Analysis of the genetic diversity 12 Iranian Damask rose (*Rosa damascena* Mill.) genotypes using amplified fragment length polymorphism markers

S Mostaffa Pirseyedi[1]*, Mohsen Mardi[1], Saied Davazdahemami[1], Maryam J. Kermani[1], S Abolghasem Mohammadi[2]

[1]*Department of Genomics, Agricultural Biotechnology Research Institute of Iran, P.O. Box 31535-1897, Karaj, I.R. Iran* [2]*Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Tabriz, Tabriz, I.R. Iran*

Abstract

In this study, the genetic diversity of 12 Iranian Damask rose (Rosa damascena Mill.) genotypes was studied using amplified fragment length polymorphism (AFLP) markers. Twelve AFLP primer combinations generated 483 polymorphic bands and showed extreme variability and genetic complexity among the studied genotypes. The AFLP analysis revealed a specific amplified fragment for the genotypes collected from the Meymand region. Despite the geographical distance between Kashan and Kazeroon regions, the results of cluster analysis showed that the genotypes collected from these districts regions were genetically related. The low genetic similarities were observed among the genotypes belonging to Tabriz, Meymand and Kashan regions suggesting that there was relatively less geographical relocation of the genotypes between these regions. Hence, for breeding of the Damask roses taken in this study, it is necessary to conjunct the molecular data with agronomic characters.
Keywords: Genetic diversity; Damask rose; Rosa damascena Mill.; AFLP

INTRODUCTION

The Damask rose (*Rosa damascena* Mill.) is the most important rose species used to produce rose water, attar of rose, and essential oils in the perfumery industry. Genetic research on determining the origin of Damask rose started since 1940s. Hurst (1941) classified the today Damask roses into two types according to their flowering habit: summer Damask that blooms only in early summer, and autumn Damask that blooms again in autumn. According to Iwata *et al*. (2000), three parental species (*R. moschata* J. Herm., *R. gallica* L. and *R. fedtschenkoana* Reg.) have been identified as ancestors of *R. damascena*.

The Damask rose is an important essential oil yielding crop in Iran. For the essential oil business, highly scented summer Damask roses are cultivated in commercial rose gardens in different regions of the country. Since Damask roses of Iran show some morphological diversities (Tabaei-Aghdaei *et al.*, 2005a), it is not clear whether the plant materials are derived from a few or many genotypes. The differences could be either attributed to the different origins of the Damask roses or they may be the results of natural hybridizations and mutations. Therefore, it is of great importance for the future breeding of *R. damascena* genotypes with improved horticultural characteristics to elucidate the genetic diversity among Damask roses of Iran.

Study of genetic diversity in the genus *Rosa* is complicated due to a large number of natural as well as man-made crosses. Many of the morphological characteristics used in rose genetic diversity determinations are difficult to evaluate (Millan *et al.,* 1996). It is obvious that rapid and reliable methods for the study of the genetic variation and genetic relationships of roses are of the outmost importance. The study of the genetic diversity of Iranian Damask rose genotypes has been based on morphological characteristics (Tabaei-Aghdaei *et al.,* 2003; Tabaei-Aghdaei and Rezaei, 2003; Tabaei-Aghdaei *et al.,* 2004a,b; 2005a,b; Yousefi *et al.,* 2005).

Molecular markers have been used to study genetic diversity and relationships among wild and modern varieties and cultivars of roses (Hubbard *et al.,* 1992;

Correspondence to:* **S. Mostaffa Pirseyed, M.Sc.
Tel: +98 261 2703536; Fax: + 98 261 2704539
E-mail: m_pirseyedi@abrii.ac.ir

Figure 1. Five selected regions where 12 Damask rose genotypes were collected.

