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Taxonomic analysis of volatiles emitted by ornamental crabapple flowers

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To identify and classify volatile compounds in the fragrances of crabapple flowers from ornamental and original species in the peak April Tai'an, Shandong flowering season. Volatile components from flowers were identified using dynamic-headspace sampling, purge and trap and GC/MS. Eighty compounds were detected in the 17 ornamental crabapple cultivars, while sixty-eight compounds were found in the nine original species, including forty-four compounds in both the original species and cultivars. The main volatile components and common components of crabapple flower aroma include: 3-methyl-1-butanol, ocimene, benzyl alcohol, 3-methyl-4-oxo-pentanoic acid and heptane. Based on the relative volatile content the 26 taxa could be classified into seven different groups through UPGMA cluster analysis. The different volatile contents, such as myrcene and benzaldehyde, result in the extraordinary aroma of different crabapple varieties. *M.* 'Dolgo', *M.* 'Eleyi', *M.* 'Hopa', *M.* 'Liset', *M.* 'Makamik' and *M.* 'Royalty' are good breeding varieties of sweet flowering crabapples which could be used to breed additional ornamental cultivars with excellent fragrance and high value.

1. Introduction

Crabapple is the designation given to a small diameter wild or half-wild plant in the apple genus, which has high anti-adversity, adaptability and close affinity with the main cultivars. The crabapple fruit is commonly used for processing or preserving jellies or wine [16]. Crabapples also have a long history as an ornamental plant, and fragrance is one essential factor in the evaluation of the ornamental value of crabapple varieties.

With the development of gas chromatography–mass spectrometry (GC–MS), many analytical tests have been conducted on the fragrances of different plants around the world [1,18,7,17,3]. In *Michelia Alba*, (White Sandalwood) the typical fragrance compounds are linalool, germacrene-D, 2-methylbutanoic acid methyl ester, elemene, 2-phenyl ethyl alcohol and ocimene [13]. The compositions of the essential oils of the leaves and flowers of *Tithonia diversifolia* (Hemsl) A. Gray, the Mexican sunflower, have been reported to be due to germacrene-D (20.3%), b-caryophyllene (20.1%) and bicyclogermacrene (8.0%) in the flower oil [14]. Study of the regulation of scent production and emission in *Rosa hybrida* cv. demonstrates the complexity of the diurnal regulation of scent emission: though the daily emission of most scent compounds is synchronized, various independently evolved mechanisms control the production, accumulation and release of different volatiles [8]. To date there has been no report on the volatile compounds in crabapple fragrance. Here we report an investigation of the volatile compounds of *Malus* flowers using GC–MS. We selected varieties which contain special fragrances, thus indicating new breeding materials for the apple genus. As a second aspect of the research, the ornamental crabapple varieties were classified by volatile compound UPGMA cluster analysis.

2. Materials and methods

2.1. Plant materials

The ornamental crabapples (*Malus* sp.) were from the ornamental orchard in Shandong Agriculture University, China. The ornamental crabapples are grafted onto *M. robusta*, irrigated and well maintained. For each variety, three trees were selected and 12 individual flowering stems per tree were randomly collected from all orientations. Flowering stems were obtained when flowers were characterized by rapid petal elongation and a high level of scent emission. Flowering stems were brought into the laboratory and cultivated in water. The varieties (Table 1) were sampled separately to avoid fragrance contamination. The flower volatile compound analysis was carried out within 24 h.

2.2. GC–MS analysis

For fragrance analysis, 2.0 g fresh flowers from each variety were placed in a 22 ml headspace vial. The vial was placed into the Perkin–Elmer HS-40XL headspace sampler and thermostated

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Table 1
Crabapple varieties used in this study.

No variety	No variety
Y1 *M. baccata* (L.) Borkh	P5 *M.*'Eleyi'
Y2 *M. coronaria* (L.) Mill.	P6 *M.*'Evereste'-a
Y3 *M. domestica* subsp.Chinensis var.binzi Li Y.N.	P7 *M.*'Flame'
Y4 *M. domestica* subsp.Chinensis var.xiangoeLi Y.N.	P8 *M.*'Evereste'-b
Y5 *M. florentina* (Zuccagni) Siebold.	P9 *M.*'Golden Hornet'
Y6 *M. halliana* Koehne	P10 *M.*'Hopa'
Y7 *M. kirghisorum* Al.Fed.	P11 *M.*'Indian Magic'
Y8 *M. platycarpa* Rehder.	P12 *M.*'Indian summer'
Y9 *M. prattii* (Hemsl.) Schneid.	P13 *M.*'Jewelberry'
P1 *M.* 'Adirondack'	P14 *M.*'Kelsey'
P2 *M.* 'Dolgo'	P15 *M.*'Liset'
P3 *M.* 'Dongbai'	P16 *M.*'Makamik'
P4 *M.* 'Donghong'	P17 *M.*'Pink Spir

Y: original species; P: cultivars.

Table 2
Perkin–Elmer HS 40XL equilibrium headspace sampler conditions.

Carrier gas	Helium	Transfer line temp.	160 °C
Carrier pressure	10 psi	Thermostat time	20 min
Sample shaker	On	Pressurize time	1.0 min
High pressure sampling	35 psi	Injection time	0.15 min
Oven temperature	35 °C	Withdraw time	0.4 min
Needle temperature	150 °C	Backflush	Off

under the conditions listed in Table 2. The vapors in the headspace were transferred directly into the GC column for chromatographic separation and detection with the SHIMADZU Q2010 Gas Chromatograph interfaced to the SHIMADZU Q2010 Mass Spectrometer. Tables 3 and 4 present the column conditions and GC/MS conditions for this application.

