

DIVERSITY OF MORPHOLOGY AND OIL CONTENT OF *ROSA DAMASCENA* LANDRACES AND RELATED *ROSA* SPECIES FROM PAKISTAN

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For the perfume industry, *Rosa damascena* is the most important species used in the production of rose attar which is made by distilling volatile oils from the petals of flowers. It is also used widely in the production of rose water, a flavoring agent. Other species like *R. gallica* L., *R. centifolia* L., *R. bourboniana* and Gruss an Teplitz also exhibit the fragrance that is sought by perfumeries in the world. Eight landraces of Damask rose along with related *Rosa* species were collected from Punjab province and evaluated to determine the diversity on the basis of morphology and oil yield. The investigated characters were flower weight, flower diameter, peduncle length, number of petals, number of stamens and oil content. Pearson's coefficients showed a negative association of flower weight with peduncle length ($r = -0.3348$) and a positive and strong correlation was observed for flower weight with the all other morphological characters. Flower diameter showed a positive but weak correlation with peduncle length, number of petals and number of stamens with the values of $r = 0.0733$, $r = 0.5302$ and $r = 0.1241$, respectively. Oil content (%) was measured from the *Rosa* species by using Soxhlet extractor with N-hexane. *R. damascena* landrace from Choha Syedan Shah produced the highest oil content of absolute oil (0.147%) on petal basis while the lowest value for absolute oil content was 0.36% from *R. indica*. Dendrogram created by cluster analysis for morphological characters, indicated no relationship of genetic variation with their collection sites. This study has revealed that the selection of landraces for their specific characteristic could be possible for future breeding program. It also provides practical information for the future collection of Damask rose germplasm and breeding program.

Keywords: *Rosa damascena*, Damask rose, essential oil, morphological characters, solvent extraction, rose oil

INTRODUCTION

Roses have gained the title of the king of flowers (Peter Bealis, 1990) and it is the most popular ornamental plant that has been cultivated systematically (Wylie, 1995). Genus *Rosa* consists of approximately 200 species and up to 18,000 cultivars (Gudin, 2000). Roses are mainly used for showy purposes (both flowers and hips) and oil extraction but they are also used for straight utilization or creation of a variety of food stuff like tea, jam and confectionary. They are a rich source of Vitamin C and used in the making of medicinal stuff. From ages, humans have been involved to make new scent by using the foliage and bloom for the enhancement of his surroundings. The utilization of Damask rose dates back to 1500 years ago. The Iranian people call it the flower of Prophet Muhammad (SAW) due to its nice fragrance (Nikbakht and Kafi, 2008).

The species mainly used for oil production are *R. damascena* Mill., *R. gallica* L., *R. moschata* Herrm., *R. centifolia* L. and *R. bourboniana* Desp. (Tucker and Maciarello, 1988; Kaul *et al.*, 2009). Rose oil is the most exclusive essential oil in the world due to its low content and unique scent. About 3000 kg of rose petals can produce only one kg of rose oil (Baydar and Baydar, 2005; Baser, 1992). The price of the rose oil has been rising during the last few years getting 5000

EUR/kg (Rusanov *et al.*, 2009). It is true that there is no alternate of rose oil present in nature nor has it been prepared synthetically (Baydar and Baydar, 2005). Other than rose oil, a number of significant raw stuff for the perfume and cosmetics industry is attained which includes rose concrete, rose absolute and rose water (Nilgun *et al.*, 2004). The oil content and constituents of the rose oil differ among species used for the production of rose oil.

In Pakistan, the rose oil is being produced from *R. damascena*, *R. centifolia*, *R. bourboniana* and a cultivar Gruss an Teplitz. *Rosa damascena* produces flowers only in April each year so farmers prefer to grow other scented species like *Rosa centifolia*, *Rosa bourboniana* and Gruss an Teplitz. These species are known for their continuous blooming throughout the year. The rose oil industry in Pakistan is at its infancy. Small size farmers are interested in exploring new sources of income and they have started to cultivate *Rosa* species as a crop to obtain rose oil. The main rose cultivation regions in Punjab are Kallar Kahar, Chakwal, Choha Syedan Shah, Pattoki, Islamabad, Shahiwal (Sargodha) and Faisalabad. Some essential oil *Rosa* species are also being cultivated in Sindh Province. Pakistan has a very diverse climate, which ranges from subtropical to temperate and allows the cultivation of almost all kinds of

plants. Therefore, we cannot negate the possibility of a large diversity in local Damask roses grown in Pakistan.

