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Original Article

Seasonal Variation in Floral Scent of Persian Musk Rose (*Rosa moschata* Hermm.)

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Abstract

The seasonal variation of volatile oil compositions (VOCs) from fresh flowers of Persian Musk rose was investigated by Combi PAL Headspace Techniques. In this study, a total number of 21 VOCs were detected by headspace on the Combi PAL System and gas chromatography-mass spectrometry (HS-GC/MS) from *Rosa moschata* Herrm. at different seasons which was representing 92.53-99.37% of total VOCs. The analysis of VOCs at different seasons detected the major compounds: Phenyl ethyl alcohol (30.68-77.36%), 1-Nonadecene (1.01-30.42%), n-Nonadecane (4.61-14.04%), n-Heneicosane (4.47-12.07%) and 1-Tricosene (0-5.91%). Phenylpropanoids content varied significantly over time, with a low level during September and maximum content in May. In contrast to phenylpropanoids contents, the high level of fatty acid derivatives was realized during September. In all of seasons a low level of terpenoids derivatives was emitted from Persian Musk rose flowers. The results of this research suggest that the fragrance characteristics of *R. moschata* resulted from its specific composition and can be manipulated by seasonal changes and environmental conditions.

Key words: Persian Musk rose, Scent, Seasonal, Phenyl ethyl alcohol

Introduction

The genus *Rosa* L. has involved considerable attention from taxonomists and several species have been explained. Currently, about 100-250 species are usually documented [1]. Only a few species among hundreds in the genus *Rosa* L. are scented, which involve *Rosa damascena* Mill., *Rosa gallica* L., *Rosa centifolia* L., *Rosa moschata* Herrm., *Rosa borboniana* N.H.F.Desp., *Rosa chinensis* Jacq., and *Rosa alba* L. [2-4]. *Rosa* has sixteen wild species in Iran of which *R. moschata* Herrm. (Syn: *R. moschata* var. *nastarana* H. Christ) with the common names of Persian Musk rose, Nastran-e Shiraz and Anbar rose, is distributed in many local regions of the Iran [4, 5]. Its wild origins are uncertain but are suspected to lie in the western Himalayas [4]. As, Persian Musk rose (*R. moschata*) has not been confirmed clearly in history, but the supposition is that it is a parent of Damask rose [6]. The quantity and composition of the rose oil distilled from the rose petals are strongly affected by the genotypes, the climatic conditions, the time of rose petals harvesting, and the technology used for processing and distillation. Comparative GC and GC/MS analyses of rose oil compositions have also been used for characterization of rose oils distilled from flowers of *R. damascena* cultivated in different geographical regions and/or different genotypes [7].

In traditional medicine, rose water of Persian Musk rose has been used to strengthen heart muscles, stomach, liver, spleen, nerves, and gums and to strengthen intelligence [6]. However the hydrodistilated essential oil composition of fresh and dried samples was studied by GC and GC/MS techniques, there is no report on the headspace

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VOCs of this species in the world. Therefore, in this research, the seasonal variation of VOCs of fresh flowers was studied by CombiPAL Headspace Techniques.

Material and Methods

Plant Material

The fresh flowers of Persian Musk rose were collected from rose gardens (Shiraz – 59° 35′ E, 29° 43′ N, Altitude 1810 m) during their flowering period (10 May, 01 June and 10 September 2014). A specimen (Collector Number: PC 87-23) has been deposited in the Herbarium of the Faculty of Sciences, Shiraz University.

Volatiles Headspace Screening

Three to five branches of Musk rose were harvested in each time and immediately placed inside a sealed bottle, and then quickly transported to the laboratory for testing. Five flowers from each branch were randomly plucked and cut. Up to 3 g of each sample were promptly placed in 20 ml headspace vials, and immediately sealed with silicone rubber septa and aluminum caps. The vials were then transferred to the headspace tray. The headspace analysis was carried out by Combi PAL System which was provided with headspace auto-sampler, heater and agitator. The vial was heated to 45 °C and retained for 30 min while being agitated. The temperature of the sampling needle and transmission line was 85 °C.

Volatile Oil Analysis Procedure

GC analysis was done using an Agilent gas chromatograph series 7890-A with a flame ionization detector (FID). The chromatography analysis was carried out on fused silica capillary HP-5 column (30 m × 0.32 mm i.d.; film thickness 0.25 μm). The injector and detector temperatures were kept at 250 °C and 280 °C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 ml/min. Oven temperature program was 60-210 °C at the rate of 4 °C/min and then programmed to 240 °C at the rate of 20 °C/min, and finally held isothermally for 8.5 min, while the split ratio was 1:50. GC/MS analysis was completed by using Agilent gas chromatograph, equipped with fused silica capillary HP-5MS column (30 m × 0.25 mm i.d.; film thickness 0.25 μm) coupled with 5975-C mass spectrometer. Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 230 °C and 280 °C, respectively. Mass range was from 45 to 550 amu. Oven temperature program was given the same as above for the GC.

