Phylogenetic Relationships among Seven Old Rose Species Grown in Iran Revealed by ISSR Markers

Article · June 2010

CITATIONS
0

READS
55

4 authors, including:

Morteza Khosh-Khui
Shiraz University
139 PUBLICATIONS **860** CITATIONS
SEE PROFILE

Hassan Salehi
Shiraz University
88 PUBLICATIONS **516** CITATIONS
SEE PROFILE

Adel Saberivand
University of Tabriz
27 PUBLICATIONS **118** CITATIONS
SEE PROFILE

Hort. Environ. Biotechnol. 51(3):189-192. 2010.

Phylogenetic Relationships among Seven Old Rose Species Grown in Iran Revealed by ISSR Markers

Zohreh Jabbarzadeh[1,2*], Morteza Khosh-Khui[2], Hassan Salehi[2], and Adel Saberivand[3]

[1]*Institute of Science and Biotechnology, Urmia University, Orumieh, Iran*
[2]*Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran*
[3]*Department of Clinical Science, College of Veterinary Medicine, Urmia University, Orumieh, Iran*

Abstract. Roses are among the most economically important ornamental crops. Inter-simple sequence repeat (ISSR) markers were used to study phylogenetic relationships among 7 rose species. Among 9 ISSR primers, 6 primers showed acceptable polymorphism and amplified 66 bands of which 50 were polymorphic. Banding patterns were transformed into binary data of presence-absence and matrices were processed using NTSYS pc 2.02 software program. The dendrogram was constructed using Jaccard coefficient and UPGMA algorithm. The 7 rose species were classified into 3 major groups with within-group similarity values of >0.58. Group 1 included *R. banksiae*; group 2, *R. canina*, *R. chinensis*, *R. damascena*, *R. moschata* and *R. hybrida*; and group 3, *R. foetida*. None of the species used in this study clustered within group 1, indicating that there is no direct relationship between these species. The species that were placed with *R. hybrida* in group 2 are the ancestors of *R. hybrida* and because of other species except these four species have contributed to the gene pool of modern garden roses, 59% similarity between these roses in this study was expected. In this phylogenetic tree *R. foetida* clusters in the lower part of the tree showed 49% similarity with group 2.

***Additional key words*:** phylogenetic tree, polymorphism, primer, rose species, similarity

Introduction

The genus *Rosa* comprises more than 150 different species distributed over the temperate regions of the northern hemisphere (Ritz et al., 2005). Rose cultivation dates back to a time period around 3000 BC in China and Egypt (Gudin, 2000). About ten different wild rose species contributed to the genome of present cultivars, leading to a wide diversity among cultivated roses. Genetic relationships within the genus *Rosa* are confusing due to the variability of species and the weak barriers to intraspecific hybridization (Matsumoto et al., 1998).

Plant systematics has been usually based on morphological characters, as an expression of the genetic phenotype. In contrast, DNA polymorphisms offer direct observation of the plant genotype (Matsumoto et al., 1997). A large number of marker protocols that are rapid and require only small quantities of DNA have been developed. Three widely-used PCR-based markers are RAPDs (Williams et al., 1990), SSRs or microsatellites (Tautz et al., 1989), and AFLPs (Vos et al., 1995). Each marker technique has its own advantages and disadvantages. RAPD markers are very quick and easy to develop (because of the arbitrary sequence of the primers) but lack reproducibility (Hansen et al., 1998; Jones et al., 1997; Virk et al., 2000). AFLP has medium reproducibility but is labour intensive and has high operational and development costs (Bornet and Branchard, 2001). Microsatellites are specific and highly polymorphous (Bornet and Branchard, 2001), but they require knowledge of the genomic sequence to design specific primers and, thus, are limited primarily to economically important species.

Since 1994, a new molecular marker technique called inter simple sequence repeat (ISSR) has been available (Zietkiewicz et al., 1994). ISSRs are semiarbitrary markers amplified by PCR in the presence of one primer complementary to a target microsatellite. Such amplification does not require genome sequence information and leads to multilocus and highly polymorphous patterns (Nagaoka and Ogihara, 1997; Tsumura et al., 1996; Zietkiewicz et al., 1994). Each band corresponds to a DNA sequence delimited by two inverted microsatellites. Like RAPDs, ISSRs markers are quick and easy to handle, but they seem to have the reproducibility of SSR markers because of the longer length of their primers (Bornet and Branchard, 2001).

In this study, phylogenetic relationships among seven old rose species grown in Iran were investigated using inter-simple sequence repeat (ISSR) markers.

