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Investigation of Volatile Organic Compounds in Aromatic *Phalaenopsis* Cultivars Using Gas Chromatography–Mass Spectrometry

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Abstract. ***Phalaenopsis*** **is a globally popular potted plant possessing a few aromatic cultivars, but analysis of volatile organic compounds (VOCs) in these cultivars is limited. Here, using nonaromatic cultivar** ***Phal.*** **Big Chili as a control, flower VOCs of four aromatic cultivars were investigated by headspace solid-phase microextraction in conjunction with gas chromatography–mass spectrometry (GC-MS). The results revealed that 43 VOCs classified into seven categories were identified in the nonaromatic** ***Phal.*** **Big Chili and four aromatic cultivars. Hexyl acetate and hexan-1-ol were common VOCs in aromatic cultivars. On the basis of partial least squares discriminant analysis, the five cultivars were classified into three groups, the nonaromatic** ***Phal.*** **Big Chili (group 1) and the strong-aromatic** ***Phal.*** **Cherry Tomato (group 2) were easily distinguished from the other three aromatic cultivars (group 3). Moreover, 17 key VOCs with the different aromatic thresholds and characteristics were identified in the four aromatic cultivars, and the types and relative contents of key VOCs varied among the aromatic cultivars, resulting in different characteristics and intensities of floral fragrance in aromatic cultivars. In aromatic cultivars, the types and relative contents of key VOCs in** ***Phal.*** **Cherry Tomato significantly exceeded those in the other three cultivars. Eight key VOCs belonging to terpenoids, olefins, and alcohols had the highest relative contents in** ***Phal.*** **'Cherry Tomato', which led to a strong and mixed aromatic type containing cedarwood, camphor, and mint fragrances.**

Phalaenopsis, indigenous to tropical regions spanning from Asia to Australia, boasts more than 70 native species categorized into four subgenera, some of which exhibit aromatic traits (Hsiao et al. 2008a). Renowned for its captivating flower posture and long flowering period, *Phalaenopsis* has been the most popular orchid and the leading potted ornamental plant in the global market (Hsu et al. 2018). Commercial *Phalaenopsis* cultivars are hybrid offspring derived from the original species. In crossbreeding, selection of large-flowered cultivars with diverse colorations is the main aim, while aromatic cultivars have received limited attention. Additionally, the genetic stability of floral fragrance degenerates during artificial crossbreeding, leading to a scarcity of *Phalaenopsis* cultivars that retain the aromatic trait in the current market (Hsu et al. 2018). Nevertheless, the fragrance emitted by flowers represents a fundamental characteristic of ornamental plants, enriching their value, product quality, and economic significance (Meng et al. 2021). Thus, the selection and breeding of aromatic *Phalaenopsis* have emerged as prevailing trends in contemporary breeding (Ahmad et al. 2020; Hsu et al. 2018).

Floral fragrance encompasses volatile organic compounds (VOCs) that serve various functions, such as repelling herbivores (Pichersky and Gershenzon 2002; Unsicker et al. 2009), combating pathogens (Arimura et al. 2004), warding off insects (Li et al. 2017), attracting pollinators (Dudareva et al. 2004), communicating with other plants (Raguso 2008; Schiestl 2010), and even captivating tourists (de Vega et al. 2014). Previous studies revealed that specific VOCs of flowers led to distinct fragrance types among different *Phalaenopsis* cultivars. Van der Pijl and Dodson classified the floral fragrances of *Phalaenopsis* into rose-scented, aromatic-scented, sweet-scented, and spicy-scented types (Hsiao et al. 2011). Until now, the investigation of VOCs in aromatic *Phalaenopsis* mainly focused on native species. For instance, the main VOCs of light-scented *Phal. schilleriana* were neryl acetate, nerol, citronellol, and citronellyl acetate (Awano et al. 1997), whereas the key VOCs of strong-scented *Phal. Bellin* and *Phal. Violacea* were linalool, geraniol, and their derivatives (Hsiao et al. 2008b). However, the reports of VOC analysis in aromatic *Phalaenopsis* cultivars were limited.

To explore the diversity of VOCs and identify the key VOCs in different aromatic *Phalaenopsis* cultivars, we investigated VOCs of four aromatic cultivars in *Phalaenopsis* through gas chromatography–mass spectrometry (GC-MS) by using nonaromatic cultivar *Phal.* Big Chili as a control. Then the obtained VOC data were subjected to analysis using partial least squares discriminant analysis (PLS-DA) combined with heat map. Our investigation will be beneficial to explore the metabolic pathways of aromatic compounds in *Phalaenopsis* cultivars and will also provide valuable information for crossbreeding of aromatic *Phalaenopsis*.

Materials and Methods

Plant materials. Five cultivars of *Phalaenopsis* were obtained from Yuxi Yunxing Biotechnology Co., Ltd. (Yunnan, China). The nonaromatic *Phal*. Big Chili was used as a control, and the other four cultivars with

Table 1. Flower characteristics and aromatic intensity of five *Phalaenopsis* cultivars.

