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Floral volatiles identification and molecular differentiation of *Osmanthus fragrans* by neutral desorption extractive atmospheric pressure chemical ionization mass spectrometry

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ABSTRACT:

RATIONALE: Floral volatiles are commonly present only at trace amounts and can be degraded or lost during vapor collection, which is often challenging from the analytical standpoint. *Osmanthus fragrans* Lour. is a widely cultivated plant known for the highly distinct fragrance of its flowers. The identification of specific volatile organic compounds (VOCs) and molecular differentiation of *O. fragrans* without any chemical pretreatment and VOC collection are important.

METHODS: Twenty-eight VOCs released by the flowers from ten different cultivars of *O. fragrans* were identified using neutral desorption extractive atmospheric pressure chemical ionization mass spectrometry (ND-EAPCI-MS) without any chemical pretreatment or VOC collection. Chemical identification was performed by high-resolution MS^n analysis and whenever possible was confirmed by the analysis of standards.

RESULTS: According to our literature search, nine of the identified VOCs, 3-buten-2-one, cyclohexadiene, 2-methylfuran, 3-allylcyclohexene, cuminyl alcohol, hotrienol oxide, epoxy-linalool oxide, N-(2-hyrdoxyethyl) octanamide, and 3-hydroxy-dihydro-β-ionone, have not been reported in *O. fragrans* in earlier studies. Confident differentiation between ten different cultivars of *O. fragrans* was achieved by the principal component analysis of the mass spectrometric results. **CONCLUSIONS:** The results of our ND-EAPCI-MS analysis substantially increase our knowledge about the chemistry of the *O. fragrans* floral fragrance and demonstrate the power of this technique for direct molecular profiling for plant recognition or in biotechnological applications.

Keywords: volatiles; principal component analysis; characterization; neutral desorption extractive atmospheric pressure chemical ionization mass spectrometry.

INTRODUCTION

Osmanthus fragrans Lour., commonly referred to as sweet osmanthus, is a plant species widely cultivated in Eastern Asia and well known for the very distinct and pleasant fragrance of its flowers [1,2]. *O. fragrans* is of high economic value in China [2,3]. The highly distinct floral fragrance of *O. fragrans* has been the subject of extensive research [4,5,6,7,8]. A variety of volatile organic compounds (VOCs) have been identified in *O. fragrans* flowers, including alcohols, aromatic compounds, ketones, terpenoids, and esters [4]. Some investigations have shown the contributions of certain VOCs to the specific fragrances of particular cultivars [5,6,7]. The identity of the chemicals responsible for the distinct osmanthus fragrance is of great interest, not only for plant and fragrance studies but also for the development of cost-effective synthetic formulations with the sweet osmanthus fragrance. Hence there is sustained interest in research on *O. fragrans* floral VOCs using emerging analytical approaches.

The conventional analytical method for the molecular analysis of *O. fragrans* VOCs is gas chromatography mass spectrometry (GC/MS) [2,4,6,7,9,10,11], which has a generally high analytical power for VOCs analysis [12,13]. However, heat-sensitive, labile and reactive compounds commonly remain undetected in GC/MS because the analysis requires sample collection, extraction and heating processes. Therefore, direct analytical methods for VOCs detection without, or with minimal, sample pretreatment present an interesting potential alternative for the study the *O. fragrans* volatiles.

Popular methods for the direct MS analysis of VOCs include selected ion flow tube mass spectrometry (SIFT-MS) [14,15], proton transfer reaction mass spectrometry (PTR-MS) [16], direct analysis in real-time mass spectrometry (DART-MS) [17], direct sampling probe (DSP) [18], and extractive electrospray ionization mass spectrometry (EESI-MS) [19] as well as methods based on atmospheric pressure chemical ionization mass spectrometry (APCI-MS)

[20,21,22]. In this study, we employed neutral desorption extractive atmospheric pressure chemical ionization mass spectrometry (ND-EAPCI-MS), an analytical technique developed in our research laboratory, for the direct characterization of the VOCs emitted by the freshly collected flowers from ten different cultivars of *O. fragrans*, entirely without collection of the VOCs, ancillary heating or organic solvent extraction.

EXPERIMENTAL

Chemicals and Reagents

Linalool, 2-methylfuran, 2-ethylfuran, toluene, cyclohexadiene and cuminyl alcohol standards were purchased from Sun Chemical Technology (Shanghai, China) Co, Ltd, with a purity > 99%. Ultra-purity nitrogen (> 99.99%) was obtained from Jiangxi Guoteng Gas Co. Ltd (Nanchang, China).

Materials

All the fresh *O. fragrans* flowers from ten different cultivars were gifted at the same time from trees cultured at a trial field organised by the Jiangxi Academy of Forestry during the time of the day with the highest floral fragrance emission (8:00-10:00). The flower chemotypes were identified prior to sample collection by Professor Xiaochun Huang (Jiangxi Academy of Forestry, Jiangxi, China). The flowers were placed separately in a 2-mL centrifuge tube (Solarbio, Beijing, China) and analyzed by mass spectrometry *ca* 10 min after collection. The voucher specimens were preserved in the Jiangxi Key Laboratory for Mass Spectrometry and Instrumentation, East China University of Technology, Nanchang, China. Two flower samples from each tree were randomly collected from three different trees in the same cultivar. A total of 60 samples from 10 cultivars were analyzed by ND-EAPCI-MS.

Each sample was analyzed 2 times, and an empty centrifuge tube was used as control.

Stock solutions of linalool, cuminyl alcohol, and 2-methylfuran were prepared at 3 μg/mL. Another stock solution was prepared for the equimolar mix of linalool, cuminyl alcohol, and 2-methylfuran. The calibration solutions were prepared by appropriate dilution with methanol/water (v/v, 30:70) to the necessary concentration just before use.

