A Unique Germplasm of Damask Roses in Iran

M. Kiani
Present Address:
Department of Ornamental Plants,
Research Center for Plant Sciences,
Ferdowsi University of Mashhad, Mashhad,
Iran

Z. Zamani, A. Khalighi and R. Fatahi

Department of Horticultural Sciences,
Faculty of Agriculture,
University of Tehran, Karaj,
Iran

D.H. Byrne
Department of Horticultural Sciences,
Texas A & M University, College Station,
USA

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Abstract

Damask rose (*Rosa damascena* Mill) is widely cultivated throughout Iran and therefore the local Damask varieties in Iran may comprise a rich gene pool for this species waiting to be explored. To assess the diversity of the Iranian Damask germplasm, 41 damask rose genotypes were collected from the most important cultivation regions for this species which includes Fars, Isfahan, East Azerbaijan, Kerman and Khorasan provinces. Genetic markers including 50 quantitative and qualitative morphological traits, 31 RAPD and 38 SSR primers were involved in the genetic diversity evaluation of the studied genotypes. Results showed a high diversity of morphological characters especially those that were related to flower, leaf and prickle morphology. Molecular markers also revealed high levels of polymorphism, 190 and 225 polymorphic bands from selected RAPD and SSR primers were amplified. The basic relationships were similar among evaluation methods, although the degree of discrimination was much less for the morphological data. The data suggests that Iran is a center of diversity of Damask rose and gives us some clues about unknown center of origin of this species.

INTRODUCTION

Flowers have long been associated with Iranian culture and history. Dating back as far as 500 B.C. we can see flower designs in stone carving of Persepolis. Within scented flowers, *Rosa damascena* Mill., an important species as a source for rose oil, is one of the oldest roses grown in Iran. The breeding of this species has been greatly limited by the sparse information on its genetic background. In recent genetic diversity studies using RAPDs and SSRs with Bulgarian Damask roses (Agaoglu et al., 2000; Rusanov et al., 2005) and AFLPs with Turkish Damask roses (Baydar et al., 2004) no variation was revealed among the Damask roses cultivated for oil in these two countries. As analysis of genetic diversity and relatedness between or within different populations, species and individuals is a central task for many disciplines of biological sciences. A breeding project was initiated at the Horticulture Department of Tehran University in 2004 for this purpose. The objective of this study was to study the genetic diversity

represented in the cultivated rose oil germplasm collected throughout the production zones of Iran using both morphological and molecular markers.

MATERIALS AND METHODS

Forty-one damask rose genotypes were collected from Fars, Isfahan, East Azerbaijan, Kerman and Khorasan provinces of Iran which are the most important cultivation regions for this species(Table 1). The rose oil variety Khazanlik no. 42 from Bulgaria was also used in this study. Genetic markers including 50 quantitative and qualitative morphological traits, 31 RAPD and 38 SSR primers were involved in the genetic diversity evaluation of the studied genotypes. The CTAB-based method of Vroh Bi et al. (1996) was used to extract DNA from leaf samples.

RAPD marker

One hundred and thirty decamer oligonucleotide primers (TIBMOLBIOL and Operon, Germany) were screened for polymorphism on DNA samples from four geographically distinct *R. damascena* genotypes. PCR reactions were performed with a 25 µl total volume containing 5 ng of each template DNA, 1x PCR buffer (SinnaGen, Iran), 1.75 mM MgCl2, 200 mM of each dNTPs, 0.2 mM of a single decamer primer and 1 U Taq DNA polymerase (SinnaGen, Iran). Amplifications were performed in a thermocycler (iCycler, Bio Rad, USA) programmed for 4 min at 94 °C, 35 cycles for 1 min at 92 °C, 1 min at 37 °C and2 min at 72 °C, followed by a final extension cycle of 72 °C for5 min. Amplified products were separated by electrophoresis in a 1.2% agarose gel stained with ethidiumbromide and photographed under UV light in a gel doc system (Ultraviolet Products, CA, USA).