MATERIALS AND METHODS

Plant material: A total of eight *R. damascena* landraces, three related scented species (*R. centifolia*, *R. bourboniana*, *R. Indica*) and a cultivar 'Gruss an Teplitz' were collected from different rose production areas of Pakistan (Table 1). These plants were grown in 2007 in the research area of Rosa Project, Institute of Horticultural Sciences (IHS), University of Agriculture, Faisalabad, Pakistan (Latitude 31° 25' 0" N, longitude 73° 5' -1" E). Total 30 plants of each *Rosa* species were planted using randomized complete block

design with three replications.

Local climate: The climate of Faisalabad is extreme with the maximum temperature in summer 50°C (122°F) and a winter temperature of -1°C (30.2°F). The average highest and lowest temperature in summer season is 39 and 27°C, respectively. It goes down to 21-6°C in winter season. The duration of summer season is April to October with the most extreme months of May, June, and July. Whereas the winter season starts from November and goes up to March with the extreme months of December, January, and February (Pakistan Meteorological Department, 2006).

Morphological characters: Five morphological characters were examined on harvesting time on three-year-old plants propagated with cuttings. The data were recorded from nine

Table 1. Rose species collected from different regions of Pakistan

Code	Name of species	Collection site	Latitude (N)	Longitude (E)	Altitude (m)	Rainfall	Summer	Winter
G1	<i>R. damascena</i>	Faisalabad	30°31	73°74	184	Semi arid	Hot	Mild
G2	<i>R. damascena</i>	Pattoki-1	31°1	73°50	186	Semi arid	Hot	Mild
G3	<i>R. damascena</i>	Pattoki-2	31°1	73°50	186	Semi arid	Hot	Mild
G4	<i>R. damascena</i>	Islamabad	33°43	73°04	507	Sub-humid	Hot	Mild
G5	<i>R. damascena</i>	Kallar Kahar	32°78	72°70	634	Semi arid	Hot	Mild
G6	<i>R. damascena</i>	C. S. Shah	32°43	72°59	676	Semi arid	Hot	Mild
G7	<i>R. damascena</i>	Chakwal	32°56	72°54	189	Semi arid	Hot	Mild
G8	<i>R. damascena</i>	Ch. Sahiwal	32°08	73°7	187	Semi arid	Hot	Mild
G9	<i>R. centifolia</i>	Faisalabad	30°31	73°74	184	Semi arid	Hot	Mild
G10	Gruss an teplitz	Faisalabad	30°31	73°74	184	Semi arid	Hot	Mild
G11	<i>R. Bourboniana</i>	Faisalabad	30°31	73°74	184	Semi arid	Hot	Mild
G12	<i>R. Indica</i>	Tando jam	25°25	86°31	13	Semi arid	Hot	Mild

*Yearly mean temperature for Hot, Warm, and Mild summer is 32°C, 21-32°C and 10-21°C, respectively while for mild and cool winter is 10-21°C and 0-10°C, respectively. Semi-arid areas receive rainfall 10-20 inches (200-500mm), while the Sub-humid areas receive 20-30 inches (500-700mm) rainfall annually (The New Oxford Atlas for Pakistan, 1998).



Figure 1. Map of Pakistan showing the production areas of *Rosa damascena* and related essential oil *Rosa* species in Punjab and Sindh Province

representative plants from each replication of the experiment. The morphological characters examined include flower weight (g), flower diameter (cm), peduncle length (cm), number of petals and number of stamens.

Oil Extraction: The oil was extracted with the help of Soxhlet Apparatus, using N-hexane as a solvent. The oil contents were calculated as proportion (%) of flower weight. To conduct this experiment, completely randomized design was used with three replications. For each replication, 500 g of petals were used for solvent extraction. Rotary evaporator was used to remove the remaining hexane from the extract obtained from soxhlet extractor and concrete oil was obtained. To get absolute oil from concrete oil, alcohol was added and filtered until wax free rose oil obtained. The percentage of concrete and absolute oil was measured on petal weight basis.