Instruments and working conditions

MS experiments were performed on an Orbitrap-XL mass spectrometer (Thermo Scientific, San Jose, CA, USA) using a home-made APCI source as detailed in our earlier studies [20,21,23,24] coupled to a home-made ambient neutral desorption (ND) device, which is used to extract the fragrance compounds from the *O. fragrans* flowers. The experimental ND-EAPCI-MS protocol is shown schematically in Figure 1. The fresh *O. fragrans* flowers were sampled in centrifuge tube. The VOCs of *O. fragrans* flower and ambient air (relative humidity - 60%) were continually blown into the ionization region *via* a PEEK tube (ID 0.5 mm) assisted by a nitrogen gas flow (0.1 MPa). The ambient corona discharge was optimized to +4.5 kV *via* a stainless steel needle (OD 150 μm) with a sharp end (curvature radius ~7.5 μm). The angle between the outlet of the sample tubing and discharge needle was 30^{o}. The distance from the inlet of the mass spectrometer capillary to the tip of the ion probe was 5 mm. The mass spectrum was averaged over 30 scans obtained during the 0.5 min sampling period in the range of *m/z* 50 to 400. The instrument was operated at a high-resolution of up to 200000. In the MS/MS experiments, the isolation width of the selected precursor ions was 1 *m/z* unit, and the normalized collision energy for the selected ions was set at the range of 20% to 30% with helium being used as the collision gas.

Multivariate data analysis

Mass spectra were collected in the mass range of *m/z* 50-400 in positive ion mode. Principal component analysis (PCA) was performed to recognize the pattern based on the raw mass spectral fingerprints using Matlab software (version 7.8.0, Mathworks, Inc., Natick, MA, USA). The fingerprint mass spectra (MS^1) data were exported to Microsoft Excel and arranged using *m/z* values in the range of *m/z* 50 to 400 as independent variables and the signal intensity of MS^1 as dependent variables. Six samples for each cultivar were collected, and every sample was analyzed two times. Hence, there were 120 mass spectra per data set. The data sets were assembled into a N×120 matrix, where N represents the total number of *m/z* values collected in the mass spectrum. The matrix could be directly used for PCA analysis. Note that the total number of *m/z* values was not the same for different samples. All the mass spectral data expressed as signal intensities of different ions were further loaded into the Matlab software for PCA analysis. The molecular information from different samples was presented visually in the PCA score plots. When exporting mass spectra data into Microsoft Excel, a total of 120 data points from 60 samples (two data points per sample on average) were interpreted in the score graphs of the PCA.

RESULTS AND DISCUSSION

The identification information of the VOC signals from the flowers of ten studied *O. fragrans* cultivars (Figure S1, supporting information) is listed in Table 1. The chemical assignment of the MS signals was based on the high resolution mass measurement of the $[M+H]^+$ precursor ions in full MS mode and product ions in MS/MS mode. Comparison with reference standard compounds or with the online chemical database Plant Metabolic Network (http://www.plantcyc.org) was performed whenever possible, as indicated in Table 1. In total, twenty-eight VOCs were identified from the studied ten cultivars of *O. fragrans* flowers (Figure 2). Fourteen of the identified VOCs were terpenoids. Nine of the reported VOCs, 3-buten-2-one, cyclohexadiene, 2-methylfuran, 3-allylcyclohexene, cuminyl alcohol, hotrienol oxide, epoxy-linalol oxide, N-(2-hyrdoxyethyl) octanamide, 3-hydroxy-dihydro-β-ionone, have not been identified in *O. fragrans* flowers in previous GC/MS studies [2,7,9,10,11,25]. The identities of these nine new chemicals were tentatively assigned based on their exact masses, collision-induced dissociation (CID) product ion spectra, and literature MS search for the VOCs reported from other plant flowers [26,27,28,29,30,31,32,33].

Although there are apparent differences in the floral fragrance of the studied cultivars, their "core" osmanthus fragrance could still be clearly distinguished by nose in every cultivar. Therefore, it is reasonable to believe that the chemicals responsible for the "core" floral fragrance will be emitted by every cultivar. Our comparative analysis indicates that there are about twenty common chemicals in the spectra of ten studied cultivars of *O. fragrans* using ND-EAPCI-MS (Figure 3 and Table 1). Of these chemicals, five (2-methylfuran, cuminyl alcohol, hotrienol oxide, N-(2-hyrdoxyethyl) octanamide, 3-hydroxy-dihydro-β-ionone) have not been reported in *O. fragrans* in earlier GC/MS studies (Table 1). These newly discovered VOCs may play an important role in forming the distinct core fragrance of *O. fragrans* flowers. For instance, 2-methylfuran creates sweet-gassy aromas [28,34]. Cuminyl alcohol was found to be the main compound in *Rhodiola* essential oil, having a specific spicy fragrance, which emphasizes the flower scent of rose root rhizomes [32]. Our olfactory tests showed that cuminyl alcohol has a very intense fragrance somewhat similar to that of *O. fragrans* flowers. Therefore, this compound may play an important role in the fragrance of *O. fragrans* flowers. Although there is no related report of the fragrance of N-(2-hyrdoxyethyl) octanamide, this compound was found in a large amount in *Indigofera cordifolia* seed oil [29].