Cultivar name	*Phal.* Big Chili	*Phal.* Purple Butterfly	*Phal.* Tzu Chiang Balm	*Phal.* Peter's Pride	*Phal.* Cherry Tomato
					
Height (cm)	9.0 ± 0.7	5.0 ± 0.2	3.9 ± 0.3	5.7 ± 0.4	4.1 ± 0.1
Width (cm)	11.3 ± 0.4	6.1 ± 0.2	4.6 ± 0.5	6.4 ± 0.4	4.6 ± 0.3
Aromatic intensity	Nonaromatic	*	**	***	****

The level of aromatic intensity is represented by the number of asterisks (*)—the more asterisks, the stronger the aromatic intensity.

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different aromatic characteristics are shown in Table 1. Among the four aromatic cultivars, *Phal.* Cherry Tomato possessed the strongest aromatic intensity, whereas *Phal.* Purple Butterfly exhibited the lightest aromatic intensity. Flowers on the fifth day of blooming were collected at 10:00 AM for investigation of VOCs.

Sample collection and headspace solid-phase microextraction. A single blooming flower was picked from the *Phalaenopsis* plant, and 1 g of fresh flower was immediately put into 20-mL solid-phase microextraction (SPME) bottles with a 20-mm opening, then sealed using PTFE/silicone septum and an aluminum gland (Thermo Fisher Scientific, Waltham, MA, USA). Three independent biological replicates were used for each *Phalaenopsis* cultivar.

For headspace SPME (HS-SPME) extraction, a manual SPME injector (Supelco, Bellefonte, PA, USA) equipped with 75-μm polydimethylsiloxane SPME fibers was carefully inserted into the injection port of GC-MS system (Trace GC Ultra/ITQ 900, Thermo Fisher Scientific). The fiber was thermally conditioned at 250 °C for 30 min. One microliter of ethyl caprate (CAS No. 110-38-3) with a concentration of 1‰ (v/v) was added to the SPME bottle containing the flower as an internal standard substance. The bottle was promptly capped, and the SPME fiber was inserted into the capped vial with the fiber positioned 1 cm above the flower. The floral compounds were adsorped at 30 °C for 30 min (Xiao et al. 2020).

GC-MS analysis. Once the adsorption process was complete, the SPME fiber was carefully withdrawn and inserted into the GC-MS injection port for desorption at 250 °C for 1 min. Subsequently, GC-MS was employed to collect data. In GC phase, a capillary column of HP-5MS (30 m × 250 μm × 0.25 μm; Agilent J&W, Santa Clara, CA, USA) was used, with helium (99.999%) serving as the carrier gas at a flow rate of 1.0 mL/min. The split ratio was set at 10:1. The heating program was executed at an injection port temperature of 250 °C and initial column temperature of 40 °C. The temperature was then gradually increased to 80 °C at a rate of 3 °C/min, followed by a further increase to 250 °C at a rate of 5 °C/min, and the conditions were held for 5 min. MS conditions were as follows: ionization source with an ionization energy, 70 eV; ion source temperature, 230 °C; quadrupole temperatures, 150 °C; scan mass, 35 to 450 amu.

Data analysis. VOCs (initial threshold of peak ≥21.5) were identified by comparing their mass spectra to the National Institute of Standard and Technologies (NIST) database (matching rate ≥90%). The retention indices of VOCs were then calculated relative to the n-alkanes (C6–C40) and compared with those of VOCs in the NIST online database (https://webbook.nist.gov/chemistry/cas-ser.html) to confirm identification of VOCs. The concentration of each VOC was calculated using the following formula (Xie et al. 2023):