Statistical analysis: The data were analyzed statistically to find the significance of the results of morphological characters and oil content of *R. damascena* landraces and related species and means were compared by Duncan's multiple range (DMR) test at 5 % probability (Steel *et al.*, 1996). Correlation among the chemical components was analyzed according to Pearson's coefficient. The cluster analysis was done by using Statistica 7, Statsoft. Inc. (1984-2007).

RESULTS

Morphological characters: Highly significant differences were observed among all landraces for all morphological characters. The landrace of *R. damascena* from Choha Syedan Shah showed the highest value (1.807g) for flower

weight, followed by landrace from Faisalabad (1.739g), *R. damascena* from Patooki-1 (1.657g) and Patooki-2 (1.615g) (Table 2). Whereas, Gruss an Teplitz and *R. indica* showed the lowest values (1.358g and 1.388g, respectively) for flower weight. The landraces of *R. damascena* from Faisalabad and Pattoki-2 got the maximum and similar (statistically non-significant) value for flower diameter (4.633cm) followed by *R. centifolia* (4.600cm) and *Rosa damascena* landrace from Chakwal (4.620cm). The data showed that the Damask rose landrace from Pattoki-2 obtained the highest value (2.350cm) of peduncle length while *Rosa centifolia* showed the highest value (48.00) for number of petals. *R. damascena* landrace from Islamabad obtained maximum number of stamens (96.33) followed by Damask rose from Pattoki-1 (94.22) and *R. centifolia* (86.22). Dendrogram showed 4 main groups at linkage distance of 10, dividing all *R. damascena* landraces and related species into four clusters (Figure 2). In the first group, G-1 (*Rosa damascena* landrace from Faisalabad), G-8 (*R. damascena* landrace from Chota Sahiwal), G-11 (*R. bourboniana*), G-12 (*R. indica*), G-7 (*R. damascena* landrace from Chakwal) and G-10 (Gruss an Teplitz) were included while in the second group G-3 (*Rosa damascena* landrace from Pattoki-2) and G-9 (*R. centifolia*) were gathered. *R. damascena* landrace from Kallar Kahar (G-5) and Choha Syedan Shah (G-6) were gathered in the same group. The last group consisted of *R. damascena* landrace from Pattoki-1 (G-2) and Islamabad (G-4). At linkage distance of 6, the first group further divided into a new single group of *R. bourboniana*.

Oil yield: The *R. damascena* landrace from Choha Syedan Shah produced the highest concrete oil content (0.197%) on

Table 2. Comparison of morphological characters of different rose species

Code	Flower weight (g)	Flower diameter (cm)	Peduncle length (cm)	No. of Petals	No. of stamens
G-1	1.739±0.014b	4.633±0.009a	2.310±0.006b	42.00±0.577de	81.22±0.618f
G-2	1.657±0.009c	4.470±0.006c	2.280±0.006c	39.00±0.577f	94.22±0.890b
G-3	1.615±0.012d	4.633±0.009a	2.350±0.006a	43.00±1.150cd	84.78±0.223d
G-4	1.631±0.001cd	4.453±0.003cd	2.220±0.006d	45.67±0.333b	96.33±0.333a
G-5	1.566±0.003ef	4.420±0.006ef	2.130±0.006f	40.33±0.333ef	71.00±0.384i
G-6	1.807±0.031a	4.460±0.006c	1.827±0.009h	44.33±0.333bc	70.55±0.223i
G-7	1.595±0.009de	4.620±0.006ab	1.937±0.007g	42.00±0.577de	78.22±0.294h
G-8	1.570±0.009ef	4.437±0.007de	1.817±0.009h	40.33±0.333ef	83.44±0.443e
G-9	1.552±0.005f	4.600±0.010b	2.190±0.006e	48.00±0.577a	86.22±0.294c
G-10	1.358±0.009h	4.407±0.009f	2.300±0.006b	36.00±0.577g	79.00±0.193gh
G-11	1.451±0.008g	4.330±0.006h	2.200±0.006e	40.00±0.577f	81.22±0.443f
G-12	1.388±0.018h	4.367±0.009g	2.273±0.003c	38.67±0.333f	80.11±0.675fg

Means sharing similar letter in a column are statistically non-significant (P>0.05).

