Volatile Compound Profiles of Malus baccata and Malus prunifolia Wild Apple Fruit

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Abstract. Volatile compounds have a tremendous impact on fruit quality. We evaluated the volatile compound profiles of ripening wild apple fruit (10 Malus baccata accessions and three Malus prunifolia accessions) in the National Field Genebank for Hardy Fruits at Gongzhuling, China. Alcohols, esters, aldehydes, terpenes, hydrocarbons, ethers, heterocycles, carboxylic acids, and ketones were detected in the M. baccata and M. prunifolia fruit, with the first four being the main volatile compounds present. Of the 92 volatiles detected, esters were the most diverse (49 compounds). This wide range of abundant volatile compounds suggests that M. prunifolia is a good resource for breeding apple cultivars with novel and interesting flavors. The M. baccata accession ‘Zhaai Shandingzi’ and the M. prunifolia accession ‘Bai Haitang’ had the widest range of volatile compounds and the highest volatile compound contents of the accessions examined, and will therefore be good breeding materials for developing commercial lines with enhanced flavor and for widening the genetic diversity. The number of different ester compounds present was significantly positively correlated (r = 0.877) with the cube root of the weight of an individual ripe fruit. Principal component analysis (PCA) showed that the contents of ester compounds could be used to distinguish between M. baccata and M. prunifolia species. Therefore, ester compounds could be used as a reference of parental choice in apple breeding.

Apple (Malus pumila) is a fruit crop of major economic importance. In worldwide apple breeding efforts, apple breeders typically intercross elite or commercial cultivars, such as Red Delicious, Golden Delicious, and Jonathan, which results in commercial apple cultivars with a narrow genetic base, low adaptability, and reduced resistance to biotic and abiotic stresses (Kumar et al., 2010; Noiton and Alspach, 1996). Therefore, there is a need to develop breeding strategies that broaden the genetic base, enhance resistance and tolerance to biotic and abiotic stresses, and improve fruit quality, including flavor (Forsline et al., 2010; Sedov and Serova, 2013). Wild apples, with their high genetic diversity and antioxidant activity, disease resistance, and stress tolerance, are important resources for apple breeding (Chen et al., 2007; Emeriewen et al., 2014; John et al., 2014; Jurick et al., 2011; Zhang et al., 2007, 2003). To collect and protect hardy fruit resources, the National Field Genebank for Hardy Fruits, Gongzhuling, China, was established at Jilin Province (Zhang et al., 2003). Phenotypic properties, biologic characteristics, cold resistance, disease resistance, and fruit quality have been described and

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evaluated in this nursery (Zhang, 2008). Malus baccata and Malus prunifolia are the hardiest wild apple species in this nursery and also the main wild cold hardy species in northern China. These cold hardy fruit can tolerate temperatures below −42 °C (Li et al., 2015; Wang et al., 2013; Zhang et al., 2003), and their hardy rootstocks are widely used for apple grafting and apple breeding in northern China. M. baccata and M. prunifolia accessions with excellent traits were formed by human selection over many years, and were cultivated for their fruit and as rootstocks (Kuznetsova et al., 2010; Meng et al., 1997; Zhang, 2008).

Volatile components have a huge impact on fruit quality, and are thus an important factor in apple breeding (Laurens, 1999). In apple, volatile components are composed of esters, alcohols, aldehydes, ketones, and ethers (Dimick and Hoskin, 1983; Kakiuchi et al., 1986). Research by Kakiuchi et al. (1986) showed that esters are the most abundant flavor components in apple cultivars (Hatsukai, Kogyoku, Golden Delicious, Musu, and Fuji). Similar results were found for ‘Annurca’ and ‘Delicious’ apple fruit (Mattheis et al., 1991; Scalzo et al., 2001). In wild apple, aldehydes, esters, and alcohols are the main volatile compounds (Li et al., 2008). In Malus sieversii, alcohols, aldehydes, and esters are the main volatile compounds (Chen et al., 2007). However, the volatile compounds present in M. baccata and M. prunifolia are unknown.

In this work, we identified the volatile compounds present in 10 accessions of M. baccata and three accessions of M. prunifolia in the National Field Genebank for Hardy
Fruits. The results of this study represent a resource for selecting and breeding cultivars with a desired volatile phenotype.

**Materials and Methods**

**Plant Materials.** *Malus baccata* and *M. prunifolia* trees were planted in the National Field Genebank for Hardy Fruits (lat. 43°50’N, long. 124°82’E). The average temperature for January is –13.6 °C and extreme low temperature is –38 °C for the nursery. The management level of the nursery is good and consistent. The fully ripened apple fruit with detectable fragrance and intrinsic color of 10 *M. baccata* accessions (‘Duanba Shandingzi’, ‘Xiaosuanguo’, ‘Zhaai Shandingzi’, ‘Benyuandaguo Shandingzi’, ‘Benzaodaguo Shandingzi’, ‘No. 3 Shandingzi’, ‘No. 5 Shandingzi’, ‘No. 6 Shandingzi’, ‘No. 7 Shandingzi’, and ‘No. 8 Shandingzi’) and three *M. prunifolia* accessions (‘Bai Haitang’, ‘Xiaohuang Haitang’, and ‘Bian Haitang’) were harvested between 20 Aug. 2014 and 20 Sept. 2014 (Fig. 1). Three trees were sampled for each accession and 50 fruit were harvested for each tree. Apple fruit were harvested one time from each tree, all with about equal conditions of maturity and of about the same size and stature. All the fruit from three trees of the same accession were mixed and divided into three groups, and each group was a biological replicate. The apples were packed and shipped overnight to the State Key Laboratory for Crop Biology at Shandong Agricultural University, Tai’an, China, for evaluation.