Materials and Methods

Plant material

For this study, seven old species of *Rosa* : *Rosa banksiae* Ait., *Rosa canina* L., *Rosa chinensis* Jacq. Var. *minima*, *Rosa damascena* Mill., *Rosa foetida* Herm, *Rosa* × *hybrida* L. and

***Corresponding author:** z_jabbarzadeh@yahoo.com
Received February 13, 2010; accepted March 6, 2010.

Table 1. The ISSR primers used to asses genetic variation among seven rose species.

No.	Primer	Sequences (5′-3′)	Sequences (5′-3′)
1	ISSR-1	5′-$(AG)_8$YT-3′	5′-AGAGAGAGAGAGAGAGYT-3′
2	ISSR-2	5′-$(GA)_8$C-3′	5′-GAGAGAGAGAGAGAGAC-3′
3	ISSR-3	5′-CCA$(CT)_8$-3′	5′-CCACTCTCTCTCTCTCTCT-3′
4	ISSR-4	5′-$(AC)_8$YT-3′	5′-ACACACACACACACACYT-3′
5	ISSR-5	5′-CCA$(AG)_8$T-3′	5′-CCAAGAGAGAGAGAGAGAGT-3′
6	ISSR-6	5′-$(CT)_8$AC-3′	5′-CTCTCTCTCTCTCTCTAC-3′
7	ISSR-7	5′-$(GA)_8$ACC-3′	5′-GAGAGAGAGAGAGAGAACC-3′
8	HB11	5′-$(GT)_6$CC-3′	5′-GTGTGTGTGTGTCC-3′
9	HB15	5′-$(GTG)_3$GC-3′	5′-GTGGTGGTGGC-3′

Y = Pyrimidine

Rosa moschata Herm. were used.

DNA extraction

Samples (fresh mature leaves 4 g) were rinsed in tap water followed by sterilized distilled water. They were then air-dried and stored at -80℃ if not used immediately. The leaves were ground in liquid nitrogen in a sterile prechilled mortar and pestle. Extraction buffer (120 mM Tris-HCl pH 8.0; 80 mM EDTA pH 8.0; 4% β-mercaptoethanol; 2% CTAB) (at a ratio of 5 ml per gram of fresh sample) was added to each tube, incubated at 60℃ water bath for 40 min. and vortexed vigorously at 5 min. interval. The tubes were centrifuged at 8000 g for 5 min. and the supernatant discarded. 20 ml clean up buffer (120 mM Tris-HCl pH 8.0; 10 mM EDTA pH 8.0; 2% β -mercaptoethanol; 2% PVP; 1.5 M NaCl; 0.2% CTAB) was added to each pellet and after resuspension, were incubated at 60℃ water bath for 30 min. with occasional inversion. After the tubes were cooled to room temperature, 20 ml of chloroform-isoamyl alcohol (24:1 v/v) and 2 ml of 5M potassium acetate was added and the tubes were shaken vigorously to form an emulsion. Tubes were centrifuged at 8000 g at room temperature for 20 min. After centrifugation the aqueous phase transferred to a new tube and added 20 ml of chloroform-isoamyl alcohol (24:1 v/v), centrifuged; then, the aqueous phase was transferred to a new tube and added 20 ml cold isopropanol with 2 ml of 3M sodium acetate, mixed and incubated at 20℃ for 30 min. The DNA was precipitated by centrifugation at 8000 g at room temperature for 10 min.

Resulting pellets were washed 3 times with an equal volume of 70% ethanol and dried the pellet and resuspended in 2 ml of TE buffer with 10 µl of RNAase and incubated at 37℃ for 4 hr.

Primers

Primers were purchased in lyophilized form from Cinnagen Co. In this research 9 ISSR primers were tested (Table 1).

DNA amplification

DNA amplification was carried out in 25 µl reactions consisting of 30 ng of template DNA, 1 U of *Taq* DNA polymerase enzyme (Fermentase Co.), 0.2 mM of each dNTP (Cinnagen Co.), 1 × *Taq* amplification buffer (Fermentase Co.), 2 mM $MgCl_2$ and 1µM primer (Cinnagen Co.). Amplifications were carried out by using a DNA thermal cycler (Eppendorf, mastercycler gradient) programmed as: 94℃ for 5 min, 35 cycles at 94℃ for 30 s, 45-50℃ for 45 s and 72℃ for 2 min. and a final extension at 72℃ for 10 min. PCR products were separated on 2% agarose gel and stained in ethidium bromide, observed under UV light and photographed using gel documentation unit.

