Genetic Diversity of Rosa Damascena Mill. in Latakia Province as Reveled by ISSR Analysis

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Abstract

The genetic relationships among 20 Rosa damascena Mill. accessions from various cultivation areas of Latakia province (Syria) and two accessions from type locality Al-Marah were analyzed using 10 ISSR primers. The ISSR primers produced 63 bands across the studied genotypes, of which 46 were polymorphic. The number of amplified bands varied from three to eight. The average numbers of bands per primer was 6.3 band. Percentage polymorphism ranged from 50% (ISSR-R9) to 100% (ISSR-D4, ISSR-R2), with an average percentage polymorphism of 73.02% across all the genotypes. Unweighted pair group method cluster analysis based on similarity values revealed 3 groups at the distance of dissimilarity 0.38. One includes 16 genotypes all from Latakia city, second cluster comprised all Gablah and Alkardaha genotypes, while Damascus genotypes (Al-Marah) formed the third group and appeared to be distinct from all others.

The wide genetic variation seen for R. damascena Mill. *genotypes included in our study is a promising future for the breeding programs of rose.*

Keywords

Rosa damascena, Molecular characterization, ISSR, Cluster analysis.

I. INTRODUCTION

Rose is one of the most important cultivated ornamental plants in the world, belongs to the family Rosaceae and the genus *Rosa* which comprises about 200–300 species and thousands of cultivars ([1], [2], [3]). *Rosa damascena* Mill. (Damask rose) the best commercial species of the fragrant roses, is a shrub growing to 2.5 meters tall, the stems densely armed with stout, curved prickles and stiff bristles. The leaves are pinnate, with five leaflets. The roses color are varied between light pink to light red. It is probable resulted from the interaction between conscious selection and opportunity for the movement and recombination of many roses in the eastern Mediterranean region [4]. *R. damascena* Mill. is a temperate plant indigenous to Europe and Middle East countries of Iran and Turkey. It is believed that the Damask rose has originated from Damascus and introduced in Mediterranean region and European countries especially Bulgaria, Turkey, France, Italy and Russia where it is cultivated in large-scale for oil production, medicinal use, ornamentation as well as manufacture of perfumes [5].

The most common method for Rose cultivar identification is based on morphological characters such as number and size of petals, pilositri of leafs, color of corollas, but in fact, many cultivars cannot be readily distinguished by morphological indicates if they are closely related and sometimes morphological characters are widely affected by environmental conditions [6].

Molecular markers have proven to be valuable tools for identification of cultivars, characterization and evaluation of the genetic diversity within and between species ([7], [8]). The exploration of rose genetic diversity has been mainly carried out using random amplified polymorphic DNA (RAPD), Simple sequence repeat (SSR), inter-simple sequence repeat (ISSR), and amplified fragment length polymorphism (AFLP) molecular markers ([9], [10], [11], [12]).

ISSR-PCR is a very simple, cost effective, highly discriminative, reliable and require small quantity of sample DNA technique ([13], [3]). It is rapidly being used in various fields of plant improvement [14]. The technique is useful in areas of phylogenetic studies, genome mapping and evolutionary biology in a wide range of plant species ([15], [16]). ISSR markers have been extensively used to distinguish genetic variation and detecting polymorphisms in ornamental plants [8], as well as to detect hybridization within Rosa species [2].

Previous investigations of some Syrian *R. damascena* Mill. genotypes collected from Damascus (5 samples) and Aleppo (2 samples) depends on RAPD and ISSR markers was achieved by [9], the results was mostly identical in both techniques and divided the samples in a similar way in the two dendrograms. The ISSR analysis depends on 21 primers generated 197 lines of bands of which 161 were polymorphic (81.7%).

In a case study by [3], the fresh leaves of seven Rosa species were collected and analyzed for ISSR markers. A total of 66 bands from nine specific primers were obtained from which 50 were polymorphic. The results showed that ISSRs can be used to study the systematic relationship among closely related Rosa species.

[17] used ISSR, SSR and RAPD markers to assess the genetic relationship and aromatic amino acids contents between Three Taif-roses genotypes from Saudi Arabia and nine rose genotypes that are grown in Syria and Egypt. Out of 8 ISSR primers used, clear and repeatable band profile of 6 primers was obtained. Total of 64 bands with polymorohism of 90.6 % were obtained. The results has led to efficient grouping of the 12 rose genotypes, among them Syrian-Gory rose shown the highest genetic similarity of 92 % with the three Taif-roses genotypes.