SSR markers

A total of 166 microsatellite “SSR” primer pairs (Esselink et al. 2003; Zhang et al. 2006; Hibrand St. Oyant et al, 2008) were initially assayed using the DNA samples of 4 distinct Damask rose genotypes. Thirty seven primers that showed good banding patterns were selected for further analysis using either 3.5% MetaPhore gel electrophoresis (for 14 primer pairs) or capillary electrophoresis using a ABI 3100 genetic analyzer for 23 labeled primer pairs (Applied Biosystems Inc., Foster City, CA, USA). PCR was performed in a final volume of 10 µl, consisting of 8 µl of GoTaq Master Mix from Promega (200 µM dNTPs, 1.5 mM MgCl2 and 1.5 U of Taq), 2.8 pmol of each primer and 5 ng of genomic DNA.. PCR cycling was: 3 min initial denaturation at 94°C, 25 cycles of amplification (30 sec. at 94°C, 1 min at optimized annealing temperature and 1 min elongation at 72°C) followed by a final extension of 25 min at 72°C. Products of labeled primers were run on an ABI 3100 genetic analyzer and scored using Genotyper software (Applied Biosystems Inc., Foster City, CA, USA.). For 14 unlabeled primers the PCR amplification was performed with the same conditions as for labeled ones, except that the number of cycles and final extension time were 35 cycles and 8 min respectively.

Data analysis

Squared Euclidean distances were calculated for morphological data and the Ward method was used to construct a dendrogram using SPSS software. For molecular markers, bands and peaks visualized on MetaPhor gels and with the Genotyper software respectively, were scored as either present or absent. For each primer, the number of polymorphic bands (n), polymorphism percentage (p), number of banding patterns (i.e.

the different combinations of bands obtained) and their frequencies were calculated. Cluster analysis was performed using the unweighted pair-group method (UPGMA) based on Dice's similarity coefficient (Nei and Li, 1979) using NTSYS-

RESULTS AND DISCUSSION

Analysis of variance showed significant differences of characters accounting for genetic variation of this species. Cluster analysis using ward's method separated genotypes into 7 groups(Fig.1, A). Fourteen genotypes located in the first group indicated the high similarity of the most studied genotypes. These genotypes were mainly from Kerman, Fars and Isfahan provinces. However, genotypes from East Azerbaijan made a distinct and separate group. Within other genotypes one with unique bushy growth habit (G3 from Tehran) and one with few prickles (G24 from Razavi Khorasan) were recognized.

Molecular markers also revealed high level of polymorphism, 190 and 225 polymorphic bands from selected RAPD and SSR primers were amplified. The dendograms generated from SSR markers and the RAPD data were similar with a few exceptions (Fig.1, B, C). The SSR data separated the genotypes into 11 groups, one more than those seen in the RAPD derived analysis. In the SSR analysis as compared to the RAPD analysis, the accessions G41 (unknown) and G28 (Fars) were removed from the main groups of the dendrogram, while, G38 from Fars was included in main group. The three accessions (G5, G9 and G30) consistently clustered separately from all other genotypes with both morphological, RAPD and SSR data. The Bulgarian genotype 'Khazanlik' G42 clustered with the majority of accessions in the first group in all three dendrograms. The results of this study revealed a high level of polymorphism of R. damascena in Iran as indicated by a recent study (Babaei et al., 2007). This contrasted by the lack of diversity revealed in previous studies of this species in Turkey (Agaoglu et al., 2000; Baydar et al., 2004) and Bulgaria (Rusanov et al., 2005).Therefore, the germplasm of *R. damascena* in Iran is more diverse than that found in Bulgaria or Turkey and that the *R. damascena* in these countries was probably obtained from Iran. Furthermore, given the high diversity found for this species in Iran, this region is a major center of diversity for the species

