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Variations in floral characteristics and scent composition and the breeding potential in seed-derived oil-bearing roses (*Rosa damascena* Mill.)

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Abstract: Oil-bearing rose (*Rosa damascena* Mill.) is one of the most strongly scented rose species used in the perfumery and cosmetics industries. This research ultimately aimed to reveal the variation in and evaluate the breeding potential of plants whose seeds were derived from *R. damascena*, which has only been propagated vegetatively for centuries. The seeds extracted from the mature fruits of open-pollinated plants were stratified at 4 °C for 3 months and sown into vials. Seedlings in pots were grown under greenhouse conditions, and a total of 83 seed-derived plants were finally planted in the experimental field. The 3-year-old progenies were examined for floral characteristics and scent composition by using HS-SPME combined with GC-MS. A wide variation in flower characteristics was identified, e.g., with different petal colors from white to red and petal numbers from 5 to 115. Considerable variability in floral scent molecules such as phenylethyl alcohol (23.26%–74.54%), citronellol (5.57%–31.59%), and geraniol (3.09%–26.93%) was recorded among the seed-derived plants. As a result, the genetic variations resulting from the segregation of the alleles at heterozygous loci were appropriate for the clonal selection of novel oil-bearing rose varieties.

Key words: Seed propagation, segregating population, flower characteristics, HS-SPME analysis

1. Introduction

Oil-bearing rose or Damask rose (*Rosa damascena* Mill. f. *trigintipetala* Dieck) with about 30 pink petals is one of the most strongly scented rose species with characteristic floral scent molecules such as citronellol, geraniol, nerol, and phenylethyl alcohol. Its main industrial products are rose oil, rose water, rose concrete, and rose absolute oil, which are produced by hydrodistillation and solvent extraction processes. *R. damascena* Mill. is an allotetraploid species (2n = 4x = 28) as a hybrid of *R. gallica* L. and *R. moschata* J.Herm. (for summer Damask roses) or *R. gallica* L. and *R. phoenicia* Boiss. (for autumn Damask roses) (Gudin, 2000). It is also thought to be of triparental origin from *R. gallica* L., *R. moschata* J.Herm., and *R. fedtschenkoana* Reg. (Iwata et al., 2000).

Turkey, Bulgaria, and Iran are the main countries producing Damask rose in the world. Although wide genetic diversity was determined by molecular markers among the *R. damascena* plants collected from Iran and its neighboring areas including Pakistan (Kiani et al., 2010; Farooq et al., 2013), studies on molecular analyses with RAPD, AFLP, and SSR markers did not show any polymorphism among *R. damascena* plants cultured in Turkey (Agaoglu et al., 2000; Gokturk Baydar et al., 2004) and Bulgaria (Rusanov et al., 2005b). These results all show that industrial oil-bearing roses under cultivation in Turkey and Bulgaria have little to no genetic diversity among individuals and populations and therefore contain many genetically identical accessions.

Since oil-bearing roses have been propagated vegetatively for hundreds of years, the current roses in the rose valleys of Turkey and Bulgaria are most probably clonal progenitors of the first planted oil-bearing roses, which have been maintaining their primitive features to date. On the other hand, the phenotypic homogeneity caused by continuous vegetative reproduction makes it possible to produce rose oil with international standards (ISO 9842:2003) describing the ranges of chemical and physical parameters. Thus, the perfumery and cosmetics industries, the main consumers of rose oil, are quite insistent on demanding the preservation and maintenance of the current quality of rose oil (i.e. its chemical and physical properties) (Rusanov et al., 2009). In conclusion, it is necessary to improve the new varieties without any significant changes that go beyond the standard specifying the limits of floral scent molecules.

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So far, superior or outstanding types of oil-bearing rose have not been selected through breeding in Turkey due to the lack of genetic variation among the plants under culture. There is a need for different methods such as hybridization (inter- and intraspecific crosses) and induced mutation (physical or chemical mutagens) to create genetic variation in roses (Gudin, 2000). The other quite practical and effective methods causing genetic variation in oil-bearing rose are progenies derived from the seeds of open- and cross-pollinated plants grown only by vegetative propagation with their cuttings (Rusanov et al., 2005a). However, the plants of oil-bearing rose produce no or few viable and nondormant seeds owing to high self-incompatibility, allogamous sexual reproduction, and complex allotetraploid nature (Rusanov et al., 2009). Nevertheless, it is possible to collect a few seeds and to break their dormancy despite all challenges (Kazaz et al., 2010). Many of the seedlings derived from the seeds of open-pollinated flowers may differ genetically due to the segregation of the alleles at heterozygous loci during meiosis (Gudin, 2003).

The ultimate goals of this research were to create genetic and/or phenotypic variations in the flower characteristics and floral scent molecules of *R. damascena* by means of seed propagation and to evaluate the potential variation for different agroindustrial uses.

