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Genetic diversity of nine elite *Rosa damascena* genotypes using molecular markers

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ABSTRACT

The existence of high genetic diversity among different genotypes of a species is essential for breeding studies, as it enables researchers to use genetic diversity to achieve optimal hybrids and maximum yields. *Rosa damascena* is one of the most important commercial plants used in the food, pharmaceutical, perfumery, and cosmetic industries. This study aims to investigate the genetic relationships of nine elite genotypes of *R. damascena* proposed for introduction as new cultivars in Iran. Leaf samples from the nine elite genotypes were collected from the Rose collection (Research Institute of Forests and Rangelands). DNA was extracted using a modified CTAB method, and Polymerase Chain Reaction (PCR) was performed with 30 SSR markers. The results showed that 23 out of 30 SSR markers successfully amplified DNA from our samples. The RD18 and RD5 markers yielded the highest number of polymorphic alleles, with 16 alleles each, while the RD7 marker produced the lowest, with just one polymorphic allele. Molecular data analysis was conducted using NTSYS software. Clustering of the nine genotypes based on the UPGMA method revealed that they were grouped into four main clades, with a similarity coefficient of 0.85: Clade I contained genotype A104; Clade II included genotype Yazd 94; Clade III encompassed six genotypes—D231, B211, A105, Kashan 93, B215, and D237; and Clade IV was represented by genotype D234.

***Key words*:** Damask rose, Genetic diversity, Molecular markers, NTSYS, SSR.

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INTRODUCTION

Rosa is an important genus in the Rosaceae family, comprising about 200 species and 18,000 cultivars worldwide (Gudin, 2010). This genus is well-known not only as a scented ornamental plant but also as a vital source for rose oil and rose water production. Four species—*Rosa damascena* Mill., *Rosa moschata* Herrm., *Rosa centifolia* L., and *Rosa gallica* L.—are primarily used for oil production (Babaei *et al*., 2008). Among these species, *R. damascena* is the most significant for oil production in Iran, Turkey, Bulgaria, and globally (Baydar *et al*., 2004).

While there is some debate regarding the origin of *R. damascena*, Iran is recognized as one of its principal centers of origin (Boskabady *et al*., 2011). *R. damascena* is the national flower of Iran and is cultivated in many regions across the country. The genotypes of *R. damascena* cultivated in Iran exhibit various morphological, physiological, biochemical, and phenological differences, such as plant height, canopy structure, spine density, spine length, branch angle, and leaf count (Tabaei *et al*., 2004). Additional traits include flower yield, flower weight, dry matter percentage, and petal weight-to-flower weight ratio (Tabaei-Aghdaei and Rezaee, 2004), as well as essential oil yield and the number of petals, stamens, and pistils (Tabaei-Aghdaei *et al*., 2005). Other reported variations include kaempferol and quercetin content (Jaimand *et al*., 2010), thorn length and density, leaflet dimensions, stipule size, branch angles, and receptacle dimensions (Tabaei-Aghdaei *et al*., 2004).

Molecular markers have increasingly been employed to assess the genetic diversity of *Rosa* spp. in various regions. Several studies have utilized RFLPs, RAPDs, AFLPs, SSRs, SCoT, and URP markers. However, the application of AFLP, SSR, and RAPD markers did not effectively reveal the genetic diversity of *R. damascena* genotypes from Turkey (Agaoglu *et al*., 2000; Baydar *et al*., 2004) and Bulgaria (Rusanov *et al*., 2005). These results suggest that the Turkish and Bulgarian genotypes may have originated from one or a few common genotypes, or that the markers employed were insufficient to distinguish among them. In contrast, high genetic diversity has been reported among *R. damascena* genotypes in Iran, as determined by RAPD, AFLP, and SSR analyses (Pirseyedi *et al*., 2005; Babaei *et al*., 2007; Kiani *et al*., 2008).

Based on comprehensive morphological, biochemical, physiological, and functional studies conducted on 40 *R. damascena* genotypes from various regions of Iran (Tabaei-Aghdaei *et al*., 2010), nine elite genotypes were selected for further investigation. This study aims to evaluate the genetic diversity among these elite genotypes of *R. damascena* using SSR markers.