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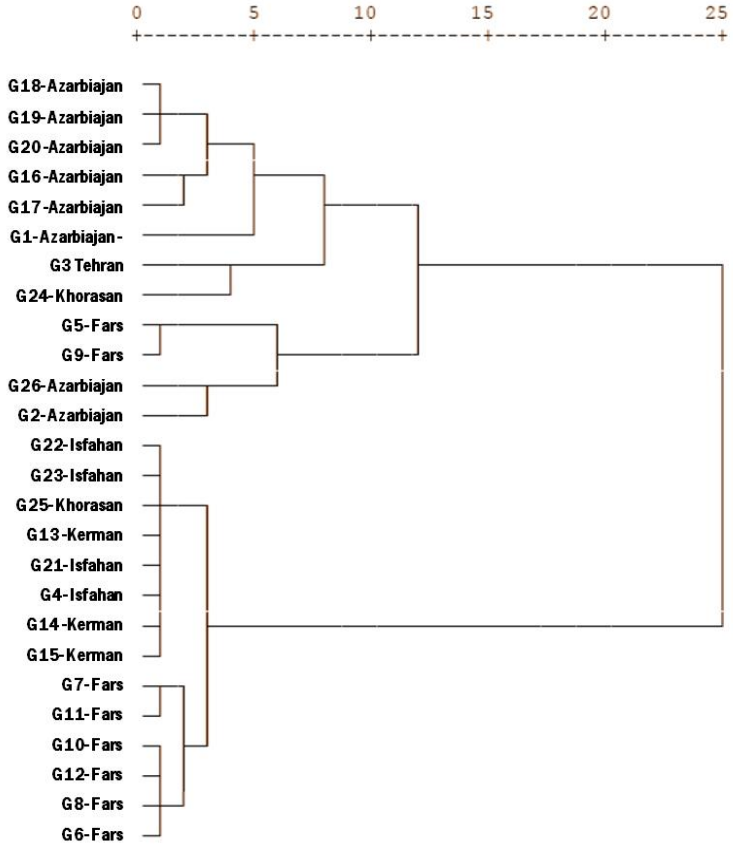
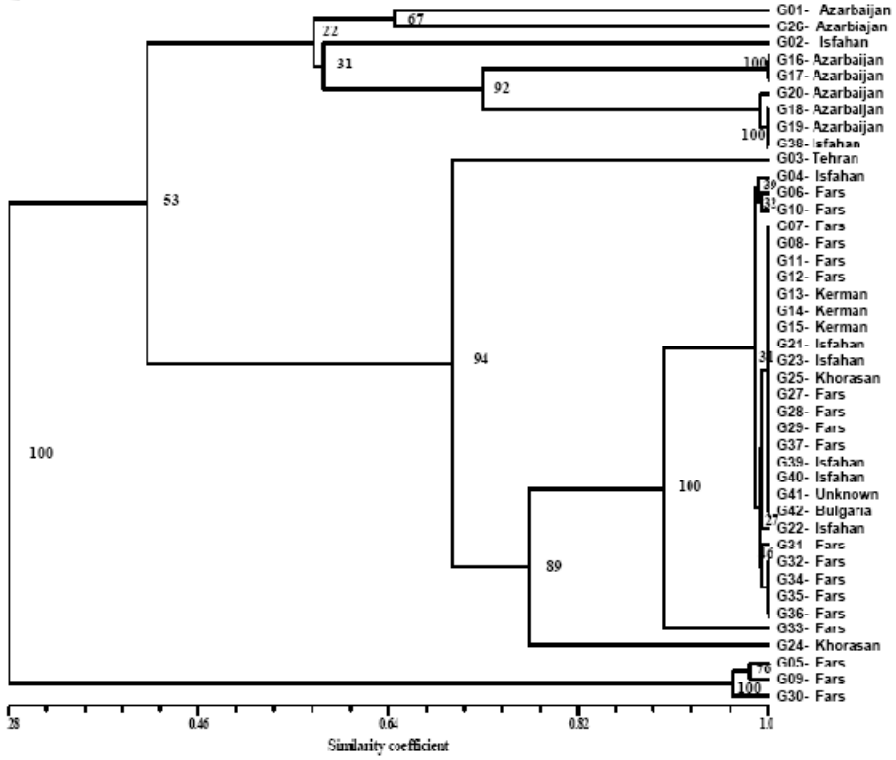
Table 1. Damask rose accessions included in this study, their province of origin and location of sample collection