2. Materials and methods

2.1. Plant materials and greenhouse and field experiments

This research was conducted at the Department of Field Crops of the Faculty of Agriculture, Süleyman Demirel University, in Isparta – a city in the southwestern part of Turkey, which is called the "Rose Valley" of Turkey because of the advanced industrial oil-bearing rose cultivation under favorable climatic and soil conditions. The seeds were extracted from the mature fruits of *Rosa damascena* Mill. f. *trigintipetala* Dieck (2n = 4x = 28) collected from the populations or plantations found in the Isparta region in October 2007. A total of 1000 seeds were stratified at 4 °C for 3 months and sown into vials in March 2008. Healthy seedlings were transferred to pots first, then grown under greenhouse conditions in 2009 and eventually planted with 1.5 m within rows and 3 m between rows with drip irrigation applied in the experimental field (an altitude of 1050 m) in March 2010 (Figure 1). A total of 83 seed-derived progenies (coded as 'Rd-1', 'Rd-2', 'Rd-3', etc.) together with their clonally derived parent coded as 'Rd-Isparta' were the same age and grown considering the recommended agricultural practices for oil-bearing rose in the following years.

2.2. Floral and flower traits

The segregated population with 3-year-old plants was examined in terms of flowering time (date), flowering duration (days of flowering between the start and end of flowering), number of petals per flower, flower weight (g per flower), petal ratio (%, the part of petal weight in the total fresh weight of a whole flower), flower diameter (cm per flower), hypanthium width and length (mm per flower), numbers of anthers and stigmas per flower, pedicel/hypanthium hairiness (present or not), and fruit set scoring fruit numbers per plant (0, 1–25, 26–50, 51–75, 76–100, and >100) in the blooming season of May and June 2013, when climatic conditions were similar to the long-term averages of the site. Five flowers from each plant were measured on the condition that the plant produced enough flowers. Petal color was assessed on recently opened flowers and measured with a portable colorimeter (Model CR-300, Minolta Camera Ltd., Osaka, Japan) to obtain L*, a*, and b* values. The value L* represents brightness and darkness, a* represents greenness and redness as the value increases from negative to positive, and b* represents blueness and yellowness. Color was measured in the middle of each petal (three replicates per flower) to ensure equal measurement conditions (Schmitzer et al., 2010).

2.3. Floral scent analysis

The floral scent molecules of the fresh flowers were detected using headspace solid-phase microextraction (HS-SPME) combined with gas chromatography/mass spectrometry (GC-MS). The flowers subjected to SPME analyses were collected around 0800 hours from flowers at stages 5 or 6 (inner and outer whorls open, reproductive organs visible), according to the proposed stages of *R. damascena* flower development as suggested by Rusanov et al. (2011). Due to the differences of flowering dates of the each seed-derived *R. damascena* plant, SPME analyses were performed on the days when each plant bloomed. The flowers before SPME extraction were stored in closed glass jars at 4 °C.

The fresh flowers were subjected to SPME (Supelco, Germany) with a fiber precoated with a 75-μm-thick layer of Carboxen/polydimethylsiloxane (CAR/PDMS) and 2.5 g of newly hand-picked fresh flowers was put into a 15-mL vial, which was then immediately sealed with a silicone septum cap. After heating at 60 °C for 30 min, the SPME fiber was pushed through the headspace of a sample vial to adsorb the volatiles for 30 min and then inserted directly into the injection port of the GC-MS instrument (Shimadzu 2010 Plus GC-MS with capillary column, Restek Rx-5 Sil MS 30 m × 0.25 mm, 0.25 μm) at 250 °C for desorption (5 min). Helium (99.99% purity) was the carrier gas at 1.5 mL/min gas flow, and the split ratio was 1:10. GC oven program: 40 °C initial hold for 2 min with a ramp of 4 °C/min to 250 °C hold for 5 min; run time 60 min. Acquisition parameters: GC inlet 250 °C; MS dedector 250 °C; ion source 200 °C; MS gain: 70 eV with scan mode. The compounds were identified with