MATERIALS AND METHODS

Plant material

Nine elite genotypes were collected from the *R. damascena* collection at the Research Institute of Forests and Rangelands (RIFR) in Tehran, Iran. These genotypes exhibit higher flowering periods, flower yields per hectare, and essential oil yields per hectare compared to the control plant (Table 1).

Table 1. Features of nine elite genotypes used in the study (Tabaei-Aghdaei *et al.,* 2010).

Genotype name	Origin site	FY/ha (Kg)*	Essence gain
A104	Isfahan	2784	0.031
A105	Isfahan	2228	0.024
B211	Khuzestan	2418	0.026
B215	Baluchestan	2340	0.032
Kashan 93	Kashan	2533	0.031
Yazd 94	Yazd	2857	0.027
D231	Yazd	2256	0.027
D234	Isfahan	2383	0.029
D237	Isfahan	2557	0.028

* FY/ha: Flower yield per hectare.

DNA extraction and PCR amplification

Young leaves were collected from each genotype, frozen in liquid nitrogen, and then stored at -80 °C. DNA extraction was performed using the CTAB method with slight modifications (Saghai-Maroof *et al*., 1984). The quantity and quality of DNA samples were assessed using agarose gel electrophoresis and spectrophotometry (NanoDrop™ 2000, Thermo Fisher Scientific, USA). After quantification, 25 ng/µl of DNA was used for the PCR process. This study utilized 30 pairs of microsatellite primers to screen the nine selected genotypes of *R. damascena*. The names and sequences of these primers are provided in Table 2 (Zhang *et al*., 2006).

The polymerase chain reaction (PCR) was conducted using a Mastercycler Gradient thermal cycler (Eppendorf, Germany) in a total volume of 20 microliters. The PCR conditions included one cycle of initial denaturation at 94 °C for 5 minutes, followed by 35 cycles with denaturation at 94 °C for 1 minute,

Table 2. SSR primers and their amplification results.

Primer	Primer sequence (5′ → 3′)	Ta (°C)	TAB	NPB	PPB	PIC	MI
RD2	F: ATCATGTGCAGTCTCCTGGT R: AATTGTGGGCTGGAAATATG	55	7	7	100	0.21	1.47
RD3	F: AGAGAATTGAAAAGGGCAAG R: GAGCAAGCAAGACACTGTAA	53	5	5	100	0.25	1.25
RD4	F: CAGGTAATTTGCGGATGAAG R: GATCCGCCGTTTCCAGT	58	8	8	100	0.27	2.16
RD5	F: GTGGATTTTCAGAGATACGC R: TCACAGACAGGACCACCTAT	50	16	16	100	0.36	5.76
RD6	F: GCCATCACTAACGCCACTAAA R: GCGTCGTTCGCTTTGTTT	56	3	3	100	0.19	0.57
RD7	F: ACAGGCCTCTGTTCACCATC R: ACACATGCACAACTCAGAGAA	55	1	1	100	0.19	0.19
RD8	F: CGGTGGAGAGGATGATGTG R: GCAACAAGAACCAGCACAGA	55	4	4	100	0.19	0.76
RD9	F: ACTCCTCCAAAGCTTCACCA R: CCTCATCGACAGAGTCGTCA	59	2	2	100	0.19	0.38
RD15	F: ACAACCAACCCAAGAACTCG R: TGCCAGCTTCAGTCTCACCT	60	13	13	100	0.27	3.51
RD16	F: CAACTGGGTTGGGTCAGTCT R: TCAAATGTACCTTGCGCTTG	60	6	6	100	0.24	1.44
RD17	F: AGAGGTTTAGGGCAGCCATT R: GCGAATGATGGTGGAGAGTT	60	2	2	100	0.19	0.38
RD18	F: CTACTCCAATGTCCGCTTCC R: GTTGGAGAAGAAGCCGTGAG	59	16	16	100	0.20	3.2
RD19	F: CGAGGAAAAACCCAAAATCC R: TGGAAGCAAGAAAAGGCAGT	60	12	12	100	0.24	2.88
RD20	F: GGCGTCTCTCACATCTCAAA R: AAGATCTTCTCTCCGGCCTT	59	3	3	100	0.19	0.57
RD21	F: GCCGTAATTCGTGGAAAGAA R: ATGCCACCAGAACCTTGAAC	60	4	4	100	0.19	0.76
RD22	F: CCTAAAGCTTAAGCCCCCAA R: GCAATAGACTTGGCAGCCTC	60	6	6	100	0.22	1.32
RD23	F: TCTGAGCACGACTCAACAGG R: AGGCATGTAATGCTGTGGGT	60	5	5	100	0.19	0.95
RD25	F: AGAAAAGCGAAAGCACAAGC R: CTTAAATGCGCCACCAATTT	59	6	6	100	0.24	1.44
RD26	F: GCCACCATAGCCAGAGACAT R: GGGCAGAGAAGAAGTTGACG	55	7	7	100	0.21	1.47
RD27	F: GACATCACCACCACCACAAG R: AACCAAGGTTTCCAGTTCCA	55	2	2	100	0.19	0.38
RD28	F: TGGTTTGGGGTTTTGTGTCT R: GCACAGTCTCCACCTGACAA	55	6	6	100	0.19	1.14
RD29	F: TTAATCCAAGGTCAAAGCTG R: TCTCTTTCCCTCCTCACTCT	53	4	4	100	0.25	1
RD30	F: AGAGAATTGAAAAGGGCAAG R: GAGCAAGCAAGACACTGTAA	52	13	13	100	0.32	4.16
Mean			6.56	6.56	100	0.23	1.61

