GENETIC VARIATION ANALYSIS OF DIFFERENT POPULATIONS OF ROSA DAMASCENA IN NW. IRAN USING RAPD MARKERS

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Random amplified polymorphic DNA (RAPD) markers have been used to characterize the genetic diversity among 12 Damask rose landraces from northwest of Iran. The 22 primers used in this study amplified 262 scorable RAPD loci among which 180 were polymorphic (67%). The average number of bands was 11.9 for each primer. Genetic similarity calculated from the RAPD data ranged from 0.261 to 0.995 with an average of 0.63. A dendrogram was prepared on the basis of a similarity matrix using the UPGMA algorithm and separated the 12 landraces into two groups. There was a good correspondence between genetic divergence and climate condition, though some landraces (such as L2 and L3) with diverse geographical condition were clustered in one group. The results showed a possible application of RAPD markers in assessment of genetic diversity in *Rosa damascena* landraces.

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Key words. *Rosa damascena* Mill., genetic similarity, RAPD analysis

بررسی تنوع ژنتیکی جمعیت های مختلف گل محمدی شمال غرب ایران با استفاده از نشانگر RAPD

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در این پژوهش نشانگر مولکولیRAPD برای تعیین تنوع ژنتیکی ۱۲ جمعیت گل محمدی شمال غرب ایران مورد استفاده قرار گرفت. با استفاده از ۲۲ آغازگر، تعداد ۲۶۲ باند قابل تشخیص ایجاد شد که ٦٧٪ آنها (۱۸۰ باند) در بین جمعیتهای مختلف چند شکلی نشان دادند. تعداد متوسط باندها به ازای هر آغازگر ۱۱/۹ بود. شاخص تشابه Dice برای تعیین همسانیهای ژنتیکی بین جمعیتها مورد استفاده قرار گرفته و با استفاده از الگوریتم UPGMA یک دندروگرام بر مبنای ماتریس تشابه تهیه شد که ۱۲ جمعیت گل محمدی را در۲ گروه مختلف قرار داد. نتایج بدست آمده ارتباط خوبی بین تنوع آب و هوایی و مولکولی نشان دادند، گرچه جمعیتهای با تنوع جغرافیایی (مانند L2 وL3) نیز در یک گروه قرار گرفتند. نتایج این پژوهش بر امکان بکارگیری نشانگرهای RAPD در ارزیابی تنوع ژنتیکی ژرم پلاسم گل محمدی تاکید داشت.

Introduction

Rosa L. as a major genus in the family of *Rosaceae* comprises 200 species with more than 18000 cultivars (Gudin 2000). Roses are the most important economically ornamental crop, because of their popularity as garden, landscape, pot plants or cut flowers, and their use as a source of essential oils for the perfume industry and medicinal purposes. One of

the most important *Rosa* species is *Rosa damascena* Mill. which some of its varieties are very important for oil production and their medicinal properties (Mahmood *et al.* 1996; Ardogan *et al.* 2002; Achuthan *et al.* 2003; Basim and Basim 2003; Ozkan *et al.* 2004). *Rosa damascena* Mill. is native in Caucasus, Syria, Morocco and Andalusia. Iran has also been mentioned as one of its origins (Chevallier 1996). Therefore, a large diversity is expected to be found in Iranian Damask rose landraces.

Genetic diversity can be distinguished from pedigree analysis, morphological traits or using molecular markers. Molecular markers provide the best estimate of genetic diversity since they are independent of the confounding effects of environmental factors.

In recent years, several molecular assays have been applied to assess genetic diversity among different rose species (Rajapakse *et al.* 1992; Ballard *et al.* 1995; Debener *et al.* 1996; Debener *et al.* 2000; Baydar *et al.* 2004; Esselink *et al.* 2004; Rusanov *et al.* 2005). These molecular methods are different in principle, application, type, amount of polymorphism detected and in task and time requirements.