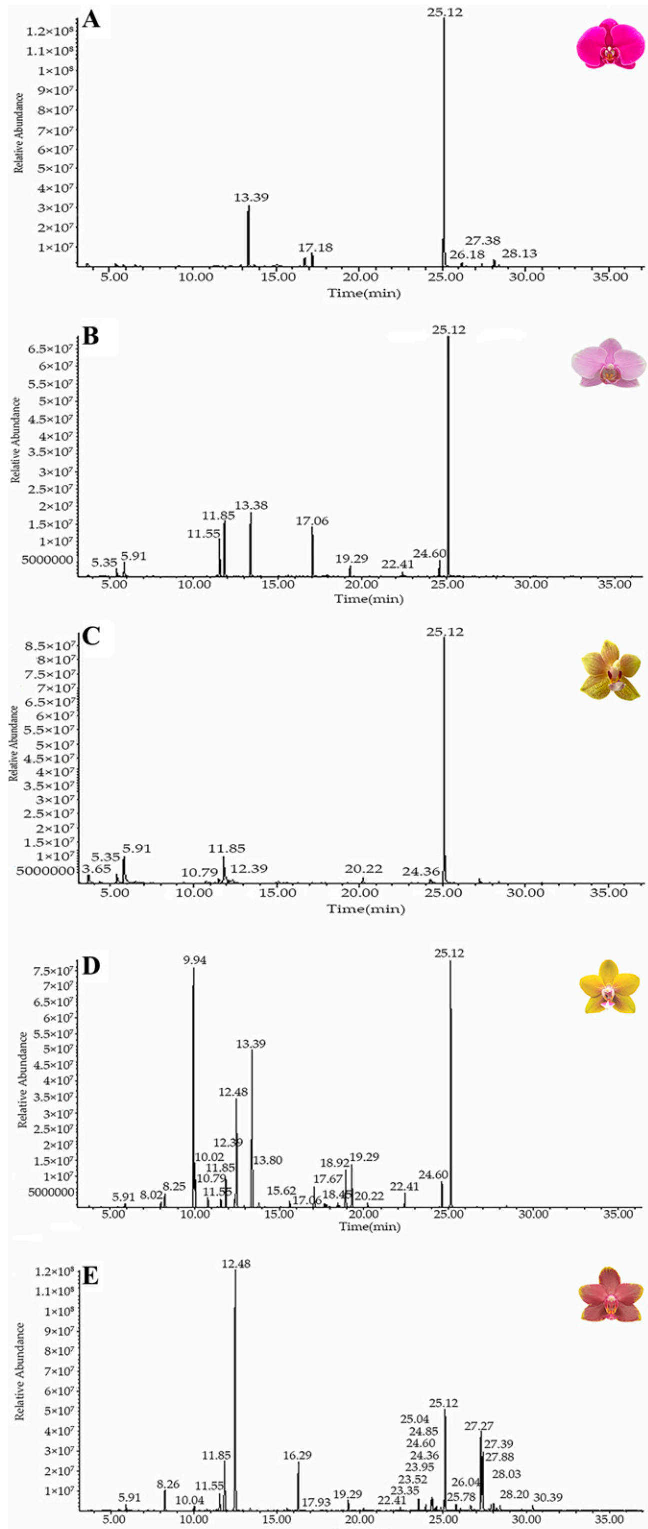

Fig. 1. The gas chromatography–mass spectrometry total ion chromatograms of volatile organic compounds (VOCs) in five *Phalaenopsis* cultivars: (**A**) Big Chili, (**B**) Purple Butterfly, (**C**) Tzu Chiang Balm, (**D**) Peter's Pride, and (**E**) Cherry Tomato. The peak at RT 25.12 is the internal standard (ISTD), ethyl caprate.

$$\text{VOC concentration}\,(\text{ng}\cdot\text{g}^{-1}) = \frac{\text{peak area of VOC}}{\text{peak area of internal standard}} \times \frac{\text{concentration of internal standard}\,(\text{ng}\cdot\mu\text{L}^{-1}) \times \text{volume of internal standard}\,(\mu\text{L})}{\text{Sample Weight}}$$

All data were analyzed by using Microsoft Excel 2021. TBtools software was employed to calculate eigenvector loading values for hierarchical cluster analysis, and MetaboAnalyst (www.metaboanalyst.ca) was used for PLS-DA analysis. Aroma characteristics and aroma types were obtained from The Good Scents company web database (www.thegoodscentscompany.com), and aroma thresholds were collected from the literature (Du et al. 2022; Jagella and Grosch 1999; Nie et al. 2020; Ong and Acree 1999; Padrayuttawat et al. 1997; Pino and Quijano 2012; Sun et al. 2014; Xiao et al. 2021; Zhang et al. 2022).

Results

Identification of VOCs. To identify VOCs in *Phalaenopsis*, the nonaromatic cultivar *Phal.* Big Chili and four aromatic cultivars were subjected to GC-MS analysis (Fig. 1). A total of 43 VOCs were identified in five *Phalaenopsis* cultivars (Table 2), but the VOC number in these cultivars differed. In the control of nonaromatic *Phal.* 'Big Chili', only five VOCs were detected. In light-aromatic *Phal.* 'Purple Butterfly' and *Phal.* 'Tzu Chiang Balm', more compounds were detected, with nine and eight VOCs identified, respectively. The number of VOCs was increased significantly in strong-aromatic cultivars; 21 and 23 VOCs were identified in *Phal.* Peter's Pride and *Phal.* Cherry Tomato, respectively.

In addition to the difference in the number of VOCs the types and concentrations of main VOCs were also different in five *Phalaenopsis* cultivars. In nonaromatic *Phal.* 'Big Chili', (Z)-β-ocimene exhibited the highest concentration (26.03 $ng\cdot g^{-1}$, Table 2). Hexyl acetate was the most abundant VOC in light-aromatic *Phal.* 'Purple Butterfly' and *Phal.* 'Tzu Chiang Balm', with respective concentrations of 97.34 $ng\cdot g^{-1}$ and 89.02 $ng\cdot g^{-1}$ (Table 2). In strong-aromatic *Phal.* 'Peter's Pride', sabinene exhibited the highest concentration at 1237.2 $ng\cdot g^{-1}$, followed by (Z)-β-ocimene at 648.55 $ng\cdot g^{-1}$ and 1,8-cineole at 497.68 $ng\cdot g^{-1}$ (Table 2). Similarly, in strong-aromatic *Phal.* Cherry Tomato, 1,8-cineole showed the highest concentration at 1242.77 $ng\cdot g^{-1}$ (Table 2). Additionally, γ-muurolene (405.26 $ng\cdot g^{-1}$), (+)-germacrene-D (273.28 $ng\cdot g^{-1}$), and hexyl acetate (225.29 $ng\cdot g^{-1}$) also present in notable concentrations (Table 2).

Moreover, we found that hexyl acetate and hexan-1-ol were detected in four aromatic cultivars at the same time but absent in nonaromatic cultivar *Phal.* Big Chili. These two VOCs were believed to contribute to the floral fragrance.