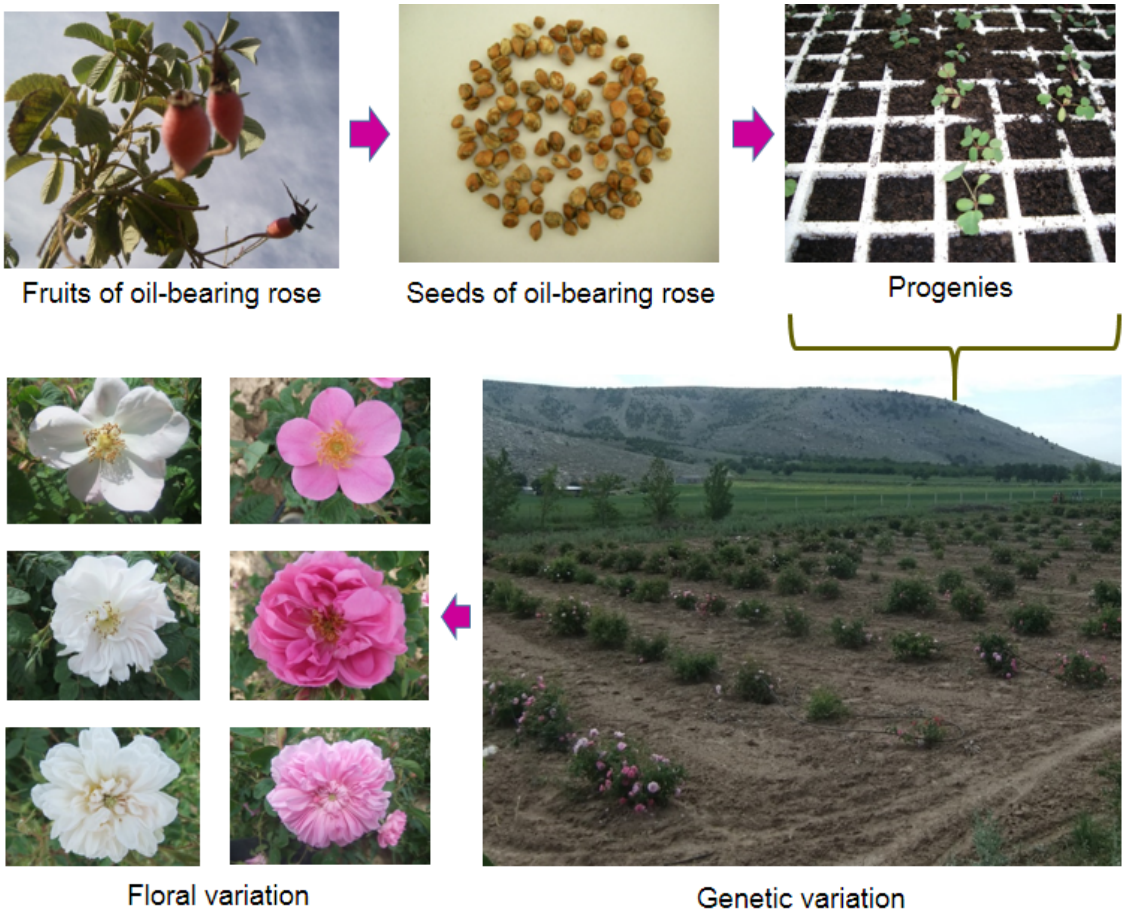

Figure 1. Variations in petal numbers and petal colors through the seed propagation of *Rosa damascena.*

the help of the retention times of standard substances by composition of mass spectra with the data given in the Wiley, NIST, Tutor, and FFNS libraries. The linear retention indices were calculated by using a series of the standards of C_7–C_{30} saturated n-alkanes (Sigma-Aldrich Chemical Co., USA) for reference in the same column and conditions as above for GC-MS analyses. The analyses of the samples were carried out in duplicate and the two results were averaged. Typical HS-SPME/GC-MS chromatograms of scent compounds in the fresh flowers of *R. damascena* are shown in Figure 2.

2.4. Statistical analysis

For floral characteristics and scent compounds, means ± standard errors and correlation coefficients at the 5% ($P \leq 0.05$) and 1% ($P \leq 0.01$) levels of significance were calculated by using SAS Version 7.0 (SAS/STAT, Cary, NC, USA).

3. Results and discussion

The floral characteristics of the plants derived from the seeds of open-pollinated *R. damascena* together with their clonally derived parent 'Rd-Isparta' are given in Table 1. Twelve plants among the segregating population with a total of 83 seed-derived plants budded but did not bloom in all flowering seasons of the years (2010 to 2013). All the remaining plants bloomed once in the season of May and June (nonrecurrent blooming) and did not have any subsequent cycles of flowering as recurrent roses. As the results revealed, the flowering dates of the seed-derived plants were changed from 7 May to 1 July, with those for parental genotype 'Rd-Isparta' being between 12 May and 18 June. The extension of the flowering season is one of the most important goals in rose breeding for both agricultural and industrial production. The flowering duration lasted more than 40 days for 6 plants and even longer than 50 days for 'Rd-29', whereas that of the parental genotype was around 35 days (Table 1). According to our observations in the research field, the plants with smaller numbers of petals or single flowers blossomed earlier and shed their petals more easily than the plants with larger numbers of petals or double flowers.

The coloration of rose flowers is mainly caused by the accumulation of anthocyanins such as pelargonidin and cyanidin in the petal cells (Schmitzer et al., 2010).