Random amplified polymorphic DNA (RAPD) markers (Welsh and McClelland, 1990; Williams *et al.* 1990) can be used to detect DNA polymorphism without requirement previous knowledge of the target genome. This method has been used for the assessment of genetic diversity in rose species (Debener *et al.* 1996; Jan *et al.* 1999) and has shown its usefulness in rapid detection of genetic variation. Debener *et al.* (1996) reported large genetic diversity among the cultivated roses as compared to the wild species using 13 different RAPD primers. They proposed utilization of this high genetic variability by breeders for future breeding programs.

The objective of this study was to understand the extent and pattern of genetic diversity in 12 Damask rose landraces, from northwest of Iran, using RAPD markers.

Materials and Methods

Plant material

A total of 12 Damask rose landraces from different regions of northwest of Iran (Figure 1 and Table 1) were selected for this study. These landraces have been planted in experimental field of the Research Institute of Forests and Rangelands (RIFR), Tehran, Iran.

DNA isolation and RAPD assays

DNA was extracted from young leaves according to CTAB extraction method with some modification in extraction buffer and stored in 1x TE solution at +4° C. The purity and quality of genomic DNA were determined by spectrophotometry (CECIL 2000) and agarose gel electrophoresis. Only UV absorption ratio A_{260}/A_{280} = 1.8-2 were considered. Polymerase chain reactions (PCR) were carried out in a 20 µl reaction mixture containing 1.9 m*M* $MgCl_2$, 0.5µl primer, 1.25x PCR Buffer, 0.2 m*M* of each dNTPs, approximately 100 ng genomic DNA and 1 U Taq DNA polymerase. Amplifications were performed in a thermal cycler (Eppendorf) programmed as follows: 4 min at 94° C, followed by 40 cycles of 94° C/1 min, 36° C/1 min, 72° C/2 min and a final of 72° C for 5 min.

Amplified products were separated by gel electrophoresis in 1.2% agarose and TBE buffer. PCR products were detected by ethidium bromide staining and photograghed under UV light, with a Gel Doc camera system (uvi-tec). Molecular size of the amplified fragments was estimated by 1kb DNA ladder (ROTCH, XVI).

Data scoring and statistical analysis

Data were scored as 1 for the presence and 0 for the absence of a DNA band for each landrace. The data matrix was entered into the NTSYS program (Rohlf 1993) and analyzed using the qualitative routine to generate Dice similarity index as in Nei and Li (1979). Dendrogram showing genetic relationships of the 12 landraces was constructed using the unweighted pair-group method with arithmetic averages (UPGMA).

Results and discussion

Twenty two random 10 and 12mer primers (Table 2) that resulted in very clear RAPD patterns (Figure 2) were selected to amplify fragments from the DNA templates of 12 landraces. A total of 262 bands were screened (average of 11.90 bands per primer) among which 180 were polymorphic (67%). Debener *et al.* (1996) in their studies on a group of rose cultivars along with wild rose species, using 13 different RAPD primers, found 104 polymorphic DNA fragments. Detection of high level of genetic diversity of *Rosa damascena* landraces is very important and leads us to think how to conserve such a high level of variation for the breeding programs and also for the easy management of genetic resources. In two previous studies with RAPD (Agaoglu *et al.* 2000), AFLP and SSR markers (Baydar *et al.* 2004) have not detected polymorphism among the *R. damascena* plants grown in Isparta, Turkey.

The number of fragments generated per primer varied between 4 and 22. Also, the number of polymorphic bands varied with primer type, ranging from 0 (primer OPA03) to 19 (primer OPA04). In the

present study, the average number of fragments per primer (11.90) was higher than that reported by Agaoglu *et al*. (2000) for *R. damascena* landraces. These differences might be related to the utilization of *R. damascena* genotypes from different geographical origins, as well as the use of different primer sequences.