Spectrometric data was compared with the NIST original library mass-spectra and Wiley library, combined with manual resolution of mass spectra and relative reports. Only results identified with positive and negative matching values more than 800 of 1000 were reported in this study. From the UPGMA cluster analysis (DPS2000), the taxa were classified according to the relative content of volatile compounds.

3. Results

3.1. Analysis of volatile compounds of ornamental crabapple flower fragrance

We identified a total of 104 compounds from the samples of *M.* 'Adirondack', 17 other ornamental crabapple cultivars and nine original species (Table. 1). The volatiles consisted of a blend of twenty-eight esters, twenty-six alcohols, twenty-four straight-chain alkanes, nine alkenes, seven acids, five ketones, three aromatic hydrocarbons and two aldehydes. Volatiles showed high variation among species and cultivars. For names and the relative

Table 3
SHIMADZU Q2010 gas chromatographic conditions.

Column	rtx-1, 30 m × 0.25 mm ID × 0.25 μm film thickness
Carrier	Helium, constant flow at 1.06 mL/min
Injector temperature	200 °C, PSS (programmable split/splitless) with narrow bore liner
Oven temperature	35°C for 2 min, 8 °C/min to 180 °C, hold for 4 min, then 15°C /min to 230°C, hold for 1 min
Split flow	10 mL/min

Table 4
SHIMADZU Q2010 mass spectrometer conditions.

Mode	EI
Electron energy	70 eV
Ion-source temperature	200 °C
Filament current	0.25 mA
Scan rate	Range: 45–450 amu, Scan time: 0.20 s, Inter-scan 0.05 s

contents of compounds, eighty compounds were detected in flowers of the 17 ornamental crabapple cultivars, while sixty-eight compounds were found in the flowers of the nine original species, including forty-four compounds detected both in the original species and the cultivars.

As shown in Table 5, all flowers of ornamental crabapple cultivars contained 3-methyl-1-butanol, benzyl alcohol, 3-methyl-4-oxo-pentanoic acid, heptane, ocimene and nonane. The highest relative content of 3-methyl-1-butanol was in *M.* 'Red Splendor' (32.93%) and the lowest was in *M.* 'Dolgo' (1.27%). The highest relative content of benzyl alcohol was observed in *M.* 'Dolgo' (39.12%) and the lowest in *M.* 'Professor Sprenger' (0.91%). The relative content of 3-methyl-4-oxo-pentanoic acid was highest in *M.* 'Professor Sprenger' (38.88%) and lowest in *M.* 'Red Splendor' (0.1%). The relative content of heptane was highest in *M.* 'Strawberryparifit' (11.07%) and lowest in *M.* 'Makamik' (1.00%). The relative content of ocimene was highest in *M.* 'Golden Hornet' (35.67%) and lowest in *M.* 'Makamik' (4.32%), while the relative content of nonane was highest in *M.* 'Strawberryparifit' (5.32%) and lowest was in *M.* 'Makamik' (0.20%).

All flower fragrances of the nine original species contained 3-methyl-1-butanol, 1-hexanol, linalol, benzyl alcohol, 2.4-hexadiene, ocimene, 3-methyl-4-oxo-pentanoic acid, heptane and nonane. The highest relative content of 3-methyl-1-butanol was in *M. halliana* Koehne (9.26%) and the lowest was in *M. prattii* (Hemsl.) Schneid. (0.60%). The highest relative content of 1-hexanol was in *M. platycarpa* Rehder. (11.54%) and lowest was in *M. sieversii* Reom. (0.90%). The highest relative content of linalol was in *M. halliana* Koehne (7.01%) and the lowest was in *M. florentina* Siebold.(1.00%). The highest relative of benzyl alcohol was in *M. sieversii* Reom. (71.95%) and the lowest was in *M. florentina* Siebold. (2.50%). The highest relative content of 2.4-hexadiene was in *M. florentina* Siebold. (4.40%) and the lowest was in *M. sieversii* subsp. *Xinjinensis* Y. N. Li (0.70%). The highest relative content of ocimene was in *M. florentina* Siebold. (20.11%) and the lowest was in *M. sieversii* Reom. (1.40%). The highest relative content of 3-methyl-4-oxo-pentanoic acid was in *M. coronaria* (L.) Mill. (15.51%) and the lowest was in *M. sieversii* Reom. (4.43%). The highest relative content of heptane was in *M. florentina* Siebold. (6.03%), and the lowest in *M. sieversii* Reom. (0.48%). The highest relative content of nonane was in *M. florentina* Siebold. (10.31%) and the lowest in *M. prattii* (Hemsl.) Schneid. (1.20%). Amongst the 104 volatiles identified, 3-methyl-1-butanol, benzyl alcohol, heptane, nonane, and ocimene were common to all fresh flowers. The variety of different volatile compounds, including benzaldehyde and myrcene, produced an extraordinary range of aromas in the different varieties of crabapple tested in this study.

3.2. Flower fragrance taxonomic analysis

Differences were observed in the numbers and abundance of the compounds emitted by the flowers from the original species and cultivars. Based on the relative content of the volatiles from the UPGMA cluster analysis, the 26 taxa could be classified into seven groups.