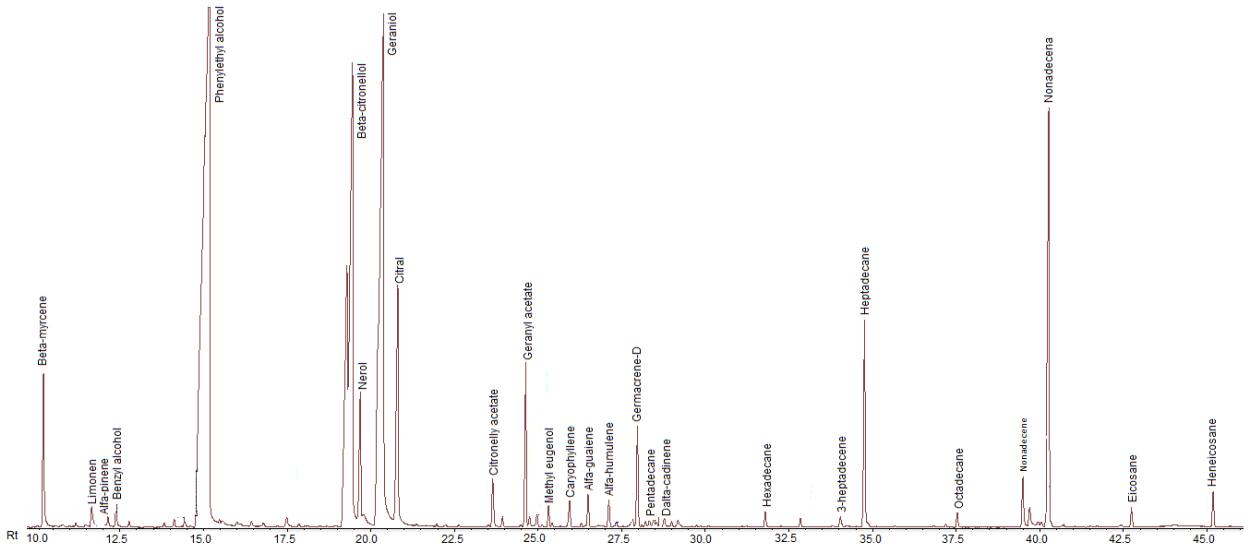

Figure 2. Typical HS-SPME/GC-MS chromatogram showing main scent compounds in the fresh flowers of *R. damascena*.

Table 1. Floral characteristics of *R. damascena* plants derived from the seeds of clonally derived genotype 'Rd-Isparta'.

Floral characteristics	Clonally derived genotype 'Rd-Isparta' (parental genotype)	Seed-derived plants (progenies from the seeds of 'Rd-Isparta')
Start of flowering (date, 2013)	12–14 May	7–29 May
End of flowering (date, 2013)	16–18 June	30 May to 1 July
Flowering duration (days)	34 to 36	12 to 50
Petal color (visual)	Pink	White to red, mainly pink
Colorimetric parameter a*	58.1 ± 5.1*	40.8 ± 5.0 to 88.7 ± 1.2
Colorimetric parameter b*	50.9 ± 4.9	4.5 ± 0.1 to 63.1 ± 3.8
Colorimetric parameter L*	–13.5 ± 1.1	–20.2 ± 0.1 to 3.8 ± 2.2
Number of petals per flower	33.5 ± 4.0	5.0 ± 0.0 to 114.8 ± 9.1
Flower weight (g)	2.57 ± 0.47	0.59 ± 0.11 to 4.22 ± 0.57
Flower diameter (cm)	6.80 ± 0.44	4.55 ± 0.05 to 8.05 ± 0.05
Petal ratio (%)	75.7 ± 5.0	44.6 ± 9.2 to 89.0 ± 6.8
Hypanthium width (mm)	4.61 ± 0.47	3.23 ± 0.49 to 7.98 ± 0.27
Hypanthium length (mm)	10.04 ± 0.55	6.11 ± 1.51 to 13.32 ± 0.93
Number of anthers per flower	95.3 ± 5.5	0 ± 0.0 to 144.0 ± 14.8
Number of stigmas per flower	33.3 ± 2.1	17.0 ± 1.0 to 88 ± 3.0
Number of fruits per plant	1–25	0 to over 100
Number of seeds per fruit	1	0 to over 10
Pedicel/hypanthium hairiness	Hairy	Hairy to hairless or naked

*The values are the means ± standard errors (n = 4 for the clonally derived genotype and n = 71 for seed-derived genotypes).

Although wild roses usually have pink or white flowers, they are devoid of the blue color because of lacking the flavonol 3',5'-hydroxylase (F3'5'H) activity to generate dihydromyricetin, a precursor of delphinidin (Tanaka et al., 2008). The petals of industrial oil-bearing roses cultured in the world are characteristically pink. In our study, the seed progenies of *R. damascena* gave a vast variation in the petal color spectrum from red ('Rd-60') to white ('Rd-12', 'Rd-

23', 'Rd-33', 'Rd-34', 'Rd-52', 'Rd-54', and 'Rd-61'); however, the majority of them were pink like the parent 'Rd-Isparta' grown in the experimental field (Figure 1; Table 1). This finding supports the idea that the pink color is a dominant character over other petal colors. The pink color has been shown to be inherited codominantly, with white being homozygous recessive, pink being heterozygous, and darker pink being homozygous dominant, as previously confirmed by Jones (2013).

According to the Minolta color parameters, while the L*, a*, and b* values of white 'Rd-12' were 86.9, 9.4, and –3.0, the same values of red 'Rd-60' were 40.8, 63.1, and –10.1, respectively (Table 1). As a result, L* and b * values decreased and a* values increased when the color darkened from white to red. Although the majority of seed-derived genotypes were pink or pinkish, some of them had different petal colors from white to red. Karami et al. (2012) found a high positive correlation (r sq. linear = 0.812) between essential oil content and anthocyanin concentration, which can be used as an essential oil quantity index in *R. damascena*.