3-Hydroxy-dihydro-β-ionone, a derivative of β-ionone, is not widely reported in the literature and has been previously tentatively assigned to lutein degradation [33]. The fragrance of 3-hydroxy-dihydro-β-ionone may be similar to that of β-ionone, which is a well-known raw material in the perfume industry, possessing an important violet fragrance and widely present in *Rosa species*, *O. fragrans*, and others [35]. Thus, it can be assumed that even a trace of 3-hydroxy-dihydro-β-ionone in *O. fragrans* flowers may still be of olfactory significance. Further research using an orthogonal technique will be needed to pinpoint which of the identified VOCs make a major contribution to the fragrance of *O. fragrans* flowers and their sensory threshold.

There may be several reasons why some the compounds detected in this study for the first time have not been previously observed by GC/MS. These could include VOC degradation upon GC-injection and/or separation, different growing conditions, the high sensitivity of ambient corona discharge ionization MS, etc. Ambient corona discharge ionization MS makes it easy to detect high proton-affinity (PA) VOCs, in particular volatile amines and minor components. Ambient ionization corona discharge ionization MS of VOCs occurs in a ladder-like fashion in which protons are gradually transferred from compounds with lower PA values to compounds with high-PA values, which favors the observation of high-PA compounds, such as N-(2-hyrdoxyethyl) octanamide, even when present at a trace level undetectable by GC/MS. Similarly, in our previous study using ambient corona discharge ionization MS we identified eleven nitrogenous volatiles emitted by the flowers of three different angiosperm plants with semen-like fragrance, including 1-pyrroline, 1-piperideine, 4-amino-butanal, 2-ethylpyridien, which have not been reported by earlier GC/MS studies [23]. Altogether, this shows good prospects for ambient floral VOCs analysis as an alternative or complement to GC/MS. It appears that ND-EAPCI-MS and GC/MS complement each other well for the rigorous profiling of the VOCs in floral fragrances.

Other “core” VOCs of *O. fragrans* identified in our analysis have been previously reported in earlier GC/MS studies. Their chemical assignments are described in Table 1, and Figures 2 and 3. The shared signals at *m/z* 137.1322 ($C_{10}H_{17}^+$), *m/z* 171.1380 ($C_{10}H_{19}O_2^+$), *m/z* 193.1584 ($C_{13}H_{21}O^+$), *m/z* 195.1741 ($C_{13}H_{21}O^+$), *m/z* 197.1897 ($C_{13}H_{23}O^+$), and *m/z* 209.1533 ($C_{13}H_{21}O_2^+$) were assigned to protonated ocimene/myrcene/limonene, 8-hydroxylinalool, β-ionone, β-ionol, dihydro-β-ionol, and 3-hydroxy-β-ionone, respectively (Figure 2). These identifications were based on the accurate MS data and their CID spectra (Figures S2-S4, supporting information), which have also been reported in *O. fragrans* flowers [2,5,8,11]. The minor peak at *m/z* 211.1689 ($C_{13}H_{23}O_2^+$) was 2 *m/z* units higher than protonated 3-hydroxy-β-ionone (*m/z* 209.1533). Based on earlier literature reports[33], this peak was tentatively assigned to protonated 3-hydroxy-dihydro-β-ionone, which has not been previously reported in *O. fragrans* flowers. From a previous study of the constituents in *O. fragrans* flowers [10], the peak at *m/z* 169.1220 ($C_{10}H_{17}O_2^+$) was assigned to be protonated hotrienol oxide (Figure S5, supporting information). Based on previous reports, the minor shared peaks at *m/z* 177.1634 ($C_{13}H_{21}^+$) and *m/z* 188.1642 ($C_{10}H_{22}NO_2^+$) were assigned to protonated 1,4-diisopropyl-2-methylbenzene[7] and N-(2-hyrdoxyethyl) octanamide [29], respectively, with these assignments being supported by their high resolution and CID data (Table 1).

The peaks at *m/z* 155.1428 ($C_{10}H_{19}O^+$), *m/z* 153.1276 ($C_{10}H_{19}O^+$), *m/z* 143.1064 ($C_8H_{15}O_2^+$), and *m/z* 83.0489 ($C_5H_7O^+$), are attributed to protonated linalool,[8] hotrienol [6], 3-hexenyl acetate [10], 2-methylfuran [28,36], which were found in all the cultivars except for "ziyinggui". Upon CID, protonated linalool generated major product ions at *m/z* 137, 127, 113, 109, 99, 95, and 85 (Figure S6B, supporting information), by loss of H_2O, $CH_2{=}CH_2$, $CH_3\text{-}CH{=}CH_2$, $H_2O{+}CH_2{=}CH_2$, C_4H_8, $CH_3\text{-}CH{=}CH_2{+}H_2O$, and C_5H_{10}, respectively, in accordance with the ions detected from authentic linalool (Figure S6A, supporting information); the detailed fragmentation mechanism is depicted in Figure S7 (supporting information). Another major shared peak at m/z 153.1276 ($C_{10}H_{17}O^+$) was 2 m/z units lower than protonated linalool, and the fragmentation patterns of these two ions were similar, with a mass shift of 2 m/z units (Figure S6C, supporting information). Therefore, the major peak observed at m/z 153.1276 was tentatively identified as the $[M+H]^+$ ion of hotrienol, and the postulated fragmentation mechanism of protonated hotrienol is depicted in Figure S8 (supporting information). Hotrienol has been found in many flowers using GC/MS [6,10], with its flavor being described as sweet and flowery [37]. Several other compounds were identified from different cultivars by analogous procedures. Starting from linalool, an array of compounds can be formed, as shown in Figure S9 (supporting information). Direct hydroxylation of linalool at the C8 position forms 8-hydroxylinalool which can give hotrienol as a product *via* dehydration. It was reported that 8-hydroxylinalool is formed from linalool after hydroxylation in the presence of P-450 hydroxylase [38]. On the other hand, myrcene, ocimene, and limonene are mainly derived by the dehydration of linalool *via* different enzymes. It was reported that a variable amount of linalool could be easily converted to myrcene [39]. The relative abundances of the peak at m/z 153.1276 recorded from the Aurantiacus-group were significantly higher than those from the Semperfloren-group (Figure 3).

