TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2017) 41: 347-355

doi:10.3906/bot-1608-32

Assessment of genetic diversity of rose genotypes using ISSR markers

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Received: 22.08.2016 • **Accepted/Published Online:** 10.03.2017 • **Final Version:** 18.07.2017

Abstract: The genus *Rosa* L. (Rosaceae) is an important ornamental crop with a high economic value in floriculture, cosmetics, and pharmaceutical industries. Turkey is an important country in rose cultivation with unique species and cultivars, especially for rose oil production. Breeding programs of different rose genotypes are crucial for the development of new commercial varieties. In this study, genetic relationships among the old rose cultivars locally grown in Isparta were assessed by DNA fingerprinting. Inter-simple sequence repeats markers (ISSRs) were used to investigate the genetic diversity among nineteen locally grown rose genotypes. Nineteen ISSR primers yielded a total of 413 scorable amplified fragments. Nei's genetic identity values ranged from 0.545 to 0.951. Nei's genetic distance was used to construct a dendrogram using the unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis. The dendrogram grouped the nineteen rose genotypes into four distinct clusters, and the principal coordinate analysis revealed similar genetic groups. The results confirmed the usefulness of ISSR markers to assess the genetic diversity among the selected old rose genotypes for genetic conservation and plant improvement.

Key words: *Rosa* spp., rose, DNA fingerprinting, ISSR-PCR, genetic diversity

1. Introduction

The genus *Rosa* L. belongs to the family Rosaceae, which is included in an important group of ornamental and aromatic plants (Gudin, 2000). Domesticated rose varieties have been cultivated for more than two thousand years. The family Rosaceae is a large plant family containing more than 18,000 commercial rose cultivars, and approximately 200 species of generally shrubs and partly herbs are reported worldwide (Nilsson, 1972). Rose cultivars have been mostly used for landscaping and rose oil production (Gault and Synge, 1971).

Turkey is one of the most important rose germplasm centers with unique genetic diversity, and 45 rose species have been defined (Özcelik, 2010). Rose is one of the economically important ornamental plants in the country (Baser et al., 2003) that provides almost 70% of rose oil production in the world. There is widespread cultivation of *R. odorata* (Andrews) Sweet and *R. gallica* in Europe, and both species are also well grown in Turkey (Özcelik et al., 2012). Flowers of some rose species such as *R. gallica*, *R. centifolia* L., *R. damascena*, and *R. moschata* have also been used for rose oil and rose water production in Anatolia since the 17th century. The city of Isparta and its surroundings (the Lakes Region) are the most important rose cultivation areas for rose oil production. There are 26,000 rose plantation areas in the region and 8000 t of rose flowers are processed each year for rose oil. Interspecific hybridization between rose genotypes has played an important role for generation of novel varieties. Most of the modern rose cultivars have been derived through interspecific hybridizations between different rose species (Gudin, 2000). Wild rose species have a great potential to broaden the gene pool of rose breeding programs. In addition to natural mutations, somaclonal variations created by chemical mutagens, radiation, and callus culture have also expanded genetic diversity in roses (Schum and Hoffman, 2001). Thus, the geographical distribution of roses, polyploidy, cross-species, and interspecies hybridization are important phenomena for characterization. Development of new cultivars may contribute towards increased quality and productivity in hybrid rose varieties. Old garden roses are grown as ornamental plants in private or public gardens. They are extremely disease-resistant plants with charming and fragrant flowers. Modern hybridizers use these old roses to create new, healthy, and disease-resistant roses for different climates. The development of new varieties by initiating breeding studies in many ornamental plant

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species, as well as in roses, is very important for the future of the ornamental plants sector. In recent years, molecular biology techniques have also played a role in the development of new varieties (Ağaoğlu et al., 2000; Gudin, 2001).

The registration and protection of modern rose varieties are generally performed based on physiological and morphological characters as described in the Union Internationale pour la Protection des Obtentions Végetales (UPOV) guidelines (UPOV guideline TG/11/8). Standard characterization methods of the UPOV can fail as the number of varieties increases and phenotypic discrimination among the varieties becomes difficult.