In *Rosa* sp., the number of flower organs, and especially that of petals, greatly affects flower architecture. The number of petals was classified as either single flowers (fewer than or equal to 7 petals) or double flowers (more than 7 petals), and the inheritance of the double flower form is controlled by a dominant allele (Debener, 2003), with additive genes contributing to different levels of doubleness (Jones, 2013). Although wild or old species generally have 5 petals, classic Damask roses have around 30 petals per flower (*trigintipetala* means thirty-petalled). The basic petal number of the seed-derived plants of *R. damascena* was 5, whereas that of the parent 'Rd-Isparta' was 33.5 ± 4.0. There was an increase in the number of petals by multiples of 5, and they finally reached 114.8 ± 9.1 in the seed-derived plant 'Rd-24' (Table 1). Our results demonstrated that one of the possible parents of *R. damascena* Mill. f. *trigintipetala* should have a single flower with 5 petals, whereas the other(s) should have more layered flowers (Figure 1).

There was no significant correlation between the number of petals per flower and flowering duration (r = 0.07), as seen in Table 2. In addition, the flowering period of the single-flowered (5 petals per flower) plants lasted shorter than that of the semi- (7–10 petals per flower) or double- (>10 petals per flower) flowered plants. These results all showed that the double flowering form in *R. damascena* was more favorable than the single flowering form for the improvement of flower yield, flowering duration, and flower picking by hand. Although considerable variation was created in terms of petal numbers in the study, it is not easy to decide what the optimum number of petals for oil-bearing rose should be for agroindustrial production. This decision is related to not only basic floral characteristics but also quality characteristics such as the volatile oil content and composition described in the standards.

The weights of individual flowers ranged from 0.59 ± 0.11 g to 4.22 ± 0.57 g, while the flower diameter per flower varied between 4.55 ± 0.05 cm and 8.05 ± 0.05 cm (Table 1). The flower weight and the petal ratio of the single flowers were less than those of double- or multilayered flowers, although there was no exact relationship between the number of petals and flower diameter (r = –0.07), as seen in Table 2. This indicates that flower diameter is principally determined by petal size but not by the number of petals. Moreover, flower weight correlated positively with the numbers of petals (r = 0.72**) and stigmas (r = 0.51**) but negatively with the number of anthers (r = –0.56**). It was reported by Tabaei-Aghdaei et al. (2007) that flower weight and the number of flowers, displaying high rates of positive correlations with flower yield in oil-bearing rose, were the most important criteria for selection.

Table 2. Correlation coefficients between the evaluated floral traits in *R. damascena*.

Floral traits	2	3	4	5	6	7	8	9
1. Flowering duration	0.17	0.11	–0.03	0.07	–0.02	–0.19	–0.12	0.09
2. Flower weight		0.31*	0.58**	0.72**	–0.56**	0.51**	0.73**	0.50**
3. Flower diameter			–0.05	–0.07	0.33**	0.17	0.13	0.25*
4. Petal ratio				0.68	–0.65**	0.18	0.40**	0.34**
5. Number of petals					–0.80**	0.42**	0.59**	0.35**
6. Number of anthers						–0.33**	–0.54**	–0.20
7. Number of stigmas							0.65**	0.10
8. Hypanthium width								0.40**
9. Hypanthium length								

*: P ≤ 0.05 (r = 0.23), **: P ≤ 0.01 (r = 0.33).

The main oil sources of a rose flower are the petals. About 80% of the oil recovered by distillation came from the petals and the rest from sepals, stamens, and carpels in our previous research (Baydar et al., 2013). This means that oil yield increases with the increasing petal ratio in the flower. Therefore, it is possible to select genotypes with a higher petal ratio in attempts to improve the essential oil content. The petal ratio was found between 44.6 ± 9.2% ('Rd-29' with 8 petals) and 89.0 ± 6.8% ('Rd-20' with over 80 petals). Our results demonstrated that 32 seed-derived plants had higher petal ratios than the parental genotype 'Rd-Isparta' with a petal ratio of 75.7 ± 5.0% (Table 1). As shown in Table 2, flower weight positively correlated with hypanthium width and hypanthium length (r = 0.50** and 0.73**, respectively). The hypanthium thus contributed significantly to the increase in the weight of a flower.

The numbers of anthers and stigmas significantly varied in the plants derived from the seeds of open-pollinated *R. damascena*. While 8 of them did not generate any anther in their flowers, 13 of them generated over 100 anthers per flower. On the other hand, the number of stigmas per flower ranged from 17.0 ± 1.0 to 88 ± 3.0, showing a negative relationship with the number of anthers (r = –0.33**) (Table 2). Meanwhile, a significant and negative correlation between the number of stamens and oil content detected by Tabaei-Aghdaei et al. (2007) may allow the indirect selection of plants with a small number of stamens and therefore a large amount of oil content.

A significant negative correlation (r = –0.80**) was obtained between the number of petals and the number of anthers (Table 2). This finding may account for the classical ABC model of flower organogenesis or development (Bowman et al., 2012). As known in this model, the formation of the floral organs is by three sets of functional genes from the MADS-box gene family as A (sepal and petal formation), B (petal and stamen formation), and C (stamen and carpel formation) that are expressed in certain regions of the developing flower (Causier et al., 2010). While wild roses have simple flowers typically with 5 petals per flower, modern roses have double flowers consisting of >10 petals (Bendahmane et al., 2013). The roses with double flowers might be due to a homeotic change of stamens into petals through the concept of a sliding boundary, which is also responsible for the morphological diversity of rose flowers (Dubois et al., 2010).