Apart from the common MS signals in most cultivars described above, there are several signals specific to particular groups or cultivars of *O. fragrans*. These signals correspond to the different volatile metabolites of the corresponding cultivars. Our comparative analysis indicates that there are about eight VOCs unique to specific cultivars of *O. fragrans* (Figure 3 and Table 1). Of these chemicals, four (3-buten-2-one, cyclohexadiene, 3-allylcyclohexene, epoxy-linalool oxide) have not been reported in *O. fragrans* by earlier GC/MS studies (Table 1). These newly discovered trace VOCs may also play important roles in the characteristic aroma of *O. fragrans*. Further studies are needed to investigate their possible role in forming the fragrance of *O. fragrans* flowers.

The peaks at *m/z* 127.1115 ($C_8H_{15}O^+$) and *m/z* 165.1271 ($C_{11}H_{17}O^+$) are attributed to 6-methyl-5-heptene-2-one [9] and jasmone [10], respectively, which were found in all the cultivars except for "chenghongdangui". 6-methyl-5-heptene-2-one was possibly formed fby the degradation of carotene, which has been reported to have a mushroom-like fragrance [28].

Based on previous reports, the minor shared peaks at *m/z* 135.1166 ($C_{10}H_{15}^+$), and *m/z* 151.1114 ($C_{10}H_{15}O^+$) were assigned to protonated *p*-cymene [9] and cuminyl alcohol [32], respectively, with these assignments supported by their high resolution and CID data (Table 1); cuminyl alcohol has not been previously reported in *O. fragrans* floral fragrance. Upon CID, protonated *p*-cymene generated major product ions at *m/z* 119, 107, 93, and 79, by the elimination of CH_3, C_2H_4, C_3H_6, $H_2O+C_2H_4$, and C_4H_8, respectively (Figure S10, supporting information). The fragmentation pattern of protonated *p*-cymene was very similar to that of protonated cuminyl alcohol. It was earlier reported that *p*-cymene is responsible for the off-fragrance of deteriorated lemon [40]; that is to say, the higher the concentration of *p*-cymene, the weaker is the fragrance. In addition, the relative abundance of *p*-cymene in "zhushadangui" (Aurantiacus group) is higher than in other cultivars (Figure 3). Furthermore, the Thunbergii and Latifolius groups display a sweet fragrance and an elegant fragrance, respectively, whereas the Aurantiacus group has no apparent fragrance[11]. The differences in aroma are due to the differences in the chemical composition between these groups. Thus, it can be concluded that the scent of "zhushadangui" is weaker than that of other cultivars, which is also in accordance with earlier studies[11].

Three peaks at *m/z* 99.0802 ($C_6H_{11}O^+$), *m/z* 97.0646 ($C_6H_9O^+$), and *m/z* 187.1325 ($C_{10}H_{19}O_3^+$) were obtained mainly in the cultivars of "liuyedangui" and "zhushadangui", and could be assigned to protonated 2-hexenal [6], 2-ethylfuran [2,41], and epoxy-linalool oxide [30].

In a similar procedure, other peaks at *m/z* 71.0489 ($C_4H_7O^+$), *m/z* 81.0696 ($C_6H_9^+$), *m/z* 93.0697 ($C_7H_9^+$), *m/z* 123.1166 ($C_9H_{15}^+$), *m/z* 175.1478 ($C_{13}H_{19}^+$) were tentatively assigned as protonated 3-buten-2-one [31], cyclohexadiene [26], toluene [2], 3-allylcyclohexene [27], and 6-ethyl-2-methyl-decane [7], based on their high resolution and CID data (Table 1). Our results demonstrate that ND-EAPCI-MS spectra provide rich chemical information about the inter-cultivar differences between plants at the VOCs level.

The main limitation of the ND-EAPCI-MS approach as well of many other ambient ionization methods is the difficulty of quantitative analysis. This is related to their being no chromatographic separation stage, which means that the signal intensity of floral VOCs is strongly affected by matrix effects. Ionization of VOCs by corona discharge occurs in a ladder-like fashion, whereby protons are gradually transferred from compounds with a low PA to compounds with a higher PA. Therefore, the presence of higher-PA VOCs suppresses the ionization of lower-PA VOCs. Low-PA VOCs are sometimes not observed even at very high concentrations whereas high-PA VOCs, in particular nitrogen-containing compounds, can be observed even at trace concentrations. Figure S11 (supporting information) reveals significant variation in the signal intensity of three VOCs from *Osmanthus* flowers (linalool, cuminyl alcohol, and 2-methylfuran) in the concentration range from 0.1 to 1000 μg/mL obtained from single standard analyte solutions (Figure S11A, supporting information) and from a standard mixture (Figure S11B, supporting information). It can be seen that the signal intensity of linalool cuminyl alcohol is increased by ca 2 times due to the presence of cuminyl alcohol and 2-methylfuran at a concentration of 1000 μg/mL. Interestingly, at low concentrations (0.1, 1 μg/mL), signal suppression was observed for linalool and cuminyl alcohol and signal enhancement for 2-methylfuran, whereas at higher concentrations (10, 100, 1000 μg/mL), signal suppression was observed for cuminyl alcohol while signal enhancement was observed for linalool and 2-methylfuran (Table S1, supporting information). A common approach to cope with the matrix effects in quantitative analysis is the use of standard additions. However, the application of this method to VOCs analysis by ND-EAPCI-MS is rather cumbersome and was not attempted in this study.