Estimates of genetic similarity using genetic fingerprinting data are a useful tool in plant breeding, allowing breeders to make better decisions regarding the selection of germplasm to be used in crossing schemes (Milbourne *et al*. 1997; Russel *et al*. 1997). Estimates of genetic similarity of RAPD based on 180 polymorphic markers between 12 landraces of *Rosa* ranged from 0.261 for L11/L12 (Dastjerde / Kooshkan) to 0.995 for L7/L8 (Ramin / Zanjan) (Table 3) with an average of 0.63. Genetic similarity values were used for cluster analysis through UPGMA, resulting in a dendrogram (Figure 3). The landraces were grouped into two clusters, with first cluster comprising 10 accessions and second cluster consisting of two accessions. Both accessions in second cluster (L10 from Gilvan and L11 from Dastjerde) belong to sources (origin sites) with arid-warm climate and also with the lowest altitude. The 10 accessions in the first cluster were again grouped into two sub-clusters. The second sub-cluster contains only one genotype (L4). First sub-cluster contains 9 landraces located in the sources with relatively high altitude. Of course relationship between genetic divergence and climate condition was not strong enough, as landraces from different climates (L2 from arid and L3 from semi arid temperate) were relatively unique and tend to be clustered in one part of the dendrogram. The dendrogram indicated that 2 accessions (L7 and L8) were identical based on Dice genetic similarity coefficient calculated from the RAPD data using 20 random primers. These 2 accessions, from Ramin and Zanjan, are probably duplications and it seems that they have been cultivated by different farmers.

In conclusion, our results show that RAPD could be useful markers for genetic diversity assessment in Damask rose landraces. There, however, are some limitations for RAPD analysis such as the problems of reliability and transferability of RAPD data among laboratories, its dominant nature and low reproducibility in amplification of RAPD markers (Jones *et al.* 1997, Naghavi *et al.* 2004). However, reliable RAPD data can be generated following a standard protocol, replication of amplification reactions and a conservative criterion of band selection (Belaj *et al.* 2003). Also, the RAPD technique is quick cost effective and the ability to perform analysis without the need for prior sequencing of the genome (Huff *et al.* 1993).

It can be concluded from the results of the present study that there is a high level of genetic variation among *R. damascena* landraces grown in Iran. As a set of robust microsatellite markers of rose are available (Esselink *et al*. 2003) and these markers are chromosome specific and scored as codominant markers. Therefore, the use of these markers for variety identification and reference collection management of the Iranian *Rosa damascena* landraces is proposed.

Table 1. Geographical origin of Damask rose landraces.

Landrace no.	Source	Climate[a]	Latitute (N)	Longitude (E)	Altitude (m)
L1	Oskoo	Arid - Temperate	37° 55'	48° 08'	1515
L2	Salmas	Arid - Temperate	38° 12'	44° 46'	1337
L3	Meshkinshahr	Semi arid - Temperate	38° 23'	47° 40'	1568
L4	Qazvin	Semi arid - Temperate	36° 16'	50° 00'	1278
L5	Abhar	Semi arid - Temperate	36° 08'	49° 13'	1536
L6	Goltappe	Semi arid - Temperate	36° 36'	48° 10'	1650
L7	Ramin	Semi arid - Temperate	36° 37'	48° 32'	1700
L8	Zanjan	Semi arid - Temperate	36° 39'	48° 29'	1620
L9	Dotappe	Semi arid - Temperate	36° 07'	48° 57'	1990
L10	Gilvan	Arid - Warm	36° 48'	49° 07'	350
L11	Dastjerde	Arid - Warm	36° 50'	48° 56'	400
L12	Kooshkan	Semi arid - Temperate	36° 41'	48° 20'	1600

[a] Annual mean temperature in warm, temperate and cool climates are 15-25°C, 10-15°C and 0-5°C, respectively. Annual mean precipitation in semi humid, semi arid and arid climates are 600-1400 mm, 300-600 mm and 100-300 mm, respectively.

Table 2. Oligonucleotide primers selected for amplification and polymorphism revealed.