Hairiness of the hypanthium and the pedicel is a characteristic of rose flowers. In the present study, the majority of the plants with a standard genotype had a hairy hypanthium and a hairy pedicel, while 13 of them were hairless or naked (Table 1). It was a significant observation that the plants with hairless hypanthia and pedicels were quite resistant to rose aphid (*Macrosiphum rosae* L.), one of the most economically harmful insects for oil-bearing roses. On the other hand, the plants with densely hairy hypanthia and pedicels were more odorous than the genotypes with hairless hypanthia and pedicels. Consequently, hypanthium and pedicel hairiness can be a useful and effective selection criterion for the screening of genotypes with rose aphid resistance or high volatile oil content. As is known, the absence of thorns on stems is a big advantage in terms of hand-picking the flowers. Although no detailed data about the thorns on the stems of the plants tested in our study were collected, less prickly plants are generally smaller and weaker than more prickly plants according to our field observations. Hence, it can be stated that the dominant gene responsible for thorn formation (Debener, 2003) may influence the other genes affecting plant growth and development in rose. Nevertheless, this prediction needs to be supported by making advanced genetic research.

The rose fruit, called a "hip", is an edible fruit that is particularly rich in ascorbic acid (vitamin C). In one of our previous studies, the hips of *R. damascena* and *R. canina* were compared in terms of such nutritional values as fatty acids, ascorbic acid, α-tocopherol, β-carotene, and mineral elements, and the results showed that Damask rose fruits could be used as food and food additives as rose hip fruits (Kazaz et al., 2009). If Damask rose varieties with high hip yield and quality are developed through breeding efforts, they will be utilized in not only the perfumery industry but also the food industry. In the present study, there was also high variability in fruit set and seed (achene) formation (Table 1). The parental genotype 'Rd-Isparta' produced a few fruits, which all carried only one seed. Of the seed-derived plants, only 24 produced fruits with a minimum of one seed (the average weight of 100 seeds was about 2 g; not tabulated). Among the plants setting fruits and seeds, 'Rd-61' produced around 10 seeds per mature fruit. This result showed that polycarpic fruits could also occur through genetic segregation. It was an interesting finding that the plants with single flowers (<7 petals) as a wild-type character produced more fruits and seeds than semi- and double-flowered plants. This result may arise from the relationships between the number of petals and other reproductive organs, as discussed previously. It is probable that the roses with simple or single flowers were less sterile and more compatible than the roses with double flowers.

HS-SPME is a technique that identifies the scent compounds in the natural forms secreted from rose flowers (Hethelyi et al., 2010). The use of this technique was also shown to be a convenient and effective analytical tool for the sampling of floral compounds of oil-bearing rose flowers by Jirovetz et al. (2005), Dobreva (2013), and Karami et al. (2013), and also oil-bearing rose products by Kiralan (2015). In the present study, a total of 41 floral compounds of the fresh rose flower were identified by HS-SPME combined with GC-MS system using the Carboxen/polydimethylsiloxane (CAR/PDMS) fiber, and no significant difference in the number of scent compounds identified was found between seed-derived plants and their parental genotype 'Rd-Isparta' (Table 3). With respect to the concentrations of the main

Table 3. Volatile oil compounds and their ratios (%) by HS-SPME/GC-MS in the fresh flowers of *R. damascena* plants.