The preceding analysis indicates that, compared with nonaromatic *Phalaenopsis*, the aromatic cultivars exhibit a greater diversity types and higher concentrations of VOC, which led to their distinct fragrance profiles.

Classification of VOCs. Forty-three VOCs identified in five *Phalaenopsis* cultivars could be classified into seven categories: terpenes,

Table 2. Volatile organic compounds (VOCs) and their concentrations in the five *Phalaenopsis* cultivars.

				Retention index		VOC concentrations ($ng\cdot g^{-1}$)				
No.	Family	VOCs	RT (min)	HP-5	NIST	*Phal.* Big Chili	*Phal.* Purple Butterfly	*Phal.* Tzu Chiang Balm	*Phal.* Peter's Pride	*Phal.* Cherry Tomato
1	Terpene	(-)-β-caryophyllene	25.78	1423	1419	nd	nd	nd	nd	27.22
2		γ-terpinene	13.80	1056	1060	nd	nd	nd	79.86	nd
3		β-myrcene	10.79	985	991	nd	nd	34.95	67.99	nd
4		β-pinene	10.04	967	979	nd	nd	nd	nd	20.70
5		β-cadinene	27.88	1505	1518	nd	nd	nd	nd	37.58
6		β-elemene	25.04	1395	1391	nd	nd	nd	nd	52.10
7		α-curcumene	27.38	1486	1483	1.61	nd	nd	nd	nd
8		α-thujene	8.02	916	929	nd	nd	nd	20.97	nd
9		(Z)-β-ocimene	13.39	1047	1038	26.03	nd	nd	648.55	nd
10		Sabinene	9.94	964	974	nd	nd	nd	1237.2	nd
11		Copaene	24.60	1379	1376	nd	32.66	nd	193.79	19.93
12		(-)-β-bourbonene	24.85	1388	1384	nd	nd	nd	nd	15.25
13		γ-muurolene	27.27	1481	1477	nd	nd	nd	nd	405.26
14		D-limonene	12.39	1024	1018	nd	nd	14.52	89.29	nd
15		2,6-dimethyl-2,4,6-octatriene	17.18	1142	1144	9.59	nd	nd	nd	nd
16		(+)-α-pinene	8.26	922	929	nd	nd	nd	76.11	99.92
17		(1S,5S)-4,6-dimethyl-6-(4-methylpent-3-enyl) bicyclo[3.1.1]hept-3-ene	26.18	1439	1435	4.62	nd	nd	nd	nd
18		(E)-β-ocimene	13.38	1047	1037	nd	85.07	nd	nd	nd
19		(-)-β-pinene	10.02	966	943	nd	nd	nd	245.35	nd
20	Terpene	rel-(1R,2S,6S,7S,8S)-8-isopropyl-1-methyl-3-methylenetricyclo [4.4.0.02,7]decane	26.04	1433	1432	nd	nd	nd	nd	4.95
21		(+)-cyclosativene	24.36	1370	1368	nd	nd	13.06	nd	58.89
22		(+)-germacrene-D	27.39	1486	1481	nd	nd	nd	nd	273.28
23	Alcohol	Guaiol	30.39	1611	1596	nd	nd	nd	nd	96.67
24		α-terpineol	18.92	1190	1189	nd	nd	nd	125.92	nd
25		Citronellol	20.22	1230	1228	nd	nd	63.91	50.84	nd
26		Hexan-1-ol	5.91	850	868	nd	20.66	67.85	15.10	24.89
27		1,8-cineole	12.48	1026	1032	nd	nd	nd	497.68	1242.77
28		4-terpineol	18.45	1177	1177	nd	nd	nd	30.20	nd
29		3-hexen-1-ol	5.36	831	856	nd	10.48	24.43	nd	nd
30		Cis-3-nonen-1-ol	17.67	1155	1156	nd	nd	nd	23.17	nd
31	Olefin	(E)-4,8-dimethyl-1,3, 7-nonatriene	16.29	1117	1116	nd	nd	nd	nd	190.21
32		(+/−)-δ-elemene	23.52	1340	1338	nd	nd	nd	nd	62.36
33	Alkane	Dodecane	19.29	1200	1200	nd	23.28	nd	175.13	41.85
34		Tridecane	22.41	1300	1300	nd	5.72	nd	54.13	13.72
35		Undecane	15.62	1099	1100	nd	nd	nd	33.83	nd
36	Ester	Hexyl Acetate	11.85	1011	1011	nd	97.34	89.02	163.08	225.29
37		(3Z)-hex-3-en-1-yl acetate	11.55	1004	1005	nd	47.50	nd	49.84	68.40
38		Citronellol acetate	23.95	1356	1354	nd	nd	nd	nd	22.24
39	Aldehyde	Hexanal	3.65	800	800	nd	nd	36.25	nd	nd
40	Others	2,6-di-tert-butyl-4-metylphenol	28.13	1516	1513	4.90	nd	nd	nd	nd
41		phenylacetonitrile	17.06	1138	1144	nd	76.97	nd	160.64	nd
42		(+)-δ-cadinene	28.03	1512	1524	nd	nd	nd	nd	27.92
43		(+)-γ-cadinene	28.20	1519	1513	nd	nd	nd	nd	15.33

The retention time (RT) for the ISTD (ethyl caprate) was 25.12. nd = the substance was not detected in the analysis.