No.	Primer	Sequence (5' – 3')	Scored bands	Polymorphic bands	Monomorphic bands	Polymorphism rate
1	OPA01	CAGGCCCTTC	16	8	8	50
2	OPA02	TGCCGAGCTG	9	4	5	44
3	OPA03	AGTCAGCCAC	4	0	4	0
4	OPA04	AATCGGGCTG	22	19	3	86
5	OPA05	AGGGGTCTTG	10	7	3	70
6	OPA06	GGTCCCTGAC	13	11	2	85
7	OPA07	GAAACGGGTG	16	7	9	44
8	OPA08	GTGACGTAGG	10	7	3	70
9	OPA09	GGGTAACGCC	16	15	1	94
10	OPA10	GTGATCGCAG	10	5	5	50
11	OPA11	CAATCGCCGT	13	9	4	69
12	OPA14	TCTGTGCTGG	10	3	7	30
13	OPA17	GACCGCTTGT	14	10	4	71
14	OPE05	TCAGGGAGGT	10	7	3	70
15	OPE18	GGACTGCAGA	6	4	2	67
16	OPB05	TGCGCCCTTC	13	9	4	69
17	OPB10	CTGCTGGGAC	11	11	0	100
18	OPC05	GATGACCGCC	14	11	3	78
19	OPC06	GAACGGACTC	14	12	2	86
20	OPC11	AAAGCTGCGG	7	3	4	43
21	A15	ATCGCGGAATAT	10	8	2	80
22	A16	ATTTGGATAGGG	14	10	4	71
Total			262	180	82	-
Percentage			-	68.7	31.3	-
Average			11.9	8.2	3.7	84.9
Range			4-22	0-19	0-9	0-100

Table 3. Data matrix of pair-wise genetic similarity between the 12 landraces of *Rosa damascena* based on Nei and Li' s coefficient

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12
L1	1											
L2	0.707	1										
L3	0.674	0.914	1									
L4	0.596	0.509	0.509	1								
L5	0.860	0.734	0.766	0.560	1							
L6	0.835	0.709	0.773	0.546	0.967	1						
L7	0.836	0.702	0.755	0.540	0.967	0.981	1					
L8	0.842	0.708	0.761	0.548	0.972	0.986	0.995	1				
L9	0.812	0.684	0.729	0.497	0.948	0.943	0.962	0.958	1			
L10	0.352	0.368	0.406	0.375	0.413	0.401	0.407	0.415	0.396	1		
L11	0.315	0.292	0.303	0.376	0.311	0.299	0.317	0.315	0.302	0.877	1	
L12	0.800	0.592	0.612	0.483	0.773	0.769	0.782	0.778	0.766	0.308	0.261	1

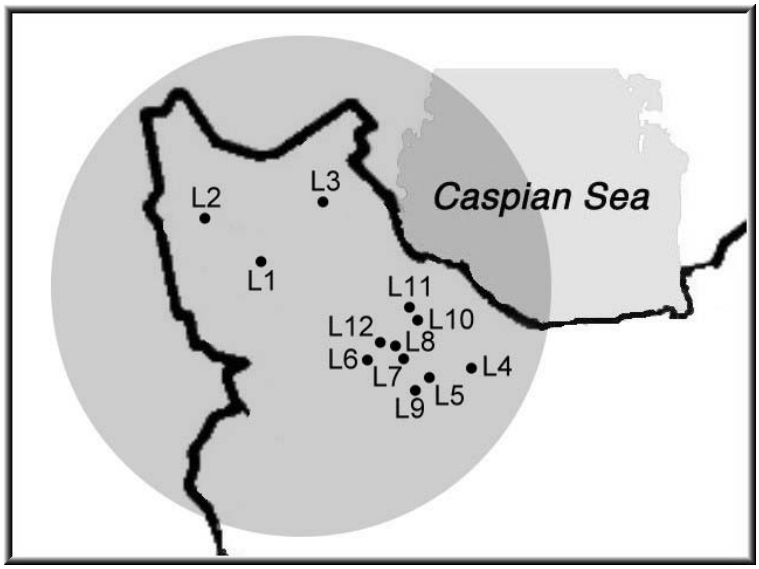

Fig. 1. Distribution of investigated Damask rose landraces regions.