**Static headspace extraction.** Ten to 50 apple fruit were rapidly cut into slices of about 3 mm wide and mixed. Six grams of sample was placed in 10 mL sample bottles, and 10 μL 3-nonenone (0.4 mg mL⁻¹) was injected into each bottle and used as an internal standard. The sample bottles were then sealed using polytetrafluoroethylene butyl rubber cap isolation. A headspace sampler (PerkinElmer TurboMatrix 40 Trap; GenTech, Arcade, NY) was used to extract the volatile component of fruit and inject the sample. Samples were extracted after 30 min of incubation at 50 °C, with the temperature of the static sample probe and transfer line being maintained at 80 °C, and then the sample bottle was pressured to 15 Pa for 5 min. Then, 500 μL of the volatile component was captured for analysis.

**Fig. 1.** Ripe fruit of (A) *Malus baccata* and (B) *Malus prunifolia* and (C) their weights. *M. baccata* accessions are abbreviated as follows: B1 = ‘Duanba Shandingzi’, B2 = ‘Beyanandaguo Shandingzi’, B3 = ‘Benzaodaguo Shandingzi’, B4 = ‘No. 3 Shandingzi’, B5 = ‘No. 5 Shandingzi’, B6 = ‘No. 6 Shandingzi’, B7 = ‘No. 7 Shandingzi’, B8 = ‘No. 8 Shandingzi’, B9 = ‘Xiaosuanguo’, and B10 = ‘Zhaai Shandingzi’; *M. prunifolia* accessions are abbreviated as follows: P1 = ‘Bai Haitang’, P2 = ‘Xiaohuang Haitang’, and P3 = ‘Bian Haitang’. The vertical bar represents ±SE of the mean of 30 fruit. Different letters above the bars indicate significant differences at P ≤ 0.05 based on Duncan’s test.
Table 1. The main volatile compounds and their contents in fruit from 10 *Malus baccata* accessions by static headspace and gas chromatography–mass spectrometry.  

| Compounds | Duabna Shandingzi | Benyuandauguo Shandingzi | Benzaodauguo Shandingzi | No. 3 Shandingzi | No. 5 Shandingzi | No. 6 Shandingzi | No. 7 Shandingzi | No. 8 Shandingzi | Xiaousanguo | Zhaai Shandingzi |
|-----------|-------------------|-------------------------|-------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 1-Hexanol | 0.0983 a          | 0.1455 a                | 0.1998 a                | 0.1083 c       | 0.0644 b       | 0.0408 b       | 0.3733 c       | 0.5181 b       | 0.1503 a      | 0.3310 b       |
| Cyclohexanol | 0.0654 b        | 0.1066 b                | 0.1633 b                | —              | —              | —              | —              | —              | —              | 0.3256 b       |
| 3-Nonanol | 0.0269 c         | 0.0102 d                | 0.0061 e                | 0.0123 f       | 0.0044 de      | 0.0021 f       | 0.0148 e       | 0.0151 f       | 0.0072 e      | 0.0111 fg       |
| 1-Penten-3-ol | — w              | —                       | —                       | 0.0035 e       | 0.0066 g       | 0.0024 e       | 0.0026 f       | 0.0066 f       | 0.0148 f      | 0.0025 f       |
| 3-Hexen-1-ol | —                | —                       | —                       | 0.4206 b       | —              | —              | —              | —              | —              | —              |
| (E)-4-Hexen-1-ol | 0.0781 b       | 0.1369 a                | —                       | 0.1436 a       | 0.1150 a       | 0.6701 b       | —              | 0.1233 a       | —              | —              |
| (E)-2-Hexen-1-ol | —                | —                       | —                       | —              | 0.0682 b       | 0.0214 c       | —              | —              | —              | 0.0543 b       |
| Ethyl hexanoate | 0.0196 cd      | —                       | 0.0277 d                | —              | —              | —              | —              | —              | —              | —              |
| Pentyl acetate | —                | —                       | —                       | 0.0059 g       | —              | —              | —              | 0.0228 e       | —              | 0.0055 g       |
| Hexyl acetate | 0.0175 cd       | 0.0150 d                | 0.0029 e                | 0.1343 c       | —              | —              | 0.1961 d       | 0.1985 de      | 0.0135 d      | 0.1943 c       |
| (Z)-3-Hexen-1-ol acetate | 0.0704 b      | —                       | —                       | 0.0083 d       | 0.0073 d       | 0.8677 a       | 0.3696 bc      | 0.0383 c      | —              |
| (Z)-2-Hexen-1-ol acetate | —              | —                       | —                       | —              | 0.0899 e       | —              | —              | —              | —              |
| (4E)-4-Hexen-1-ol acetate | —              | —                       | —                       | 0.0073 fg      | —              | —              | —              | —              | 0.4244 b      | —              |
| (Z)-3-Hexenyl formate | —              | —                       | —                       | —              | —              | —              | —              | 0.4027 b      | —              |
| (E)-3-Hexenyl acetate | —              | 0.0515 c                | —                       | 0.7941 a       | —              | —              | —              | —              | —              |
| (Z)-3-Hexenyl acetate | —              | —                       | —                       | —              | —              | —              | —              | —              | —              |
| (Z)-2-Hexenyl acetate | 0.0073 d       | 0.0070 e                | —                       | —              | —              | —              | —              | —              | 0.3817 b      | —              |
| Hexanal | —                | 0.0289 d                | 0.0773 c                | 0.1247 c       | 0.0260 c       | 0.1699 a       | 0.2121 cd      | 0.2560 d       | 0.0234 cd      | 0.1720 c       |
| (E)-2-Hexenal | 0.0185 cd       | 0.0766 c                | 0.1885 a                | 0.0617 d       | 0.0428 c       | 0.1473 a       | 0.1062 d       | 0.8009 a       | 0.0313 c       | 0.6354 a       |
| α-Longipinene | —                | —                       | —                       | 0.0398 e       | —              | —              | —              | —              | —              | —              |
| α-Famesene | 0.0098 d         | 0.0118 d                | 0.0713 c                | 0.0392 e       | 0.1379 a       | 0.0557 b       | 0.0119 e       | 0.1146 e       | 0.0202 cd      | 0.0449 ef       |
| 1,3,5,7-Cyclooctatetraene | —              | —                       | —                       | —              | —              | —              | —              | —              | —              | —              |
| 1-Methylnaphthalene | —              | —                       | —                       | —              | —              | —              | —              | —              | —              | —              |
| Toluene | —                | —                       | —                       | —              | —              | —              | —              | —              | —              | —              |
| Di-n-butyl ether | 0.0133 d        | 0.0059 e                | —                       | 0.0122 f       | 0.0064 d       | 0.0040 e       | 0.0047 f       | 0.0075 g       | 0.0046 f      | 0.0121 fg       |
| 2-Pentylfuran | —                | —                       | —                       | —              | —              | —              | —              | —              | —              | 0.0067 g       |