The instrument response curves for linalool, cuminyl alcohol, and 2-methylfuran standard solutions in the concentration range from 0.1 to 1000 μg/mL revealed linearity coefficient of 0.9999, 0.9995, and 0.9991, respectively (Figure S11A, supporting information); the limit of detection (LOD) for linalool, cuminyl alcohol, and 2-methylfuran was estimated to be about 0.001 μg/mL for all three compounds. The relative standard deviations (RSDs) for linalool, cuminyl alcohol, and 2-methylfuran were estimated to be < 10%, <10%, and <15%, respectively, in the concentration range of 0.1-1000 μg/mL. Of course, these estimates for LOD and linearity range based on single-compound measurements could be significantly affected by matrix effects, as demonstrated in Figure S11 (supporting information).

The MS fingerprints of the ten cultivars of *O. fragrans* flowers (Figure 3) showed notable differences. To easily visualize an overview of correlations among different cultivars, a multivariate statistic tool, PCA, was employed to process the MS fingerprints data using Matlab software. Figure 4 shows the PCA score plot obtained using mass spectra from 60 individual *O. fragrans* samples (120 data points), in which the samples were separated from each other before analysis. A total of 120 data points of the *O. fragrans* samples were clustered regularly in the PCA plots. However, samples "tianxiangtaige" and "tiannvsanhua" occupied adjacent area in Figure 4A, indicating that these two cultivars display similar VOCs profile. The first three PCs accounted for 90.4% of the total variance, with PC1 at 41.6%, PC2 at 30.2%, and PC3 at 18.6% (Figure 4B). It can be seen from the PCA loading plots that the peaks at *m/z* 137, 153, 169, 171, 188, 193, and 195 are the main contributors to the differentiation (Figure 4C). For instance, PC1 was positively correlated with *m/z* 153 (loading

0.7), and negatively correlated with *m/z* 137 and *m/z* 193 (loadings -0.6 and -0.4). For PC2, *m/z* 171 showed a very high positive loading value at 0.7.

CONCLUSIONS

Twenty-eight VOCs emitted by the freshly collected flowers from ten different cultivars of *O. fragrans* were identified using a home-made ND-EAPCI-MS platform without any sample pretreatment. Nine of the reported VOCs have not been identified in *O. fragrans* flowers in previous GC/MS studies and may be significant for the highly distinct fragrance of *O. fragrans* flowers. The results also suggest that ND-EAPCI-MS of floral volatiles is a promising analytical tool for rapid inter-cultivar differentiation using non-destructive VOC analysis. Further research is needed to pinpoint the exact role of the discovered VOCs in the characteristic floral fragrance and in the metabolism of *O. fragrans*.

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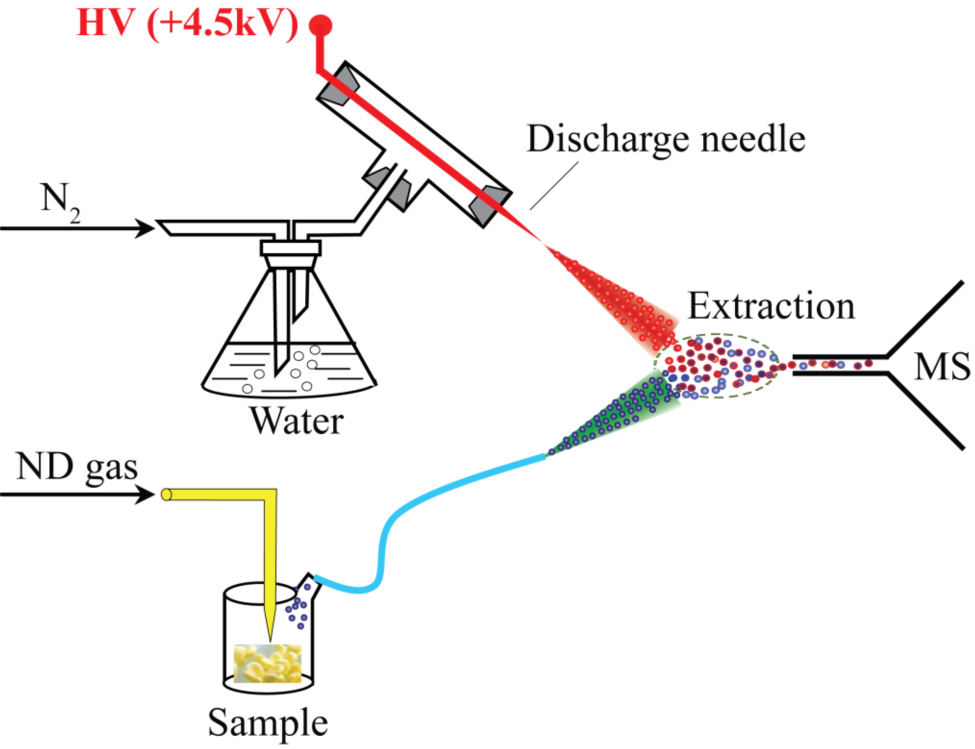

Figure 1. Schematic illustration of the concept and protocol of ND-EAPCI-MS for the analysis of *O. fragrans* flowers.