Ta: Temperature annealing, TAB: Total amplified bands, NPB: Number of polymorphic, PPB: Percentage of polymorphism, PIC: Polymorphism information content, MI: Marker index.

annealing at 50–60 °C for 1 minute, and extension at 72 °C for 1 minute. A final extension cycle was performed at 72 °C for 7 minutes. Amplified fragments were separated using Bio-Rad vertical electrophoresis (Sequi-Gene GT) with 8% polyacrylamide gel (PAG).

Data analysis

Due to the complexity of co-dominant scoring of markers in polyploid plants such as roses (Esselink *et al*., 2003), dominant scoring was implemented, where bands were scored as '1' for the presence of an

allele and '0' for its absence. Genetic distances were calculated using the Dice coefficient (Nei and Li, 1979), which is more suitable when SSR markers are scored dominantly (Engqvist and Becker, 1994; Link *et al*., 1995). NTSYS-pc version 2.2 (Numerical Taxonomy and Multivariate Analysis System, Exeter Software) (Rohlf, 2000) was employed for cluster analysis of the nine genotypes using the UPGMA method. For each primer, annealing temperature, total amplified bands, number of polymorphic bands, percentage of polymorphism, polymorphism information content, and MI marker index were calculated (Anderson *et al*., 1993).

RESULTS AND DISCUSSION

Microsatellite analysis

In this study, nine elite genotypes of *R. damascena* were analyzed using 30 microsatellite markers. Based on the results of vertical electrophoresis of PCR products (Figure 1), 23 out of the 30 SSR markers were successfully amplified. In total, 151 polymorphic alleles were identified across the nine elite genotypes of *R. damascena*. The RD18 and RD5 markers each exhibited 16 polymorphic alleles, representing the highest number, while the RD7 marker presented only one polymorphic allele, indicating the lowest number. On average, 6.56 polymorphic alleles were detected per SSR marker (Table 2).

Genetic distance analysis

After scoring the alleles, the Dice similarity coefficient matrix was calculated, as shown in Table 3. The highest genetic similarity was observed between genotypes D237 and B215, with a coefficient of 0.988. Conversely, the lowest genetic similarity was found between A104 and Yazd 94, with a coefficient of 0.23.