The assessment of genetic diversity is one of the key steps in any plant breeding program as the classical methods become less efficient for the identification of different varieties. Molecular as well as morphological characterization need to be conducted to clarify the relationships between genotypes. These data should then be applied as inputs to the breeding process of the desired traits suchas color, flower shape, and fragrance (Zeinali et al., 2009). Studies on natural and cultural genotypes indicated that there have been some critical issues to be solved for identification and genetic conservation of rose genotypes. Obviously, identification of hybrids and progenitors requires firm evidence, which is a preliminary step for further uses in plant improvement (Qiu et al., 2012). To fulfill the need for effective, accurate, and fast identification tools for rose varieties, application of several molecular marker systems has been performed. Different DNA fingerprinting techniques such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), and intersimple sequence repeats (ISSRs) have been used for effective characterization of rose varieties (Zhang et al., 2006; Crespel et al., 2009). In recent years, as a microsatellite-based technique, ISSRs are widely used PCR-based marker systems that provide valuable tools for genetic characterization of different organisms, especially in plants. The ISSR markers have detected a sufficient degree of polymorphism with reproducible fingerprinting profiles for evaluating genetic diversity (Zietkiewicz et al., 1994; Rajapakse et al., 2001; Machkour-M'Rabet et al., 2009; Najaphy et al., 2012). ISSR-PCR has been reported as a rapid, reproducible, and cheap fingerprinting technique based on the variation found in the regions between microsatellites (Zhang et al., 2006; Golkar et al., 2011). Some parallel studies were also performed using RAPD and ISSR assays in the literature. It was concluded that ISSR profiling is more informative, discriminant, and powerful than RAPD assay data for molecular identification of the screened varieties (Atienze et al., 2005; Panwar et al., 2015).

The present study is the first molecular fingerprinting analysis of the locally grown old garden rose genotypes in Isparta with a unique primer set. The objectives of the study can be summarized as: 1) to determine the genetic diversity among nineteen rose genotypes using a molecular fingerprinting method based on ISSR markers, and 2) to get a better understanding of the genetic relationships among these genotypes for breeding new rose cultivars.

2. Materials and methods

2.1. Plant materials

The rose genotypes used in the study were collected from the Botanical Garden of Süleyman Demirel University located in the city of Isparta. The botanical garden harbors a rich diversity of rose germplasms that are either native to the Isparta region or obtained from other rose-growing regions of the country. The selected old garden roses (Table 1) are propagated for different purposes. Rose genotypes

Table 1. The list of the selected rose genotypes. Their use is: garden rose (G), landscape (L), oil rose (O), or fruit for industry (F).

Plant no.	Name of genotype	Use	Plant no.	Name of genotype	Use
R1	*Rosa alba* L.	G, O	R11	*Rosa borboniana* Desp.	G
R2	*Rosa damascena* Mill.	O	R12	*Rosa noisettiana* Thory	*L*
R3	*Rosa damascena* Mill.	O	R13	*Rosa odorata* (Andrews) Sweet	L
R4	*Rosa damascena* Mill.	O	R14	*Rosa beggeriana* Schrenk	F, L
R5	*Rosa damascena* Mill.	O	R15	*Rosa borboniana* Desp.	*G*
R6	*Rosa damascena* Mill.	O	R16	*Rosa odorata* (Andrews) Sweet	L
R7	*Rosa semperflorens* (Loisel. & Michel) Özçelik & Yıldırım	L, G	R17	*Rosa odorata* (Andrews) Sweet	L
R8	*Rosa damascena* Mill.	O	R18	*Rosa odorata* (Andrews) Sweet	L
R9	*Rosa versicolor* (Weston) Özçelik & Yıldırım	G	R19	*Rosa foetida* J. Herrmann 2491	L, F
R10	*Rosa borboniana* Desp.	G			

were evaluated for the potential of ISSR-PCR assays for future rose breeding programs.

2.2. DNA isolation

The genomic DNA extraction protocol of nineteen locally grown rose genotypes was performed based on the modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990). Young leaf tissue (100 mg) was harvested, frozen in liquid nitrogen, and pulverized to a fine powder using an electric grinder. Following the grinding, 600 µL of prewarmed (60 °C) CTAB buffer [2% CTAB, 100 mM Tris-HCl (pH 8), 10 mM EDTA, 1.4 M NaCl, 2% PVP] and 0.2% β-mercaptoethanol were freshly added into each tube. The tubes were then incubated at 60 °C for 40 min, mixed by inverting gently from time to time, and kept at room temperature for 10 min. Afterwards, 500 µL of chloroform-octanol (24:1) was added to the solution, and the supernatant was centrifuged at 13,000 rpm for 10 min and transferred into a fresh tube. Furthermore, 350 µL (half the volume of the supernatant) of 5 M NaCl and 700 µL (2 volumes of the supernatant) of cold absolute ethanol were added to the supernatant, allowed to sit for 10 min at 20 °C, and centrifuged at 13,000 rpm for 10 min. The DNA pellet was gained and washed twice with 500 µL of 70% ethanol. The pellet was dried and resuspended in 100 µL of TE buffer (pH 8.0). RNA was removed by digestion with 2 U of DNase-free ribonuclease A (10 mg mL^{-1}). The DNA quantity and quality were analyzed by NanoDrop spectrophotometer. The quality and integrity of the DNA were also checked by visualization on 0.8% agarose gel.