*The table showed that the content of volatile compound was more than 0.02 μg.g⁻¹ 3-nonanone. Values are given with 3-nonanone as the internal standard.

Volatiles components and contents were measured as three technical replicates each with three independent biological replicates.

Means followed by different letters with the same column for volatile compounds contents in the same accession are significantly different at $P \leq 0.05$ by Duncan’s test.

Not found or not exist.
Table 2. The main volatile compounds and their contents in fruit from three *Malus prunifolia* accessions by static headspace and gas chromatography–mass spectrometry.

| Compounds                      | Bai Haitang | Xiaohuang Haitang | Bian Haitang | (µg·g⁻¹·3-nonanone) |
|-------------------------------|-------------|-------------------|--------------|---------------------|
| Cyclohexanol                  | —           | —                 | 0.0607 d     |
| 1-Hexanol                     | 0.1602 c    | 0.2956 b          | 0.1684 c     |
| 1-Butanol                     | 0.0129 g    | —                 | —            |
| 2-Methyl-1-butanol            | 0.0303 ef   | 0.0127 f          | —            |
| Methyl butanoate              | 0.0465 e    | —                 | 0.0097 ef    |
| Methyl 2-methylbutanoate      | 0.0573 d    | —                 | 0.0250 e     |
| Methyl hexanoate              | 0.0689 d    | —                 | 0.0239 e     |
| Ethyl propionate              | 0.0547 d    | —                 | 0.0080 f     |
| Ethyl hexanoate               | 0.4518 b    | 0.0662 c          | 0.2872 b     |
| Ethyl butanoate               | 0.5837 a    | 0.0498 d          | 0.1365 c     |
| Ethyl 2-methylbutyrate        | 0.4151 b    | —                 | 0.1153 c     |
| Ethyl tiglate                 | —           | —                 | 0.0362 e     |
| Propyl butyrate               | 0.1138 c    | —                 | —            |
| Propyl hexanoate              | 0.0488 d    | —                 | —            |
| Propyl 2-methyl butyrate      | 0.0699 d    | —                 | —            |
| Butyl butyrate                | 0.0527 d    | —                 | —            |
| Butyl 2-methylbutyrate        | 0.0483 e    | —                 | —            |
| Hexyl hexanoate               | 0.0253 f    | 0.0039 g          | 0.0064 f     |
| Hexyl butyrate                | 0.0578 d    | 0.0403 d          | 0.0211 e     |
| (2Z)-2-Hexenyl acetate        | —           | 0.0053 g          | —            |
| Hexyl acetate                 | —           | 0.0241 e          | —            |
| Hexyl 2-methylbutyrate        | 0.0412 e    | 0.0104 f          | 0.0119 ef    |
| (4E)-4-Hexenyl acetate        | —           | 0.0450 d          | —            |
| Isopentyl 2-methylbutanoate   | 0.0137 g    | 0.0030 g          | —            |
| (2E)-2-Hexenyl butyrate       | 0.0098 g    | 0.0233 e          | 0.0107 ef    |
| Hexanone                      | —           | 0.0216 e          | —            |
| 2-Hexenal                     | —           | 0.0769 c          | —            |
| α-Curcumene                   | 0.0034 h    | 0.0127 f          | 0.0138 ef    |
| α-Farnesene                   | 0.5465 a    | 0.5262 a          | 0.4233 a     |

*The table showed that the content of volatile compound was more than 0.02 µg·g⁻¹ 3-nonanone. Values are given with 3-nonanone as the internal standard.*

*Volatile components and contents were measured as three technical replicates each with three independent biological replicates.*

*Not found or not exist.*

*Means followed by different letters with the same column for volatile compounds contents in the same accession are significantly different at P ≤ 0.05 by Duncan’s test.*

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY. A gas chromatography–mass spectrometry [GC–MS (GCMS-QP2010; Shimadzu, Kyoto, Japan)] instrument was used to analyze the volatile composition of substances released from the apple slices. GC conditions were as follows: the chromatographic column used was Rtx-5 (30 m × 0.32 mm × 1.0 µm; Restek, Bellefonte, PA); the injection port temperature was 200 °C; and the column temperature was maintained at 35 °C for 2 min, at 8 °C·min⁻¹ to 180 °C for 4 min, and then at 15 °C·min⁻¹ to 230 °C for 1 min. MS conditions were as follows: helium was used as the carrier gas at a flow rate of 1.06 mL·min⁻¹. The MS was operated in the electron impact ionization mode with an electron energy of 70 eV. The scan range was 45–450 atomic mass units. Splitless injection of 1 µL of solution was performed and the temperature of the ion source was 200 °C.