Cluster analysis of genotypes

Considering a similarity coefficient threshold of 0.85, four main clades were formed. Clade I included genotype A104; Clade II included genotype Yazd 94; Clade III encompassed six genotypes—D231, B211, A105, Kashan 93, B215, and D237; and Clade IV contained genotype D234 (Figure 2).

This research measured the genetic diversity among the nine *R. damascena* genotypes using SSR markers. Our results demonstrated significant genetic diversity within these genotypes, with the proportion of polymorphism estimated to be 100% for all SSR primers (Table 2). Thus, SSR markers exhibit great potential for identifying *R. damascena* genotypes. Babaei *et al*. (2007) similarly reported 100% polymorphism when using SSR markers in *R. damascena*, and Kiani *et al*. (2008) found the same level of polymorphism among 41 Damask rose genotypes from various

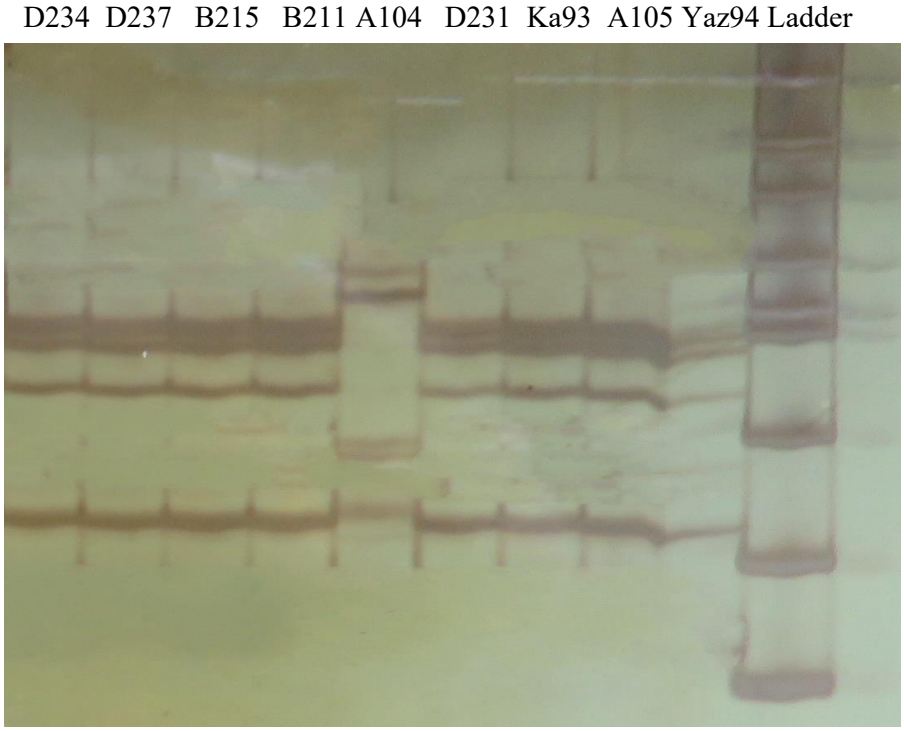

Figure 1. A sample of a polyacrylamide gel showing amplified fragments related to nine genotypes of *Rosa damascena* using the SSR marker RD8. Each lane represents a different genotype, demonstrating the variation in amplification patterns.

Table 3. Genetic distances among nine genotypes of *rosa damascena* calculated with the dice coefficient.

Rows\Cols	D234	D237	B215	B211	A104	D231	Kashan93	A105	Yazd94
D234	1								
D237	0.7820	1							
B215	0.7820	0.9882	1						
B211	0.8734	0.9069	0.9069	1					
A104	0.2091	0.3113	0.3113	0.3313	1				
D231	0.6754	0.8969	0.9090	0.8143	0.3086	1			
Kashan93	0.7875	0.9770	0.9770	0.9090	0.3391	0.8875	1		
A105	0.8101	0.9767	0.9767	0.9310	0.3076	0.8862	0.9772	1	
Yazd94	0.5853	0.8321	0.8175	0.7625	0.2302	0.8421	0.8226	0.8057	1