Data analysis

The observed bands in the gel were evaluated based on the presence (coded 1) or absence (coded 0) of polymorphic fragments for each primer. Cluster analysis was performed with NTSYS-pc Version 2.02, a numerical taxonomy and multivariate analysis software package using an unweighted pair-group method, arithmetic average (UPGMA).

Results and Discussion

ISSR amplification

ISSR amplification of 7 DNA samples extracted from rose species resulted in multiple banding profiles by 6 primers. Representative banding patterns observed with primer ISSR-7 (5′ -$(GA)_8$ ACC-3′) are shown in Fig. 1. The amplified fragment sizes ranged from 100 to 3000 bp with the scorable region being from 150 to 2500 bp. The number of fragments per primer ranged from 6 (5′-$(AG)_8$ YT-3′) to 11 (5′-$(GA)_8$ACC-3′). Of the total 66 scorable fragments, 50 were polymorphic among the samples (Table 2). Based on these polymorphic bands a similarity dendrogram (Fig. 2) and a similarity coefficient were constructed using UPGMA cluster analysis.

Phylogenetic relationships among *Rosa* species

On the basis of similarity dendrogram, the 7 samples of rose species could be classified into 3 major groups with within-

group similarity value of >0.58 (Fig. 2). Group 1 consisted of *Rosa banksiae* which was placed independently, very distant from the rest of the species. None of the species used in this study clustered within group 1, indicating that there is no direct relationship between these species. This species was the most dissimilar in the dendrogram of Matsumoto et al. (1998) and Atineza et al. (2005). By RAPD-analysis Millan et al. (1996) assigned *R. banksiae* as a member of subgenus *Rosa*. This species is belonging to the Sect. *Banksianae*. Morphologically the section is characterized by free and deciduous stipules, nonpubescent receptacles and branchlets, and reflexed and deciduous sepals (Wissemann and Ritz, 2005).

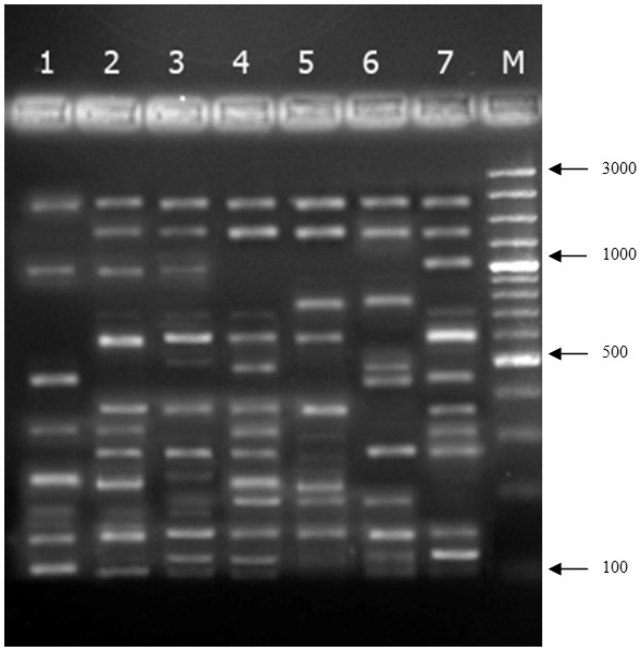

Fig. 1. ISSR-PCR band profiles generated by the primer ISSR-7 with the sequence 5′-$(GA)_8$ACC-3′ used in seven rose species included in this study. Lane 1: *Rosa banksiae*, lane 2: *Rosa canina*., lane 3: *Rosa chinensis*, lane 4: *Rosa damascena*, lane 5: *Rosa foetida*, lane 6: *Rosa* × *hybrida* and lane 7: *Rosa moschata* and M: Weight marker.

Group 2 included *R. canina*, *R. chinensis*, *R. damascena*, *R. moschata* and *R. hybrida* (Fig. 2). In this group, *R. damascena* and *R. moschata* were laid much closed to each other. This agrees with previous results about these two species e. g. Takeuchi et al. (2000) in their molecular research showed that *R. moschata* and *R. damascena* were grouped into a cluster. Koopmann et al. (2008) with AFLP analysis reported that the species of Sect. *Rosa* (which *R. damascena* was placed in this Sect.) and the species of Sect. *Synstylae* (which *R. moschata* was placed in this Sect.) were in the same clade.

In group 2, *R. canina* and *R. chinensis* had similarity coefficient of 0.74. Matsumoto et al. (1997) in their molecular classification of wild roses using organelle DNA probes showed that there was 61% similarity between *R. canina* and the species of Sect. *Indicae* (*R. chinensis* was placed in this Sect.). Also, Millan et al. (1996) in their studies with RAPD markers reported that there was 80% similarity between these two species.