To asses genetic variability among six Rosa genotypes of which four belongs to *R. damascena* Mill. grown in different plantations in Saudi Arabia, SSR and ISSR markers were used, The tested SSR primers showed low level of variation comparing with ISSR primers which generated high levels of polymorphism ranging from 66.7 to 100% [18].

Similar type of work has been done by [12] used SSR and ISSR markers to assess genetic variation between Almadinah and Taif *R. damascena* Mill. and to investigate if both Roses are derived from one original plant. Both cultivars were successfully distinguished by both markers and showed similar banding pattern. They suggested that each cultivar plants came from the same plant.

The objectives of the present study were to determine genetic relationships and identify molecular fingerprints in twenty two *R. damascena* Mill. varieties from which twenty varieties from different locations from Latakia province and two varieties from Damascus (Al-Marah) using ISSR markers.

II. MATERIALS AND METHODS

A. Plant materials

Twenty *R. damascena* Mill. genotypes from different locations from Latakia province, and two original genotypes from Al-Marah (Damascus) were used in this study (Table 1).

TABLE 1: Genotypes used in this study, altitude, and annual rainfall

Genotype	Origin	Altitude	Annual rainfall
R1 - R2	Latakia,- Alshabatliyah	25	850
R3 - R4	Latakia- Al-adra	30	850
R5 -R6	Latakia- Qastal Maaf, Rmadeea	300	1130
R7 -R8	Latakia-Qastal Maaf	320	1130
R9 -R10	Latakia- Kasab, Alrabuah	850	1270
R11 -R12	Latakia- Mazar alqtrea	130	1120
R13 -R14	Latakia- Kasab, Alshagarah	650	1270
R15 -R16	Latakia- Kasab	800	1270
R17 -R18	Latakia- Alqardaha	750	1067
R19 -R20	Damascus- Al-Marah	1400	120
R21 -R22	Latakia- Gablah	500	550

B. DNA extraction

Fresh leaf material was collected and preserved on silica gel. Total DNA was isolated from silica gel dried material using a cetyltrimethyl ammonium bromide (CTAB) extraction method as described in [19] as follows:

The leaves were ground in liquid nitrogen in a sterile prechilled mortar and pestle. Extraction buffer contained 2% (w/v) CTAB, 1.4 mM NaCl, 100 mM Tris-HCl, pH 8.0, 20 mM EDTA, 2% (w/v) PVP and 2% (v/v) β-mercaptoethanol was added to each tube, the tubes were incubated at 65°C water bath for 30 min and vortexed vigorously at 5 min interval. The tubes were centrifuged at 10000 g for 5 min and the supernatant was transferred to a new tube. 600µL of chloroform-isoamyl alcohol (24: 1 v/v) was added and the tubes were shaken vigorously to form an emulsion. Tubes were centrifuged at 10000 g at room temperature for 10 min. After centrifugation, the aqueous phase was transferred to a new tube and 600µL of chloroform-isoamyl alcohol (24: 1 v/v) were added and centrifuged. The aqueous phase obtained after centrifugation was transferred to a new tube and 400µL cold isopropanol were added, mixed and incubated at 20°C for 2 h. The DNA was precipitated by centrifuging at 10000 g at room temperature for 10 min. Resulting pellets were washed 3 times with an equal volume of 70% ethanol and the pellets were dried and re-suspended in 200µL of TE (Tris + ethylenediaminetetraacetic acid (EDTA)) buffer with 3 µl of RNAase and incubated at 37°C for 30 min, then stored at 4°C until use. The concentration and purity of the isolated DNA was determined using a Qubit® 3.0 Fluorometer and the quality verified by electrophoresis on a 0.8% agarose gel.

C. PCR-ISSR amplification

The PCR settings were as follows: 25 µL reaction containing 2,5 µL of 10% Taq buffer, 2 µL of 25 mM MgCl2 , 4 µL of dNTP mix (each 1,25mM), 1 µL of ISSR primer (20pmol/µL) (Primer formulas and annealing temperatures are listed in table 2), and 0,2 µL taq polymerase (5 units/µL). Amplification were carried out by using a DNA thermal cycler (Flexi gene - Germany) programmed as: 94°C for 5 min, 35 cycles at 94°C for 30 s, specific annealing temperature for 45 s and 72°C for 2 min and a final extension at 72°C for 10 min. The amplification products were analyzed on a 1.8% agarose high resolution gel (Roti®) with a 100-bp DNA ladder. The gel was stained by ethidium bromide 0.5 µg/ml, visualized under ultraviolet light and photographed using a gel documentation system (CAMAG Reprostar3 – Switzerland).