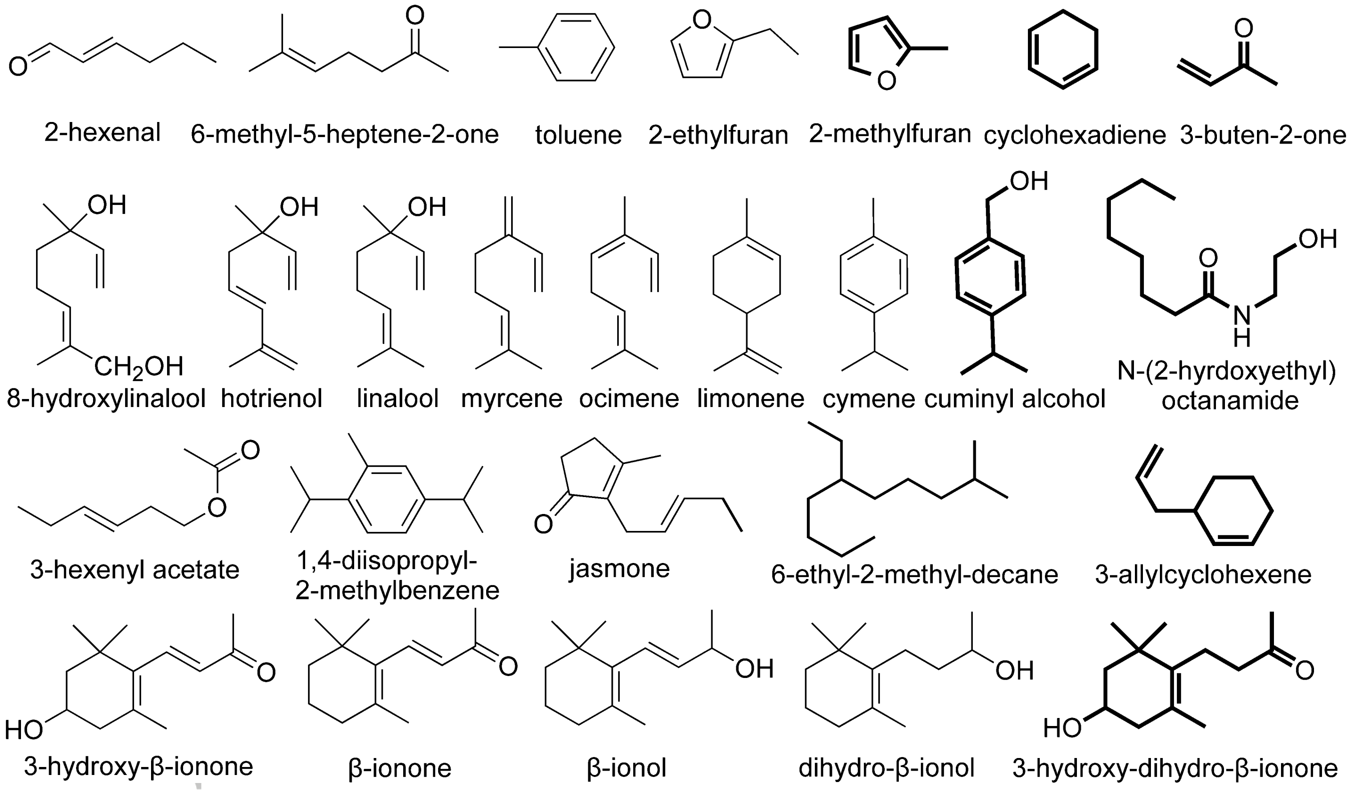

Figure 2. Structures of compounds identified in ten different cultivars of *O. fragrans* flowers. The bold-line structures correspond to the VOCs that have not been reported in the earlier studies of *O. fragrans.*