Genotype no.	Province of origin	Collection site
G1	**East Azarbaijan**	**Kashan, Collection of Taghtiran company**
G2	**Isfahan**	**Kashan, Collection of Taghtiran company**
G3	**Tehran**	**Kashan, Collection of Taghtiran company**
G4	**Isfahan**	**Kashan, Collection of Taghtiran company**
G5	**Fars**	**Kashan, Collection of Taghtiran company**
G6	**Fars**	**Darab- Lyzangan**
G7	**Fars**	**Darab- Rostagh**
G8	**Fars**	**Darab- Ghale Biaban**
G9	**Fars**	**Darab- Lyzangan**
G10	**Fars**	**Maimand- Sahra sefid**
G11	**Fars**	**Maimand- Kang**
G12	**Fars**	**Maimand**
G13	**Kerman**	**Bardsir**
G14	**Kerman**	**Bardsir**
G15	**Kerman**	**Mahan**
G16	**East Azarbaijan**	**Osco**
G17	**East Azarbaijan**	**Tabriz**
G18	**East Azarbaijan**	**Osco**
G19	**East Azarbaijan**	**Osco**
G20	**East Azarbaijan**	**Ahar**
G21	**Isfahan**	**Kashan**
G22	**Isfahan**	**Kashan**
G23	**Isfahan**	**Kashan**
G24	**Razavi Khorasan**	**Mashhad**
G25	**Razavi Khorasan**	**Mashhad**
G26	**East Azarbaijan**	**Tabriz**
G27	**Fars**	**Darab- Lyzangan**
G28	**Fars**	**Darab- Lyzangan**
G29	**Fars**	**Darab- Lyzangan**
G30	**Fars**	**Darab- Lyzangan**
G31	**Fars**	**Darab- Lyzangan**
G32	**Fars**	**Darab- Lyzangan**
G33	**Fars**	**Darab- Lyzangan**
G34	**Fars**	**Darab- Lyzangan**
G35	**Fars**	**Darab- Lyzangan**
G36	**Fars**	**Darab- Lyzangan**
G37	**Fars**	**Darab- Lyzangan**
G38	**Isfahan**	**Karaj**
G39	**Isfahan**	**Karaj**
G40	**Isfahan**	**Karaj**
G41	**Unknown**	**Karaj**
G42	**Bulgaria**	**Kerman**

A


0
5
10
15
20
25
G18-Azarbiajan
G19-Azarbiajan
G20-Azarbiajan
G16-Azarbiajan
G17-Azarbiajan
G1-Azarbiajan-
G3 Tehran
G24-Khorasan
G5-Fars
G9-Fars
G26-Azarbiajan
G2-Azarbiajan
G22-Isfahan
G23-Isfahan
G25-Khorasan
G13-Kerman
G21-Isfahan
G4-Isfahan
G14-Kerman
G15-Kerman
G7-Fars
G11-Fars
G10-Fars
G12-Fars
G8-Fars
G6-Fars

