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ORIGINAL PAPER

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ESSENTIAL OIL CONTENT AND COMPOSITION IN VARIOUS ECOTYPES OF DAMASK ROSE FROM DIFFERENT ECOLOGICAL REGIONS

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ABSTRACT

Rosa damascena as a holy ancient plant with modern uses in perfumery and therapeutic processes, should be more investigated due to its utilization in food ingredients, preclinical and clinical studies, and cosmetics industry. Here, we have evaluated the proline content, total phenol of sepal and petal, oil content, and essential oil (EO) composition in different damask rose ecotypes [(Oroumieh; OR), (Golab; GB), (Oskou; OS), (London; LN), and (Mahallat; MT)]. The highest proline and oil content produced in GB ecotype. There was a positive correlation between oil content and proline production in ecotypes ($r^2 = 0.8064$). The major compounds of rose EO in OR, GB, OS, and MT ecotypes were nonadecane, heneicosane, citronellol, and geraniol. Whereas the main compounds in LN oil were heneicosane (11.43%), *Z*-5-nonadecene (10.34%), citronellol (8.84%), and geraniol (6.97%). The highest content of terpenes + sesquiterpenes were produced in GB followed by MT, while the lowest terpenes + sesquiterpenes content were in OR and LN, respectively. Based on the uses of rose oil for cosmetics, medicine, and/or therapeutic processes, the specific ecotype with distinct oil profile can be proposed.

Key words: 2-phenethylalcohol, citronellol, gc-ms, proline, rose oil

INTRODUCTION

Rosa damascena Mill. (Damask rose), which is known as *Gol-e-Mohammadi* in Iran, is one of the most important essential oil (EO) sources. It's a perennial bush rose which has a pink flower with 30 petals and heavy scent. *Rosa damascena* plant was originally brought to Europe from Damascus and therefore, called Damask rose. The genus *Rosa* comprises more than 200 species with 18 000 cultivars from *Rosaceae* family originated in the temperate regions, is now widespread all over the globe [Nedeltcheva-Antonova et al. 2017]. Rose oil is the most widely used EO in perfumery and cosmetics as well as in therapeutic possessing bacteriostatic, antihystological, antispasmodic, gall curative, and relaxing [Nazzaro et al. 2017]. In traditional medicine, *Rosa damascena* is used for the treatment of abdominal and chest pains, menstrual bleeding, and digestive problems [Mahboubi 2016]. Basim and Basim [2003] which is reported that EO of *Rosa damascena* is a potential control agent for *Xanthomonas axonopodis* spp. *Vesicatoria* bacteria. In the Unani system of medicine, *Rosa damascena* has been widely used as an ingredient of various polyherbal formulation for the treatment of obesity [Government of India 1981].

The chemical composition of *Rosa damascena* oil has been reported by many researchers [Pellati

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et al. 2013, Koksall et al. 2015, Mirzaei et al. 2016]. Monoterpene alcohols content, including citronellol, geraniol, nerol, linalool, and 2-phenetheylalcohol characterize the rose oil. The daily in the morning hand-picked flowers of damask rose (5:00–10:00 am) are using for distillation. Hydro-distillation is a widely used method for producing EO from damask rose. Due to the low oil content in rose petals, (~1 kg from 3000 kg petals), rose oil is one of the most expensive oils in the world markets. Temperature, cloudiness, humidity, and precipitation in the flowering season (May and June) are main factors affecting rose oil quality. Therefore, the worldwide production of rose essential oil is currently centered in Turkey (Isparta province), Bulgaria (Kazanluk Valley), and Iran (Kashan, Isfahan Province) [Zeinali et al. 2010]. Recently, China, France, and Romania have been trying to develop their own *Rosa damascena* EO. However, due to the specific climate condition, they did not have significant success [Nedeltcheva-Antonova et al. 2017].