2.3. ISSR analysis

ISSR-PCR assays were carried out using 15–18-mer ISSR primers (Table 2). Initially, twenty-five ISSR primers were tested for ISSR amplifications of the genotypes. Fifteen ISSR primers were selected for further analyses based on their ability to perform distinct and strong amplification of polymorphic fragments. The amplification reaction was performed in a reaction volume of 25 µL containing 1X PCR buffer (10 mM Tris-HCl, pH 9.0), 0.2 µM dNTPs, 10 pmol of primer, 50 mM $MgCl_2$, 50 ng of genomic DNA, 0.5 U of Taq polymerase, and PCR-grade dH_2O. After the initial denaturation step at 94 °C for 4 min, the ISSR-PCR was programmed for 35 cycles of three steps: denaturation at 94 °C for 30 s; annealing at 53 °C (or specific T_a for a primer) for 60 s and extension at 72 °C for 45 s; and a final extension at 72 °C for 10 min before holding the tubes at 4 °C. The amplification products were resolved by electrophoresis on 1.8% agarose gel using 1X TBE buffer, visualized with ultraviolet light, and photographed. All PCR assays were performed twice and gel images with scorable band profiles were used for data analyses.

Table 2. List of ISSR primer sequences, annealing temperature, and total number of ISSR-PCR amplified bands. R: purines (A or G); Y: pyrimidines (T or C).

Primer no.	Primer code	Nucleotide sequence (5'–3')	T_a (annealing temperature)	Number of bands
1	UBC*811	(GA)8 C	54 °C	17
2	UBC 816	(CA)8 T	53 °C	15
3	UBC 818	(CA)8 G	53 °C	16
4	UBC 822	(TC)8A	53 °C	22
5	UBC 834	(AG)8YT	59 °C	29
6	UBC 835	(AG)8YC	59 °C	28
7	UBC 836	(AG)8YA	57 °C	35
8	UBC 840	(GA)8 YT	59 °C	34
9	UBC 843	(CA)8 RA	55 °C	47
10	UBC 845	(CT)8 RG	55 °C	17
11	UBC 848	(CA)8 RG	56 °C	25
12	UBC 850	(CT)8 YC	58 °C	26
13	UBC 855	(AC)8 YT	57 °C	36
14	UBC 868	(GAA)6	54 °C	37
15	UBC 881	(GGGTG)3	61 °C	29
Total number of bands				413

*UBC: The University of British Columbia, Canada.

2.4. Data analysis

ISSR data analyses were performed based on PCR amplified band profiles of nineteen rose cultivars. The amplified ISSR fingerprinting profiles were converted into a numeric database using scorable ISSR-PCR banding patterns of the genotypes. A binary matrix was generated by scoring the presence (1) or absence (0) of each individual band in all lanes of the agarose gels. The pairwise Nei's genetic distance (Nei, 1972) and genetic identity estimates were done by using NTSYS software (Numerical Taxonomy System, Applied Biostatistics, Inc.) version 2.02 (Rohlf, 1998). The statistics were calculated using 100 simulated samples. Moreover, the unweighted pair group method with arithmetic averages (UPGMA) method and Nei's standard genetic distance were used for the phylogenetic tree construction based on the ISSR data matrix of the nineteen rose genotypes. Furthermore, principal coordinate analysis (PCA), as implemented in the NTSYS-pc software, was also employed to analyze the spatial clustering of the nineteen rose genotypes.

