African Journal of Biotechnology Vol. 10(55), pp. 11373-11377, 21 September, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.861
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Genetic relationships among *Rosa* species based on random amplified polymorphic DNA (RAPD) markers

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Accepted 21 July, 2011

To investigate the genetic diversity of *Rosa* accessions, random amplified polymorphism DNA (RAPD) approach was employed. Nine of ten primers amplified 138 scorable RAPD loci with 111 polymorphic bands (80%). Percentages of polymorphic bands ranged from 75 to 100%. Sizes of amplified DNA fragments ranged from 250 to 6000 bp and were used for statistical analyses. Cluster analysis based on presence-absence of bands used Jaccard similarity coefficient and the unweighted pair group method with arithmetic averages (UPGMA). Genetic similarities between *Rosa* cultivars ranged from 0.42 to 0.84. The dendrogram revealed two main clusters, revealing considerable genetic diversity among these cultivars. Cluster I was divided into two subgroups. RAPD proves a useful tool for evaluating genetic diversity and relationships among different rose cultivars.

Key words: Genetic diversity, (random amplified polymorphism DNA) RAPD markers, polymorphism, Rosaceae.

INTRODUCTION

The plant family, Rosaceae consists of over 100 genera and 3,000 species that include many important fruits, nuts, ornamental and wood crops (Dirlewanger et al., 2002). *Rosa* L. is a major genus in the Rosaceae family which comprises of 200 species with more than 18000 cultivars (Gudin, 2000). The *Rosa* genus is cultivated in many areas of the world such as Bulgaria, Turkey, and India (Tabaei et al., 2006). Fossil records of *Rosa* date back 35 million years to the Oligocene. Iran has also been mentioned as a genus Center (Kiani et al., 2008; Yousefi et al., 2009). Chromosome numbers of *Rosa* vary from 2x = 14 to 8x = 56, but most species are diploid or tetraploid (Shulaev et al., 2008). Many members of this family are high value nutritional foods and contribute in desirable aesthetic and industrial products. For example some varieties of *Rosa damascena* Mill are important for oil production and medicinal properties (Mahmood et al., 1996). Despite common origin, *R. damascena* grown in different locations show some morphological differences such as flower shape, flower color, leaf and plant size. It seems these morphological features are much influenced by the environment (Teng et al., 2002). Estimation of genetic diversity to identify groups with similar genotypes is important for conserving and utilizing genetic resources. Therefore, markers independent of the environment are necessary for reliable identification and discrimination of genotypes and cultivars. Different types of marker systems have been used for genetic analysis and genotyping, including morphological, cytological, biochemical and DNA markers. The value of DNA markers depends on their heritability and level of polymorphism that they can reveal (Sarkhosh et al., 2006). Thus, the establishment of genetic relationships within *Rosa* species based on molecular data may provide a better solution to taxonomic problems. Molecular markers have been applied in various studies to evaluate genetic relationships in *Roses (*Baydar, 2004; Kaur et al.,2007).

They represent a significant resource for creating genetic and physical genome maps, distinguishing individuals, investigating genetic relatedness and studying genome organization (Debener et al., 1998). Various types of DNA markers are now available. The main ones

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Abbreviations: RAPD, Random amplified polymorphic DNA; **ISSR,** inter-simple sequence repeat; **RFLPs,** restriction fragment length polymorphism; **PCR,** polymerase chain reaction; **SSRs,** simple sequence repeats; **AFLP,** amplified fragment length polymorphism.

Table 1. *Rosa* species used in this study.

Accession	Specie	Sample	Collection location
M1	*Rosa damascena*	Leaf	Isfahan
M6	*Rosa damascena*	Leaf	Isfahan
7	*Rosa damascena*	Leaf	Zanjan
22	*Rosa damascena*	Leaf	Kohkiluye and Boyer ahmad
3	*Rosa damascena*	Leaf	Ardebil
Yellow	*Rosa pimpinellifolia*	Leaf	Urmia
Pink	*Rosa canina*	Leaf	Urmia

include restriction fragment length polymorphic DNAs (Sarkhosh et al., 2006), random amplified polymorphic DNAs (Shasany et al., 2005), amplified fragment length polymorphic (Pezhmanmehr et al., 2009), simple sequence repeats (Darvishzade et al., 2010), and inter-simple sequence repeats (Jabbarzadeh et al., 2010).