Organic compounds such as proline, which is a sign of plant adoption to unfavorable conditions [Kashefi et al. 2012], play an important role in osmotic adjustment in all plant species under stress conditions [Garcia-Caparros et al. 2016], and help to maintain the balance of osmotic potential in the vacuole [Munns and Tester 2008]. Phenolic compounds synthesized at low concentrations in plant organs and have many medicinal effects and antioxidant activities [Kovatcheva-Apostolova et al. 2008]. Phenolic compounds as plant secondary metabolites produce from phenylpropanoid pathway [Kanani and Nazarideljou 2017] and pentose phosphate pathway [Lin et al. 2016] and are usually related to defense responses in the plant.

Plant ecotypes are distinct genotypes or populations within a species. Ecotypes resulting from adaptation to local environmental conditions, capable of interbreeding with other ecotypes or epitypes [resulting from adaptation to a specific (local) genetic background] of the same species [Hufford and Mazer 2003]. Ecotypes, from different geographical regions, could have evolved different gene complexes favoring adaptation to local environmental conditions. The translocation of plant species during the restoration of native ecosystems provoke new questions concerning many growth aspects as well as modifications in chemical composition between locally adapted and transplanted genotypes. The geographical origin, production method, and environmental conditions are among the factors affecting EOs composition [Koksall et al. 2015]. Researchers have reported different EOs composition from different part of the world. In the central part of Iran, as one of the main grower and producer of *Rosa damascena* and derivatives, the major EOs components have been reported as citronellol, geraniol, and nonadecane [Sadraei et al. 2013]. In the northern part of Iran, triacosane, 1-nonadecene, *n*-tricosane, and geraniol was introduced as major compounds of rose oil. In Pakistan, 2-phenethylalcohol was reported as the main component of rose oil [Khan and Rehman 2005]. In another study, citronellol, nonadecane, geraniol, ethanol, heneicosane, nerol, and 1-nonadecene have been reported as the major components in rose EOs [Bayrak and Akgül 1994]. Considering the importance of damask rose oil in industry, cosmetics, health, and modification of EOs content in different growth conditions, here, efforts have been made to compare essential oil content and composition of damask rose ecotypes after restoration in order to choose the best ecotype for producing essential oil between the studied ecotypes.

MATERIAL AND METHODS

Plant materials. Different ecotypes of *Rosa damascena* (OR: Oroumieh, West Azerbaijan: North West of Iran; GB: Golab, Kashan: Central Iran; OS: Oskou: East Azerbaijan: North West of Iran; LN: London, UK; MT: Mahallat: Markazi: Central Iran) were supplied from different regions, translocated to the Oroumieh site [38 S 517767 4142124, H = 1300 m] and cultivated outdoor in the prepared site. During the first year, the morpho-physiological parameters tracked, and then, during the second year after restoration, biochemical parameters including proline content, total phenolic content, and essential oil composition were studied in a completely randomized block design with three repetitions including three plants at each block.

Proline assay. Bates et al. [1973] method with some modifications applied to measure proline content. Briefly, 50 mg of dried leaf tissue was homogenized in 1 ml sulfo-salicylic acid 3% and then centrifuged for 10 min at 6000 rpm. Afterwards, 2 ml of supernatant + 2 ml ninhydrin (Merck, Germany) reagent + 2 ml

glacial acetic acid were mixed and then transferred to the heat bath (100°C) for 1 h. The reaction was stopped using ice water bath. Then, 2 separate layers were formed with adding 8 ml toluene (Merck, Germany) to the mixture. The absorbance of supernatant was read at 520 nm with spectrophotometer (Jenway 6705 UV/Vis, UK). Proline content calculated based on a standard curve (Fig. 1A).

Formula 1: [(µg proline/ml) ×
× ml Toluene) / 115.5 µg/mmole] / (g sample/5)

Extraction for phenol assay. Sepals and petals of damask rose were fine dried and ground in lab condition. Afterward, 1 g of each sample was transferred to a falcon and 10 ml methanol 80% was added (sample: methanol = 1 : 10 ratio). After 24 h, the mixture filtered and methanol evaporated using a rotary set. The extract was centrifuged (Rotofix 32A-Zentaifugen, Germany) for 5 min at 2500 rpm. The supernatant used for total phenol determination.