Rt[a]	LRI[b]	Compounds	Clonally derived genotype (Rd-Isparta)		Seed-derived plants			
					Single-flowered		Double-flowered	
			Range	Mean ± SE[c]	Range	Mean ± SE	Range	Mean ± SE
5.828	866	Hexenol	0.00–0.24	0.12 ± 0.06	0.02–0.42	0.19 ± 0.12	0.04–0.79	0.16 ± 0.03
5.946	867	n-Hexanol	0.18–0.92	0.46 ± 0.16	0.20–2.59	1.39 ± 0.69	0.14–8.88	1.21 ± 0.35
7.988	933	α-Pinene	0.00–0.56	0.40 ± 0.13	0.00–0.43	0.23 ± 0.20	0.03–2.60	0.33 ± 0.12
8.951	964	Benzaldehyde	0.19–0.53	0.41 ± 0.08	0.21–0.51	0.39 ± 0.09	0.05–0.60	0.31 ± 0.05
10.176	991	β-Myrcene	0.64–3.03	1.66 ± 0.55	0.72–1.24	0.94 ± 0.15	0.53–7.50	1.99 ± 0.36
11.396	1025	*p*-Cymene	0.00–0.20	0.10 ± 0.05	0.00–0.04	0.04 ± 0.00	0.03–1.48	0.30 ± 0.09
11.583	1030	Limonene	0.21–0.56	0.35 ± 0.08	0.29–0.58	0.43 ± 0.08	0.24–1.87	0.67 ± 0.11
11.751	1031	Benzyl alcohol	1.02–1.80	1.39 ± 0.19	1.59–2.39	2.18 ± 0.30	0.33–3.47	1.36 ± 0.14
11.928	1035	(Z)-β-Ocimene	0.15–0.51	0.27 ± 0.08	0.20–0.28	0.25 ± 0.03	0.13–1.19	0.39 ± 0.07
12.066	1045	Phenylacetaldehyde	0.09–0.24	0.13 ± 0.04	0.12–0.26	0.18 ± 0.04	0.07–0.29	0.15 ± 0.01
12.328	1046	(E)-β-Ocimene	0.16–0.73	0.38 ± 0.12	0.19–0.40	0.31 ± 0.06	0.04–2.12	0.51 ± 0.11
13.739	1086	Terpinolene	0.00–0.10	0.06 ± 0.02	0.00–0.12	0.08 ± 0.05	0.03–0.35	0.12 ± 0.02
14.014	1090	Rosefuran	0.06–0.17	0.10 ± 0.02	0.00–0.07	0.07 ± 0.00	0.03–0.79	0.34 ± 0.05
14.344	1101	Linalool	0.08–0.18	0.13 ± 0.02	0.07–0.55	0.30 ± 0.14	0.08–0.64	0.23 ± 0.03
15.019	1113	Phenethylalcohol	32.89–47.41	42.01 ± 3.16	33.17–48.06	41.03 ± 4.32	23.26–74.54	38.76 ± 2.05
15.354	1125	Rose oxide	0.00–0.07	0.05 ± 0.02	0.00–0.01	0.01 ± 0.00	0.04–0.18	0.13 ± 0.04
15.457	1128	2.4-Dimethylanisole	0.00–0.07	0.20 ± 0.12	0.06–0.09	0.08 ± 0.01	0.05–0.61	0.25 ± 0.05
15.895	1140	2.4-Xylenol	0.00–0.20	0.18 ± 0.03	0.10–0.24	0.17 ± 0.04	0.04–0.84	0.29 ± 0.05
16.310	1165	β-Citronellal	0.05–0.27	0.14 ± 0.05	0.04–0.52	0.26 ± 0.14	0.04–0.46	0.22 ± 0.04
17.397	1179	Verbenol	0.11–0.16	0.13 ± 0.01	0.00–0.13	0.11 ± 0.02	0.05–0.44	0.17 ± 0.02
19.137	1212	Linalylformate	4.47–12.19	8.41 ± 1.64	5.55–10.28	7.62 ± 1.40	0.53–19.35	10.34 ± 0.95
19.293	1232	Citronellol	5.81–16.55	11.48 ± 2.20	7.13–21.98	12.15 ± 4.91	5.57–31.59	12.89 ± 1.49
19.654	1238	Nerol	0.58–2.64	1.66 ± 0.43	1.61–1.49	1.67 ± 0.13	0.06–2.51	1.41 ± 0.16
20.161	1250	Geraniol	10.29–21.97	14.52 ± 2.58	11.68–21.98	15.79 ± 3.15	3.09–26.93	14.56 ± 1.28
20.695	1268	Geranial	0.00–2.39	2.07 ± 0.18	1.37–2.32	1.95 ± 0.29	0.49–3.90	1.91 ± 0.17
23.538	1350	Citronellyl acetate	0.14–1.43	0.62 ± 0.30	1.13–1.36	1.21 ± 0.07	0.36–1.88	0.90 ± 0.10
23.545	1357	Eugenol	–	–	–	–	0.51–2.10	1.20 ± 0.34
24.516	1361	Neryl acetate	0.14–5.81	2.90 ± 1.16	1.79–7.16	4.75 ± 1.57	0.07–10.97	3.43 ± 0.52
25.204	1397	Methyl eugenol	0.00–0.89	0.50 ± 0.39	0.07–0.34	0.23 ± 0.08	0.03–0.54	0.17 ± 0.04
25.837	1418	β-Caryophyllene	0.05–0.20	0.10 ± 0.04	0.06–0.69	0.32 ± 0.19	0.05–0.61	0.14 ± 0.03
26.393	1438	Aromadendrene	0.00–0.22	0.12 ± 0.05	0.00–0.83	0.47 ± 0.37	0.03–0.17	0.12 ± 0.04
26.999	1454	α-Humulene	0.03–0.14	0.08 ± 0.02	0.00–0.71	0.71 ± 0.00	0.03–0.13	0.08 ± 0.01
28.591	1500	Pentadecane	0.47–1.07	0.82 ± 0.13	0.25–0.41	0.32 ± 0.05	0.21–3.18	1.00 ± 0.17
31.677	1600	Hexadecane	0.09–0.21	0.14 ± 0.03	0.00–0.09	0.08 ± 0.01	0.04–0.39	0.14 ± 0.03
33.916	1680	Tetradecanol	0.14–0.46	0.28 ± 0.07	0.00–0.13	0.13 ± 0.00	0.07–1.39	0.45 ± 0.09
34.624	1700	Heptadecane	1.02–3.49	2.25 ± 0.60	0.56–1.33	1.00 ± 0.23	0.16–6.15	1.83 ± 0.33
37.384	1800	Octadecane	0.08–0.13	0.10 ± 0.01	–	–	0.03–0.10	0.08 ± 0.01
39.365	1884	Hexadecanol	0.54–1.09	0.84 ± 0.12	0.20–0.30	0.24 ± 0.03	0.05–1.87	0.65 ± 0.13
40.079	1900	Nonadecane	3.31–3.64	3.49 ± 0.07	0.81–1.22	0.99 ± 0.12	0.13–4.91	1.70 ± 0.26
42.585	2000	Eicosane	0.09–0.21	0.14 ± 0.02	0.00–0.00	–	0.03–0.14	0.08 ± 0.01
45.049	2100	Heneicosane	0.00–0.63	0.50 ± 0.07	0.00–0.06	0.04 ± 0.01	0.07–0.57	0.29 ± 0.11