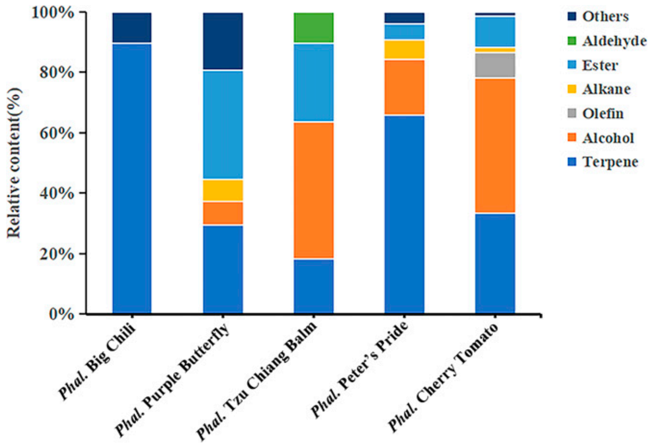

Fig. 2. Relative contents of seven categories of volatile organic compounds in five *Phalaenopsis* cultivars.

alcohols, esters, olefins, aldehydes, alkanes, and others. Terpenes were detected in all five cultivars, whereas alcohols and esters were only found in the four aromatic cultivars (Fig. 2).

The relative contents of these seven categories varied with different cultivars (Fig. 2). In aromatic cultivars *Phal.* Tzu Chiang Balm and *Phal.* Cherry Tomato, alcohol VOCs showed the highest relative content, accounting for 45.41% and 44.78%, respectively (Fig. 2). Ester VOCs possessed highest relative content (36.24%) in aromatic cultivar *Phal.* Purple Butterfly (Fig. 2). Moreover, the strong-aromatic *Phal.* Cherry Tomato contained the most abundant categories, including six categories except aldehydes VOCs, which contributed to its distinct aroma.

Stoichiometric analysis of VOCs. According to a PLS-DA of 43 VOCs, the five cultivars included in this study were divided into three groups (Fig. 3A). The nonaromatic *Phal.* Big Chili (group 1) and strongest aromatic *Phal.* Cherry Tomato (group 2) were easily distinguished from the other three aromatic cultivars (group 3).

Combining the position guidance of the three groups in score plot (Fig. 3A) and the relative content heat map of 43 VOCs (Fig. 4), VOCs determining groups 1 and 2 classification were identified in a loading plot (Fig. 3B). In the nonaromatic *Phal.* Big Chili (group 1), four VOCs—(7) α-curcumene, (15) 2,6-dimethyl-2,4,6-octatriene, (17) (1S,5S)-4, 6-dimethyl-6 -(4-methylpent-3-enyl) bicyclo [3.1.1]hept-3-ene, and (40) 2,6-di-tert-butyl -4-methylphenol—distinguished this cultivar from the four aromatic cultivars (Fig. 3B). Seven VOCs distinguished the strongest aromatic *Phal.* Cherry Tomato (group 2) from nonaromatic *Phal.* Big Chili (group 1) and the other three aromatic cultivars (Fig. 3B). The four VOCs in the nonaromatic *Phal.* 'Big Chili' (group 1) and seven VOCs in strongest aromatic *Phal.* Cherry Tomato (group 2) showing high relative contents belonged to clusters 1-1 and 2-1, respectively (Fig. 4). Although the VOCs distinguishing group 3 from other groups was not identified clearly, the three aromatic cultivars in group 3 had some VOCs in common, and thus they were categorized into one group.

Analysis of key VOCs. In the PLS-DA model, the VOCs with variable importance in projection (VIP) values >1 were considered as the key VOCs. In 43 VOCs of five *Phalaenopsis* cultivars, 18 VOCs had VIP values >1 (Fig. 5). However, the key VOC 2,6-dimethyl-2,4,6-octatriene was found in only nonaromatic cultivar *Phal.* Big Chili, so there were 17 VOCs identified in the four aromatic cultivars. These 17 VOCs had different aromatic thresholds and characteristics (Table 3), which affected the type and intensity of floral fragrance in the four aromatic *Phalaenopsis* cultivars. The key VOCs with a low aromatic threshold were easily perceived olfactorily.

The types and relative contents of key VOCs varied among the different aromatic cultivars (Fig. 5). In the four aromatic cultivars, the types and relative contents of key VOCs in *Phal.* Cherry Tomato significantly exceeded than those in the other three aromatic cultivars. Among 17 key VOCs, eight belonging to terpenoids, olefins, and alcohols obtained the highest relative content in *Phal.* Cherry Tomato (Fig. 5), which led to a strong and mixed aromatic type containing cedarwood, camphor, and mint fragrances (Table 3). Five key VOCs—hexyl acetate, β-myrcene, (Z)-β-ocimene, phenylacetonitrile, and D-limonene—had the highest relative contents in *Phal.* Peter's Pride (Fig. 5), giving the flowers a strong aromatic mix of citrus, mint, and fruit fragrances (Table 3). *Phal.* Tzu Chiang Balm exhibited high relative contents of 3-hexen-1-ol, citronellol, hexan-1-ol, and hexanal, which contributed to a mixed aromatic type including green (herb), rose, citrus, and fruit fragrances. Although the relative contents of both 3-hexen-1-ol and phenylacetonitrile were high in *Phal.* Purple Butterfly (Fig. 5), phenylacetonitrilec had high aromatic threshold (1 mg·kg^{-1}, Table 3), and therefore *Phal.* Purple Butterfly mainly exhibited a green (herb) fragrance from 3-hexen-1-ol.