The Folin–Ciocalteu (Merck, Darmstadt, Germany) method based on Slinkard and Singleton [1977] with some modifications was used to determine total phenolic content in leaves and petals of *Rosa damascena*. Total phenol content expressed as µg GAE g^{-1} DW based on the prepared standard curve (Fig. 1B).

Oil content. Oil content was measured after water distillation using Clevenger apparatus and separation of oil from water using hexane.

Essential oil assay. Damask rose petals were handpicked in the early morning, transferred to the laboratory and hydro-distillation was done using Clevenger apparatus and oil content was measured. The collected essential oil injected to a GC-MS and chemical composition identified. The extract was injected into a 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a 5975C mass detector (Agilent Technologies). The injector was in splitless mode and operated at 250°C with a purge flow of 50 ml min^{-1} for 2 min. For separations, a 30 m × 0.25 mm (internal diameter) column with 0.25 µm film thickness (HP-5 MS column; Agilent Technologies) used. Helium gas was used at a flow rate of 1.5 ml min^{-1}. The temperature of the column was held at 80°C for 3 min then increased at a rate of 8°C min^{-1} to 180°C and held for 3 min before increasing to a final post-run temperature of 240°C for 5 min. The parameters of the HP 5975C mass detector were as follows: electron impact ionization (EI) source temperature: 230°C; interface temperature: 280°C; ionization energy: 70 eV; mass range: 40-500 amu; quadrupole temperature: 150°C. The compounds identified using the Mass Spectral Library (edition 7n.1; Wiley, Hoboken, NJ, USA). Their retention indices compared, and the area under the curve of each compound peak measured to determine their relative abundances.

Statistical analysis. Data analysis was performed using SAS V9.2 software (SAS Institute Inc., Cary, NC, USA). Means were compared using Duncan's multiple range test at $P \leq 0.01$ and $P \leq 0.05$.

RESULTS

Proline. A significant difference ($P \leq 0.01$) was observed among ecotypes in proline content. The highest and lowest proline content produced in GB and OR ecotypes, respectively (Tab. 1).

Phenol of sepal and petal. Total phenol content in damask rose sepals did not show a significant difference between treatments, although the highest content of total phenol in sepals was produced in OR ecotype (Tab. 1). The highest and lowest content of phenol in damask rose petals were produced in OR and GB ecotypes. Ecotypes that produced the highest content of proline, produced the lowest petal phenols and there was a negative correlation between proline content and petal phenol ($r^2 = 0.9968$; Fig. 2B).

Essential oil content and composition. The highest oil content was produced in GB (0.24 ±0.001 a) and MT (0.23 ±0.001 a) ecotypes and there was a significant difference between ecotypes ($P < 0.01$). The lowest oil content resulted in LN ecotype (Tab. 1).

Essential oil analysis was done qualitatively and GC-MS chromatograms were studied (Fig. 3). Results revealed that the main compounds of damask rose oil in OR, GB, OS, and MT ecotypes were nonadecane, heneicosane, citronellol, geraniol, and *Z*-5-nonadecene, while the main compounds in LN ecotype were heneicosane, *Z*-5-nonadecene, citronellol, and geraniol (Tab. 2). The highest and lowest content of terpenes were in GB (Central Iran) and OR (North-West of Iran) ecotypes, respectively (Tab. 2). OR ecotype produced the highest content of aliphatic hydrocarbons followed