Torres *et al.,* 1993; Ben-Meir and Vainstein, 1994; Debener *et al*., 1996; Millan *et al.,* 1996; Reynder-Aloisi and Bollereau, 1996; Vosman *et al.,* 2004; Rusanov *et al.,* 2005). Moreover, different molecular genetic linkage maps for roses have been reported (Debener and Mattiesch, 1999; Debener *et al.,* 2001; Rajapakse *et al.,* 2001; Yan *et al.,* 2005). The purpose of the present study was to analyze the genetic diversity of 12 Iranian Damask rose (*Rosa damascena* Mill.) genotypes using AFLP markers.

MATERIALS AND METHODS

Plant material: Twelve Damask rose genotypes (*R. damascena* Mill.) belonging to 12 commercial rose gardens were used in this study. Eight of these gardens (Naragh, Kamo, Mashad-Ardehal, Mah-Golab, Sad-Abad, Vidooj, Varkan and Vidooja) were within the Kashan regions and the other four were in the Lavasanat, Meymand, Kazeroon and Tabriz regions. The geographical locations of the collection regions are shown on the map (Fig. 1).

AFLP analysis: The AFLP analysis was conducted as described by Vos *et al.* (1995), but with minor modifications.

DNA extraction: For each genotype, young leaves of 15 mature plants were harvested and pooled. Total genomic DNA was extracted as described by Suhl and Korban (1996) and its quantity and quality were evaluated with a UV-Photometer.

DNA digestion: 500 ng of DNA was digested with 2.5 units of *Mse*I and *Eco*RI restriction enzymes in a 40 µl final reaction volume using 4 µl of 10X One-Phor-All buffer (10 mM Tris-HAc pH 7.5, 10 mM MgAc, 50 mM KAc, 5 mM DTT, 50 ng/µl BSA; Boehringer Mannheim) and incubated at 37ºC for 1.5h.

Ligation of adaptors: The *Eco*RI and *Mse*I enzyme restriction sites of genomic DNA fragments were ligated to 50 mM *Mse*I-adaptor and 5 mM *Eco*RI-adaptor (MWG Biotech Ebersberg, Germany) using 2 unites of T4 DNA ligase (Boehringer Mannheim) and 1 mM ATP per reaction. The volume of reactions mixtures were adjusted to 50 µl and incubated at 37ºC for 3h. The resulting reaction product was diluted with 75 µl of distilled water.

Preamplifications: Preamplification was performed with the *Eco*RI/*Mse*I primer combination having no selective nucleotide at the 3´ end (MWG Biotech, Ebersberg, Germany). The PCR preamplification was carried out in a 25 µl reaction volume with 0.3 µM *Eco*RI -primer, 0.3 µM *Mse*I-primer, 0.2 mM of each dNTP, 1.5 mM MgCl$_2$, 1 X PCR buffer (10 mM Tris-Hcl pH 8.3 50 mM KCl, gelatin solution 1%), 0.5 units of *Taq* polymerase and 3.75 µl of diluted ligated DNA. For preamplification, the following cycle profile was used: 2 min at 72°C, 20 cycles: 30 sec at 94°C, 1 min at 60°C and 2 min at 72°C. The PCR product was diluted with 75 µl of distilled water.

Selective amplifications: Twelve *Eco*RI+NNN/*Mse*I

Table 1. The number of polymorphic bands and polymorphic information content (PIC) of 12 AFLP selective primer combinations.