The preceding analysis indicates that the type, relative content, and aromatic threshold of key VOCs could determine the qualities and intensity of floral fragrance in aromatic *Phalaenopsis* cultivars.

Discussion

In the same genus, different aromatic species or cultivars possesses common or different VOCs. The common VOC categories of flowers (i.e., terpenes, alcohols, and esters) have been reported in *Cymbidium faber* (Omata et al. 1990), *Dendrobium* (Yang et al. 2022), and *Rosa* (Jiao et al. 2022) and were also detected in the native species *Phal. violacea* (Chuang et al. 2017), *Phal. bellina* (Mus et al. 2020), and the four aromatic *Phalaenopsis* cultivars studied here. In *Phalaenopsis*, the common VOCs of the native species *Phal. violacea* (Chuang et al. 2017) and *Phal. bellina* (Chuang et al. 2018) were linalool and geraniol, whereas that of the four aromatic *Phalaenopsis* cultivars in this study were hexyl acetate and hexan-1-ol. The VOC differences between native species and cultivars were also observed in *Freesia genus* (Bao et al. 2023), *Calanthe sylvatica* (Delle-Vedove et al. 2011), and *Chimonanthus praecox* (Meng et al. 2021), which might be related to allelic gene variations of fragrance biosynthesis (Bao et al. 2023).

There was also significant difference in the number of VOCs among different aromatic cultivars. The stronger the fragrance of the aromatic cultivars, the greater the number of VOCs in *Lilium* hybrid (Kong et al. 2017)

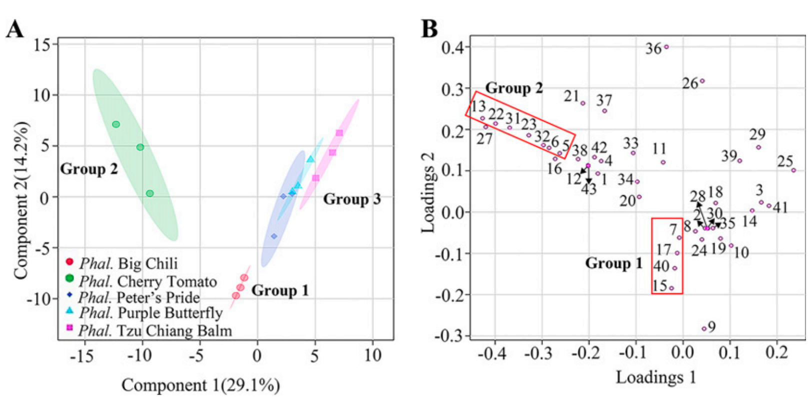

Fig. 3. Stoichiometric analysis of 43 volatile organic compounds (VOCs) in the five *Phalaenopsis* cultivars. (**A**) Score plot; (**B**) loading plot; numbers 1–43 indicate the 43 VOCs detected in the five *Phalaenopsis* cultivars listed in Table 2.