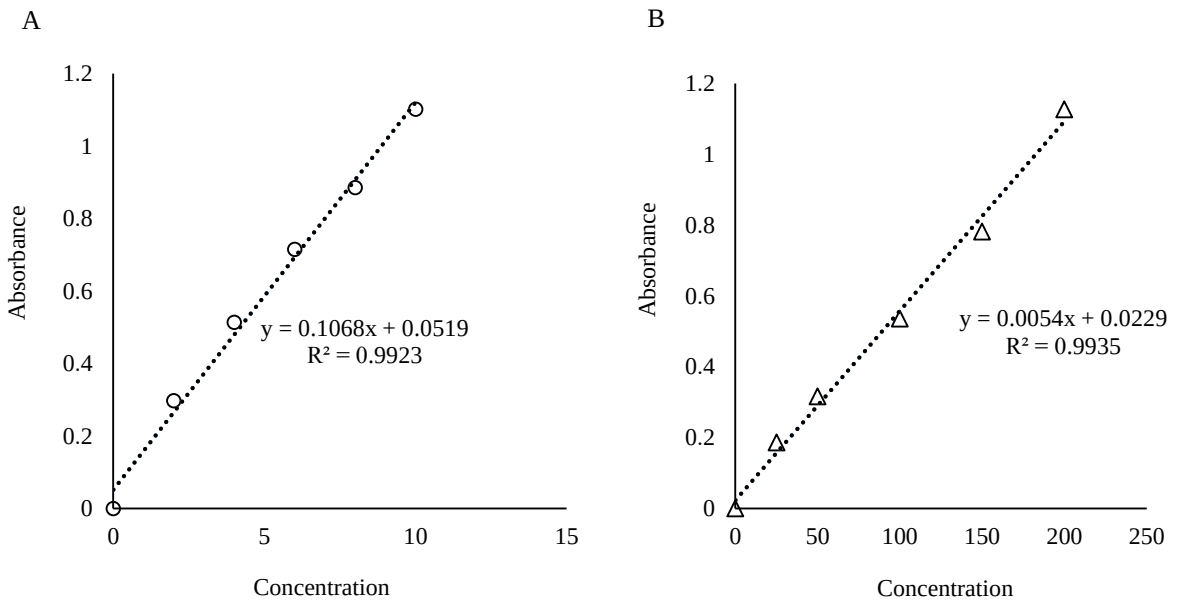

Fig. 1. A standard curve of proline (A) and gallic acid (B) for determination of *Rosa damascena* proline and phenolic content, respectively

