each experiment was performed in triplicate and the means ± SE value of their content were shown in the line chart.

Figure S2 | Different appearance of three types of melon (Cucumis melo). (A) Oriental melon (C. melo var. makuwa Makino) cultivar “Chai Hong” (CH). (B) Oriental melon (C. melo var. makuwa Makino) cultivar “Chai Hong” (CH). (C) Oriental melon (C. melo var. makuwa Makino) cultivar “Cui Bao” (CB). (D) Snake melon (C. melo L. var. flexuosus Naud) “Cai Gua” (CG).

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