Primers combination*	Sequence of primers	Number of polymorphic bands	PIC
M50 / E76	5'-GATGAGTCCTGAGTAACAT-3' / 5'-GACTGCGTACCAATTCGTC-3'	28	0.17
M65 / E76	5'-GATGAGTCCTGAGTAAGAG-3' / 5'-GACTGCGTACCAATTCGTC-3'	30	0.12
M52 / E76	5'-GATGAGTCCTGAGTAACCC-3' / 5'-GACTGCGTACCAATTCGTC-3'	32	0.15
M47 / E41	5'-GATGAGTCCTGAGTAACAA-3' / 5'-GACTGCGTACCAATTCAGG-3'	34	0.019
M50 / E41	5'-GATGAGTCCTGAGTAACAT-3' / 5'-GACTGCGTACCAATTCAGG-3'	36	0.002
M65 / E38	5'-GATGAGTCCTGAGTAAGAG-3' / 5'-GACTGCGTACCAATTCACT-3'	38	0.306
M47 / E76	5'-GATGAGTCCTGAGTAACAA-3' / 5'-GACTGCGTACCAATTCGTC-3'	39	0.11
M47 / E25	5'-GATGAGTCCTGAGTAACAA-3' / 5'-GACTGCGTACCAATTCTG-3'	39	0.228
M65 / E41	5'-GATGAGTCCTGAGTAAGAG-3' / 5'-GACTGCGTACCAATTCAGG-3'	42	0.19
M50 / E32	5'-GATGAGTCCTGAGTAACAT-3' / 5'-GACTGCGTACCAATTCAAC-3'	46	0.541
M50 / E38	5'-GATGAGTCCTGAGTAACAT-3' / 5'-GACTGCGTACCAATTCACT-3'	49	0.482
M65 / E32	5'-GATGAGTCCTGAGTAAGAG-3' / 5'-GACTGCGTACCAATTCAAC-3'	70	0.719

* The AFLP primer combinations were abbreviated according to the standard nomenclature of AFLPs proposed by KeyGene.

+NNN primer combinations with three selective nucleotides on the 3´ end of either primer were used for selective amplification (Table 1). The AFLP primer names were abbreviated according to the standard nomenclature of AFLPs proposed by KeyGene (Mardi *et al*., 2005). 3.75 µl of diluted preselective PCR products were used as DNA templates for selective amplifications. The PCR selective amplifications were carried out in the same manner as PCR preamplifications.
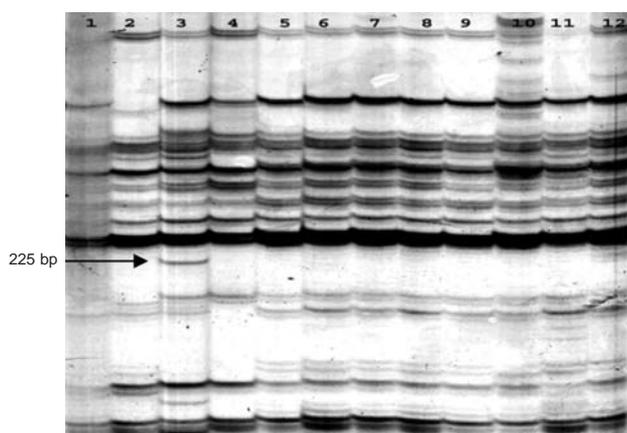

Figure 2. Amplified fragment length polymorphism (AFLP) analysis in 12 Damask rose genotypes using the M50/E41 selective primer combination. Arrow shows specific marker for genotype sampled from the Meymand region. The lane numbers cross reference to genotype plants (see Table 2).

For selective amplification, the following cycle profile was used: 2 min at 94°C, 10 cycles: 30 sec at 94°C, 30 sec at 63°C (touchdown 1°C per cycle to 54°C), 2 min at 72°C and 23 cycles: 30 sec at 94°C, 30 sec at 54°C, 2 min at 72°C. All amplifications were performed in a BioRad thermocycler (BioRad Laboratories Inc., Hercules, CA, USA).

Gel analysis: Amplification reaction products were separated on a 6% denaturing polyacrylamide gel in a 50 cm Sequi-Gen GT Sequencing Cell gel apparatus (BioRad Laboratories Inc., Hercules, CA, USA). The amplified fragments were detected by silver staining as described by Bassam *et al*. (1991). The resulting gels were scored manually.

Data analysis: The average polymorphic information content (PIC) was calculated according to Botstein *et al.* (1980) for each primer combination. Cluster analysis based on complete linkage algorithm using Jaccard's coefficient, were performed using the NTSYS-pc version 2.01 software (Rohlf, 1993).