Identification of Compounds

The constituents of the VOCs were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes (C8-C25) and the volatile oil on a HP-5 column under the same chromatographic conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds, and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature [8]. For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors.

Cluster Analysis

Cluster analysis was used to classify of 21 VOCs of *R. moschata* from different seasons according to Ward's technique with nearest neighbor measure by statistical package of SPSS (IBM SPSS Statistics 21) release.

Results and Discussion

The analysis of chromatograms of VOCs and their corresponding relative abundance at the each time is based on net values after eliminating control air background. At the selected time points, volatiles were collected for periods of 3 hrs and analyzed by GC/MS. In this study, a total number of 21 VOCs were detected by headspace on the Combi PAL System and gas chromatography spectrometry (HS-GC/MS) from *R. moschata* at different season (Table 1). The developed headspace analysis procedures present a supplementary representative volatile profile of living plants than traditional methods of solvent extraction or steam distillation. Moreover, manually derived headspace sampling methods, automated VOC analysis systems with high time resolution and on-line facility have become requisite for monitoring fast changes of volatile profiles during plant development or under stress conditions. Additionally, VOC analysis systems that are sensitive, fast and fully automated are also of rising significance to reveal the biosynthesis of plant VOCs [9-10]. Besides this technique is sensitive enough to classify plant odors from specific tissues, such as flower organs, pollen, and nectar [7, 9-13].

Table 1 Seasonal changes in volatile oil compounds (%) of *Rosa moschata* Herrm.

Components	RI*	May	June	September
α-Pinene	932	0.575	0.102	1.012
β-Pinene	975	0.109	-	-
p-Cymene	1022	0.181	-	-
Limonene	1026	0.107	0.262	-
Benzene acetaldehyde	1041	0.134	0.175	-
γ-Terpinene	1055	0.32	-	-
Phenyl ethyl alcohol	1110	77.363	54.41	30.684
2-Phenyl ethyl acetate	1254	0.462	0	0.67
Eugenol	1354	1.29	2.164	-
2-Phenyl propyl butanoate	1484	0.316	-	-
dihydro-β-Ionone	1435	-	0.545	-
(E)-β-Ionone	1482	-	2.325	1.97
n-Pentadecane	1496	-	0.42	-
1-Heptadecene	1672	-	1.524	2.26
n-Heptadecane	1695	0.82	1.419	2.895
1-Nonadecene	1865	1.017	15.282	30.429
n-Nonadecane	1890	4.611	5.783	14.047
n-Octadecanol	2072	-	0.885	0.588
n-Heneicosane	2100	12.071	4.476	7.978
1-Tricosene	2285	-	5.917	-
n -Tricosane	2297	-	3.588	-
VOCs biosynthetic origin				
Terpenoids derivatives		1.292	3.234	2.982
Phenylpropanoids derivatives		79.596	56.749	31.354
Fatty acid derivatives		18.519	39.294	58.197
Total		99.376	99.277	92.533

*RI: Retention indices analyzed on HP-5 column -: not detected

GC/FID and GC/MS analysis results of VOCs of *R. moschata* at different seasons are shown in Table 1. In total, 19, 16, and 10 components were identified and quantified in the subsequent season, respectively, which was representing 92.53–99.37% of total VOCs.

The analysis of VOCs at different season detected the major compounds: Phenyl ethyl alcohol (30.68-77.36%), 1-Nonadecene (1.01-30.42%), n-Nonadecane (4.61-14.04%), n-Heneicosane (4.47-12.07 %) and 1-Tricosene (0-5.91%). Based on VOCs biosynthetic origin, all VOCs are separated into numerous classes, including terpenoids, phenylpropanoids/benzenoids and fatty acid derivatives[13]. Phenylpropanoids content varied significantly over time, with a low level during September and maximum content in May. In contrast to phenylpropanoids contents, the high level of fatty acid derivatives was realized during September. In all of seasons a low level of terpenoids derivatives was emitted from Persian Musk rose flowers (Table1). Phenyl ethyl alcohol content as phenylpropanoids derivatives varied significantly over time, with a low level during September (30.68%) and maximum content in May (77.36%). In the June the amount of Phenyl ethyl alcohol in the Persian Musk rose flower (54.41%) was lower than May and higher than September. Phenyl ethyl alcohol is a major scent compound unrestricted from flowers of damask rose and some hybrid roses (*Rosa* 'Hoh-Jun' and *Rosa* 'Yves Piaget')[7, 14-15]. In the wild roses, from which *Rosa hybrida* Vill. is resulting, floral scent are notice to be chemical signals between the plant and insects, the second including both pollinators and predators [9]. In previously study in *R. damascena,* it was shown those petal aromas are dominated by Phenyl ethyl alcohols, which are known insect attractants for seed formation and dispersers [7, 16-17].