QUALITATIVE AND QUANTITATIVE ANALYSIS. The GC data processing system, the computer retrieval and NIST library (107,000 compounds), and the Wiley library (y320 k compounds, version 6.0), combined with artificial map matching were used to confirm the chemical composition of volatile substances. Relative content was calculated according to the peak area normalization method (Wang et al., 2014). 3-Nonanone was used as an internal standard to estimate the volatile relative content.

**Data Analysis.** Volatile components and contents were measured as three technical replicates each with three independent biological replicates. SPSS software (version 17.0; IBM Corp, Armonk, NY) was used for significant differences (P ≤ 0.05), PCA, and Pearson’s correlation evaluation.

**Results.**

**Categorization of the main volatile compounds released by *M. baccata* and *M. prunifolia* fruit.** We identified 58 and 54 volatile compounds, including alcohols, esters, aldehydes, terpenes, hydrocarbons, ethers, heterocycles, carboxylic acids, and ketones, in 10 *M. baccata* accessions and three *M. prunifolia* accessions using GC–MS (Supplemental Tables 1 and 2). The main volatile compounds identified (with contents of more than 0.02 µg·g⁻¹ 3-nonanone) are shown in Tables 1 and 2. We found that all 10 *M. baccata* accessions contained four common components: 1-hexanol, 3-nonanol, (E)-2-hexenal, and α-farnesene, with 1-hexanol being the most abundant volatile component of ‘Duanba Shandingzi’, ‘Benyuandaguo Shandingzi’, ‘Benzaodaguo Shandingzi’, and ‘Xiaosuanguo’, and (E)-2-hexenal being the most abundant volatile component of ‘No. 8 Shandingzi’ and ‘Zhai Shandingzi’. (E)-3-Hexenyl acetate, (E)-4-hexen-1-ol, hexanal, and (Z)-3-hexen-1-ol acetate were the most abundant volatile compounds present in ‘No. 3 Shandingzi’, ‘No. 5 Shandingzi’, ‘No. 6 Shandingzi’, and ‘No. 7 Shandingzi’, respectively (Table 1). In three *M. prunifolia* accessions, nine kinds of volatile compounds were common (Table 2), including 1-hexanol, ethyl hexanoate, ethyl butanoate, hexyl hexanoate, hexyl butyrate, hexyl 2-methylbutyrate, (2E)-2-hexenyl butyrate, α-curcumene, and α-farnesene. For ‘Bai Haitang’, the content of ethyl butanoate (0.5837 µg·g⁻¹ 3-nonanone) was highest among the volatile compounds, followed by α-farnesene (0.5465 µg·g⁻¹ 3-nonanone). However, α-farnesene was the most abundant volatile compound in ‘Xiaohuang Haitang’ (0.5265 µg·g⁻¹ 3-nonanone) and ‘Bian Haitang’ (0.4233 µg·g⁻¹ 3-nonanone).

Furthermore, we determined the contents of alcohols, esters, aldehydes, terpenes, and hydrocarbons in *M. baccata* and *M. prunifolia* fruit (Fig. 2). For *M. baccata* accessions, the content of alcohols was highest in ‘Duanba Shandingzi’, ‘Benyuandaguo Shandingzi’, ‘Benzaodaguo Shandingzi’, ‘No. 5 Shandingzi’, and ‘Xiaosuanguo’ and the content of aldehydes was highest in ‘No. 3 Shandingzi’, ‘No. 6 Shandingzi’, ‘No. 7 Shandingzi’, ‘No. 8 Shandingzi’, and ‘Zhai Shandingzi’. For the three *M. prunifolia* accessions, the content of terpenoids and hydrocarbons was highest in ‘Shandingzi’, ‘No. 5 Shandingzi’, ‘No. 6 Shandingzi’, and ‘Zhai Shandingzi’.
accessions examined, esters, terpenes, and alcohols were the most abundant volatile compounds present (Fig. 2).

**Volatile compound types and contents in 10 M. BACCATA and 3 M. PRUNIFOLIA accessions.** In the ‘Zhaai Shandingzi’ accession, we detected 28 kinds of volatile compounds, 10 of which were hydrocarbons, 6 of which were esters, and 4 of which were aldehydes. ‘Zhaai Shandingzi’ was found to have the highest content of volatile compounds (2.9096 μg g⁻¹ 3-nonanone) among the M. baccata fruit (Table 3 and Supplemental Table 1). A total of 38, 22, and 25 kinds of volatile compounds were detected in ‘Bai Haitang’, ‘Xiaohuang Haitang’, and ‘Bian Haitang’ from M. prunifolia, respectively; most of which were esters, and ‘Bai Haitang’ had the highest contents of volatile compounds (3.007 μg g⁻¹ 3-nonanone) among the M. prunifolia fruit (Table 3 and Supplemental Table 2). Esters (49 kinds) were the most diverse category of volatile compound, accounting for more than half of the total volatile compounds present (92 kinds).