RESULTS

Twelve AFLP selective primer combinations amplified 483 polymorphic bands. The number of polymorphic

Table 2. Pairwise genetic similarity among 12 Damask rose genotypes based on Jaccard's coefficients estimated from AFLP data.

No.	Genotypes / Collection site	N	L	M	T	VA	KA	MA	MG	S	VI	VIA	KAM
1	Naragh (N)												
2	Lavasanat (L)	0.41											
3	Meymand (M)	0.25	0.30										
4	Tabriz (T)	0.27	0.28	0.20									
5	Varkan (VA)	0.45	0.36	0.24	0.19								
6	Kazeroon (KA)	0.54	0.48	0.27	0.24	0.63							
7	Mashad_Ardehal (MA)	0.42	0.33	0.26	0.20	0.63	0.54						
8	Mah_Golab (MG)	0.51	0.45	0.26	0.19	0.56	0.70	0.56					
9	Sad_Abad (S)	0.54	0.43	0.26	0.20	0.64	0.67	0.58	0.67				
10	Vidooj (VI)	0.36	0.26	0.20	0.31	0.40	0.38	0.37	0.34	0.38			
11	Vidooja (VIA)	0.48	0.39	0.25	0.20	0.46	0.55	0.45	0.59	0.56	0.34		
12	Kamo (KAM)	0.45	0.37	0.26	0.20	0.64	0.58	0.56	0.60	0.63	0.40	0.51	

bands per assay ranged from 28 (M50/E76) to 70 (M65/E32) with an average of 40.2 (Table 1). The polymorphic information content ranged from 0.002 (M50/E41) to 0.719 (M65/E32) (Table 1). Among the 483 polymorphic bands, a 225 bp amplified band using the M50/E41 primer pair was specific for the Meymand genotype (Fig. 2). Pairwise genetic similarity between genotypes based on Jaccard's coefficient, ranged from 0.19 (in two pairs of genotypes including Tabriz/Varkan and Tabriz/Mah-Golab) to 0.70 (Mah-Golab/Kazeroon) (Table 2). Cluster analysis using complete linkage algorithm assigned the studied genotypes into four groups (Fig. 3). Cluster 1 consisted of nine and clusters 2, 3 and 4 each consisted of one

genotype. In cluster 1, the genotype collected from the Vidooj region was separated from the other seven genotypes collected from the Kashan (Naragh, Kamo, Mashad-Ardehal, Mah-Golab, Sad-Abad, Vidooja and Varkan) and Kazeroon regions. The genotypes collected from the Lavasanat, Meymand, and Tabriz regions were grouped separately in distinct clusters.

DISCUSSION

The study of the Iranian Damask rose (*R. damascena* Mill.) genotypes has been based on morphological traits (Tabaei-Aghdaei *et al.,* 2003; Tabaei-Aghdaei
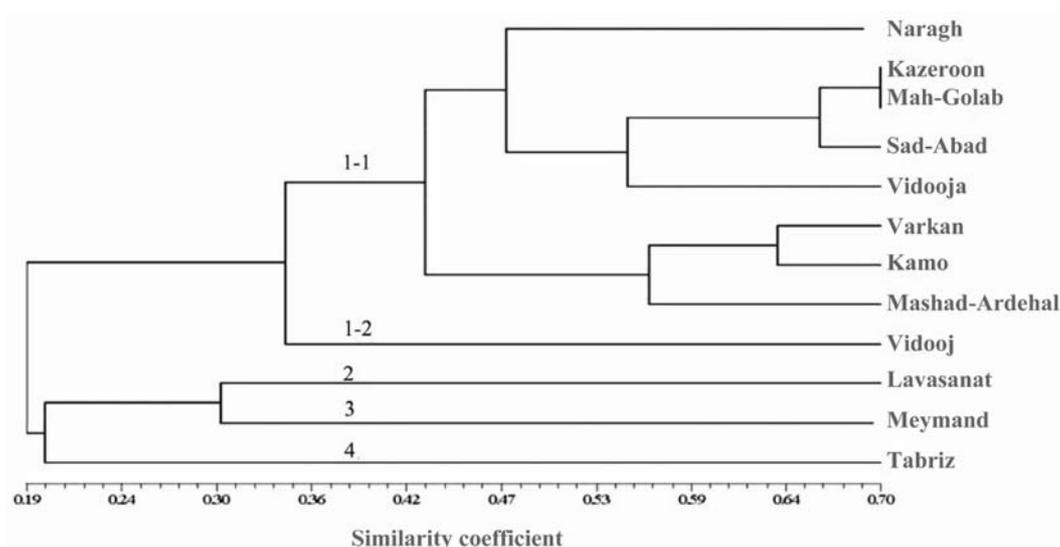