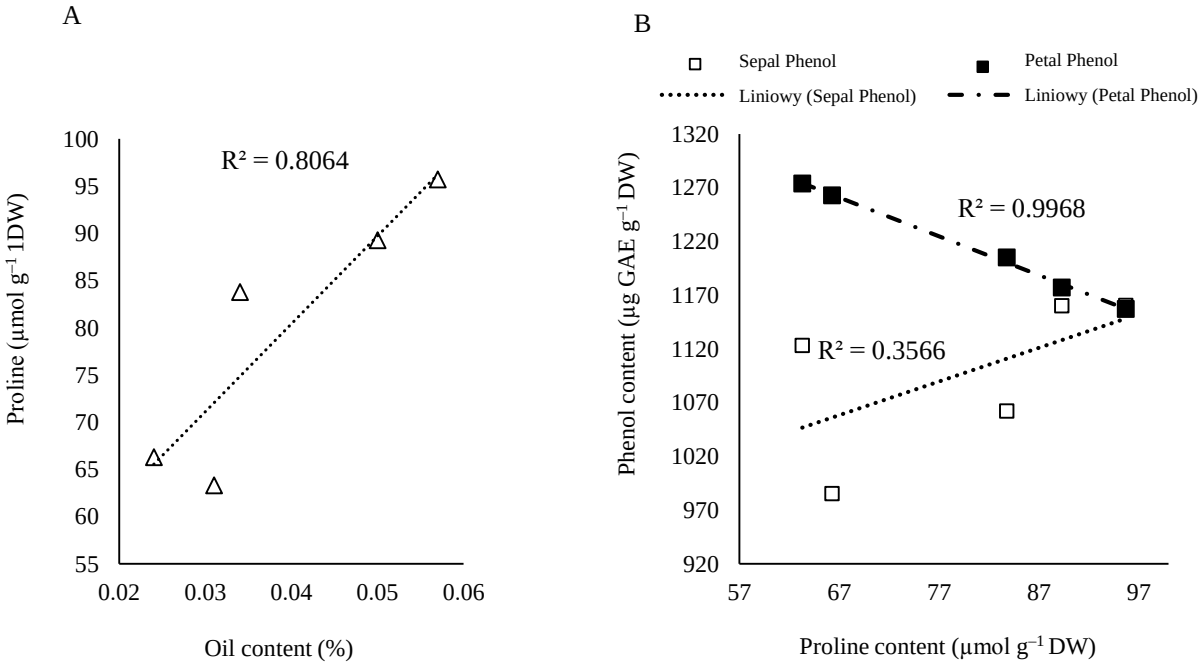

Fig. 2. Correlation between proline production and oil content (A) and phenol production (B) in *Rosa damascena* essential oil

Table 1. Comparison of the physio-biochemical parameters in various damask rose ecotypes from different ecological regions (mean ±SE, n = 3)

Ecotypes	Proline (μmol g^{-1} DW)	Oil content (%)	Sepal phenol (μg GAE g^{-1} DW)	Petal phenol (μg GAE g^{-1} DW)
Oroumieh	63.30 ±0.13 d	0.19 ±0.002 bc	1123.03 ±4.46 a	1273.65 ±1.07 a
Golab	95.71 ±0.13 a	0.24 ±0.001 a	1160.32 ±1.61 a	1157.0 ±2.48 c
Oskou	83.78 ±0.67 c	0.20 ±0.0021 b	1062.05 ±5.5 a	1205.0 ±2.43 b
London	66.29 ±0.53 d	0.16 ±0.0029 c	985.26 ±10.54 a	1262.78 ±11.82 a
Mahallat	89.29 ±1.55 b	0.23 ±0.001 a	1160.07 ±1.13 a	1177.11 ±0.85 c

Different letters in each column indicate significant differences determined using a Duncan's multiple range test ($P < 0.05$)

by OS ecotype. MT ecotype produced the highest content of sesquiterpenes followed by GB ecotype (Both from Central Iran).

DISCUSSION

Proline. Proline is a versatile metabolite accumulates in higher plants in response to a stress condition, which is a protective mechanism. Likewise, proline acts as a molecular chaperone to enhance the activity of some enzymes and protect protein integrity [Szabados and Savoure 2010]. Involvement in osmoregulation and the stabilizing of sub-cellular structures are among the main roles of proline in enhancing stress resistant [Pociecha et al. 2008]. Therefore, proline content enhances during exposure to environmental stresses and climate changes, mainly due to increased synthesis and reduced degradation. Here, OR ecotype produced the lowest proline content which could be due to cultivation in its native site, However, the GB ecotype belongs to the central Iran which translocated into the new site in West-Northern part produced the highest proline content. Surprisingly, the proline content in LN ecotype compared to some native ecotypes such as GB, MT, and OS was lower, which could be due to better adaptation and restoration in the new site.

Moghaddam and Mehdizadeh [2017] reported that stress condition, such as drought, alkalinity, high and low temperature, UV, and salinity could enhance the content of EO production in plants. High production of oil content in GB ecotype could be due to more severe exposure to stress condition. Based on Table 1, the highest proline content as an indicator of stress condition was produced in GB ecotype and there was a positive correlation between proline production and oil content in different ecotypes ($r^2 = 0.8064$; Fig. 2A).

Phenol of sepal and petal. Phenolic compounds play multiple biological activities and thus exhibit a lot of scientific attention. The difference in phenol content in different plant organs have been frequently reported [Ceccarelli et al. 2010; Elfalleh et al. 2012], which reported that the distribution of phenolic compounds may change during development.