3. Results and discussion

Roses are the most popular ornamental plants cultivated for different purposes. Breeding efforts have been undertaken routinely to develop new commercial rose cultivars in concordance with the data of molecular and morphological studies. In this study, the locally grown rose germplasms were analyzed for their genetic diversity. It is aimed to get a better understanding of genetic relationships within those genotypes in order to breed new rose varieties. Therefore, all of the nineteen old rose samples were analyzed based on an ISSR marker system using primers representing di-, tri-, and pentanucleotide repeats.

Microsatellite primers amplifying dinucleotide, trinucleotide, tetranucleotide, or pentanucleotide repeat motifs of 16–25 bp long are usually used to target multiple genomic loci in ISSR analysis (Gupta et al., 1994; Atienza et al., 2005). Usually, dinucleotide repeats having primers anchored either at the 3' or 5' end reveal high polymorphism (Joshi et al., 2000). Carvalho et al. (2009) reported that dinucleotide primers were more suitable for amplifying ISSRs in bread and durum wheat. However, all the primers analyzed in the present study showed a high percentage of polymorphism (99.52%) among the selected rose genotypes of Isparta, regardless of the repeat size of the 15 ISSR primers. In parallel to our study, a high percentage of polymorphism (93.7%) was reported in a genetic diversity study on 33 unrelated rose genotypes based on nine ISSR primers (Carvalho et al., 2009) with dinucleotide repeats. Panwar et al. (2015) also reported that ISSR markers with 94% genetic polymorphism have more potential than RAPD markers to discriminate rose cultivars. The high percentage of polymorphism is probably caused by the heterozygous nature of the polyploidy genome structure of rose species.

The amplification products of the nineteen genotypes yielded a total of 413 scorable bands with an average of 27 bands per primer in the current study. The size of clearly detectable amplified ISSR-PCR fragments ranged from 150 bp to 1100 bp (Table 2) and the number of bands generated by each primer varied. The UBC 843 primer produced the highest number of polymorphic bands (47), while the lowest number of polymorphic bands (15) was obtained with the UBC 816 primer. As an example, the PCR banding pattern of the UBC 840 ISSR primer is shown in Figure 1. The binary data matrix generated by the amplified fragments of the nineteen rose individuals in the ISSR-PCR analyses was used for the computations of Nei's genetic distances and genetic identities for every pairwise comparison of the genotypes (Table 3) for the analysis of ISSR data. The estimated genetic distance ranged from 0.0496 (between R4 and R5) to 0.5897 (R2 and R15), and genetic identity estimates ranged from 0.5545 (R2 and R15) to 0.9516 (R4 and R5). The results revealed that R5

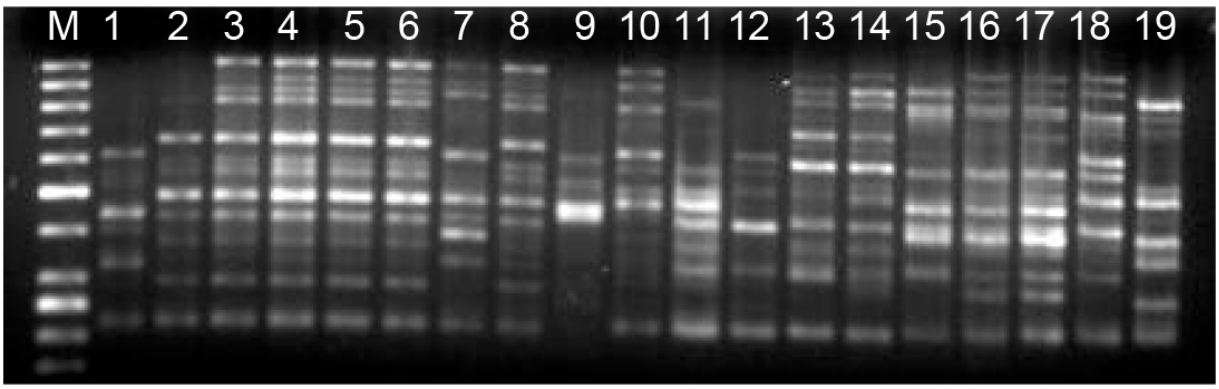

Figure 1. ISSR marker profiles of the amplified loci among the rose genotypes using primer UBC 840. Each lane contains a different rose variety (R1–R19). M: 50-bp DNA ladder as molecular weight.