Furthermore, in present phylogenetic tree, *R. hybrida* was placed in group 2. According to Hurst and Wylie (Gudin, 2000) that investigated the origin of modern garden roses and indicated that all known cultivars originated from only 10 species including: *R. foetida* Herm., *R. gallica* L., *R. gigantea* Colett ex Crep., *R. moschata* Herm., *R. multiflora* Thunb., *R. phoenicea* Boiss, *R. rugosa* Thunb. and *R. wichuraina* Crep. this similarity was expected. In the other hand, the species that were placed with *R. hybrida* in group 2 are the ancestors of *R. hybrida* and because of other species except these four species have contributed to the gene pool of modern garden roses, 59% similarity between these roses in this study was probably true.

In this phylogenetic tree *R. foetida* clusters in the lower part of the tree with 49% similarity with group 2. This relationship was seen in researches of Bruneau et al. (2007), Koopmann et al. (2008), Matsumoto et al. (1997), Millan et al. (1996) and Wissemann and Ritz (2005).

In conclusion, the results of this study showed ISSR markers as a powerful tool that can differentiate rose species. It is obviously necessary to enlarge both the number of wild rose

Table 2. List of primers, their sequence motifs, melting temperatures, annealing temperatures, number of the amplified fragments generated by ISSR primers in seven rose species.

No.	Primers	5′-3′ motif	Melting temperature	Annealing temperature	Number of polymorphic bands	Total number of bands amplified
1	ISSR-1	$(AG)_8$YT	42.3	45	6	8
2	ISSR-2	$(GA)_8$C	43.3	45	8	11
3	ISSR-4	$(AC)_8$YT	48.3	50	8	10
4	ISSR-7	$(GA)_8$ACC	51.5	50	11	12
5	HB11	$(GT)_6$CC	42.6	45	10	15
6	HB15	$(GTG)_3$GC	43.5	45	7	10
Total					66	50

Y = Pyrimidine.

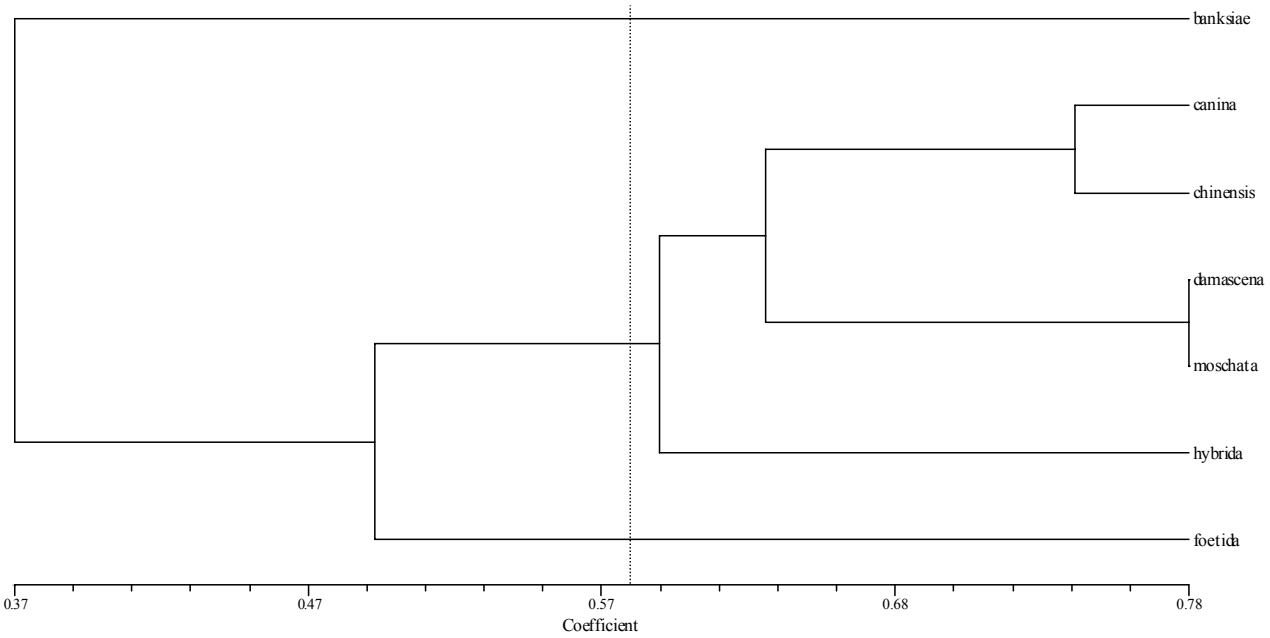

Fig. 2. Dendrogram represents the phylogenetic relationships among 7 rose species.

species and the number of modern cultivated groups to obtain a deeper insight within the genus *Rosa*. In this study roses were chosen because of their extreme variability, their genetic complexity and their relatively well-known recent history.

