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

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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 utmost 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;
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 *MseI* and *EcoRI* 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 37°C for 1.5h.

**Ligation of adaptors:** The *EcoRI* and *MseI* enzyme restriction sites of genomic DNA fragments were ligated to 50 mM *MseI*-adaptor and 5 mM *EcoRI*-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 37°C for 3h. The resulting reaction product was diluted with 75 µl of distilled water.

**Preamplifications:** Preamplification was performed with the *EcoRI/MseI* 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 *EcoRI*-primer, 0.3 µM *MseI*-primer, 0.2 mM of each dNTP, 1.5 mM MgCl₂, 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 *EcoRI*-NNN/*MseI*
+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.

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...
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-Aghdae
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, 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).

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References