Table 3. Nei's original measures of genetic identity (above diagonal) and genetic distance (below diagonal) values (Nei, 1972) of the rose varieties based on variation in ISSR markers.

pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	****	0.7022	0.6707	0.6659	0.6562	0.6659	0.6199	0.6586	0.6707	0.6223	0.5884	0.6320	0.6053	0.6295	0.5714	0.6295	0.6271	0.6392	0.6513
2	0.3536	****	0.9298	0.8910	0.8620	0.8378	0.7143	0.7530	0.6392	0.6150	0.5908	0.6150	0.6223	0.6513	0.5545	0.6077	0.5956	0.5738	0.5811
3	0.3994	0.0728	****	0.9467	0.9177	0.8499	0.7215	0.7554	0.6320	0.6077	0.5787	0.6223	0.6344	0.6634	0.5666	0.6005	0.5981	0.5811	0.5787
4	0.4067	0.1154	0.0547	****	0.9516	0.8789	0.7167	0.7748	0.6320	0.6223	0.6029	0.6320	0.6344	0.6877	0.5763	0.6150	0.6126	0.5956	0.5932
5	0.4213	0.1485	0.0859	0.0496	****	0.8935	0.7458	0.7893	0.6077	0.6271	0.6126	0.6368	0.6344	0.6828	0.5811	0.6199	0.6029	0.6053	0.5835
6	0.4067	0.1770	0.1627	0.1290	0.1127	****	0.7700	0.8281	0.6562	0.6610	0.6416	0.6707	0.6586	0.6925	0.5956	0.6441	0.6126	0.6295	0.5932
7	0.4783	0.3365	0.3264	0.3331	0.2933	0.2614	****	0.8063	0.6295	0.6828	0.6731	0.6731	0.6659	0.6755	0.6077	0.6126	0.6150	0.6416	0.5908
8	0.4176	0.2837	0.2804	0.2551	0.2366	0.1886	0.2153	****	0.6634	0.6828	0.6683	0.6634	0.6659	0.6949	0.6077	0.6416	0.6344	0.6223	0.6005
9	0.3994	0.4475	0.4589	0.4589	0.4980	0.4213	0.4628	0.4103	****	0.6174	0.5884	0.6513	0.6441	0.6538	0.5860	0.6489	0.6465	0.6392	0.5835
10	0.4744	0.4861	0.4980	0.4744	0.4666	0.4140	0.3815	0.3815	0.4822	****	0.8257	0.7240	0.6295	0.6538	0.6683	0.6683	0.6223	0.6441	0.6174
11	0.5304	0.5263	0.5470	0.5060	0.4901	0.4437	0.3958	0.4030	0.5304	0.1916	****	0.7385	0.6586	0.6538	0.6877	0.6489	0.6465	0.6731	0.6126
12	0.4589	0.4861	0.4744	0.4589	0.4513	0.3994	0.3958	0.4103	0.4287	0.3230	0.3031	****	0.7070	0.6877	0.6973	0.7070	0.6998	0.7119	0.5932
13	0.5020	0.4744	0.4551	0.4551	0.4551	0.4176	0.4067	0.4067	0.4400	0.4628	0.4176	0.3467	****	0.8450	0.6804	0.6901	0.6780	0.6707	0.5908
14	0.4628	0.4287	0.4103	0.3745	0.3815	0.3675	0.3922	0.3640	0.4250	0.4250	0.4250	0.3745	0.1684	****	0.6659	0.7046	0.6683	0.6513	0.6247
15	0.5596	0.5897	0.5681	0.5512	0.5428	0.5181	0.4980	0.4980	0.5345	0.4030	0.3745	0.3605	0.3851	0.4067	****	0.7579	0.7361	0.7337	0.6005
16	0.4628	0.4980	0.5100	0.4861	0.4783	0.4400	0.4901	0.4437	0.4325	0.4030	0.4325	0.3467	0.3710	0.3501	0.2772	****	0.8717	0.7288	0.6247
17	0.4666	0.5181	0.5141	0.4901	0.5060	0.4901	0.4861	0.4551	0.4362	0.4744	0.4362	0.3570	0.3887	0.4030	0.3064	0.1373	****	0.7554	0.6368
18	0.4475	0.5554	0.5428	0.5181	0.5020	0.4628	0.4437	0.4744	0.4475	0.4400	0.3958	0.3399	0.3994	0.4287	0.3097	0.3163	0.2804	****	0.6247
19	0.4287	0.5428	0.5470	0.5222	0.5387	0.5222	0.5263	0.5100	0.5387	0.4822	0.4901	0.5222	0.5263	0.4705	0.5100	0.4705	0.4513	0.4705	****

and R4 genotypes were closely related, having the lowest genetic distance (0.0496) and the highest genetic identity (0.9516). The highest genetic distance (0.5897) and the lowest genetic identity (0.5545) values belonged to R15 and R2 genotypes, which were the most distant genotypes (Table 3). These values can be employed in a breeding program such that the genotypes with the lowest genetic similarities could be selected as parents to improve the rose varieties.