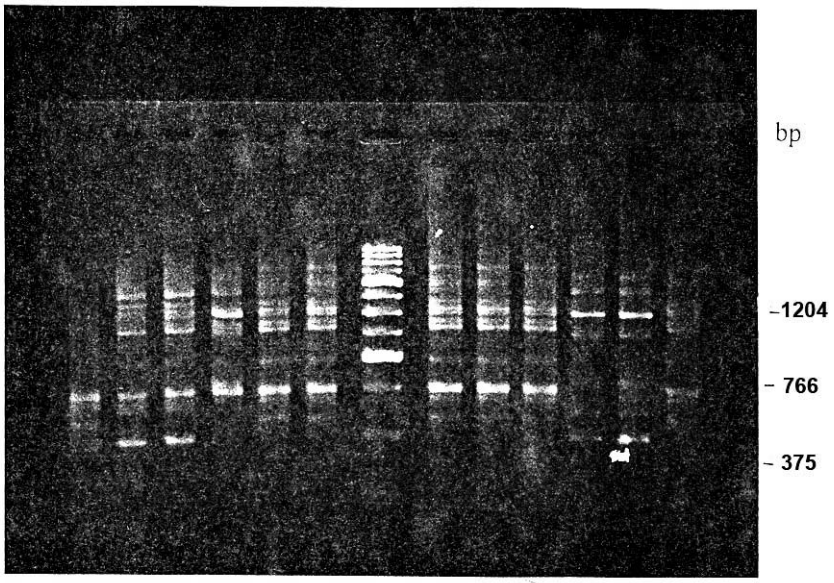

Fig. 2. RAPD pattern of twelve rose landrace amplified using primer OPA09. Lane 7 is molecular weight marker (ROTCH, XVI).

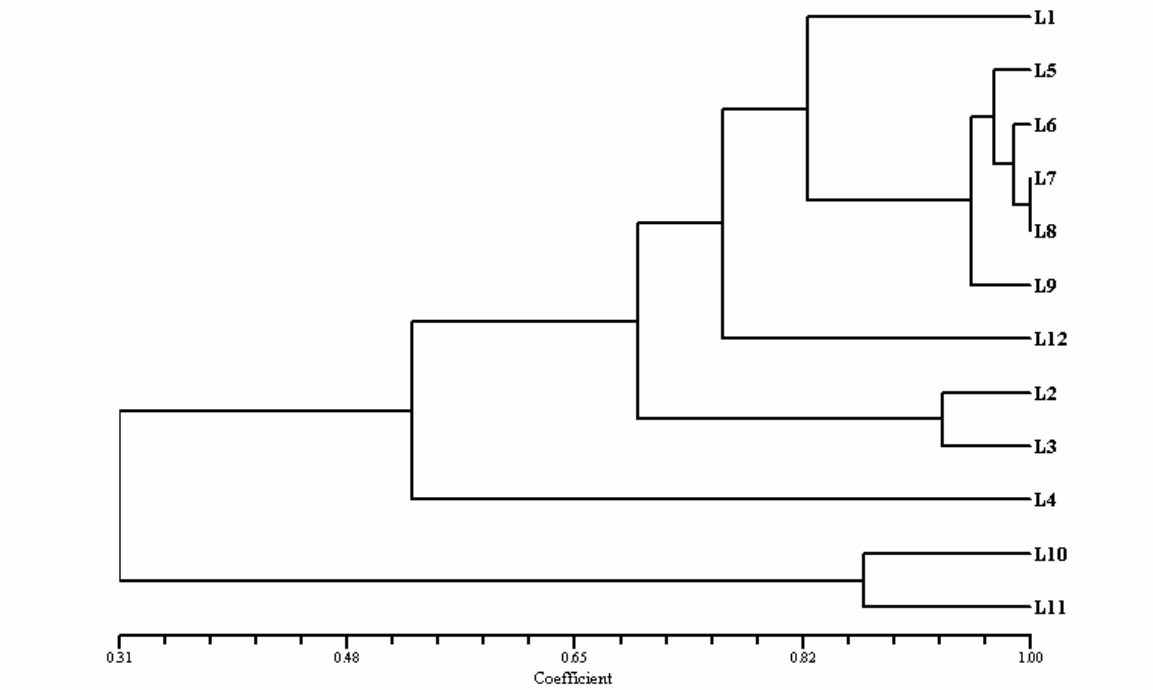

Fig. 3. UPGMA dendrogram showing genetic relationships among the 12 landraces of *Rosa* used in this study. The dendrogram was constructed based on genetic similarity calculated according to Nei and Li's coefficient.

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