Figure 3. Dendrogram depicting the genetic relationship among 12 Damask rose genotypes constructed using complete linkage analysis based on Jaccard's coefficient estimated from AFLP data.

and Rezaei 2003; Tabaei-Aghdaei *et al.,* 2004a,b; 2005a,b; Yousefi *et al.,* 2005). However, these morphological traits are largely dependent on environmental conditions. The development of DNA based marker procedures has lead to a greater understanding by the breeder of genetic diversity, genetic relationships and an ability to identify genotypes. In this context, AFLP fingerprints are more reliable and reproducible, making this a more appropriate technique for detecting genetic variation among Damask rose genotypes as reported by Baydar *et al.* (2004).

In our study, 12 AFLP primer combinations with 483 polymorphic bands showed an extreme variability and genetic complexity among the studied Damask rose genotypes. Vosman *et al.* (2004) observed remarkable genetic diversity among 83 rose varieties using 12 AFLP primer combinations with 284 polymorphic bands. Debener *et al.* (1996) observed a large genetic diversity among cultivated rose varieties and wild species from different series of the genus *Rosa* using 13 random amplified polymorphic DNA (RAPD), markers with 104 polymorphic fragments. Matsamoto and Fukui (1996) classified rose cultivars using three RAPD primers. Baydar *et al.* (2004) studied genetic relationships among *R. damascena* plants in Turkey by using 23 AFLP and 9 microsatellite markers. Rusanov *et al.* (2005) studied genetic similarity between 26 oil-bearing *R. damascena* Mill. accessions from Bulgaria, Iran, India and Europe using microsatellite markers.

In our study, the AFLP analysis revealed a specific amplified fragment for the genotypes collected from the Meymand region. Millan *et al.* (1996) and Matsamoto and Fukui (1996) found specific bands for *Rosa* spp. using RAPD markers. The AFLP marker might also be developed into primers consisting of characterized amplified regions, to identify the genotypes collected from the Meymand region. The highest PIC value was observed for the AFLP primer combination M65/E32. This value showed the discriminatory power of this primer combination for the studied genotypes.

The present investigation showed that the molecular marker technique used in this study enables to distinguish genetically particular genotypes grown in the same region or in different regions. Tabaei-Aghdaei *et al.* (2004a,b; 2005a,b) studied *R. damascena* Mill. genotypes collected from different regions of Iran and observed a considerable variation for the studied morphological traits. Despite the geographical distance between Kashan and Kazeroon regions, the similarity coefficient of 0.70 (Table 2 and Fig. 3) was observed between the genotypes collected from Mah-Golab

(Kashan) and Kazeroon regions, possibly suggesting that there was a relatively common origin for these genotypes. Baydar *et al.* (2004) by using AFLP and microsatellite markers found out that the *R. damascena* plants in Turkey were derived from the same original genotype by vegetative propagation. Rusanov *et al.* (2005) by using microsatellite markers reported that there was a very narrow gene pool for the studied oil-bearing Damask rose accessions. However, the applicability of identified microsatellite markers in genome analysis of rose primarily depends on three inherent circumstances: abundance, hypervariability and in most cases, stable Mendelian inheritance.