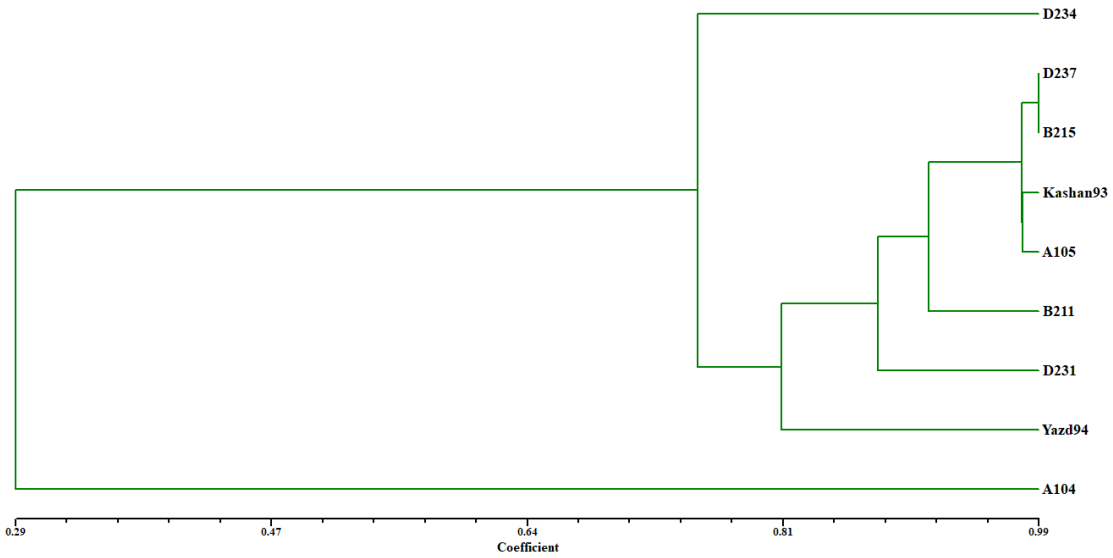

Figure 2. Dendrogram of clustering of the nine genotypes of *Rosa damascena* according to the UPGMA method based on SSR marker data.

cultivation areas in Iran and one from Bulgaria. Additionally, Mostafavi *et al*. (2021) reported 100% genetic polymorphism using URP and SCoT markers in *R. damascena*, aligning with our findings. Given the heterozygous structure of the polyploid genome in roses, a high percentage of polymorphism is expected.

In contrast, Agaoglu *et al*. (2000) and Baydar *et al*. (2004) studied the genetic diversity among cultivars in Turkey using RAPD, AFLP, and SSR molecular markers and found insufficient genetic diversity among different cultivars. These cultivars appeared to have originated from one or a few common primary genotypes, which does not align with the results of the present research. Rusanov *et al*. (2005) similarly reported an absence of genetic diversity among *R. damascena* cultivars in Bulgaria, using SSR markers to investigate this diversity. The discrepancies between their findings and those of this research may be attributed to the transfer of rose cultivars from other sources to Bulgaria.

Pirseyedi *et al*. (2005) reported high genetic diversity among 12 genotypes of *R. damascena* collected from various regions in Iran using AFLP markers, which is consistent with our results. The capability of SSR and AFLP molecular markers to distinguish between different genotypes likely explains this finding, as well as the genetic differences present among them. Babaei *et al*. (2007) evaluated genetic diversity among 40 *R. damascena* genotypes from 28 provinces in Iran, reporting considerable genetic variation. Their study revealed that SSR markers could classify the genotypes into nine distinct clusters, with the largest cluster including 27 genotypes, including cultivars from Turkey and Bulgaria. This suggests that the genotypes found in Turkey and Bulgaria may have been transferred from Iran. Kiani *et al*. (2008) demonstrated that RAPD markers could distinguish among 41 genotypes in Iran, classifying these varieties into ten

groups, with Turkish and Bulgarian cultivars included in the largest group. The results of the present study indicate that Iran can serve as a vital gene reservoir for *R. damascena*, due to its climatic diversity and the presence of various genotypes.

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