(B) RAPD


G01- Azarbaijan
G26- Azarbiajan
G02- Isfahan
G16- Azarbaijan
G17- Azarbaijan
G20- Azarbaijan
G18- Azarbaijan
G19- Azarbaijan
G03- Tehran
G04- Isfahan
G06- Fars
G10- Fars
G08- Fars
G11- Fars
G12- Fars
G13- Kerman
G14- Kerman
G15- Kerman
G23- Isfahan
G25- Khorasan
G27- Fars
G28- Fars
G37- Fars
G39- Isfahan
G40- Isfahan
G41- Unknown
G22- Isfahan
G32- Fars
G34- Fars
G35- Fars
G36- Fars
G24- Khorasan
G09- Fars
G30- Fars
Similarity coefficient

(C) SSR

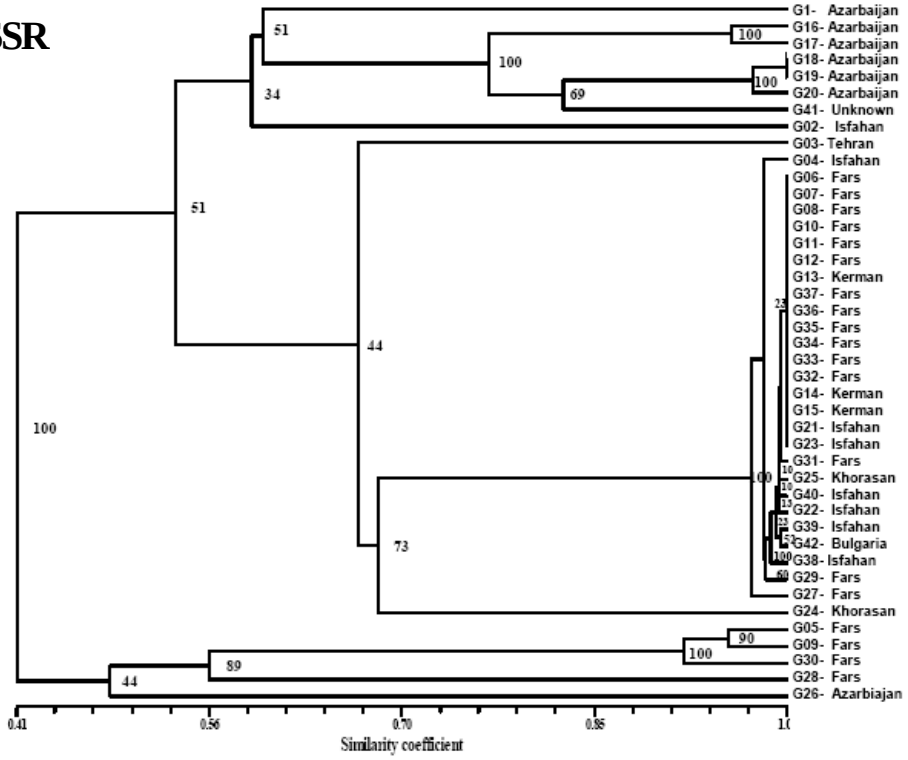

G1- Azarbaijan
G16- Azarbaijan
G17- Azarbaijan
G18- Azarbaijan
G19- Azarbaijan
G20- Azarbaijan
G41- Unknown
G02- Isfahan
G03- Tehran
G04- Isfahan
G06- Fars
G07- Fars
G08- Fars
G10- Fars
G11- Fars
G12- Fars
G13- Kerman
G37- Fars
G36- Fars
G35- Fars
G34- Fars
G33- Fars
G32- Fars
G14- Kerman
G15- Kerman
G21- Isfahan
G23- Isfahan
G31- Fars
G25- Khorasan
G40- Isfahan
G22- Isfahan
G39- Isfahan
G42- Bulgaria
G38- Isfahan
G29- Fars
G27- Fars
G24- Khorasan
G05- Fars
G09- Fars
G30- Fars
G28- Fars
G26- Azarbiajan
Similarity coefficient

Figure 1. Dendrograms of 42 Damask rose accessions obtained using morpholological traits (A).RAPD (B) and SSR (C) Numbers are bootstrap values based on 1000 re-samplings