Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 7, No. 3, p. 254-260, 2015
http://www.innspub.net

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RESEARCH PAPER
OPEN ACCESS

Assessment of genetic variation among *Rosa* species using ISSR genetic markers

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Article published on September 28, 2015

Key words: Genetic variability, ISSR markers, Polymorphism, Rosaceae, Cluster analysis.

Abstract

In this study, Inter-Simple Sequence Repeats (ISSR) markers were applied to assess genetic diversity and genetic relationships among seven species of Rosa. Six of ten used primers could amplify 81 scorable ISSR loci which 71 bands were polymorphic (86%). The 811 and 827 primers produced minimum and maximum bands, respectively. The percentage of polymorphic bands ranged from 60 to 100 with an average of 86.25 %. The size of amplified DNA fragments was ranging between 500 to 2500 bp which was used for calculation and statistical analysis. The cluster analysis based on the presence or absence of bands was performed by Jaccard's similarity coefficient, based on Unweighted Pair Group Method with Arithmetic Averages (UPGMA). Similarity coefficient ranged from 0.30 to 0.66. Based on cluster analysis, the dendrogram revealed three main clusters. First cluster was divided into two subgroups. This investigation showed that genetic distance was relatively significant among these species. The results also propose that ISSR marker is a useful tool for evaluation of genetic diversity and relationships among different *Rosa* genotypes.

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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* 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 epoch. Iran has also been mentioned as a genus Center (Yousefi *et al*., 2009). The chromosome number of *Rosa* varies from 2x = 14 to 8x = 56, but most species are diploid or tetraploid (Shulaev *et al*., 2008). 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 of coming from the same origin, *R. damascena* plants grown in different source of Iran show some morphological differences such as flower shape, flower color, leaf and plant size (Samiei *et al*., 2010). It seems these morphological features are much influenced by 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 the *Rosa* species based on molecular data may provide a better solution to taxonomic problems. Molecular markers have been applied in various studies to evaluate the genetic relationships in Roses (Kaur *et al*, 2007; Baydar *et al*, 2004).

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; Gupta *et al*., 2008). Various types of DNA markers are now available. The main ones include Restriction Fragment Length Polymorphic DNAs (Sarkhosh *et al*., 2006), Random Amplified Polymorphic DNAs (Millan *et al*., 1996), Amplified Fragment Length Polymorphic (Pezhmanmehr *et al*., 2009), Simple Sequence Repeats (Darvishzade *et al*., 2010), Inter-Simple Sequence Repeats (Chowdhary *et al*., 2002).
ISSR marker is a highly sensitive, reliable, reproducible and polymorphic which has been used to estimate genetic diversity among closely related populations. Both Inter- Simple Sequence Repeats (ISSR) and Random Amplified Polymorphic DNA (RAPD) DNA markers are easily to perform and do not require DNA sequence information. The advantage of ISSR as a dominant marker compare to RAPD is the reproducibility of ISSR methodology which has been reported for several species (Paplauskiene *et al*., 2008). Considering this advantage, ISSR marker system was used to analyze the genetic variability among *Rosa* genotypes in this study (Sofalian *at al*., 2008).

Materials and methods

Field collections

Five genotypes of *Rosa damascena* and 2 other genotypes (*Rosa pimpinelliflia and Rosa canina)* from *Rosaceae* family were sampled in July 2009 (Table 1).

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

DNA extraction and quantification

Genomic DNA was extracted from fresh young leaves. Approximately 50 mg of frozen leaf tissue was ground

in liquid nitrogen into a fine powder by mortar and pestle. Each sample consists of three different plants. The pulverized material was transferred to 2 ml eppendorf tube and DNA extracted by modified CTAB method (Tripathi *et al.*, 2007). Total DNA was quantified spectrophotometrically (Eppendorf) and samples yielding good quality (A_{260}/A_{280} ratio 1.7–1.9) were chosen. Quality of DNA was checked visually on 0.8% agarose gel electrophoresis.