Graham *et al.* (1994) described that many red raspberry cultivars, although given different names, were in fact very similar in terms of their genetic contribution. The genetic similarities varied from 0.34 to 0.64 between the genotypes belonging to Kashan regions (Table 2). Tabaei-Aghdaei *et al.* (2003) reported significant differences among nine *R. damascena* Mill. genotypes collected from three parts of the Kashan areas in terms of the measured traits. The low genetic similarities were observed among the genotypes belonging to Tabriz, Meymand and Kashan regions (Fig. 3), possibly suggesting that there was relatively little geographical relation of this species.

Our results suggest that the AFLP approach is a reliable, rapid and sensitive technique to estimate genetic diversity of Damask rose genotypes. The results showed an extreme variability and genetic complexity among 12 Iranian Damask rose (*R. damascena* Mill.) genotypes grown in the famous rose gardens of Tabriz, Meymand and Kashan regions of Iran. For breeding of Damask roses, it is necessary to conjunct molecular data with agronomic characters such as yield of rose oil and novel scent compounds. This strategy may benefit the breeding of *R. damascena* plants as proposed by Debener *et al.* (2003).

Acknowledgment

References

Bassam BJ, Caetano-Anolles G, Gresshoff PM (1991). Fast and sensitive silver staining of DNA in acrylamide gels. *Anal Biochem.* 196: 80-83.

Baydar NG, Baydar H, Debener T (2004). Analysis of genetic relationship among *Rosa damascena* plants grown in Turkey by using AFLP and microsatellite markers. *J Biotech.* 111: 263-267.

Ben-Meir H, Vainstein A (1994). Assessment of genetic relatedness in roses by DNA fingerprinting analysis. *Hort Sci.* 58: 158-

164.

Botstein D, White RL, Skolnick M, Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet.* 32: 314-331.

Debener T, Bartels C, Mattiesch L (1996). RAPD analysis of genetic variation between a group of rose cultivars and selected wild rose species. *Mol Breed.* 2: 321-327.

Debener T, Mattiesch L (1999). Construction of a genetic linkage map for roses using RAPD and AFLP markers. *Theor Appl Genet.* 99: 891-899.

Debener T, Mattiesch L, Vosman B (2001). A molecular marker map for roses. *Acta Hortic.* 547:283-287.

Debener T, Malek B, Schreiber M, Drewes-Alvarez R (2003). Marker assisted background selection for the introgression of blackspot resistance into cultivated roses. *Eur J Hort Sci.* 68: 245-252.

Graham J, Mcnicol RJ, Greig K, Vandeven WTG (1994). Identification of red raspberry cultivars and an assessment of their relatedness using fingerprints produced by random primers. *Hort Sci.* 69: 123-130.

Hubbard M, Kell J, Rajapakse S, Abbott A, Ballard R (1992). Restriction fragment length polymorphism in rose and their use for cultivar identification. *Hort Sci.* 27: 172-173.

Hurst, CC (1941). Notes on the origin and evolution of our garden roses. *J Roy Hort Soc.* 66: 77-289.

Iwata H, Kato T, Ohno S (2000). Triparental origin of Damask roses. *Gene.* 259: 53-39.

Mardi M, Buerstmayr H, Ghareyazie B, Lemmens M, Mohammadi SA, Nolz R, Ruckenbauer P (2005). QTL analysis of resistance to Fusarium head blight in wheat using a 'Wangshuibai' derived population. *Plant Breed.* 124: 329-333.

Matsumoto S, Fukui H (1996). Identification of rose cultivars and clonal plants by random amplified polymorphic DNA. *Sci Hort.* 76: 49-54.

Millan F, Osuma F, Cobos S, Torres A, Cuber JI (1996). Using RAPDs to study phylogenetic relationships in *Rosa. Theor Appl Genet.* 92: 273-277.

Prakash NS, Combes MC, Dussert S, Naveen S, Lashermes P (2005). Analysis of genetic diversity in Indian robusta coffee genepool (*Coffea canephora*) in comparison with a representative core collection using SSRs and AFLPs. *Genet Resour Crop Evol.* 52: 333–343.