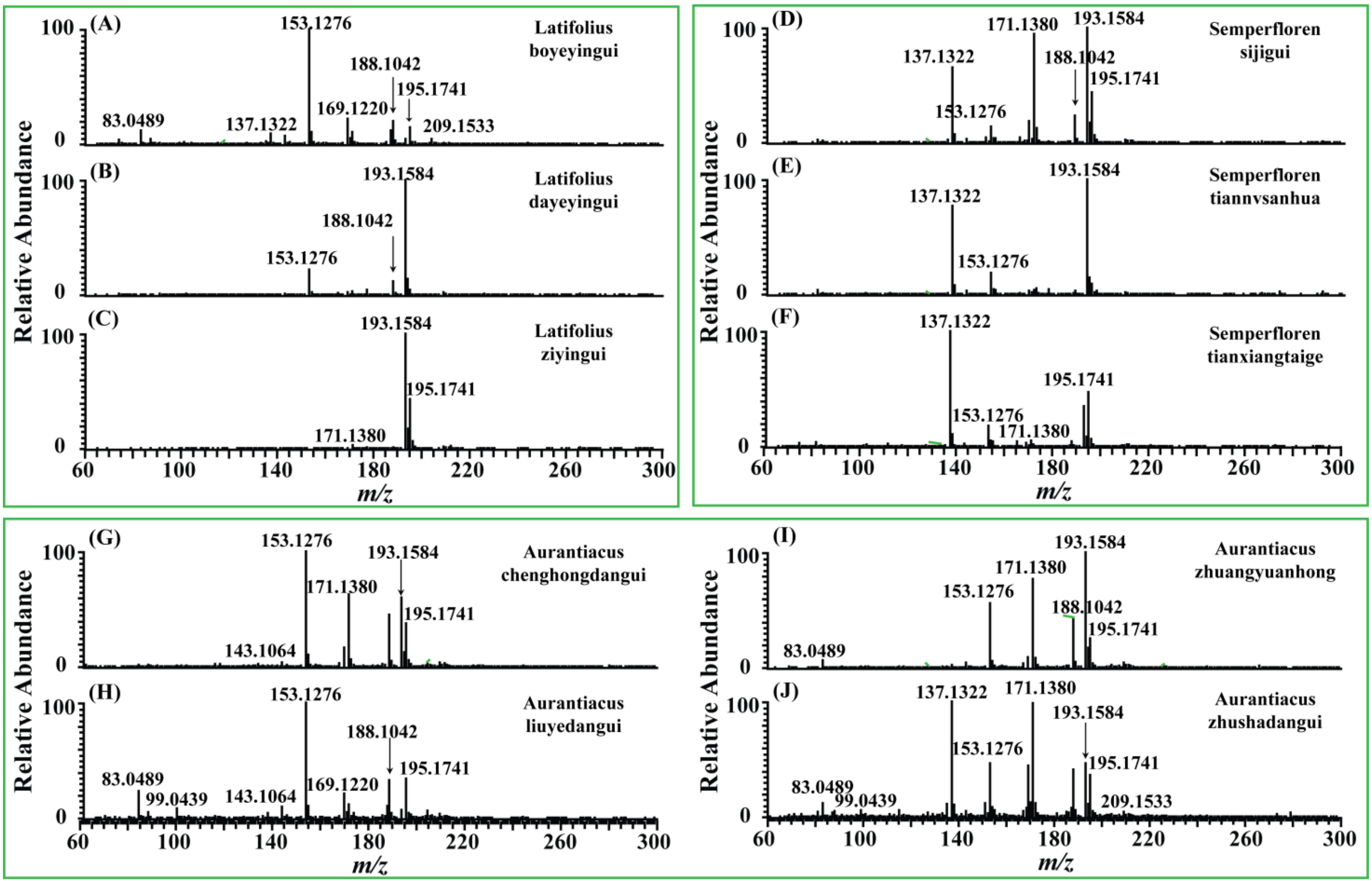

Figure 3. ND-EAPCI-MS spectra of ten different cultivars of *O. fragrans* flower samples from three different groups. Latifolius group: (A) boyeyingui, (B) dayeyingui, (C) ziyingui; Semperfloren group: (D) sijigui, (E) tiannvsanhua, (F) tianxiangtaige; Aurantiacus group: (G) chenghongdangui, (H) liuyedangui, (I) zhuangyuanhong, (J) zhushadangui.

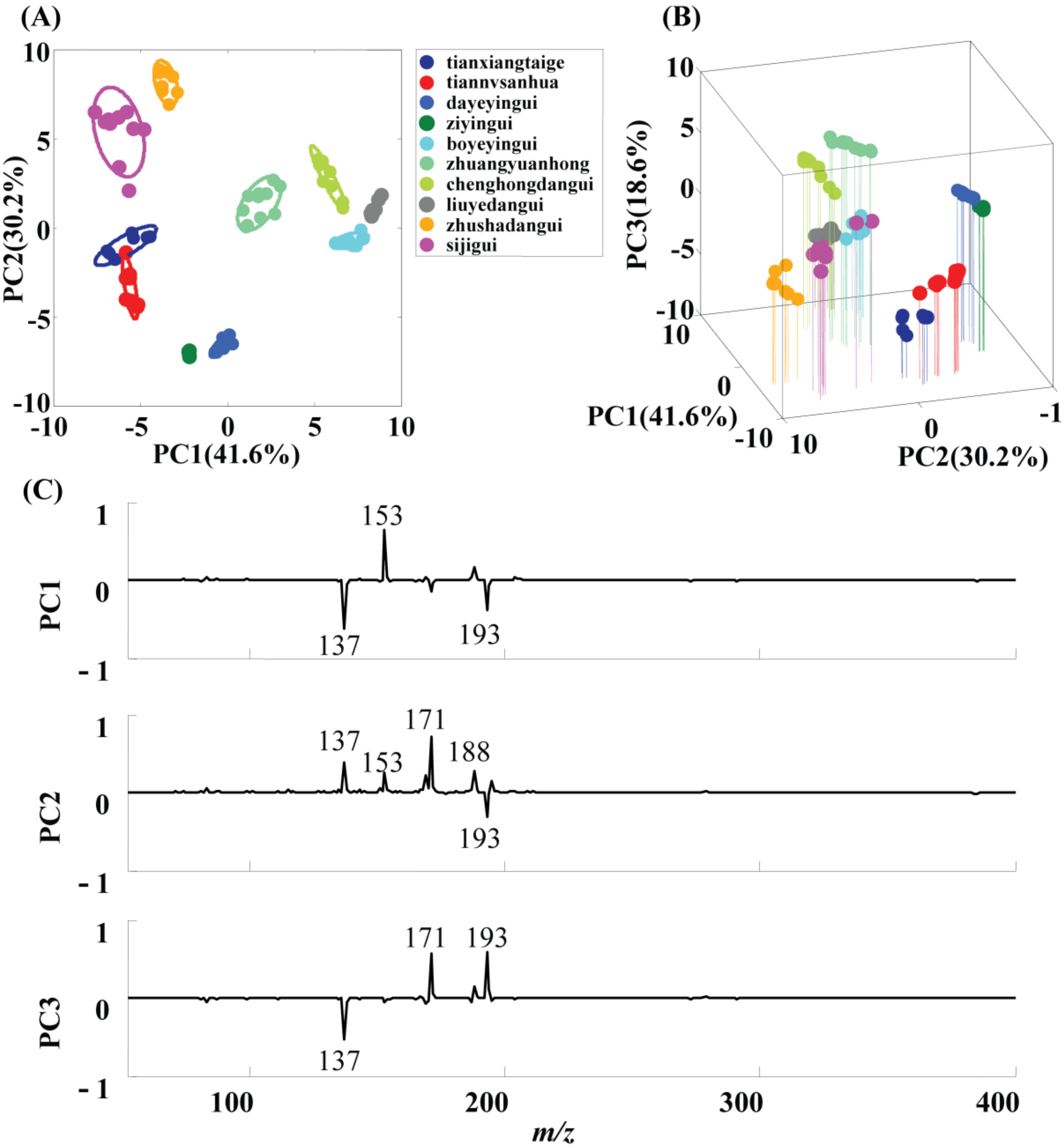

Figure 4. PCA results of ND-EAPCI-MS data of ten different cultivars of *O. fragrans* flowers. (A) 2D score plots, (B) 3D score plots, (C) corresponding PCA loading plots.