PCR amplification

A set of 10 ISSR primers was purchased from a commercial source (Cinnagen, Tehran). PCR conditions were optimized by varying concentrations of template 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, six primers were chosen for further studies (Table 2). PCR reactions were performed as follows: DNA samples of the 7 Rosa genotypes each from 3 individual plants were adjusted to 40 ng/µl and used in the amplification reactions with a final volume of 25µl containing 1µl of DNA, 0.5µl of primer (50 pmol), 11 µl deionized water and 12.5 µl of master mix including dNTPS, PCR buffer, $MgCl_2$ and Taq DNA polymerase (5 U/µl). DNA amplification was carried out using Master Cycler Gradient Thermocycler (Eppendorf) under the following conditions: The initial denaturation was in 94°C for 3 min that followed by 44 cycles of 94°C for 1 min (denaturation), 50 °C for 2 min & 20 s (annealing), 72 °C for 2 min (extension), ultimately 10 min extension at 72 °C and then cooled to 8 °C. The amplification products were separated on 1.5% agarose gel in 0.5 x TBE buffer and stained with Etidium Bromide. Gel was photographed using a gel documentation system (Gel doc 2000). Gene Ruler 1kb DNA Ladder Mix (Fermentas) was used as the DNA fragment size marker. The lists of Inter –Simple Sequence Repeat (ISSR) primers have been summarized in Table 2.

Data analysis

The ISSR bands (Fig.1) were scored by using the binary scoring system that recorded the presence and absence of bands as 1 and 0, respectively. Only bright and well separated bands were scored to avoid counting of faint artificial bands. Genetic distances among genotypes were calculated according to Jaccard (1908).

The formulas are as follow: (GDij = 1 - Sij) and 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 hired for cluster analysis and dendrogram was drawn using NTSYSpc 2.02 software (Yao *et al.*, 2007).

Results and discussion

The genetic structure of plant populations reflects the interactions of different processes including the long term evolutionary history of the species including shifts in distribution, habitat fragmentation, population isolation, mutation, genetic drift, mating system, gene flow and selection. The geographical and ecological differences are extremely common in distribution of genetic diversity in populations (Ge *et al.*, 2003).

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

Accession	Genotype	sample	Collection location
M1	*Rosa Damascena*	Leaf	Isfahan
M6	*Rosa Damascena*	Leaf	Isfahan
12	*Rosa Damascena*	Leaf	Zanjan
22	*Rosa Damascena*	Leaf	Kohkiluye & boyer ahmad
3	*Rosa Damascena*	Leaf	Ardebil
Yellow	*Rosa pimpinelliflia*	Leaf	Urumiye
Pink	*Rosa canina*	Leaf	Urumiye

On the other hand, studies on DNA polymorphism are of great relevance in plant breeding since they give a deeper insight into genetic diversity (Paplauskiene *et al.*, 2008). To our knowledge, no report has been recorded on *Rosa* using ISSR marker in Iran. In this study, ten ISSR marker primers were considered to determine suitable specific primers amplifying the genetic materials of *Rosa* genotypes. Six primers were chosen for further analysis after exclusion of four primers (814, 846, 848 and 858) which did not give the consistent result and clear bands. ISSR amplifications using six primers generated a total of 81 reliable fragments among 7 *Rosa* genotypes.

Table 2. Primers sequence and number of polymorphic bands for each ISSR primer.

primer	Sequence (50–30)	GC%	Number of bands	Number of polymorphic bands	Polymorphism (%)
811	(GA) 8C	52	10	6	60
827	(AC) 8G	52	17	15	88
834	(AG) 8CTT	47	16	14	87.5
845	(CT) 8AGG	52	14	13	92
856	(AC) 8CTA	47	11	10	90
868	CGTAGTCGT(CA)7	52	13	13	100

The number of DNA amplified fragments varied from 10 to 17 depending on the primer, with an average of 13.5 fragments per primer. The size of these fragments ranged between 500 to 2500 bp. Of the 81 DNA fragments, 71 bands (86%) were polymorphic (Table 1). Similarity coefficient (Table 3) was used as a basis to cluster the samples in dendrogram form (Fig. 2). Cluster analysis revealed the genetic relationships among the Rosa species at inter- and intra- species level. The values of similarity coefficient among samples ranged from 0.30 to 0.66 (Table 3).

Table 3. Similarity coefficient among seven *Rosa* accessions (genotypes) based on ISSR markers.