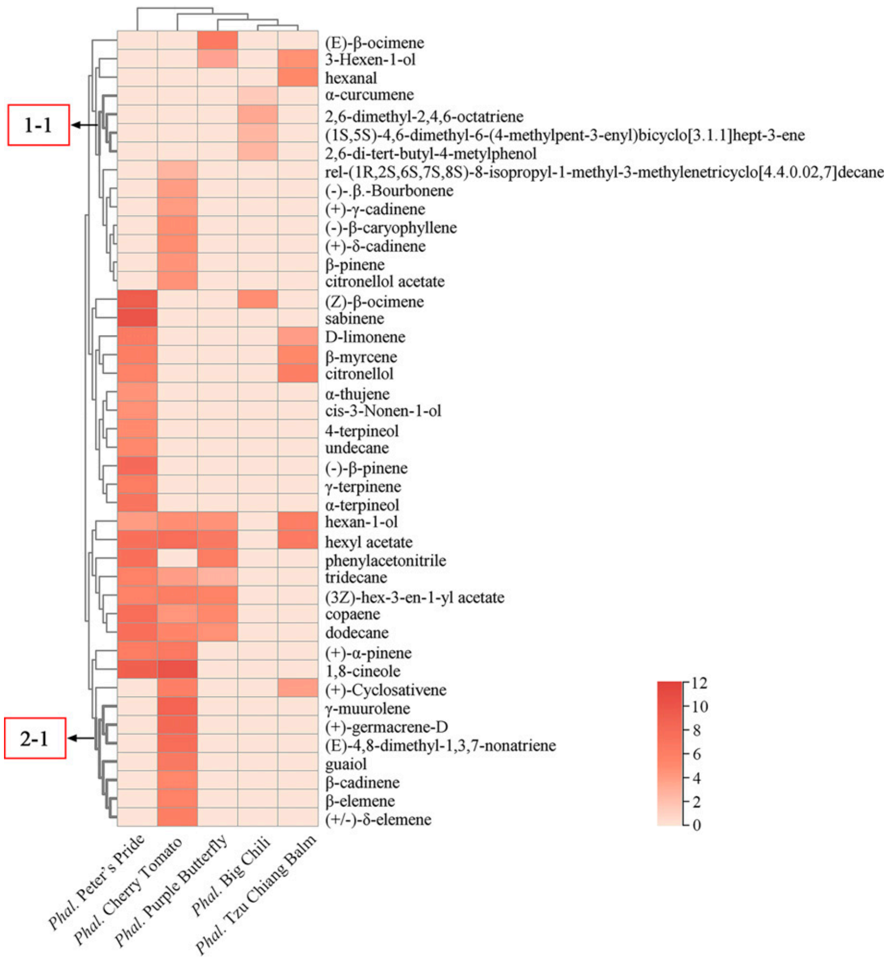

Fig. 4. The relative content heat map of volatile organic compounds (VOCs) in the five *Phalaenopsis* cultivars. The color bar indicates the relative contents of VOCs. The darker the color, the higher the relative content.

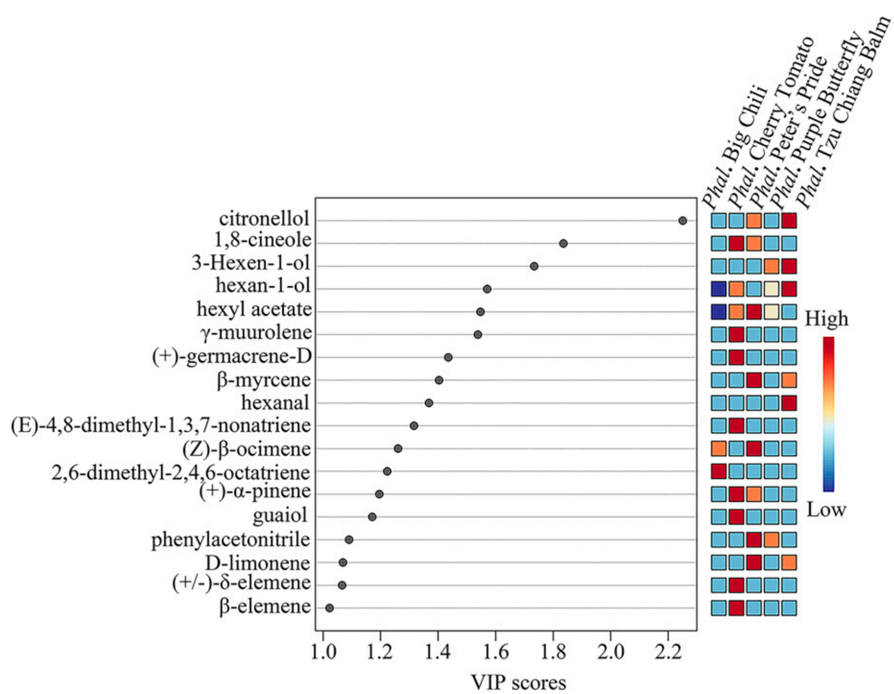

Fig. 5. Eighteen key volatile organic compounds (VOCs) in five *Phalaenopsis* cultivars determined by partial least squares discriminant analysis. The colored bar represents the relative contents of VOCs, the darker the color, the higher the relative content.

and *Paeonia lactiflora* (Song et al. 2018). In the present study, the number of VOCs in strong-aromatic *Phal.* Peter's Pride and *Phal.* Cherry Tomato studied here were 21 and 23, respectively, which was more than light-aromatic *Phal.* Purple Butterfly (nine VOCs), *Phal.* Tzu Chiang Balm (eight VOCs), and nonaromatic *Phal.* Big Chili (five VOCs). Furthermore, the same VOC exhibited different concentrations in the different cultivars. Here, the common aromatic VOC (Z)-β-ocimene was detected in strong-aromatic *Phal.* Peters Pride and nonaromatic *Phal.* Big Chili, but its concentration in *Phal.* Peter's Pride (678.55 $ng \cdot g^{-1}$) was significantly higher than that in *Phal.* Big Chili (26.03 $ng \cdot g^{-1}$). The different concentrations might be responsible for the difference in aromatic intensity in the two cultivars. Similarly, linalool and geraniol were identified in both the aromatic and nonaromatic *Phalaenopsis* species, but the concentrations of the two VOCs in the nonaromatic species were markedly lower than that in the aromatic species (Xiao et al. 2021).