Literatures cited

Atineza, S.G., A.M. Torres, T. Millan and J.I. Cubero. 2005. Genetic diversity in *Rosa* as revealed by RAPDs. Agr. Cons. Sci. 70:75-85.

Bornet, B. and M. Branchard. 2001. Nonanchored inter simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. Plant Mol. Bio. Rep. 19:209-215.

Bruneau, A., J.R. Starr and S. Joly. 2007. Phylogenetic relationships in the genus *Rosa*: new evidence from chloroplast DNA sequences and an appraisal of current Knowledge. Syst. Bot. 32:366-378.

Gudin, S. 2000. Rose: Genetics and Breeding. In: Plant Breeding Reviews, (ed. J. Janick). John Willey & Sons, 17:159-189.

Hansen, M., C. Halldén and T. Säll. 1998. Error rates and polymorphism frequencies for three RAPD protocols. Plant Mol. Bio. Rep. 16:139-146.

Jones, C.J., K.J. Edwards, S. Castaglione, M.O. Winfield, F. Sale F, C. Van de Wiel, G. Bredemeijer, M. Buiatti, E. Maestri, A. Malcevshi, N. Marmiroli, R. Aert, G. Volckaert, J. Rueda, R. Linacero, A. Vazquez and A. Karp. 1997. Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Mol. Breed. 3:381-390.

Koopman, W.J.M., V. Wissemann, K. De Cock, J.V. Huylenbroeck, J. De Riek, G.J.H. Sabatino, D. Visser, B. Vosman, C.M. Ritz, B. Maes, G. Werlemark, H. Nybom, T. Debener, M. Linde and M.J.M. Smulders. 2008. AFLP markers as a tool to reconstruct complex relationships: a case study in *Rosa* (Rosaceae). Amer. J. Bot. 95:353-366.

Matsumoto, S., H. Wakita and H. Fukui. 1997. Molecular classification of wild roses using organelle DNA probes. Sci. Hort. 68:191-196.

Matsumoto, S., M. Kouchi, J. Yabuki, M. Kusunoki, Y. Ueda and H. Fukui. 1998. Phylogenetic analysis of the genus *Rosa* using *mat*K sequence: molecular evidence for the narrow genetic background of modern roses. Sci. Hort. 77:73-82.

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

Nagaoka, T. and Y. Ogihara. 1997. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Appl. Genet. 94:597-602.

Ritz, C.M., H. Schmuths and V. Wissemann. 2005. Evolution by reticulation: European dogroses originated by multiple hybridization across the genus *Rosa*. J. Heredity 96:4-14.

Takeuchi, Sh., K. Nomura, H. Uchiyama and K. Yoneda. 2000. Phylogenetic relationship in the genus Rosa based on the restriction enzyme analysis of the chloroplast DNA. J. Japan. Soc. Hort. Sci. 69:598-604.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res. 17:6463-6471.

Tsumura, Y., K. Ohba and S.H. Strauss. 1996. Diversity and inheritance of inter-simple sequence repeat polymorphisms in Douglas-fir (*Pseudotsuga menziesii*) and sugi (*Cryptomeria japonica*). Theor. Appl. Genet. 92:40-45.

Virk, P.S., J. Zhu, H.J. Newbury, G.J. Bryan, M.T. Jackson and B.V. Ford-Lloyd. 2000. Effectiveness of different classes of molecular markers for classifying and revealing variations in rice (*Oryza sativa*) germplasm. Euphytica 112:275-284.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van der Lee, M. Hornes, A. Fritjers, J. Pot, J. Peleman, M. Kuiper and M. Zabeau. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407-4414.

Williams, J.G.K., A.R. Kubelik, K.J. Liavak, J.A. Rafalski and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.

Wissemann, V. and C.M. Ritz. 2005. The genus Rosa (Rosoideae, Rosaceae) revisited: molecular analysis of *nr*ITS-1 and *atp*B-*rbc*L intergenic spacer (IGS) versus conventional taxonomy. Bot. J. L. Soc. 147:275-290.

Zietkiewicz, E., A. Rafalski and D. Labuda. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20:176-183.