Fatty acid derivatives content were increased in subsequent seasons, herein a low level, middle and maximum content were detected during May (18.519%), June (39.294%) and September (58.197%) respectively (Table 1).

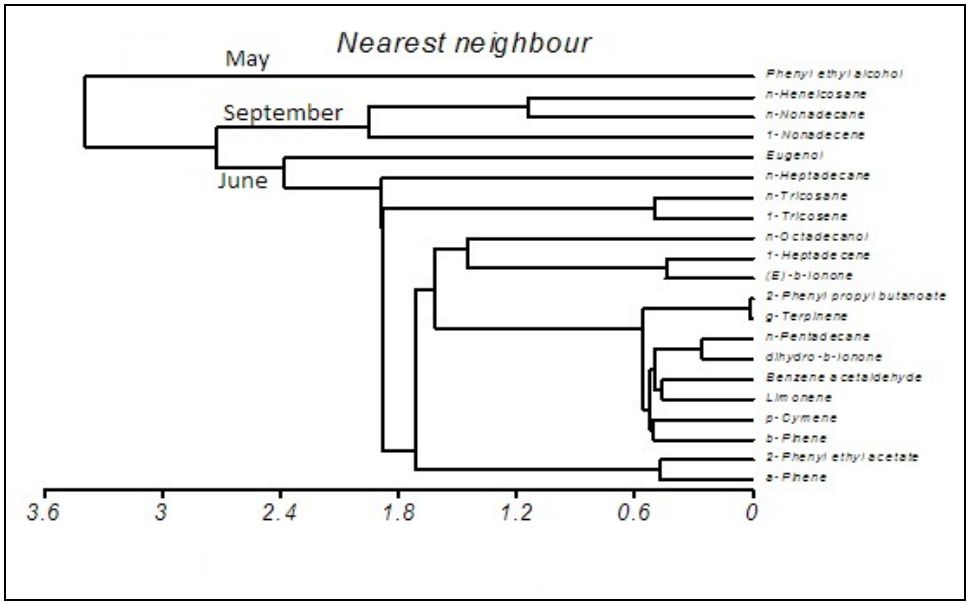

Fig. 1 Dendrogram obtained from the hierarchical cluster analysis (HCA) of 21 VOCs of *R. moschata* Herrm. in different seasons. Samples are clustered using Ward's technique with nearest neighbor measure.

Fatty acid derivatives such as 1-Nonadecene (1.01-30.42 %), n-Nonadecane (4.61-14.04%), n-Heneicosane (4.47-12.07%) and 1-Tricosene (0-5.91%) were fluctuated in different seasons. Plants produce many fatty acid derivatives, numerous of which play key regulatory functions. Additionally, fatty acids could be involved in cell death and the expression of stress-related gene, be active as intracellular mediators, extracellular signals, signals for communication between organisms and activate jasmonate signaling for inhibition of root growth and the increase of anthocyanins [13, 18].

Terpenoids derivatives content were different in each season, with a low level during May and maximum content in June and September respectively. In June the amount of (E)-β-Ionone in the VOCs was highest (2.325 %). The lowest terpenoids derivatives content occurred during May, when the (E)-β-Ionone contents in the VOCs was highest. In the Persian Musk rose VOCs, the high (E)-β-Ionone content is compensated by lowering α-Pinene, Limonene and dihydro-β-Ionone. (E)-β-Ionone and α-Pinene were present at higher levels in the Persian Musk rose VOCs in September, while the (E)-β-Ionone were not detected in May. Many procedures could be concerned in the change of terpenoids content, e.g. the rate of biosynthesis, the rate of metabolic loss, or the rate of volatilization [19]. For example, in the monoterpene group, it is important to communicate that the relationship between α-terpinene and *p*-cymene: an increase of *p*-cymene was associated with a decrease of α-terpinene [19].

Conclusion

From the results of this study, it is clear that during seasonal variation and emission timing, floral VOCs varied significantly over time. Hierarchical cluster analysis (HCA) was performed to define both similarities and differences across different seasons in Persian Musk rose flower. Cluster analysis of the different seasons showed two major clear clusters, each of seasons (Fig. 1) referred to as groups May and June/September.

Plant VOCs have the chemical scene of several ecosystems during seasonal variation. They are particularly involved in the attraction of pollinators and seed dispersers, protection against pathogens, herbivores and florivores, plant-plant signaling, operate both as long- and short-distance attractants, defense plants against biotic and abiotic stresses [13]. Therefore, seasonal variations of floral VOCs have essentially function and application in natural ecosystems and agriculture. In addition, further investigation of accurate selection of emission timing rather than emission amount will indubitably encourage our understanding in the fundamental mechanisms of plant-insect interactions and assist to reach preferred effects such as increasing pollination whereas decreasing pest attraction.

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