TABLE 2: The ISSR primers used to assess genetic variation among R. damascena Mill. genotypes.

No	Primer code	Altitude	Annual rainfall
1	ISSR-B3	CTCTCTCTCTCTCTCTTG	850
2	ISSR-B4	CACACACACACAGG	850
3	ISSR-B7	GTGGTGGTGGC	1130
4	ISSR-D4	GATAGATAGATAGATA	1130
5	ISSR-R1	AGAGAGAGAGAGAGT	1270
6	ISSR-R2	GAGAGAGAGAGAGAGAC	1120
7	ISSR-R3	CCACTCTCTCTCTCTCTCT	1270

8	ISSR-R4	ACACACACACACACACYT	1270
9	ISSR-R8	GAGAGAGAGAGAGAGAACC	1067
10	ISSR-R9	GTG TGT GTG TGT CC	120

D. Data analysis

Only clear bands were considered for data analysis. Each band was considered to be a single locus. Data were scored as "1" for presence and "0" for absence. The binary data matrix was entered into the Numerical Taxonomy and Multivariate Analysis System NTSYS [20]. The level of similarity between varieties was established as the percentage of polymorphic bands, and a matrix of genetic similarity was compiled using Jaccard's similarity coefficient (JSI). Similarity coefficients were used to construct the dendrogram using the unweighted pair group method with arithmetic average (UPGMA) and the sequential hierarchical and nested clustering routine in the NTSYS pc program, representing genetic relationship between twenty two R. damascena Mill. varieties.

III. RESULTS AND DISCUSSION

A total of 63 loci were scored for the 10 ISSR primers in the 22 accessions of *R. damascena* Mill. surveyed, of which 46 were Polymorphic (73.02%). Number of bands varied from three (ISSR-B7) to eight (ISSR-R2, ISSR-R4), and sizes ranged from 235 to 1400 bp. Average numbers of bands and polymorphic bands per primer were 6.3 and 4.6 respectively. The polymorphism in the tested primers ranged from 50% for primer (ISSR-R9) to 100% for primer (ISSR-D4, and ISSR-R2) (Table 3, Fig. 1). Some genotypes were distinguished by different bands and different primers especially Al-Marah genotypes as well as Gablah and Alkardaha genotypes (Fig. 1).

TABLE 3: Characteristics of amplified fragments obtained from 10 primers for ISSR analysis of *R. damascena* Mill. Genotypes.

No	Primer code	Total bands	Polymorphic bands	Polymorphism %
1	ISSR-B3	6	5	83.3
2	ISSR-B4	7	4	57.14
3	ISSR-B7	3	2	66.7
4	ISSR-D4	6	6	100
5	ISSR-R1	7	5	71.42
6	ISSR-R2	8	8	100
7	ISSR-R3	7	4	57.14
8	ISSR-R4	8	5	62.5
9	ISSR-R8	5	4	80
10	ISSR-R9	6	3	50
Sum		63	46	73.02

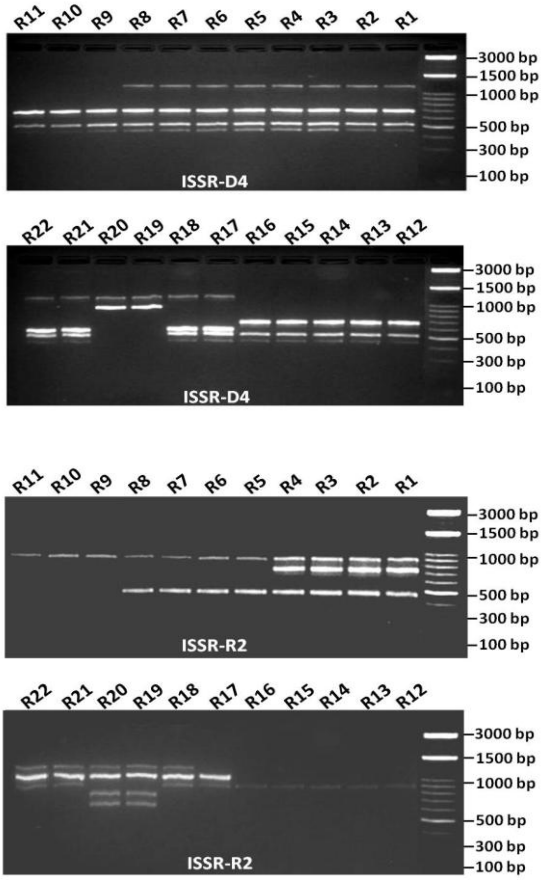

Fig 1: Example of amplification products from primers ISSR-D4 and ISSR-R2.