Proline accumulation varies among species and can be 100 times greater than in the control situation [Verbruggen and Hermans 2008]. Hardening of two *Festulolium* genotypes plants at 2°C, increased proline content as well as phenolic contents in leaves [Pociecha et al. 2008]. In plants, proline synthesized from two substrates, including glutamate and ornithine [Verbruggen and Hermans 2008]. The conversion of phenols into glutamate and proline in *Corynebacterium glutamicum* has been reported by Lee et al. [2010]. Our results suggested that during environmental stress conditions, phenols converted into the proline and plants have used the petal phenols to conversion.

Essential oil content and composition. Baydar et al. [2008] and Verma et al. [2011] have reported the citronellol, geraniol, heneicosane, and nonadecane compounds in damask rose oil. Citronellol and geraniol, as monoterpenoids, are responsible for the higher quality of the rose oil and the lower amount of these compounds, especially citronellol, led to the

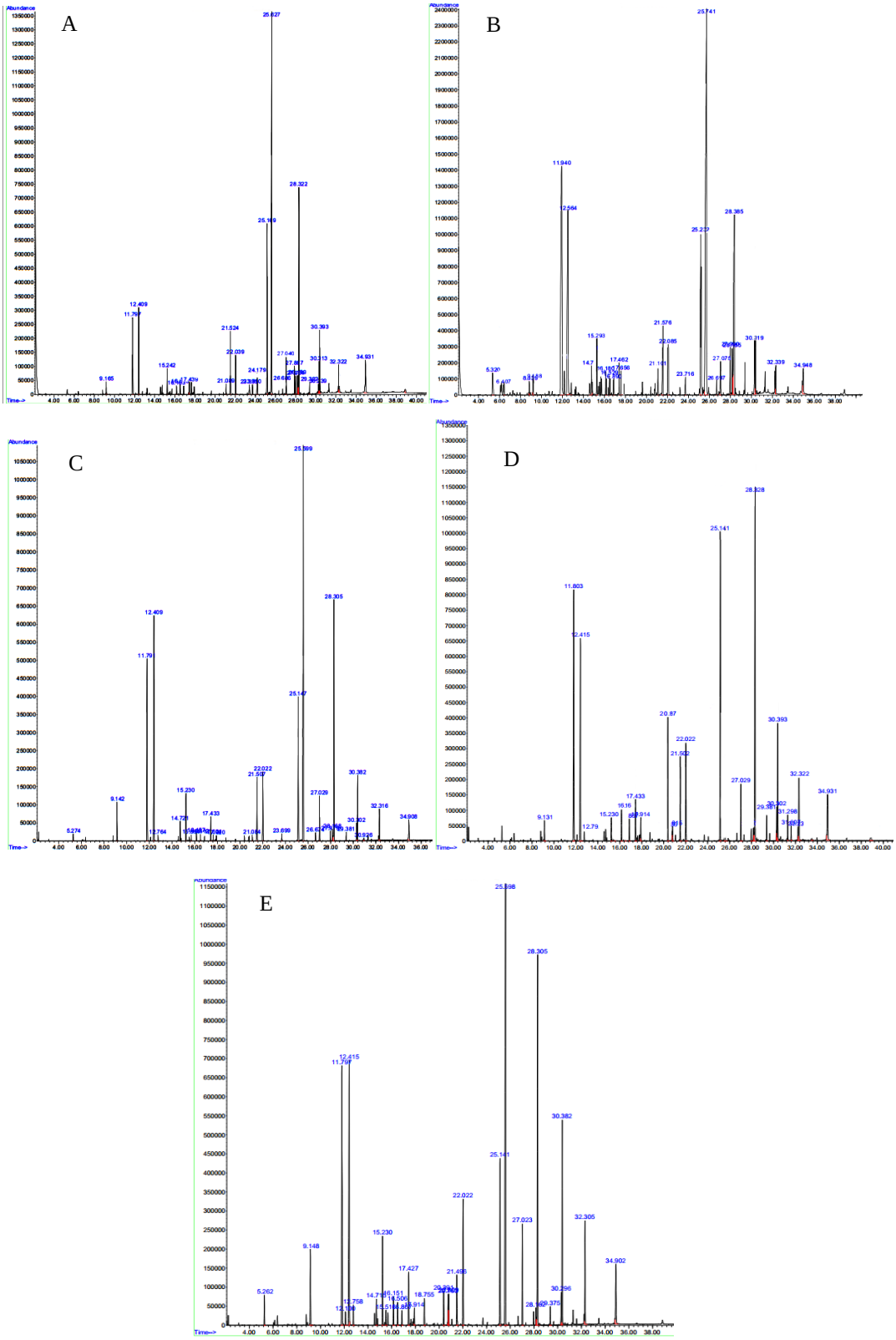

Fig. 3. GC-MS chromatograms of essential oil in various damask rose ecotypes from different ecological regions (A – Oroumieh, B – Golab, C – Oskou, D – London, and E – Mahallat)

Table 2. Essential oil composition in various ecotypes of *Rosa damascena* from different ecological regions

	Component	Formula	RI	RT	Oroumieh (%)	Golab (%)	Oskou (%)	London (%)	Mahallat (%)
1	α-pinene	$C_{10}H_{16}$	936	5.32	–	1.65	0.36	–	0.70
2	β-myrcene	$C_{10}H_{16}$	992	6.41	–	0.74	–	–	–
3	linalool l	$C_{10}H_{18}O$	1097	8.78	–	0.54	–	–	–
4	2-phenethyl alcohol	$C_8H_{10}O$	1115	9.16	0.66	1.33	1.84	0.75	2.45
5	citronellol	$C_{10}H_{18}O$	1236	11.79	5.97	13.72	10.34	8.84	10.87
6	neral	$C_{10}H_{16}O$	1242	12.11	–	–	–	–	0.39