Rajapakse S, Byrne DH, Zhang L, Anderson N, Arumuganathan K, Ballard RE (2001). Two genetic linkage maps of tetraploid roses. *Theor Appl Genet.* 103: 575-583.

Rusanov K, Kovacheva N, Vosman B, Zhang L, Rajapakse S, Atanassov A, Atanassov I (2005). Microsatellite analysis of *Rosa damasena* Mill. Accessions reveals genetic similarity between genotypes used for rose oil production and old Damask rose varieties. *Theor Appl Genet.* 111: 804-809.

Reynder-Aloisi S, Bollereau P (1996). Characterization of genetic diversity in genus *Rosa* by random amplified polymorphic DNA. *Acta Hortic.* 424: 253-259.

Rohlf FJ (1993). NTSYS-pc: Numerical taxonomy and multivariate analysis system. Vers 1.80. *Applied Biostatistics Inc,* New York.

Suhl IW, Korban SS (1996). A highly efficient method for isolating genomic DNA from plant tissues. *Plant Tissue Cult Biotechnol.* 2: 113-116.

Tabaei-Aghdaei SR, Rezaei MB (2003). Study of flower yield variation in *Rosa damascena* L. genotypes of Kashan. *Iranian J Rangeland Forest Plant Breed Genet Res.* 9:111-122.

Tabaei-Aghdaei SR, Rezaei MB, Jaimand K (2003). Evaluation of genetic variation in floral parts and essential oils concentration of *Rosa damascena* genotypes collected from Kashan/Iran. *Iranian J Rangelands and Forest Plant Breed Genet Res.* 11: 219-247.

Tabaei-Aghdaei SR, Soleimani E, Jafari AA, Rezaei MB (2004a). Evaluation of flower yield and morphological characteristics of *Rosa damascena* Mill genotypes from west parts of Iran, using multivariate analysis. *Iranian J Rangeland Forest Plant Breed Genetic Res.* 12: 203-220.

Tabaei-Aghdaei SR, Sahebi M, Jafari AA, Rezaei MB (2004b). Evaluation of flower yield and morphological characteristics of 11 *Rosa damascena* Mill. Using multivariate analysis. *Iranian J Med Aromatic Plant.* 20: 199-211.

Tabaei-Aghdaei SR, Rezaei MB, Jaimand K (2005a). Study of genetic variation in essential oils yield of *Rosa damascena* Mill genotypes from central regions of Iran. *Iranian J Med Aromatic Plant.* 21: 35-49.

Tabaei-Aghdaei SR, Soleimani E, Jafari AA (2005b). Evaluation of morphological variation in *Rosa damascena* Mill genotypes-from six central province of Iran. *Iranian J Med Aromatic Plant.* 21: 233-246.

Torres AM, Millan T, Cubero JT (1993). Identifying rose cultivars using random amplified polymorphic DNA markers. *Hort Sci.* 28: 33-334.

Vos P, Hoger R, Bleeker M, Reijans M, Van De Lee T, Hornes M, Frijteters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* 23: 4407-4414.

Vosman B, Visser D, Rouppe van der Voort J, Smulders MJM, van Eeuwijk F (2004). The establishment of essential derivation among rose varieties using AFLP. *Theor Appl Genet.* 109: 1718-1725.

Yan Z, Denneboom C, Hattendorf A, Dolstra O, Debener T, Stam P, Visser PB (2005). Construction of an integrated map of rose with AFLP, SSR, PK, RGA, RFLP, SCAR and morphological markers. *Theor Appl Genet.* 110: 766-777.

Yousefi B, Tabaei-Aghaei SR, Assareh MH (2005). Study of cutting rhizogenesis and sapling growth variation in *Rosa damascena* genotypes in Kurdistan. *Iranian J Rangeland Forest Plant Breed Genet Res.* 13: 1-27.