Development of desired genotypes requires assessment of genetic variability as the basis of breeding. Hybridization among selected genotypes may create a new gene pool with specific traits (Singh and Shukla, 1998). Hybridization can be carried out among the genotypes that belong to distance clusters. Thus, a wide range of segregants could be obtained for desired characters (Aminul Islam et al., 2016). According to the literature, highest genetic differences could be selected as breeding materials to improve new varieties (Singh, 1991). It is known that the crossing of highly statistically distant genotypes from the clusters leads to variations among the segregants (De et al., 1992). These distant genotypes could be used in breeding programs for obtaining a wide spectrum of variation.

In the present study, the level of genetic similarity between the genotypes was assessed by using Jaccard's similarity coefficient (Jaccard, 1980) and the estimated similarity values (data not shown) ranged from 0.102 (between genotypes R15 and R2) to 0.863 (between genotypes R5 and R4). The average gene diversity (h) among the 19 rose genotypes was calculated as 0.3171.

There are some genetic diversity studies on different rose species based on different markers (AFLP, RAPD, SSR, SRAP, etc.) in the literature. For example, Yang and Guo (2015) reported a low genetic diversity estimate (average h = 0.09) among five populations of *R. beggeriana* based on AFLP data. Another AFLP study reported a low genetic diversity estimate (h = 0.09) for the *R. laxa* population (Yang et al., 2013). Furthermore, Mezghani et al. (2015) reported relatively high similarity estimates (0.53–0.86) for *R. gallica* populations based on AFLP markers, which can be interpreted as low genetic diversity. In addition, Xu et al. (2011) reported a SRAP study with wild germplasms and cultivars of *R. rugosa* and reported the average gene diversity for wild germplasms as 0.1225, and for cultivars as 0.2684. Similarly, another study on *R. rugosa* based on RAPD markers revealed an average genetic diversity estimate of 0.1878. In addition, Nadeem et al. (2014) analyzed 22 hybrids (F1) of nine parents using SSR markers and reported a high average observed heterozygosity (H_{obs} = 0.887) and genetic diversity (h = 0.852) among the parents and hybrids. Although these studies used different marker systems and different rose species, we can still compare the results in some ways. In general, it is observed that cultivars and hybrids have high genetic diversity resulting from polyploidy genomes leading to high heterozygosity. Moreover, wild populations may have very low genetic diversity estimates (Yang et al., 2013, 2015), probably due to being confined to a relatively small region and having a low introgression rate.

In the context of the present study, the analyzed rose genotypes are domestic and old garden roses of Turkey. The species were identified using classical taxonomic methods. The phylogenetic tree of the rose genotypes was constructed using the UPGMA method based on the estimated Nei's genetic distances, where four clades were observed (Figure 2). The first (I) clade included genotype 1 (R1) and genotype 9 (R9). These cultivars belong to *R. alba* (R1) and *R. versicolor* (R9). The second clade (II) was divided into two subclusters with 7 taxa (R2, R3, R4, R5, R6, R7, and R8) that comprised six cultural genotypes of *R. damascena* var. *trigintipetala* (R2, R3, R4, R5, R6, R8) and one genotype of *R. damascena* var. *sempeflorens* (R7). Within this clade, the smallest genetic distance was found between R4 and R5 (0.0496) and the highest genetic distance was found between R2 and R7 (0.3365). Even though R8 belongs to *R. damascena* var. *trigintipetala*, it is genetically closer to the R7 sample (*R. damascena* var. *sempeflorens*) with a distance of 0.2153 than to the other *R. damascena* var. *trigintipetala* taxa (R2–R6). This might be due to transitions that might have occurred between R7 and R8 cultivars. The third clade (III) was divided into three subclusters with 9 taxa (R10–R12; R13–R14; R15–R18) that belonged to four different species including *R. borboniana* (R10, R11, R15), *R. noisettiana* (R12), *R. odorata* (R13, R16, R17, R18), and *R. beggeriana* (R14). Regarding the genetic similarity observed between R7 and R8 in clade II, a similar case was also observed in clade III. For example, the R15 cultivar of the *R. borboniana* species grouped with R16–R18 samples of *R. odorata* species rather than grouping with the R10 and R11 taxa of the same species (*R. borboniana*). Moreover, the R13 cultivar of the *R. odorata* species grouped with the R14 cultivar of *R. beggeriana* species rather than grouping with the taxa of its own species (R16–R18). These observations might also be due to transitions that occurred between the analyzed cultivars of different species.