7	geraniol	$C_{10}H_{18}O$	1255	12.41	6.44	11.40	12.02	6.97	11.59
8	geranial	$C_{10}H_{16}O$	1272	12.78	–	0.53	0.25	0.53	0.56
9	citronellyl acetate	$C_{12}H_{22}O_2$	1351	14.56	–	0.73	–	–	–
10	eugenol	$C_{10}H_{12}O_2$	1360	14.76	–	1.14	0.90	–	0.75
11	geranyl acetate	$C_{12}H_{20}O_2$	1381	15.24	2.06	3.43	2.17	0.73	2.62
12	*trans*-caryophyllene	$C_{15}H_{24}$	1425	16.16	0.43	0.72	0.35	1.55	0.88
13	α-guaiene	$C_{15}H_{24}$	1442	16.51	0.41	0.52	0.29	–	0.66
14	α-humulene	$C_{15}H_{24}$	1460	16.89	–	0.47	–	0.32	0.45
15	germacrene D	$C_{15}H_{24}$	1486	17.43	1.32	1.65	1.19	1.41	1.68
16	*N*-pentadecane	$C_{15}H_{32}$	1496	17.64	0.47	0.55	0.28	0.40	–
17	α -eudesmol	$C_{15}H_{26}O$	1659	20.82	–	–	–	4.16	0.98
18	8-heptadecene	$C_{17}H_{34}$	1673	21.08	0.51	0.71	0.32	0.59	–
19	heptadecane	$C_{17}H_{36}$	1696	21.52	4.32	4.05	3.40	3.18	1.70
20	1-heptadecanol	$C_{17}H_{36}O$	1720	22.03	3.35	3.55	3.87	3.23	4.70
21	octadecane	$C_{18}H_{38}$	1798	23.71	0.47	0.52	0.38	–	–
22	*Z*-5-nonadecene	$C_{19}H_{38}$	1877	25.16	11.02	9.33	8.84	10.34	5.90
23	nonadecane	$C_{19}H_{40}$	1903	25.62	27.40	20.59	26.88	–	20.29
25	(*E*)-9-eicosene	$C_{20}H_{40}$	1976	26.68	0.50	0.49	0.34	–	–
26	eicosane	$C_{20}H_{42}$	2000	27.04	3.06	2.52	2.83	1.94	2.57
27	heneicosane	$C_{21}H_{44}$	2097	28.32	14.14	10.18	13.50	11.43	13.70
28	docosane	$C_{22}H_{46}$	2199	29.39	0.44	–	0.32	0.36	0.44
29	9-tricosene	$C_{23}H_{46}$	2291	30.31	1.19	1.14	0.76	0.86	0.93
30	tricosane	$C_{23}H_{48}$	2299	30.39	4.54	3.30	3.72	3.79	4.96
31	pentacosane	$C_{25}H_{52}$	2498	32.33	2.12	1.61	1.66	2.19	3.08
32	heptacosane	$C_{27}H_{56}$	2702	34.93	2.59	1.52	1.49	1.60	2.80
Terpenes (%)		–	–	–	13.07	29.38	24.56	16.56	25.61
Sesquiterpenes (%)		–	–	–	2.16	3.36	1.83	3.28	3.67
Aliphatic hydrocarbons (%)		–	–	–	76.12	60.06	68.59	44.07	62.05
Total components (%)					93.41	98.63	98.3	65.17	95.65