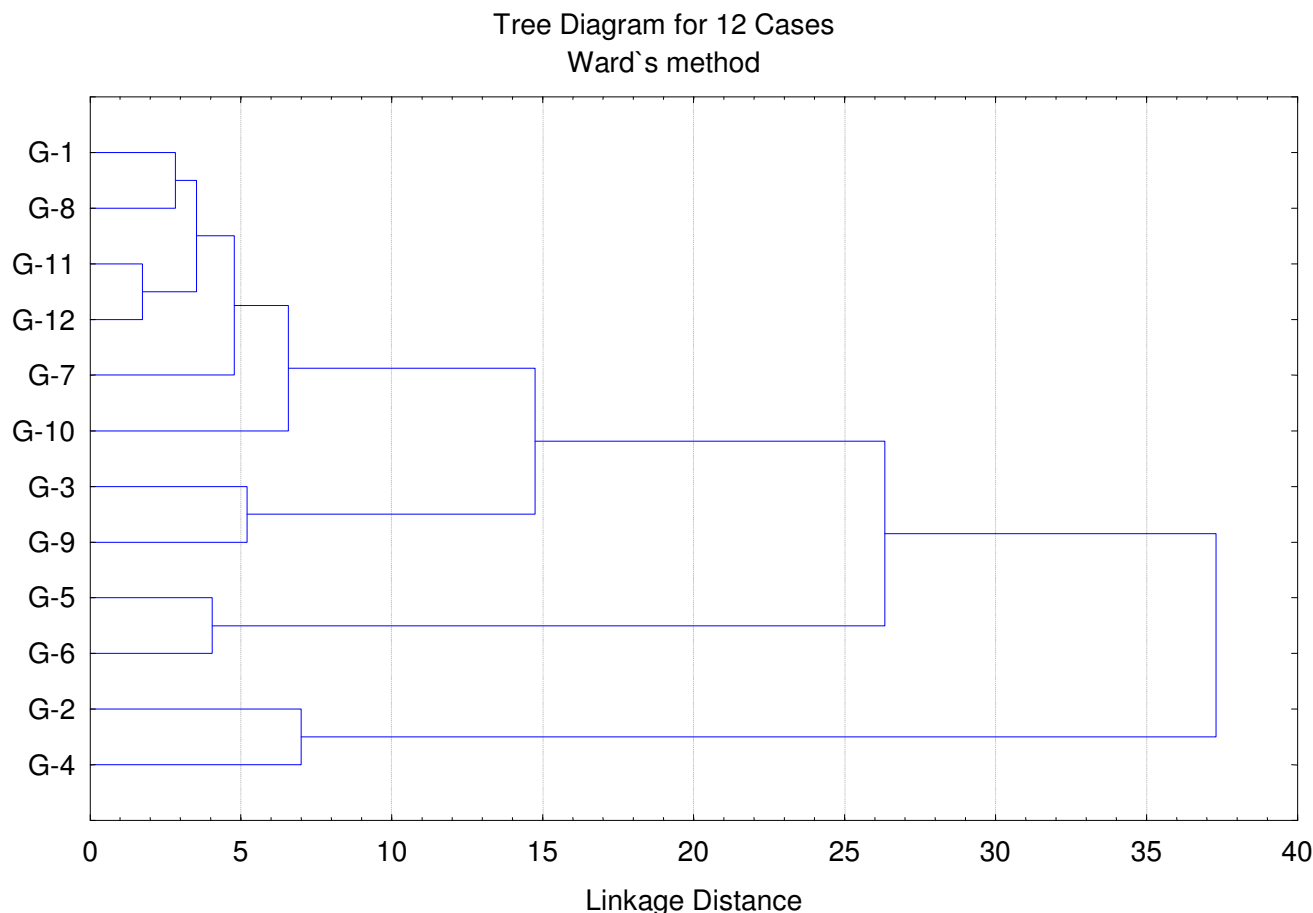


Figure 2. Tree diagram showing the grouping of Damask rose landraces (G1-G8) and related essential oil *Rosa* species (G9-G12) on the basis of morphological characters