The fourth clade (IV) comprised genotype 19 (R19), which belongs to *R. foetida*. This phylogenetic tree is an unrooted tree. However, if it were preferred to construct a rooted tree, the *R. foetida* genotype might be a good choice to be an outgroup because of different phenotypic appearance with yellow colored flowers. In general, an appropriate outgroup should be unambiguously outside the clade of interest in the phylogenetic study. In contrast, in some cases outgroups could be the members of an ingroup. These roses were the plant material of a project that aimed to get new genotypes via hybridization. Thus, we did not choose any outgroup in our study while constructing the phylogenetic tree.

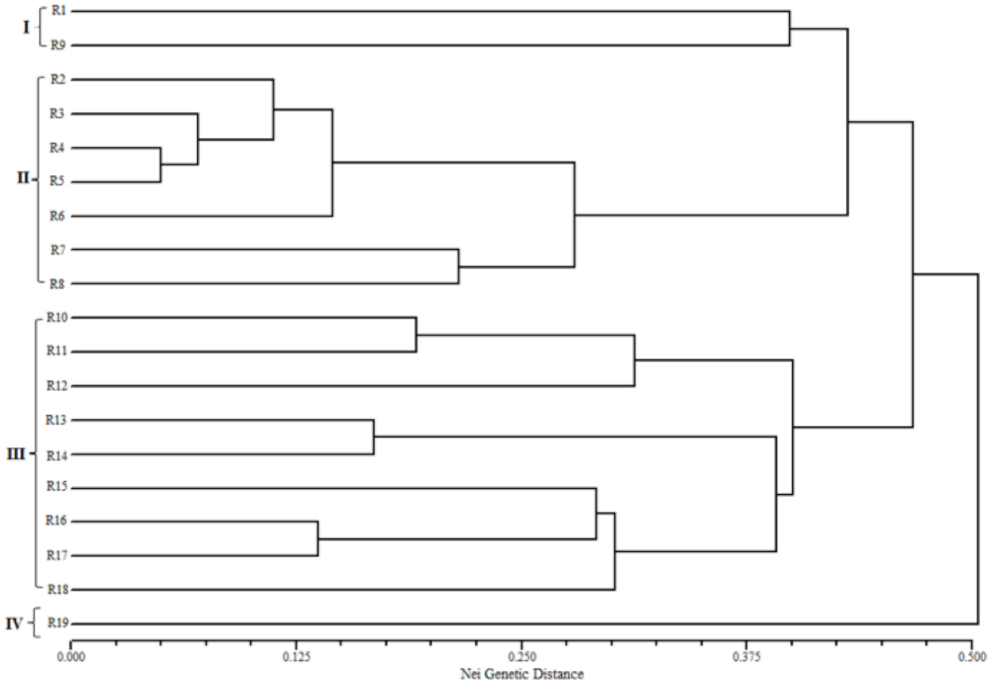

Figure 2. Dendrogram illustrating the genetic relationship among the 19 rose cultivars based on Nei's standard genetic distance. The clade numbers are shown as I–IV.

A dendrogram was constructed based on the genetic similarity estimates and showed that the rose cultivars used in the study were similar to each other in a range of 51%–100%. The tree topology indicated that species *R. borboniana*, *R. noisettiana*, *R. beggeriana*, and *R. odorata* might have a common ancestor that is different from *R. alba* and *R. foetida*.