Aromatic flowers possess various VOCs, but only the key VOCs determine the aromatic characteristics of flowers. In this study, 17 key VOCs were identified in the four aromatic cultivars via PLS-DA analysis. Eight key VOCs belonging to terpenoids, olefins, and alcohols obtained the highest relative content in strong-aromatic *Phal.* Cherry Tomato (Fig. 5), which led to the strong and mixed aromatic type containing cedarwood, camphor, and mint fragrances (Table 3). Among the eight key VOCs, 1,8-cineole and elemene were also identified as the key VOCs in *Phalaenopsis violacea* (Chuang et al. 2017; Xiao et al. 2020); (+)-germacrene-D, and (+)-α-pinene were found in some *Phalaenopsis* hybrids as well (Tong et al. 2023). Five key VOCs—hexyl acetate, β-myrcene, (Z)-β-ocimene, phenylacetonitrile, and D-limonene—had the highest relative contents in strong-aromatic *Phal.* Peter's Pride (Fig. 5), giving the flowers a strong and mixed aromatic type of citrus, mint, and fruit fragrances (Table 3). Among the five key VOCs, hexyl acetate, β-myrcene, (Z)-β-ocimene, and D-limonene were also common key VOCs in orchids (Ma et al. 2023), which has been reported in *Oncidium* (Chiu et al. 2017) and *Dendrobium* (Silva et al. 2015). However, phenylacetonitrile was not a common key VOC in orchids but was one of the key VOCs in *Hemerocallis* (Zhou et al. 2023). The light-aromatic *Phal.* Tzu Chiang Balm studied here exhibited high relative contents of 3-hexen-1-ol, citronellol, hexan-1-ol, and hexanal, which contributed to a mixed aromatic type of green (herb), rose, citrus, and fruit fragrances. Among these, citronellol was the signature VOC of the aromatic *Rosa rugosa*, whereas 3-hexen-1-ol, hexanal, and hexan-1-ol were mainly detected in nonaromatic *Phalaenopsis* hybrids (Xiao et al. 2021; Tong et al. 2023), indicating that these three key VOCs might weakly affect the aromatic characteristics of *Phal.* Tzu Chiang Balm.

Aromatic characteristics were also influenced by the aromatic threshold of key VOCs.

Table 3. Aroma thresholds and characteristics of 17 key volatile organic compounds (VOCs) in the four aromatic cultivars in *Phalaenopsis* based on partial least squares discriminant analysis.

No.	Key VOCs	Aroma threshold ($mg \cdot kg^{-1}$)	Aroma characteristics	Odor type
13	γ-muurolene	—	—	Floral
22	(+)-germacrene-D	1.8	Floral, soapy	Woody
16	(+)-α-pinene	2.1	Cedarwood, pine, sharp	Woody
32	(+/−)-δ-elemene	—	—	Woody
6	β-elemene	0.15	—	Woody
25	Citronellol	0.1	Rose, citrus	Floral
9	(Z)-β-ocimene	0.034	Floral	Floral
23	Guaiol	—	—	Woody
29	3-Hexen-1-ol	0.2	Green	Green
39	Hexanal	0.0615	Fruity, apple	Green
31	(E)-4,8-dimethyl-1,3,7-nonatriene	—	—	Green
27	1,8-cineole	0.02	Camphor, mint	Herbal
26	Hexan-1-ol	0.056	Banana, flower, grass	Herbal
36	Hexyl acetate	0.002	Pear, apple, banana, grass	Fruity
3	β-myrcene	0.0049	Balsamic, fruit, geranium, must	Spicy
14	D-limonene	0.01	Citrus	Fruity
41	Phenylacetonitrile	1	Refreshing	Refresh

In the key VOC analysis of *Dendrobium officinale*, benzyl alcohol had high concentration (19,671 $ng \cdot g^{-1}$) but was not crucial to aromatic characteristics because of its high aromatic threshold (2.55 $mg \cdot kg^{-1}$) (Yang et al. 2022). Nevertheless, 2,4-nonadienal (71 $ng \cdot g^{-1}$) with the low aromatic threshold (5×10^{-5} $mg \cdot kg^{-1}$) had significant effects on aromatic characteristics of *Camellia sinensis* (Wang et al. 2020). Similarly, (E)-β-ionone, possessing a low aromatic threshold (7×10^{-6} $mg \cdot kg^{-1}$), was considered to be a key VOC determining the aromatic characteristics of *Mangifera indica* (Pino and Mesa 2006). In the present study, the relative contents of both 3-hexen-1-ol and phenylacetonitrile were high in *Phal.* Purple Butterfly (Fig. 5), but the aromatic type of this cultivar mainly exhibited green (herb) fragrance from 3-hexen-1-ol because of its lower aromatic threshold (0.2 $mg \cdot kg^{-1}$, Table 3) compared with phenylacetonitrilec (1 $mg \cdot kg^{-1}$, Table 3).

Conclusion

In this study, we used GC-MS to investigate the flower VOCs of four aromatic cultivars in *Phalaenopsis* by using the nonaromatic cultivar *Phal.* Big Chili as a control. A total of 43 VOCs classified into seven categories were identified in the nonaromatic *Phal.* Big Chili and four aromatic cultivars. Hexyl acetate and hexan-1-ol were common VOCs in the aromatic cultivars. On the basis of the PLS-DA model, the five cultivars were classified into three groups. Seventeen key VOCs with different aromatic thresholds and characteristics were identified in the four aromatic cultivars, and the types and relative contents of key VOCs varied, resulting in the different characteristics and intensities of floral fragrance among the cultivars. Our investigation will be beneficial for exploration of the key VOCs determining the fragrance of *Phalaenopsis* and also provide valuable information for crossbreeding of *Phalaenopsis* to breed more aromatic cultivars.

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