**Relationship between the number of volatile compounds and fruit weight.** To establish if fruit weight affects the number of volatile compounds, we conducted a correlation analysis between the volatile compounds and the cube root of the weight of an individual ripe fruit. As shown in Fig. 3, the number of different types of esters had the highest linear correlation with the cube root of fruit weight ($R^2 = 0.7694$), followed by the number of different aldehydes ($R^2 = 0.63223$) and the number of total volatile compounds ($R^2 = 0.3793$). Furthermore, Pearson’s correlation analysis showed that the cube root of fruit weight exhibited a significant positive correlation with the number of types of esters ($r = 0.877$, $P \leq 0.01$), a significant negative correlation with the number of aldehydes ($r = -0.795$, $P \leq 0.01$), a significant positive correlation with the number of total volatile compounds ($r = 0.616$, $P \leq 0.05$), and was not correlated with the number of alcohols, terpenes, and hydrocarbons (Table 4). Therefore, fruit weight correlates with the number of different types of esters, aldehydes, and total volatile compounds produced.

**PCA of the volatile compound contents.** To decipher the role of volatile compounds in apple evolution, we analyzed the contents of alcohol compounds, ester compounds, aldehyde compounds, terpene compounds, and the total volatile compounds using PCA. The PCA results of alcohol compounds, ester compounds, and the total volatile compounds are shown in Fig. 4. Principal component 2 explained 18.10% of the total variance observed and separated M. baccata and M. prunifolia into two groups according to the total volatile
Table 3. The number of volatile compounds in fruit from 10 Malus prunifolia accessions and three accessions by static headspace and gas chromatography–mass spectrometry. 

| Categories | Total volatile compounds (no.) | M. prunifolia | M. baccata | No. 3 | No. 5 | No. 6 | No. 7 | No. 8 | 'Bai Shandingzi' | 'Bai Haitang' | 'Zhaai Shandingzi' |
|------------|--------------------------------|----------------|-----------|------|------|------|------|------|----------------|-------------|-------------------|
| Alcohols   | 4                              | 4              | 4         | 5    | 3    | 3    | 5    | 3    | 2              | 1           | 2                 |
| Esters     | 9                              | 7              | 4         | 6    | 13   | 5    | 3    | 6    | 2              | 1           | 4                 |
| Aldehydes  | 4                              | 3              | 4         | 4    | 3    | 3    | 4    | 1    | 2              | 0           | 5                 |
| Ketones    | 2                              | 2              | 2         | 1    | 3    | 1    | 3    | 2    | 3              | 3           | 7                 |
| Hydrocarbons | 1                             | 0              | 0         | 1    | 2    | 3    | 0    | 1    | 0              | 0           | 1                 |
| Aldehyde   | 1                              | 1              | 0         | 1    | 1    | 1    | 0    | 1    | 0              | 0           | 0                 |
| Ethers     | 1                              | 1              | 0         | 1    | 1    | 1    | 0    | 1    | 0              | 0           | 0                 |
| Heterocycles | 0                            | 0              | 0         | 0    | 0    | 0    | 0    | 0    | 0              | 0           | 1                 |
| Carboxylic acids | 0                  | 0              | 0         | 0    | 0    | 0    | 0    | 0    | 0              | 0           | 1                 |
| Ketones    | 0                              | 0              | 0         | 0    | 0    | 0    | 0    | 0    | 0              | 0           | 2                 |
| Ketones    | 0                              | 0              | 0         | 0    | 0    | 0    | 0    | 0    | 0              | 0           | 0                 |

Note: The number of volatile compounds was measured as three technical replicates each with three independent biological replicates.

Discussion

In this study, we examined the volatile compound profiles of 10 M. baccata accessions and three M. prunifolia accessions using GC–MS. Malus baccata and M. prunifolia are two cold-resistant species that are widely used for hardy rootstocks in northern China. 'Zhaai Shandingzi', a M. baccata accession, is an excellent dwarf hardy rootstock (the dwarf form is a descendant of the tall form) (Kuznetsova et al., 2010; Meng et al., 1997). We showed that 'Zhaai Shandingzi' was the M. baccata accession with the highest diversity of volatile compounds, having 28 different types of volatile compounds. Therefore, 'Zhaai Shandingzi' will be an excellent resource for enhancing the flavor of dwarf hardy apple. The M. prunifolia accession 'Bai Haitang' is widely distributed in northern China; due to its nice flavor and high level of cold resistance, it is used both for edible purposes and as a hardy rootstock. Simple sequence repeat marker analysis showed that 'Bai Haitang' was more distant related to popular commercial cultivars (such as Golden Delicious, Jonathan, and Red Delicious) than to wild accessions, according to an unweighted pair-group method with arithmetic means dendrogram and principal coordinate analysis (Zhang et al., 2012). In our study, GC–MS analysis showed that 'Bai Haitang' produced 38 volatile compounds and had the highest diversity of volatile compounds of all M. prunifolia accessions. Therefore, 'Bai Haitang' will be an excellent resource for enhancing the flavor and widening the genetic diversity of commercial cultivars of apple.

Terpenes are compounds produced by plants that ward off pathogens and herbivores (Kessler and Baldwin, 2001). α-Farnesene, a lepidopteran attractant and oviposition inducer (Bengtsson et al., 2001; Landolt et al., 2000; Sobottnik et al., 2008; Sutherland and Hutchins, 1972), is the predominant terpene volatile produced during apple fruit storage (Huelin and Murray, 1966; Meigh and Filmer, 1969). However, the oxidation products of α-farnesene can induce superficial scald disorder, which occurs in apple and pear fruit after several months of cold storage (Huelin and Coggiola, 1970; Isidoro and Almeida, 2006; Rowan et al., 2001). Wild apples have higher contents of terpene compounds than do domesticated cultivars (Chen et al., 2007; Wang et al., 2014). In our study, we found that M. prunifolia had a higher volatile terpene content than did M. baccata, and that α-farnesene was the most abundant terpene in all accessions examined. M. prunifolia fruit are difficult to store and are not commonly used for edible purposes. They are used as a prominent ornamental plant and robust rootstock for developing new apple cultivars (Igarashi et al., 2006; Rowan et al., 2001). Malus prunifolia may also be used in efforts to breed new apple cultivars with higher volatile terpene content.