The dendrogram based on UPGMA analysis of the genetic dissimilarity matrix show that the 22 accessions of *R. damascena* Mill. cluster into three main groups with Jaccard's dissimilarity coefficient ranging from 0.00 to 0.38 (Figure 1): one includes 16 genotypes all from Latakia city and is furthermore divided into two subgroups, one include all Alshabatliyah and Al-adra (R1, R2, R3, R4) and Qastal Maaf (R5, R6, R7, R8) genotypes, while the other include all Kasab and Mazar alqtrea genotypes (R9, R10, R11, R12, R13, R14, R15, R16).

Cluster II comprised all Alkardaha (R17, R18) and Gablah (R21, R22) genotypes. Damascus genotypes (Al-Marah) (R19, R20) grouped in one cluster and appeared to be distinct from all

others.

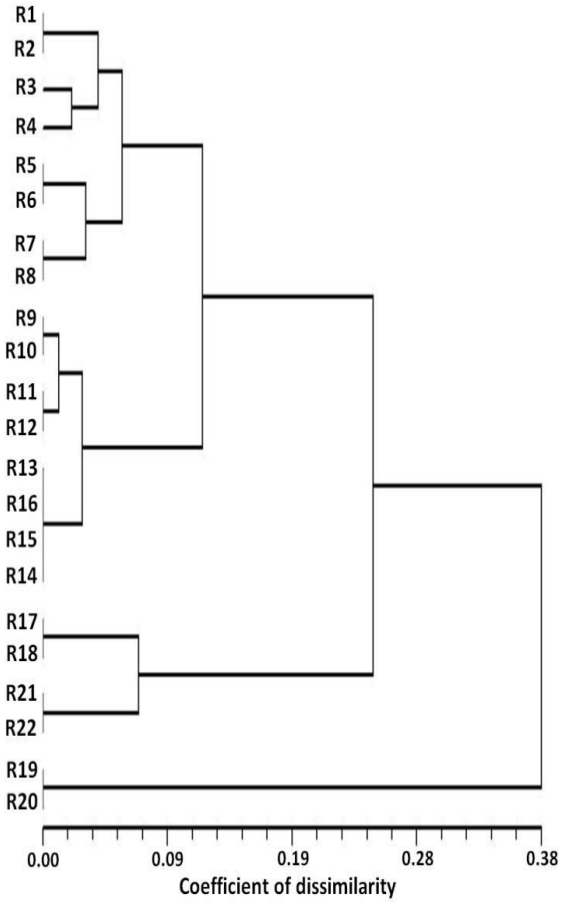

Fig 2: Dendrogram demonstrating the relationships among the 22 *Rosa damascena* Mill. genotypes based on ISSRs.

All ISSR primers generated high levels of polymorphism ranging from 50 to 100%. These results were in conformity with those of the Iranian and Syrian researchers that scored high levels of diversity among rose genotypes collected from different regions from Iran and Syria ([3], [9]).

In the current study, the ISSR analysis proved to be very simple, fast and cost effective, successfully discriminated between both Latakia province and Al-Marah Roses by using ten different ISSR loci. The genetic diversity between the studied genotypes might lead to suggest that most of them originally come from different plant scattered in different geographic and bioclimatic zones and its more or less different from the original genotype (Al-Marah). The effect of climatic conditions on genetic variability within and between Rosa species has been mentioned in further studies ([21], [18]).

In some cases no polymorphism was detected among the genotypes such as all Kasab, and Kasab-Alshagarah genotypes (R13, R14, R15, R16), in contrast to [9] who found no variation between Aleppo genotypes depends on ISSR and RAPD analysis. The conclusion from these data is that all *R. damascena* plants from this locality are derived from the same original genotype by vegetative propagation.

IV. CONCLUSIONS

This is the first report on the assessment of genetic variation in *R. damascena* Mill. varieties cultivated in Latakia compared with two local genotypes from Damascus using molecular markers. In this study, cluster analysis based on ISSR markers divided the varieties into three distinct groups with a high level of polymorphism, which suggests that this marker amplification technique can be a useful and serve as a potentially powerful tool for genotyping studies in the genus *Rosa*.

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