	R.d.M1	*R.d.M6*	*R.d.12*	*R.d.22*	*R.d.canina*	*R.d.pimpinellifia*	*R.d.3*
R.d.M1	0.00000						
R.d.M6	0.633803	0.00000					
R.d.12	0.432432	0.582090	0.00000				
R.d.22	0.408451	0.492537	0.666667	0.00000			
R.d.canina	0.423077	0.441558	0.463768	0.439394	0.00000		
R.d.pimpinellifia	0.304878	0.320988	0.405797	0.358209	0.324675	0.00000	
R.d.3	0.386667	0.386667	0.362319	0.333333	0.455882	0.338028	0.0000

At the interspecific level, the lowest similarity value was 0.30 between R. *pimpinelliflia* and *R.d.M1* while the highest value was 0.66 between *R.d.22* and *R.d.7*. The clustering result indicated that all samples could be distinguished by ISSR markers. Three major groups were revealed by UPGMA dendrogram (Fig. 2). The group I, consisted of *R.d.M1, R.d.M6 & R.d.7, R.d.22* (two subgroups), Group II included *R.canina* and *R.3* (*R.canina* was close to *R.3* with genetic similarity value of 0.45) and Group III, included *R. pimpinelliflia*. According to Fig. 2, ecological conditions could have caused intra species genetic variation. The genetic relationships based on ISSR analysis generally supported those based on morphological data, indicating ISSR is an efficient marker system which can provide information about relationships among closely related congenial species. Thus, ISSR is offered clearly the ability of studying

the genetic relationships of Rosa that is applicable for similar studies in other groups of species. Our result indicates relatively high level of genetic distance. Because of varying ecological conditions of studied locations in this research (five areas involving almost in the west north of Iran), observed genetic difference among accessions was expected. Studies on genetic diversity among *Rosa damascene* have not detected polymorphism using RAPD (Agaoglu *et al.*, 2000), AFLP and SSR (Bayder *et al.*, 2004) in Isparta, Turkey. In Iran Tabaei (2006) have reported detection about 67 % polymorphism using 22 RAPD primers among *Rosa damascene* genotypes. Study on a group of *Rosa* genotypes, using RAPD markers also indicated high level of genetic diversity among *Rosa damascene* genotypes (Debener *et al.*, 1996).

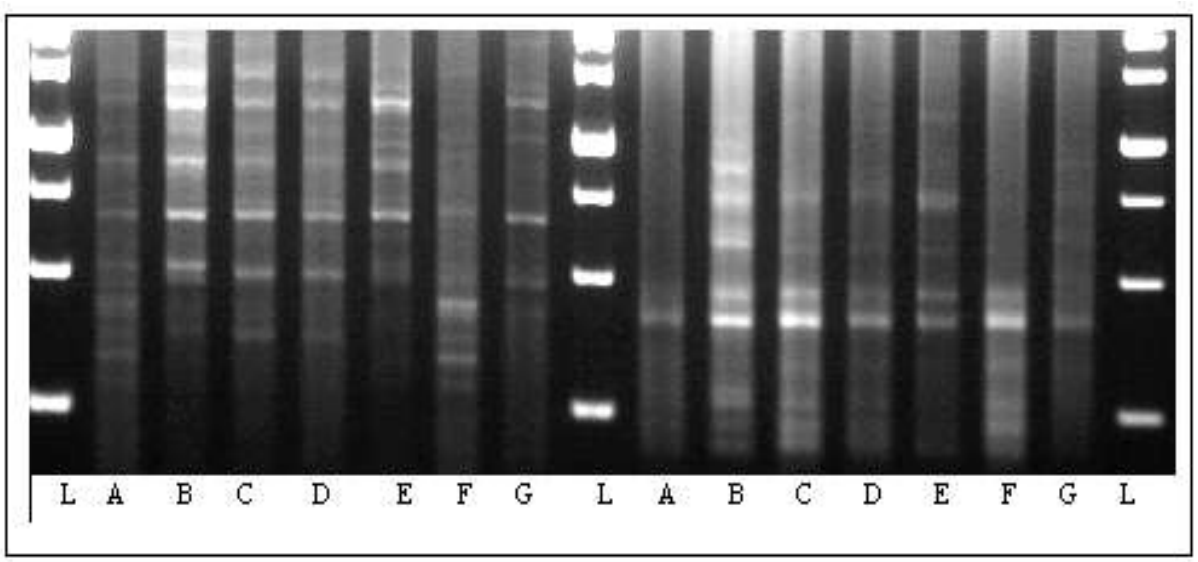

Fig. 1. ISSR amplification patterns with primers 834(left) &845(right). Lane A- B- C-D and G contain the amplified products from *R. damascena*, lane E from *R. caninaand* lane F from *R. pimpinelliflia*. Lane L is 1 kb DNA ladder.