Esters are the most abundant volatile compound in ripening apple fruit and normally account for 80% to 95% of the total volatile emission (Kakuchi et al., 1986; Paillard, 1990). Sugimoto et al. (2015) evaluated ripening apple fruit from 184 germplasm lines, and found that ester diversity tended to
increase with increasing fruit weight and was linearly
 correlated with the cube root of fruit weight ($R^2 = 0.64$).
 Our study showed that esters (49 kinds) accounted for 53.26% 
of the total volatile compounds (92 kinds) produced by wild
 apple and that ester diversity was more linearly correlated
 with the cube root of fruit weight ($R^2 = 0.7694$) than was the
 total volatile compound diversity ($R^2 = 0.3793$). Pearson’s
 correlation analysis showed that the cube root of fruit weight

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Fig. 3. Regression analysis between number of volatile compounds and the cube root of ripe fruit weight from 10 *Malus baccata* accessions (‘Duanba Shandingzi’, ‘Xiaosuanguo’, ‘Zhaai Shandingzi’, ‘Benyuandaguo Shandingzi’, ‘Benzaodaguo Shandingzi’, ‘No. 3 Shandingzi’, ‘No. 5 Shandingzi’, ‘No. 6 Shandingzi’, ‘No. 7 Shandingzi’, and ‘No. 8 Shandingzi’) and three *Malus prunifolia* accessions (‘Bai Haitang’, ‘Xiaohuang Haitang’, and ‘Bian Haitang’). Volatile compounds were detected by static headspace and gas chromatography–mass spectrometry from ripe fruit as three technical replicates each with three independent biological replicates. Fruit weight was average weight of 30 fruit.
was more significantly positively correlated with ester diversity ($r = 0.877$, $P \leq 0.01$) than with total volatile compound diversity ($r = 0.616$, $P \leq 0.05$). Furthermore, PCA showed that the ester compound contents could better separate *M. baccata* and *M. prunifolia* accessions into two groups than could total volatile compound contents. Therefore, to expand the genetic diversity of commercial cultivars, ester compounds are important indicators of parental choice in apple breeding.

At the same time, consumers perceived esters (hexyl acetate, butyl acetate, and 2-methylbutyl acetate) as “fruity” and “floral” (Aharoni et al., 2000; Plotto et al., 2000). Our study showed that hexyl acetate was detected in most *M. baccata* accessions and *M. prunifolia* accession ‘Xiaohang Haitang’. Therefore, these accessions might be good breeding materials to meet the sensory needs of consumers.