RI – retention index, RT – retention time

lower quality of rose essential oil [Erbas and Baydar 2016]. According to the international standard of rose oil (ISO 9842:2003), the content of rose oil requires citronellol (20–34%), geraniol (15–22%), and nonadecane (8–15%) [ISO 2003], where the ratio of citronellol/geraniol is considered to be 1.25–130 [Mirzaei et al. 2016], to fill the demand of the perfumery industry. The amount of citronellol and geraniol in GB (13.72% and 11.40%, respectively), OS (10.34% and 12.02%, respectively), and MT (10.87% and 11.59%, respectively) ecotypes were higher, however the OR and LN ecotypes produced the lower amount of citronellol and geraniol (although the highest ratio of citronellol/geraniol was observed in LN ecotype). Thus, the quality of rose essential oil in GB ecotype was the best and OR ecotype produced the least oil quality between studied ecotypes (Tab. 2). According to rose oil standard, GB and LN ecotypes EOs have the potential in order to be used in perfumery (Tab. 2). Further, the highest and lowest amount of total essential oil compounds produced in GB and LN ecotypes, respectively. Enhancement in the content of EO is often happen as a response due to several environmental stresses, such as high and low temperatures, drought, alkalinity, salinity, UV stress, pathogen infection [Moghaddam and Mehdizadeh 2017].

2-phenethyl alcohol (2-phenylethanol) is also responsible for the characteristic odor of fresh rose petals. The presence of this compound in rose oil, accelerate the quality. 2-phenylethanol is slightly soluble in water and in hydro-distillation method, the highest amount of this alcohol is lost during the extraction process. Here, MT ecotype produced the highest content of 2-phenylethanol, where OR ecotype produced the lowest. β-myrcene, linalool, and citronellyl acetate solely produced in GB ecotype, while docosane compound produced in all studied ecotypes except GB.

CONCLUSIONS

The result of this study revealed that the GB ecotype produced the highest oil content, as well as the highest amount of citronellol, and geraniol. Further, the highest diversity of oil compounds was in GB ecotype (28 compounds), where LN ecotype produced the least number of compounds (21 compounds). Therefore, the GB content (originated from central Iran) is a desirable ecotype for producing high-quality EO and have potential to be used in the perfumery industry among the studied ecotypes. Other studied ecotypes produced a high amount of citronellol, geraniol, 2-phenylethanol, heneicosane, and nonadecane and therefore have the potential to be used as food ingredients and medicinal practices.

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