petal basis followed by Damask rose from Kallar Kahar, Faisalabad and Chakwal with statistically non-significant values of 0.195%, 0.193% and 0.193%, respectively (Table 3). The lowest value for concrete oil is 0.060% that belongs to *R. Indica* from Tando Jam, Sindh. The results revealed that the *R. damascena* landrace from Choha Syedan Shah produced the highest oil content of absolute oil (74.48%) on concrete oil basis while the lowest value for absolute oil content is 50.62% that belongs to *R. bourboniana* from Faisalabad. For absolute oil yield on petal basis, again *R. damascena* landrace from Choha Syedan Shah produced the highest oil content of absolute oil (0.147%). This value is followed by landraces from Kallar Kahar, Faisalabad and Chakwal with 0.145%, 0.144% and 0.142% respectively. The lowest value for absolute oil content is 0.039% and 0.036% that belongs to Gruss an Teplitz (Faisalabad) and *R. Indica* (Tando Jam, Sindh), respectively.

There was a strong and positive association of flower weight with absolute oil content ($r = 0.8722$) in all Damask roses and other related *Rosa* species (Table 4). Flower diameter and number of petals also showed strong and positive correlation for absolute oil content with the values of $r = 0.6171$ and $r = 0.4666$, respectively. While the peduncle length and number of stamens were negatively correlated for absolute oil content with the values of ($r = -0.4022$) and ($r = -0.0843$), respectively. Dendrogram showed 4 main groups at linkage distance of 6. Four landraces, G-1 (*R. damascena* from Faisalabad) and G-5 (*R. damascena* from Kallar Kahar), G-7 (*R. damascena* from Chakwal) and G-6 (*R. damascena* from Choha Syedan Shah) were included in cluster I, G-1 (*R. damascena* from Pattoki), G-4 (Islamabad), G-8 from (*R. damascena* Chota sahiwal) and G-9 (*R. centifolia*) in group II, Gruss an Teplitz and *R. Indica* were found in cluster III, while cluster V included *R. bourboniana* making a separate group from others.

Table 3. Comparison of oil content in different rose species

Code	%age of concrete oil on petal basis	%age of absolute oil on the basis of concrete oil	% of absolute oil on petal basis
G-1	0.193±0.0018 b	73.96±0.199 a	0.144±0.0009 bc
G-2	0.175±0.0002 c	71.30±0.158 cd	0.125±0.0001 e
G-3	0.177±0.0002 c	71.73±0.065 bc	0.127±0.0002 d
G-4	0.160±0.0001 e	69.77±0.197 e	0.112±0.0003 g
G-5	0.195±0.0002 ab	74.14±0.125 a	0.145±0.0003 b
G-6	0.197±0.0001 a	74.48±0.069 a	0.147±0.0002 a
G-7	0.193±0.0010 b	73.75±0.105 a	0.142±0.0007 c
G-8	0.171±0.0010 d	70.84±0.138 d	0.121±0.0009 f
G-9	0.140±0.0003 f	72.36±0.467 b	0.101±0.0006 h
G-10	0.070±0.0004 h	55.42±0.159 g	0.039±0.0002 j
G-11	0.090±0.0005 g	50.62±0.537 h	0.046±0.0007 i
G-12	0.060±0.0008 i	59.35±0.364 f	0.036±0.0003 k

Means sharing similar letter in a column are statistically non-significant ($P>0.05$).

Table 4. Correlation between morphological characters and absolute oil content (%) in different rose species

	Flower weight (g)	Flower diameter (cm)	Peduncle length (cm)	No. of Petals	No. of stamens	Absolute oil
Flower weight (g)	1.0000					
Flower diameter (cm)	0.5165	1.0000				
Peduncle length (cm)	-0.3348	0.0733	1.0000			
No. of Petals	0.5462	0.5302	-0.1837	1.0000		
No. of stamens	0.0128	0.1241	0.4172	0.2130	1.0000	
Absolute oil	0.8722**	0.6171*	-0.4022	0.4666	-0.0843	1.0000

* = Significant ($P<0.05$); ** = Highly significant ($P<0.01$)