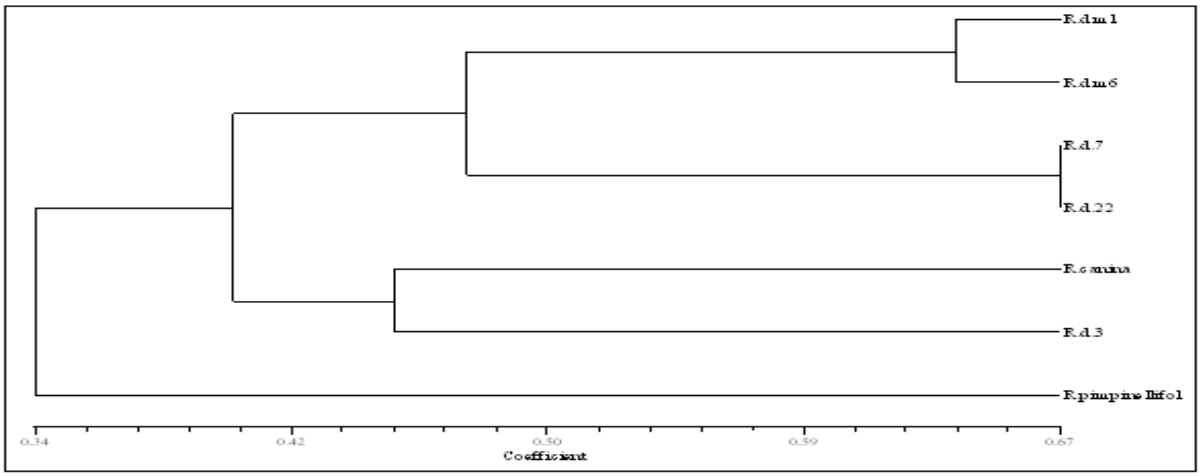

Fig. 2. UPGMA dendrogram of *Rosa* generated by similarity coefficients based on ISSR analysis.

Conclusion

There were several studies in the genus Rosa that used growth characteristics and morphologi attributes to describing genetic diversity but there is little information about using with molecular markers data to reveal genetic variation within Rosa genotypes. diversity study is very important and leads us to think how to conserve such a high level of

variation for the breeding program and also for easy management of genetic resources.

Acknowledgment

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

References

Agaoglu Y, Ergul A, Baydar N. 2000. Molecular analyses of genetic diversity of oil Rose (*Rosa damascene Mill*) grown in Isparta (Turkey) region. Biotechnology and Biotechnological Equipment **14**, 16-18.

Baydar Ng, Baydar H, Debener T. 2004. Analysis of genetic relationships among *Rosa damascena* plant grown in Turkey by using AFLP and microsatellite markers. Journal of Biotechnology **111,** 263-267.

Chowdhury MA, Vandenberg V, Warkentin T. 2002. Cultivar identification and genetic relationship among selected breeding lines and cultivars in chickpea (*Cicer arietinum* L.). Euphytica **127,** 317–325.

Darvishzade R, Azizi M, Hatamimaleki H, Bernousi I, Abdollahimandolkani B, JafariM, Sarrafi A. 2010. Molecular characterization and similarity relationships among sunflower (*Helianthus annuus* L.) inbred lines using some mapped simple sequence repeats. African Journal of Biotechnology **439,** 7280-7288.

Debener T, Mattiesch L. 1998. Effective pairwise combination of long primers for RAPD analyses in Roses. Plant Breeding **117,** 147-151.

Degenerate M, Mattiesch L. 1999. Construction of a genetic linkage map for roses using RAPD and AFLP markers. Theoretical and Applied Genetics **99,** 891-899.

Dirlewanger E, Cosson P, Tavaud M, Aranzana MJ, Poizat C, Zanetto A, Arusplaigret F. 2002. Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L). Theoretical and Applied Genetics **105,** 127-138.

Ge XJ, Yu Y, Zhao NX, Chen HS, Qi WQ. 2003. Genetic variation in the endangered Inner Mongolia endemic shrub Tetraena mongolica Maxim *(Zygophyllaceae)*.Biological Conservation **111,** 427–43.