The 2D and 3D scatter plots of PCA (Figure 3) were formed based on Nei's genetic distance estimates from the binary data matrix. The plots helped to visualize the

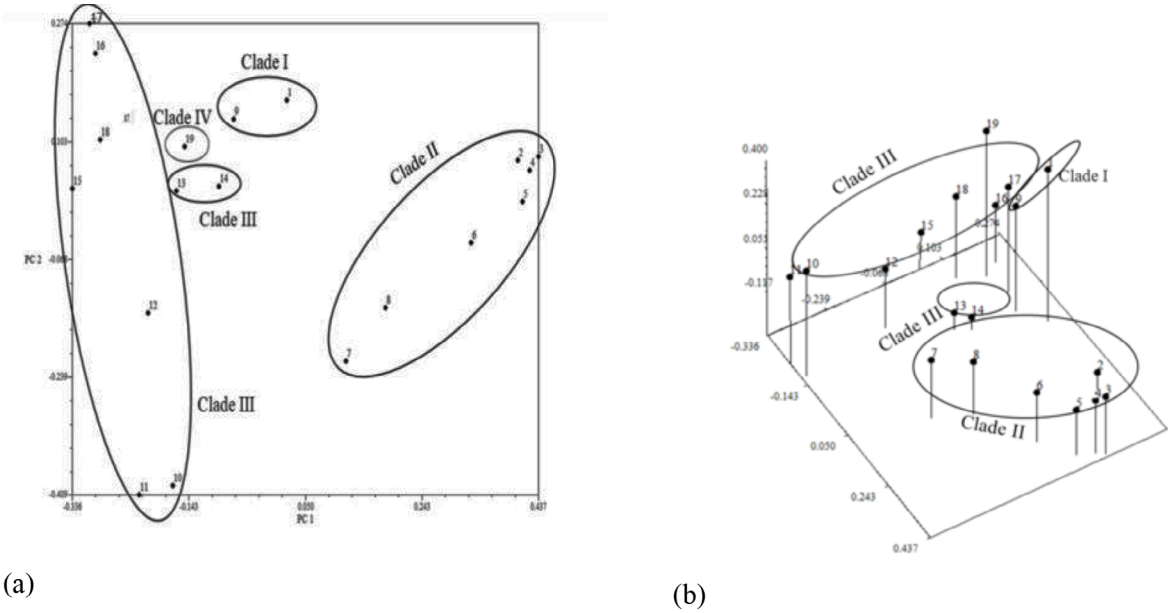

Figure 3. Two- (a) and three-dimensional (b) plots of the principal coordinate analysis (PCA) of ISSR data showing the clustering of the rose varieties. The numbers represent individual rose cultivars (R1–R19).

interspecific genetic relationships among the cultivars and supported the results obtained from the phylogenetic tree analysis. The four main clusters and subclusters observed on the phylogenetic tree were labeled on the plots of the PCA. It seems that the third principal coordinate mainly contributes to the clustering of clade II, which also separates R19 (clade IV) and R1 (clade I) cultivars from the others. Furthermore, the first principal coordinate mainly contributes to the clustering of the cultivars in clade III.

ISSR markers are currently applied in plant sciences for evaluating genetic diversity of different plant germplasms. In general, these markers detect a sufficient degree of polymorphism with reproducible fingerprinting profiles to evaluate the genetic diversity among a variety of plants including horticultural and field species (Zietkiewicz et al., 1994; Virk et al., 2000). It is even reported that ISSR primers could detect more polymorphism than mtDNA, cpDNA, RAPD, and isozyme markers in closely related plants. ISSR markers involve amplification of DNA fragments between two identical repeat regions (Arnau et al., 2002). In the present study, a high level of polymorphism was obtained among the selected rose genotypes using ISSR markers.

The high level of polymorphism among the rose genotypes suggested that domesticated rose germplasm was not limited. The presence of the polymorphic marker data is an important tool for different breeding strategies. For example, since the R2 and R15 genotypes have the lowest genetic similarity in the present study they could be selected as parents to improve the native rose varieties.

In conclusion, PCR-based marker data with unique DNA profiles are valuable sources for breeding and management of rose germplasm to develop new cultivars with new characteristics. The present study demonstrated the utility of ISSR primers to characterize the genetic diversity among nineteen locally grown genotypes of the genus *Rosa* with unique, specific, and reproducible banding patterns. These data can further serve to strengthen the applicability of rose breeding programs, also comparing with morphological data for the evaluation of rose genotypes.

Acknowledgments

This work was financially supported by the Industrial Thesis Supporting Program of the Republic of Turkey Ministry of Science, Industry, and Technology (SANTEZ No: 01177. STZ.2011-2). The authors thank Konca Bulut for her technical assistance. The authors wish to acknowledge the contributions made by staff members of İstanbul Ağaç Peyzaj Company.

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