Table 1. VOCs emitted by the flowers from ten different cultivars of *O. fragrans* by ND-EAPCI-MS.

Name	MH^+ (m/z)	Formula	Error (ppm)	Product ions (m/z)	Reference	Latifolius [d]			Semperfloren [d]				Aurantiacus [d]		
						boye yingui	daye yingui	ziyin gui	siji gui	tiannv san hua	tian xiang taige	cheng hong dangui	liuye dangui	zhuang yuan hong	zhusha dangui
3-Buten-2-one [c]	71.0489	$C_4H_7O^+$	-3.4	/	31	/	/	/	+	+	+	/	+	/	+
Cyclohexadiene [a,c]	81.0696	$C_6H_9^+$	-3.2	79	26	+	/	/	+	+	+	/	/	/	+
2-Methylfuran [a,c]	83.0489	$C_5H_7O^+$	-2.4	55	28	+	+	/	+	+	+	+	+	+	+
Toluene [a]	93.0697	$C_7H_9^+$	-1.9	91	2	+	/	+	+	+	+	/	+	/	+
2-Ethylfuran [a]	97.0646	$C_6H_9O^+$	-2.2	69	2	/	/	-	+	+	+	/	+	/	+
2-Hexenal [b]	99.0802	$C_6H_{11}^+$	-2.1	81,71	6	+	+	/	+	/	/	/	+	/	+
3-Allylcyclohexene [c]	123.1166	$C_9H_{15}^+$	-2.1	95,81	27	/	/	/	+	+	/	/	+	/	/
6-Methyl-5-heptene-2-one [b]	127.1115	$C_8H_{15}O^+$	-1.7	109,99	9	+	+	+	+	+	+	/	+	+	+
p-Cymene [b]	135.1166	$C_{10}H_{15}^+$	-1.7	119,107,93,79	9	+	+	+	+	+	+	+	+	+	+
Ocimene/myrcene/limonene [b]	137.1322	$C_{10}H_{17}^+$	-1.8	119,109,95,81	11	+	+	+	+	+	+	+	+	+	+
3-Hexenyl acetate [b]	143.1064	$C_8H_{15}O_2^+$	-1.6	125,115,97	10	+	+	/	+	+	+	+	+	+	+
Cuminyl alcohol [a,c]	151.1114	$C_{10}H_{15}O^+$	-2.3	133,123,109,95	32	+	+	+	+	+	+	+	+	+	+
Hotrienol [b]	153.1276	$C_{10}H_{17}O^+$	1.0	135,125,107,93	6	+	+	/	+	+	+	+	+	+	+
Linalool [a,b]	155.1428	$C_{10}H_{19}O^+$	-1.7	137,127,109,95	8	+	+	/	+	+	+	+	+	+	+
Jasmone [b]	165.1271	$C_{11}H_{17}O^+$	-2.3	/	10	+	+	+	+	+	+	/	+	+	+
Hotrienol oxide [c]	169.1220	$C_{10}H_{17}O_2^+$	-1.6	151,141,123,109	/	+	+	+	+	+	+	+	+	+	+
8-Hydroxylinalool [b]	171.1380	$C_{10}H_{19}O_2^+$	0.1	153,143,125,111	5	+	+	+	+	+	+	+	+	+	+
6-Ethyl-2-methyl-decane [b]	175.1478	$C_{13}H_{19}^+$	-1.9	/	7	/	/	+	+	/	/	/	/	/	/
1,4-Diisopropyl-2-methylbenzene[b]	177.1634	$C_{13}H_{21}^+$	-2.1	149,135,121,107	7	+	+	+	+	+	+	+	+	+	+
Epoxy-linalool oxide [c]	187.1325	$C_{10}H_{19}O_3^+$	-2.0	/	30	+	/	/	/	+	+	/	+	+	+
N-(2-hyrdoxyethyl) octanamide [c]	188.1642	$C_{10}H_{22}NO_2^+$	-1.7	170,160,146,132	29	+	+	+	+	+	+	+	+	+	+
β-Ionone [b]	193.1584	$C_{13}H_{21}O^+$	-1.6	175,149,135,123	11	+	+	+	+	+	+	+	+	+	+
β-Ionol [b]	195.1741	$C_{13}H_{23}O^+$	-1.1	177,163,149,135	8	+	+	+	+	+	+	+	+	+	+
Dihydro-β-ionol [b]	197.1897	$C_{13}H_{25}O^+$	-1.5	/	2	+	+	+	+	+	+	+	+	+	+
3-Hydroxy-β-ionone [b]	209.1533	$C_{13}H_{21}O_2^+$	-1.5	/	8	+	+	+	+	+	+	+	+	+	+
3-Hydroxy-dihydro-β-ionone [c]	211.1689	$C_{13}H_{23}O_2^+$	-1.7	/	33	+	+	+	+	+	+	+	+	+	+

[a] The identification was based on the reference CID-MS analysis of standard compound.

[b] The identification was based on high-resolution MS, CID and published MS data from *O. fragrans* flowers.

[c] These compounds were identified in *O. fragrans* flowers for the first time.

[d] "+" indicates detectable; "/" indicates undetectable.