Random amplified polymorphic DNA (RAPD) is one of DNA marker systems that can be used for the investigation of genetic diversity in plants. This marker system is very useful due to its rapidity, efficiency and non-requirement of sequence information (Yonemoto et al., 2007).

MATERIALS AND METHODS

Field collections

Five accessions of *Rosa damascena* and 2 other species (*Rosa pimpinellifolia* and *Rosa canina)* from the Rosaceae family were sampled in July 2009 (Table 1).

Young leaves were collected and kept in plastic bags containing Silica gel. Upon arrival in the lab, leaves were snap frozen in liquid nitrogen and then stored in -80°C for future use.

Genomic DNA extraction and quantification

Stored leaves were ground in liquid nitrogen by pestle and mortar and genomic DNA was extracted from each plant of *Rosa* species according to the Modified CTAB method. Total DNA was quantified spectrophotometrically and samples yielding good quality (A_{260}/A_{280} ratio 1.7–1.9) were chosen. The extracted DNA from three different individual plants belonging to each species were mixed. Quality of DNA was checked visually on 0.8% agarose gel electrophoresis.

PCR amplification

A set of 10 RAPD primers was purchased from a commercial source (Cinnagen, Tehran). PCR conditions were optimized by varying concentrations of genomic DNA, primers and annealing temperature. The primers with reproducible and scorable amplifications were used in the analysis of all seven genotypes. The reproducibility of PCR fragments was tested twice for each sample and each primer. After initial tests, nine primers were chosen for further studies (Table 2). PCR reactions were performed as follows: DNA samples of each *Rosa* species from 3 individual plants were adjusted to 40 ng/µl and used in the amplification reactions with a final volume of 25 µl containing 1 µl of DNA, 0.5 µl of primer (50 pmol), 12.5 µl of master mix including (dNTPS, PCR buffer, $MgCl_2$, Taq DNA polymerase (5 U/µl) and 11 µl deionized water. DNA amplification was carried out using thermocycler Eppendorf programmed with 3 min at 94°C for initial denaturation, followed by 35 cycles of 1 min at 94°C, 2 min at 43°C, 2 min at 72°C, and a final 5 min extension at 72°C. After amplification, the DNA fragments were separated by electrophoresis in 1.5% agarose gel submersed in 0.5X TBE buffer. The gels were stained with ethidium bromide solution and observed under UV light. All PCR experiments were done at least twice and the best gels of the replicates used for band scoring. Each gel was photo documented using Gel Doc 2000 system. 1 kb DNA ladder was used as a reference to allow comparison among amplified bands.

Data analysis

The RAPD bands in Figure 1 got binary scores recording presence and absence of bands as 1 and 0, respectively. Only bright and well separated bands were scored to avoid counting faint artificial bands. Genetic similarity among genotypes was calculated according to Jaccard (1908). The formula is as follows:

$$Sij = (NAB/ (NAB + NA + NB),$$

Where, NAB is the number of bands shared by samples; NA represents amplified fragments in sample A; and NB represents amplified fragments in sample B. The UPGMA (Un-weighted Pair Group Method with Arithmetic Average) was used for cluster analysis and a dendrogram was drawn using the NTSYSpc 2.02 software (Yao et al., 2007).

RESULTS AND DISCUSSION

Geographical and ecological differences are extremely common in the distribution of genetic diversity among in populations (Ge et al., 2003). Studies on DNA polymorphism are of great relevance in plant breeding since they give a deeper insight into genetic diversity (Paplauskiene and Dabkeviciene, 2008).

Our results show that nine of ten used primers amplified 138 scorable RAPD loci which 111 bands were polymorphic (80%). Figure 1 represents the amplified fragments by OPA-02, OPM-17 and OPD-02 primers. The OPI-08 and OPM-17 primers produced minimum and maximum bands, respectively.

The percentage of polymorphic bands ranged from 75 to

Table 2. Primer sequences and number of polymorphic bands derived from genomic DNA for each RAPD primer.