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| Compounds | No. 3 | No. 5 | No. 6 | No. 7 | Benyuandaqiao | Benzaodaqiao | Duanba | Xiaosuanguo | Zhai |
|-----------|------|------|------|------|---------------|-------------|-------|-------------|-----|
| Alcohols  |      |      |      |      |               |             |       |             |     |
| Benzy alcohol         | —    | —    | —    | —    |               |             |       |             |     |
| 1-Hexanol           | 0.1083 | 0.0644 | 0.0408 | 0.3733 | 0.5181         | 0.1455       | 0.1998 | 0.0983       | 0.1503 |
| Cyclohexanol        | —    | —    | —    | —    | 0.4840         | 0.1066       | 0.1633 | 0.0654       | —    |
| 3-Nonanol           | 0.0123 | 0.0044 | 0.0021 | 0.0148 | 0.0151         | 0.0102       | 0.0061 | 0.0269       | 0.0072 |
| 1-Penten-3-ol       | 0.0066 | 0.0024 | 0.0026 | 0.0066 | 0.0148         | —            | 0.0035 | —            | 0.0025 |
| 3-Hexen-1-ol        | 0.4206 | —    | —    | —    | —              | —            | —    | —            | —    |
| (E)-4-Hexen-1-ol    | —    | 0.1436 | 0.1150 | 0.6701 | —              | 0.1369       | —    | 0.0781       | 0.1233 |
| (E)-2-Hexen-1-ol    | —    | 0.0682 | 0.0214 | —    | —              | —            | —    | 0.0543       | —    |
| Esters              |      |      |      |      |               |             |       |             |     |
| Methyl hexanoate    | —    | —    | —    | —    |               |             | —    | —            | —    |
| Methyl salicylate   | 0.0068 | —    | —    | —    | —              | —            | —    | —            | —    |
| Ethyl hexanoate     | —    | —    | —    | —    |               | —            | —    | —            | —    |
| Ethyl isohexanoate  | —    | —    | —    | —    |               | —            | —    | —            | —    |
| Ethyl butyrate      | —    | —    | —    | —    |               | —            | —    | —            | —    |
| Ethyl benzenecarboxylate | —    | —    | —    | —    |               | —            | —    | —            | —    |
| (Z)-3-Hexen-1-ol propanoate | —    | —    | —    | —    |               | —            | —    | —            | —    |
| Ethyl 2-methylbutyrate | —    | —    | —    | —    |               | —            | —    | —            | —    |
| Pentyl acetate      | 0.0059 | —    | —    | —    | 0.0228         | —            | —    | —            | 0.0055 |
| Hexyl acetate       | 0.1343 | —    | —    | —    | 0.1961         | 0.1985       | 0.0150 | 0.0029       | 0.0175 |
| Hexyl butyrate      | —    | —    | —    | —    | —              | —            | —    | —            | —    |
| Cis-3-Hexenyl isobutyrate | —    | —    | —    | —    | 0.0037         | 0.0052       | —    | 0.0023       | —    |
| (E)-2-Hexen-1-ol acetate | —    | —    | —    | —    | 0.0407         | —            | —    | —            | —    |
| (Z)-3-Hexen-1-ol acetate | —    | 0.0083 | 0.0073 | 0.8677 | 0.3696         | —            | 0.0704 | 0.0383       | —    |
| (Z)-2-Hexen-1-ol acetate | —    | —    | —    | —    | 0.0899         | —            | —    | —            | —    |
| (4E)-4-Hexen-1-ol acetate | 0.0073 | —    | —    | —    | 0.4244         | —            | —    | —            | —    |
| (2E)-2-Hexenyl butyrate | —    | —    | —    | —    | 0.0082         | 0.0099       | 0.0034 | —            | —    |
| (Z)-3-hexenyl butanoic acid ester | 0.0151 | —    | —    | —    | —              | —            | —    | —            | —    |
| (Z)-3-Hexenyl formate | —    | —    | —    | —    | —              | —            | —    | —            | 0.4027 |
| (E)-3-Hexenyl acetate | 0.7941 | —    | —    | —    | —              | 0.0515       | —    | —            | —    |
| (Z)-3-Hexenyl acetate | —    | —    | —    | —    | —              | —            | —    | 0.3817       | —    |
| (2Z)-2-Hexenyl acetate | —    | —    | —    | —    |               | 0.0070       | —    | 0.0073       | 0.1521 |
| Tetrahydrofurural N-(4-pyridyl) carbamate | —    | —    | 0.0023 | —    | —              | —            | —    | —            | —    |
| Isonicotinic acid 2-tetrahydrofurfurylmethyl ester | —    | —    | —    | —    | —              | —            | —    | —            | 0.0105 |
| Aldehydes            |      |      |      |      |               |             |       |             |     |
| Benzaldehyde        | 0.0191 | —    | —    | —    | 0.0056         | —            | —    | 0.0041       | —    |
| Hexanal             | 0.1247 | 0.0260 | 0.1699 | 0.2121 | 0.2560         | 0.0289       | 0.0773 | —            | 0.0234 |
| (E)-2-Hexenal       | 0.0617 | 0.0428 | 0.1473 | 0.1062 | 0.8009         | 0.0766       | 0.1885 | 0.0185       | 0.0313 |
| Nonanal             | 0.0035 | 0.0038 | 0.0015 | 0.0050 | 0.0081         | —            | 0.0148 | 0.0064       | 0.0031 |
| Decanal             | —    | 0.0025 | —    | —    | 0.0040         | 0.0132       | 0.0081 | —            | 0.0111 |
| Compounds | No. 3 Shandingzi | No. 5 Shandingzi | No. 6 Shandingzi | No. 7 Shandingzi | No. 8 Shandingzi | Benyuandaguo Shandingzi | Benzaodaguo Shandingzi | Duanba Shandingzi | Xiaosuanguo Shandingzi | Zhai Shandingzi |
|-----------|-----------------|-----------------|-----------------|-----------------|-----------------|------------------------|---------------------|------------------|----------------------|------------------|
| *α*-Longipinene | — | 0.0398 | — | — | — | — | 0.0059 | — | — | — |
| *α*-Curcumene | — | 0.0034 | 0.0014 | — | — | — | — | 0.0059 | — | — |
| *α*-Farnesene | 0.0392 | 0.1379 | 0.0557 | 0.0119 | 0.1146 | 0.0118 | 0.0713 | 0.0098 | 0.0202 | 0.0449 |
| Limonene | — | — | — | — | 0.0036 | — | — | — | — |
| Methyl chavicol | — | — | — | — | — | 0.0056 | — | — | — | 0.0127 |
| Hydrocarbons | Cyclohexene | 0.0045 | 0.0018 | 0.0066 | — | — | — | — |
| 1-Methyl-5-(1-methylethenyl)cyclohexene | — | — | — | 0.0052 | — | — | — | — |
| 1,3,5,7-Cyclooctatetraene | — | — | — | — | 0.0107 | — | — | — | 0.0394 |
| 3,7-Dimethyldecane | — | — | — | — | — | — | — | 0.0038 | — |
| Heneicosane | — | — | — | — | — | — | — | — | — | 0.0049 |
| Nonphytane | — | — | — | — | — | — | — | — | — | 0.0054 |
| Heptadecane | — | — | — | — | — | — | — | — | — | — | 0.0046 |
| Fluorene | — | — | — | — | — | — | — | — | — | 0.0194 |
| Dibenzofuran | — | — | — | — | — | — | — | — | — | 0.0125 |
| 2,6-Dimethylnaphthalene | — | — | — | — | — | — | — | — | — | 0.0061 |
| 1-Methylnaphthalene | — | — | — | — | — | — | — | — | — | 0.0261 |
| Toluene | — | — | — | — | — | — | — | — | — | 0.0049 |
| 1-methoxy-4-(1-propenyl)-benzene | — | — | — | — | — | — | — | — | — | 0.0050 |
| Duodecane | — | — | — | 0.0046 | 0.0074 | — | — | — | — |
| Ethers | Di-n-butyl ether | 0.0122 | 0.0064 | 0.0040 | 0.0047 | 0.0075 | 0.0059 | — | 0.0133 | 0.0046 | 0.0121 |
| Heterocycles | 2-Pentylfuran | — | — | — | — | 0.0067 | 0.0125 | — | — | — | 0.0067 |
| Total content | 1.7762 | 0.5593 | 0.5768 | 2.59 | 3.3469 | 0.6287 | 0.7813 | 0.4807 | 0.472 | 2.9096 |