DISCUSSION

The landrace of *R. damascena* from Choha Syedan Shah showed the highest value for flower weight indicating a source for enough volatile compounds responsible for scent of rose oil. The flower weight of various *R. damascena* landraces and related *Rosa* species is comparable with the previous study done by Tabaei-Aghdaei *et al.* (2007) where the flower weight of *R. damascena* landraces collected from 28 provinces of Iran, got the range from 1.39g to 2.78g. *R. damascena* landraces examined for flower diameter can be compared with a previous study regarding *R. damascena* var. Jwala, *R. damascena* var. Himroz and *R. bourboniana* (Table 2). The flower width of these varieties was examined as 6.7cm, 6.5cm and 4.8cm, respectively (Kaul *et al.*, 2009). While the values for flower diameter obtained in the present study, are significantly lesser than the previous study conducted by Tabaei-Aghdaei *et al.* (2007) where it ranged from 5.29 to 6.10cm. The data regarding number of petals is similar with the landraces collected from East and West Azarbayajan, Ardabil, Tehran, Markazi, Razavi Khorasan, South Khorasan, Guilan, Mazandaran and Golestan provinces of Iran in the previous study (Tabaei-Aghdaei *et al.*, 2007). On the other hand, number of petals for *R. damascena* var. Jwala, *R. damascena* var. Himroz and *R.*

bourboniana were found 38, 32 and 47, respectively (Kaul *et al.*, 2009). The landraces with lot of petals could be the best sources for allied products like Rose jam and perfumes. Simple correlation coefficient indicates the degree of association between two variables, which are considered to be independent (Sokal and Rohlf, 1995). There was a negative association of flower weight with peduncle length in all landraces. These findings agree with the previous studies conducted in Iran (Babaei, *et al.*, 2008; Tabaei-Aghdaei *et al.*, 2007) where a positive correlation was observed between number of petals and number of stamens (Table 4). Tabaei-Aghdaei *et al.* (2007) have discussed that more number of stamens, there will be more chances for greater number of petals because stamens are being converted into petals during phenologic period.

Process of distillation to capture the aroma of flowers is getting older and it also lost very delicate aromatic compounds during extraction process so solvent extraction is preferred method. Robiquet used first time the solvent extraction in 1835. The essential oil yielded from Solvent extraction, contains relatively little wax and coloring matter (Wang, 2000) and superior essential oil obtained than the steam distillation method (Younis, 2006). The present study showed variation of oil contents among different species (Table 3). The results regarding oil content of absolute oil are

comparable with the previous study where *R. damascena* produced 0.14% of absolute oil followed by 0.11% of absolute oil from *R. centifolia* while *Rosa* 'Gruss an Teplitz' produced the lowest absolute oil content (Younis *et al.*, 2008). The oil content in the present study ranged from 0.147% to 0.036% while Kokkini and Vokon (1989) reported 0.1% oil contents in Damask rose. Oil yield of *R. damascena* in Greece, ranged from 0.1% to 0.9%. Genetic variation may be the one of the reason for the variation in essential oil contents in the examined species. This variation could be the positive indication for the proper selection for oil content (Kokkini and Papageorgion, 1998). A positive a strong correlation was found between the concrete oil on petal basis and the absolute oil yield on concrete oil basis indicating that greater the concrete oil percentage more will be the absolute oil content (Table 4).

A strong and positive association between flower weight and absolute oil content indicates that more flower weight is responsible for sufficient volatile compounds in the rose petals. Flower diameter and number of petals also showed strong and positive correlation for absolute oil content. These results agree with the previous study, where the number of stamens were negatively associated oil content percentage. So the landrace with low number of stamens might be good for the high oil content (Tabaei-Aghdaei *et al.*, 2007). But stamens, itself have some relative concentration of rose oil content. In *R. damascena* flower, the most of the oil contents are found in the petals (92.8%) and stamens (4.6%) followed by ovary with calyx 2-3% and stigma 0.3% (Anonymous 1972). While there is a non-significant correlation has been observed between oil content and floral characteristics in damask roses (Patra *et al.*, 1987).

CONCLUSION

The present study suggests that the variations among *R. damascena* landraces are caused by the combination of environmental and genetic factors. The result of the cluster analysis did not show any relationship between collection sites as landraces from the same sites showed a little variation in some characters and also landraces from different sites entered the same cluster (Figure 2). It seems that the genotypes/germplasm have been exchanged among different areas of Pakistan. The *R. damascena* landrace from Choha Syedan Shah and *Rosa centifolia* should be exploited for oil content and number of petals. Furthermore, a breeding program should be initiated to combine the specific characteristics of these species into one hybrid.

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