Primer	Number of band	Polymorphism (%)	Sequence
OPA-01	14	87.5	5'-CAGGCCCTTC-3'
OPA-02	15	83.5	5'-TGCCGAGCTG-3'
OPC-04	14	82.5	5'-CCGCATCTAC-3'
OPE-08	9	82	5'-TCACCACGGT-3'
OPB-10	19	90.5	5'-CTGCTGGGAC-3'
OPM-17	23	100	5'-TCAGTCCGGG-3'
OPD-02	16	84	5'-GGACCCAACC-3'
OPI-08	7	87.5	5'-TTTGCCCGGT-3'
OPK-10	------	------	5'-GTGCAACGTG-3'
OPO-15	20	95.5	5'-TGGCGTCCTT-3'

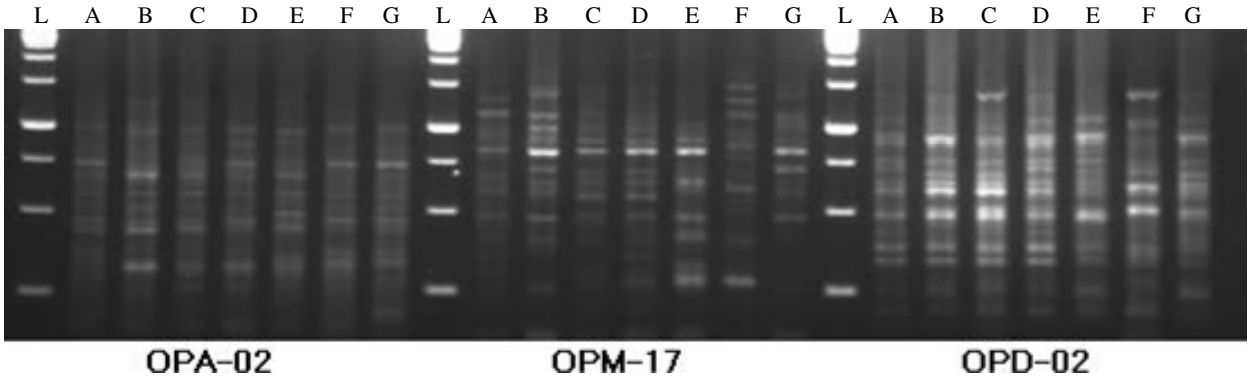

Figure 1. RAPD amplification patterns with primers OPA-02, OPM-17 and OPD-02 (left to right). Lanes A, B, C, D, E, F and G are representative of *R. damascena* M1, *R. damascena* M6, *R. damascena* 7, *R. damascena* 22, *R. canina* , *R. pimpinellifolia* and *R. damascena* 3, respectively. Lane L is 1 kb DNA ladder.

100%. The size of amplified DNA fragments ranged from 250 to 6000 bp. These fragments were used for calculations and statistical analysis.

The presence-absence data from RAPD-PCR was used to calculate Jaccard similarity coefficients (Table 3). Similarity matrices were used as a basis to cluster the samples in the dendrogram form (Figure 2). Cluster analysis showed the genetic relationships among Rosa at inter and intra species level. The dendrogram revealed two main clusters. Cluster I was divided into two subgroups. The *R.d.M1*, *R.d.7*, *R.d.22* and *R. canina* were included in subgroup I. The *R.d.M6* and *R.d.3* were grouped in subgroup II. Cluster II comprised of R.*pimpinellifolia*. The *R.d.7* and *R.d.22* accessions showed the highest genetic similarity (0.83) and the lowest genetic similarity was observed between *R.d.M6* and *R. pimpinellifolia* (0.42). The genetic relationships from this study support those based on morphological data and indicate that RAPD is an efficient marker system which can provide information about relationships among closely related congenial species.

Our results show that RAPD markers are useful for genetic diversity assessment in *Rosa* species. There 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 (Naghavi et al., 2007).

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). On the other hand, the RAPD technique is quick, cost effective and gives the ability to perform analysis without the need for prior sequencing of the genome.