*The values are given with 3-nonanone as the internal standard.*

*Volatile components and contents were measured as three technical replicates each with three independent biological replicates.*

*Not found or not exist.*
Supplemental Table 2. Volatile compounds and their contents in fruit from three *Malus prunifolia* accessions by static headspace and gas chromatography–mass spectrometry.  

| Compounds                | Bai Haitang | Xiaohuang Haitang | Bian Haitang |
|--------------------------|-------------|-------------------|--------------|
|                          | (µg g⁻¹ 3-nonanone) |                  |              |
| **Alcohols**             |             |                   |              |
| Cyclohexanol             | —           | —                 | 0.0088       |
| 1-Hexanol                | 0.1602      | 0.2956            | 0.1684       |
| 1-Butanol                | 0.0129      | —                 | —            |
| 2-Methyl-1-butanol       | 0.0303      | 0.0127            | —            |
| 1-Pentanol               | 0.0031      | —                 | —            |
| 3-Nonanol                | —           | 0.0088            | 0.0131       |
| 2-Methyl-5-(1-methylethenyl)-cyclohexanol | 0.0040 | —                 | —            |
| **Esters**               |             |                   |              |
| Methyl butanoate         | 0.0465      | —                 | 0.0097       |
| Methyl 2-methylbutanoate | 0.0573      | —                 | 0.0250       |
| Methyl hexanoate         | 0.0689      | —                 | 0.0239       |
| Methyl 5-acetyl-2-methoxybenzoate | 0.0062 | —                 | —            |
| Ethyl propionate         | 0.0547      | —                 | 0.0080       |
| Ethyl hexanoate          | 0.4518      | 0.0662            | 0.2872       |
| Ethyl isobutyrate        | 0.0050      | —                 | —            |
| Ethyl pentanoate         | —           | —                 | 0.0092       |
| Ethyl 3-hexenoate        | —           | —                 | 0.0060       |
| Ethyl 2-butenoate        | —           | —                 | 0.0022       |
| Ethyl butanoate          | 0.5837      | 0.0498            | 0.1365       |
| Ethyl 2-methylbutyrate   | 0.4151      | —                 | 0.1153       |
| 1-Ethyl-2-methylbutyl acetate | 0.0039 | —                 | —            |
| Ethyl octanoate          | 0.0135      | —                 | 0.0113       |
| Ethyl heptanoate         | 0.0096      | —                 | 0.0120       |
| Ethyl tiglate            | —           | —                 | 0.0362       |
| Ethyl isohexanoate       | —           | 0.0028            | 0.0107       |
| Propyl butyrate          | 0.1138      | —                 | —            |
| Propyl hexanoate         | 0.0488      | —                 | —            |
| Propyl 2-methylbutyrate  | 0.0699      | —                 | —            |
| Isobutyl caproate        | 0.0022      | —                 | —            |
| Butyl butyrate           | 0.0527      | —                 | —            |
| Isobutyl butyrate        | 0.0110      | —                 | —            |
| Butyl 2-methylbutyrate   | 0.0483      | —                 | —            |
| (Z)-3-Hexenyl isobutyrate| —           | 0.0073            | —            |
| Hexyl hexanoate          | 0.0253      | 0.0039            | 0.0064       |
| Hexyl propionate         | 0.0020      | 0.0040            | —            |
| Hexyl butyrate           | 0.0578      | 0.0403            | 0.0211       |
| (2Z)-2-Hexenyl acetate   | —           | 0.0053            | —            |
| Hexyl acetate            | —           | 0.0241            | —            |
| Hexyl 2-methylbutyrate   | 0.0412      | 0.0104            | 0.0119       |
| Cyclobutanecarboxylic acid, hexyl ester | — | — | 0.0037 |
| (4E)-4-Hexenyl acetate   | —           | 0.0450            | —            |
| Isopentyl 2-methylbutanoate | 0.0137 | 0.0030            | —            |
| Amyl hexanoate           | 0.0051      | —                 | —            |
| (Z)-3-Hexen-1-ol acetate | —           | 0.0082            | —            |
| (2E)-2-Hexenyl butyrate  | 0.0098      | 0.0233            | 0.0107       |
| **Aldehydes**            |             |                   |              |
| Hexanal                  | —           | 0.0216            | —            |
| 2-Hexenal                | —           | 0.0769            | —            |
| Decanal                  | 0.0035      | —                 | —            |
| Terpenes                 | —           | —                 | —            |
| α-Curcumene              | 0.0034      | 0.0127            | 0.0138       |
| α-Farnesene              | 0.5465      | 0.5262            | 0.4233       |
| Estragole                | —           | 0.0120            | —            |
| Ylangene                 | —           | —                 | 0.0035       |

Continued next page
Supplemental Table 2. Continued.

| Compounds          | Bai Haitang (μg g⁻¹ 3-nonanone) | Xiaohuang Haitang | Bian Haitang |
|--------------------|---------------------------------|-------------------|--------------|
| Hydrocarbons       |                                 |                   |              |
| Fluorene           | 0.0103                          | —                 | —            |
| Ketones            | 0.0029                          | —                 | —            |
| 6-Methyl-5-hepten-2-one | 0.0024                      | —                 | —            |
| Carboxylic acids   |                                 |                   |              |
| 2-Methyl-butanoic acid | 3.007                        | 1.2513            | 1.4167       |

The values are given with 3-nonanone as the internal standard.

Volatile components and contents were measured as three technical replicates each with three independent biological replicates.

Not found or not exist.