[a]Rt: Retention time (min).
[b]LIR: Linear retention indices calculated by using a series of the standards of C_7–C_{30} saturated n-alkanes.
[c]Values are means ± standard errors (n = 4 for the clonally derived genotype, n = 3 for single-flowered plants, and n = 19 for double-flowered plants).
–: not detected.

individual compounds, phenylethyl alcohol (23.26%–74.54%), citronellol (5.57%–31.59%), and geraniol (3.09%–26.93%) were the major volatile compounds in the seed-derived plants. The same compounds in the flowers of the parental genotype were found in the ranges of 32.89%–47.41%, 5.81%–16.55%, and 10.29%–21.97%, respectively (Table 3). Karami et al. (2013) found that the main floral headspace compound of *R. damascena* flowers was phenylethyl alcohol in genotype 1 and benzyl alcohol in genotype 2, and their relative percentages increased gradually at the floral developmental stages in both genotypes.

Although phenylethyl alcohol, or 2-phenylethanol, is the major scent compound of the fresh flower, its content is around 1% in hydrodistilled rose oil due to the high solubility in residue water or rose water, by-products of hydrodistillation (Baydar et al., 2008), which explains why the smell of rose oil does not resemble the genuine odor of a rose flower. Kiralan (2015) indicated that the most abundant volatile compounds detected by HS-SPME combined with GC-MS were phenylethyl alcohol for rose concrete and rose absolute, and β-citronellol for rose oil.

While the mean ratios of monoterpenic alcohols such as linalool, citronellol, geraniol, and nerol were quite close to each other, the double-flowered plants showed a wide range of variation in these compounds also including phenylethyl alcohol in comparison with the single-flowered plants. On the other hand, the aliphatic hydrocarbons such as pentadecane, hexadecane, heptadecane, octadecane, nonadecane, eicosane, and heneicosane were higher in the double-flowered plants. The double-flowered plants also contained more minor compounds such as rosefuran and rose oxide, which are responsible for the typical rose fragrance (Table 3). Our results demonstrated that the genotypic structure of the plants significantly changed the natural scent profile of oil-bearing rose.

Methyl eugenol is not desired above a certain concentration in the essential oils due to the negative side and allergic effects on human health (Harris, 2002). The parental clonally derived genotype contained methyl eugenol twice higher than those of the seed-derived plants (Table 3). Additionally, the single-flowered plants had a higher percentage of methyl eugenol, mainly due to their low petal ratios in comparison with the double-flowered plants. Previous studies showed that the main source of methyl eugenol was petal-less parts including stamens, carpels, and sepals (Rusanov et al., 2012; Baydar et al., 2013).

We estimated the correlation coefficients among the main compounds of volatile oils (Table 4). The geraniol content correlated positively with the citronellol content ($r = 0.50^{*}$) and linalyl formate ($r = 0.82^{**}$) but negatively with the phenylethyl alcohol content ($r = -0.63^{**}$). Moreover, the content of methyl eugenol did not correlate significantly with other volatile compounds.

Unfortunately, the research on the genetics and breeding of oil-bearing rose in the world is very limited in comparison with that on other commercial roses. The conventional propagation methods based on the cuttings from the juvenile pruning of *R. damascena* provide clonal propagation but inhibit genetic variation due to the lack of seed propagation. However, open- or self-pollinating flowers show a high degree of outcrossing and therefore lead to a high degree of heterozygosity in the alleles or genes associated with important floral characteristics and scent molecules. As a result of this study, the seed propagation of clonally propagated *R. damascena* yielded a change in the genetic segregation of the alleles. The wide variations in petal colors and petal numbers in particular are sources of selection that are important to be utilized for not only industrial oil-bearing roses but also park and home roses. Hence, the seeds of oil-bearing rose plants

Table 4. Correlation coefficients between the main scent compounds in *R. damascena* volatile oil.

Compounds	2	3	4	5	6	7	8	9
1. Phenethyl alcohol	−0.67**	−0.13	−0.25	−0.63**	−0.20	−0.08	−0.48*	−0.42*
2. Linalyl formate		−0.37	0.22	0.82**	0.10	−0.09	0.33	0.27
3. Citronellol			−0.03	0.50*	−0.40	0.18	−0.31	−0.29
4. Nerol				0.04	−0.05	0.37	−0.07	0.07
5. Geraniol					0.35	−0.02	0.36	0.33
6. Neryl acetate						0.07	0.58**	0.54**
7. Methyl eugenol							−0.22	0.13
8. Heptadecane								0.86**
9. Nonadecane								

*: $P \leq 0.05$ ($r = 0.40$), **: $P \leq 0.01$ ($r = 0.51$).

would be a crucial selection source to breed novel varieties. On the other hand, the segregating progenies derived from seeds may also enable the development of molecular markers linked to the floral and scent traits of economic importance. Such molecular markers will provide an opportunity for applying marker-assisted selection and for a quantitative trait locus analysis.

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