Previous studies on genetic diversity among *Rosa damascena* Mill grown in Isparta have detected low level of polymorphism using RAPD marker (Agaoglu et al., 2000). Tabaei et al. (2006) have reported high polymorphism detection using RAPD primers among *Rosa damascena* accessions. Molecular genetics study on a group of *Rosa* cultivars, using RAPD markers also indicated high level of genetic diversity among *Rosa damascena* cultivars (Debener et al., 1998). Our RAPD molecular markers result also supports high genetic distance at inter and intra species level among *Rosa*

Table 3. Jaccard similarity coefficient among seven *Rosa* accessions based on RAPD markers.

Parameter	*R.d.M1*	*R.d.M6*	*R.d.7*	*R.d.22*	*R.canina*	*R.pimpinellifolia*	*R.d.3*
R.d.M1	1.000000						
R.d.M6	0.489130	1.000000					
R.d. 7	0.647058	0.566666	1.000000				
R.d.22	0.682352	0.565217	0.837500	1.000000			
R.canina	0.600000	0.576087	0.608695	0.659340	1.000000		
R.pimpinellifolia	0.426829	0.421686	0.440476	0.425284	0.470588	1.000000	
R.d.3	0.625000	0.523255	0.522726	0.574712	0.586206	0.520547	1.000000

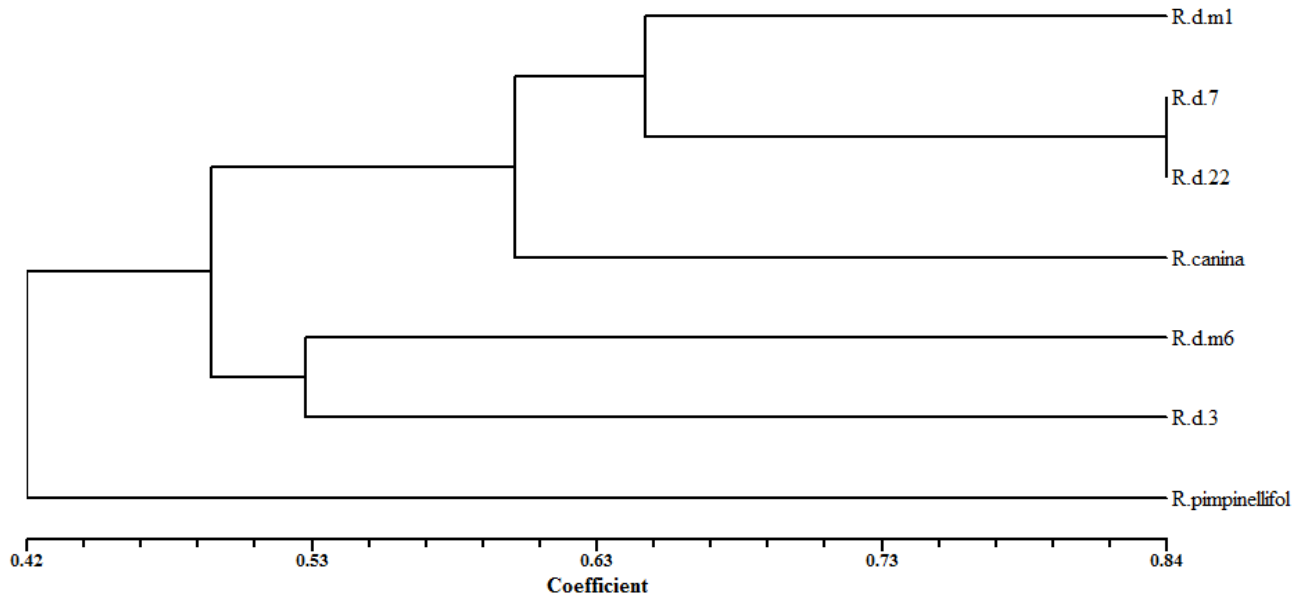

Figure 2. UPGMA dendrogram of *Rosa* generated by similarity coefficients based on RAPD analysis.

species.

The study of genetic diversity is very important and leads us to think how to conserve such a high level of variation for breeding programs and also for easy management of genetic resources.

ACKNOWLEDGMENTS

This research was financially supported by the Ministry of Sciences and carried out in the Biotechnology Research Center of the Urmia University.

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