Gudin S. 2000. Rose: genetics and breeding. Plant Breed **17,** 59-189.

Gupta SH, Srivastava M, Mishra GP, Naik PK, Chauhan RS, Tiwari SK, Kumar M, Singh R. 2008. Analogy of ISSR and RAPD markers for comparative analysis of genetic diversity among different Jatropha curcas genotypes. African Journal of Biotechnology **42,** 30-43.

Kaur N, Sharma RK, Sharma M, Singh V, Ahuja PS. 2007. Molecular Ealuation and Micropropagation of Field Selected Elites of R.damascena Gen. Plant physiology **33,** 171-186.

Mahmood N, Piacente S, Pizza C, Burke A, Khan AI, Hay AJ. 1996. The Anti-HIV Activity and Mechanisms of Action of Pure Compounds Isolated from *Rosa damascene* .Biochemistry and Biophysics Research Communication **20,** 73-79.

Millan T, OsunaF, Cobos S, Torres AM, Cubero JI. 1996. Using RAPDs to study phylogenetic relationships in *Rosa*. Theoretical and Applied Genetics **92,** 273–277.

Paplauskiene V, Dabkeviciena G. 2008.Genetic variability determination using ISSR-PCR markers in red clover varieties. Biologica **54,** 56-59.

Phillips R, Rix M. 1988. *Roses*. Random House, New York, 224 p.

Pezhmanmehr M, Hassani ME, Jahansooz F, Najafi AA, Sefidkon F, Mardi M, Pirsiedi M. 2009. Assessment of genetic diversity in some Iranian populations of *Bunium persicum* using RAPD and AFLP markers. Iranian journal of Biotechnoogy **33,** 202-204.

Porter WM, Smith DH. 1982. Detection of identification errors in germplasm collection. Crop Scientia **22,** 701–703.

SamieiL, Naderi R, Khalighi A, Shahnejatbushehri AA, Mozaffarian V, Esselink GD, Kazempourosaloo S, Smulders MJM. 2010. Genetic diversity and genetic similarities between Iranian rose. Journal of Horticultural Science & Biotechnology **85,** 231–237.

Sarkhosh A, Zamani Z, Fatahi R, Ebadi A. 2006. RAPD markers reveal polymorphism among some Iranian pomegranate genotypes. Scientia Horticulturae **111,** 24-29.

Shulaev V, Korban SS, Sosinski B, Abbott AG, Aldwinckle HS, Folta KM, Iezzoni A, Main D, Arus P, Dandekar AM, Lewers K, Brown SK, Davis TM, Gardiner SE, Potter Dand Veilleux RE. 2008. Multiple Models for Rosaceae Genomics. American Society of Plant Biologists **147,** 985–1003.

Sofalian O, Chaparzadeh N, Javanmard A, Hejazi MS. 2008. Study the Genetic Diversity of Wheat Landraces from Northwest of Iran Based on ISSR Molecular Markers. Journal of Agriculture & Biology **10,** 465–8.

Tabaeiaghdaei SR, Hosseinimonfared H, Fahimi H, Ebrahimzade H, Jebelly M, Naghavi MR, Babaei A. 2006. Genetic Variation Analysis of Different Population of *Rosa damascena* in NW.Iran using RAPD Markers. Iranian journal of Botany **12,** 121-127.

Teng Y, Tanabe K, Tamaru F, Itai A. 2002. Genetic relationship of Pyrus species and cultivar native to East Asia revealed by randomly amplified polymorphic DNA markers. Journal of the American Society for Horticultural Science **127,** 262-270.

Tripathyogendra K, Gurha P, Ghosh D, Kumar RV, Prakash V. 2007. Determination of Phytogenetic Relationships among Isoetes Species Using Random Primers. Turkish Juornal of Botany **31,** 367-372.

Yao Q, Yang K, Pan G, Romg T. 2007. Genetic Diversity of Maize (*Zea mays* L.) Landraces from Southwest China Based on SSR Data . Journal of Genetics and Genomics **349,** 851-860.

Yousefi B, Tabaei SR, Darvish F, Assareh MH. 2009. Flower yield performance and stability of *Rosa damascene* Mill.landraces under different ecological conditions. Scientia Horticulturae **121,** 333-33.