doi:10.20944/preprints202105.0305.v1

Distance Only Brings You Closer: Application of ISSR Markers to Analyze Molecular Relationships in Roses (*Rosa spp.)*- The Symbol of Love

Tanay Joshi [1,*], Santosh Kumar [2], Lalit Arya [3] and Amritbir Riar [1]

Abstract

Genetic diversity is inevitable in making any crop improvement program successful. DNA fingerprinting technology to assess the genetic relationship among the selected genotypes for identification and cataloging of different species and cultivars of roses is a promising tool for Rosa genomes. The inter-simple sequence repeats markers (ISSRs) were used to investigate the genetic diversity among twenty-one diverse Rosa genotypes belonging to two different species, *Rosa hybrida* and *R. damascena*, and three distinct groups of rose varieties, namely Hybrid Tea, Floribunda, and Damask roses. Twenty-four ISSR primers yielded a total of 280 scorable amplified fragments from 250-1800 bp in length, from which 244 were polymorphic, resulting in an average of 86.4% polymorphism. UPGMA cluster analysis based on Jaccard's pairwise similarity coefficient values ranged from 0.264 to 0.818, clearly distinguished different species and genotypes, grouping them into three distinct clusters. The results confirmed a high degree of variation in the rose germplasm studied highlighting the potential of improvement in roses for the ornamental and perfume industry.

Keyword: ISSR, *Rosa spp*., DNA fingerprinting, genotypes, genetic diversity

Dr. Tanay Joshi, Scientist, Research Institute of Organic Agriculture FiBL, Switzerland
Dr. Santosh Kumar, Professor Floriculture and Landscaping, G.B Pant University of Agriculture and Technology
Dr. Lalit Arya, Principal Scientist, National Bureau of Plant Genetic Resources | NBPGR · Division of Genomic Resources
Dr. Amritbir Riar, Sr. Scientist & Theme Leader, Research Institute of Organic Agriculture FiBL, Switzerland
Correspondence: tanay.joshi@fibl.org

1. Introduction

Flowers and gardens for a long have been imperative for three main considerations viz., aesthetic, economic, and social. Floriculture is identified as a fast-emerging industry with potential for domestic and export markets in India [1]. Rose is one of the most important crops in the floriculture industry [2], and is used as cut flowers, potted plants, and garden plants [3]. Roses have also been used in the food, perfumery, and cosmetics industries for many years [4–6]

Roses belong to the genus *Rosa* in the *Rosaceae* family and are one of the most commercially cultivated ornamental plants in the world [7]. Modern rose cultivars are generally triploid or tetraploid hybrids derived from 7-10 wild diploid rose species and a few tetraploid species. The genus Rosa consists of about 200 species and thousands of cultivars that are widely distributed in Europe, Asia, the Middle East, and North America with the greatest diversity of species found in western China [8,9] . More than 150 species have already been catalogued [10,11]. The exact numbers of the rose cultivars are unknown, whereas the number is estimated at more than 20000 rose cultivars in the world [12,13]. Also, only 11 out of 200 Rosa species have contributed to the origin of modern cultivars [14].

The present-day garden roses are complex hybrids involving interspecific hybridization, polyploidy with high female and male sterility [15]. In their study, Raymond et al. [16] reported the rose whole-genome sequencing and assembly and resequencing of major genotypes that contributed to rose domestication. A wide range of variability in flower type and plant growth has been developed in the genus Rosa due to considerable advancement in rose breeding technology for the last 200 years. Unfortunately, just a small portion of this variability has been used in the present breeding.

Traits-based morphological identification and classification of the rose species and cultivars become very difficult when the genetic distance between varieties becomes smaller [17,18]. Potential of Wild rose species help widen the rose gene pool which has not been fully exploited yet. There is always demand and the need for new varieties in floriculture, and the global flower industry prospers on novelty traits such as flower color, form, and scent which are primary novelty markers in consumer choice. For the development of a new variety, the creation of genetic variability is a prerequisite. The assessment of genetic diversity is the most important for a meaningful breeding improvement program because hybrids between genetically diverse parents manifest greater heterosis than those between closely related parents.

Molecular characterization needs to be conducted to clarify the relationships between genotypes as well as for identification and genetic conservation of rose genotypes [19]. In recent years, several molecular marker systems have been developed for fast identification which is accurate and equally effective. ISSRs, a microsatellite-based technique is used for genetic characterization of different plant organisms. Evaluating genetic diversity of reproducible fingerprinting profiles with a sufficient degree of polymorphism can be achieved using ISSR markers [13,20,21].

The present study is the molecular fingerprinting analysis of the locally grown and maintained rose genotypes in North India with a unique primer set to fulfil the gap of genetic relationships among different species and cultivar types including hybrid tea, floribunda and damask roses for breeding novel rose cultivars for better use in ornamental, medicinal and aromatic industry.

2. Results and discussion

2.1. DNA Fingerprinting by ISSR Markers

A total of 33 primers with different repeat motifs were used for initial screening with 3 genotypes, one each from Hybrid Tea, Floribunda, and Oil Content. Out of these, 9 primers gave no amplification at all, while only 24 primers were found to give clear and polymorphic patterns. In similar study Matsumoto et al. [22], also reported that out of 13 primers tested 7 primers generated clear and consistent banding patterns with clonal plants of Marina and rootstock. These twenty-four primers were subsequently employed to analyze the entire set of 21 rose genotypes, as given in **Table** 1.

Table 1. Rose genotypes representing Hybrid Tea, Floribunda and Damask groups

Sr. no	**Cultivar**		**Pedigree and Source**
1	Hapiness	(HT)	(Rome glory × Tassin)×[Charles P. Kilham × (Charles P Kilham × Capucine Chambard]
2	Kiss of Fire	(HT)	Lavena Roses cavriglia
3	Superstar	(HT)	(Seedling × Peace) × (Seedling × Alpine Glow)
4	Mirnalani	(HT)	Pink Parfait × Christian Dior
5	Lady X	(HT)	Seedling × Simone
6	Eiffel Tower	(HT)	First Love × Seedling
7	Avon	(HT)	Nocturne × Chrysler Imperial
8	Swarthmore	(HT)	(Independence × Happiness) × Peace
9	Abhisarika	(HT)	Induced Mutant × Kiss of Fire
10	President	(HT)	-
11	Delhi Prince	(FL)	IARI
12	Ice Berg	(FL)	Robinhood × Virgo
13	Pink Delight	(FL)	-
14	Laher	(FL)	IARI
15	Nimish	(FL)	-
16	Noorjahan	(OC)	Sweet Afton × Crimson Glory
17	Rani Sahiba	(OC)	CIMAP Selection
18	Gruss n Tepliz	(OC)	[((Sir Joseph Paxton × Fellenberg) × Papa Gontier) × Gloredes Rosomanes]
19	Himroz	(OC)	IHBT
20	Jawala	(OC)	IHBT
21	Chati Gulab	(OC)	CIMAP Selection

The amplification products of the 24 primers screened on 21 genotypes yielded a total of 280 scorable bands among these 244 were polymorphic with an average of 11.66 bands per primer in the current study (**Figure 1**). The size of clearly detectable amplified ISSR-PCR fragments ranged from 250 bp to 1800 bp and the number of bands generated by each primer varied. The UBC 825 primer produced the highest number of polymorphic bands (17), followed by P-21 (15 bands) and primer (P-02, P-03 & UBC 826) 14 bands each, while the lowest number of polymorphic bands (7) were obtained with the P-18 primer (**Table *2***).

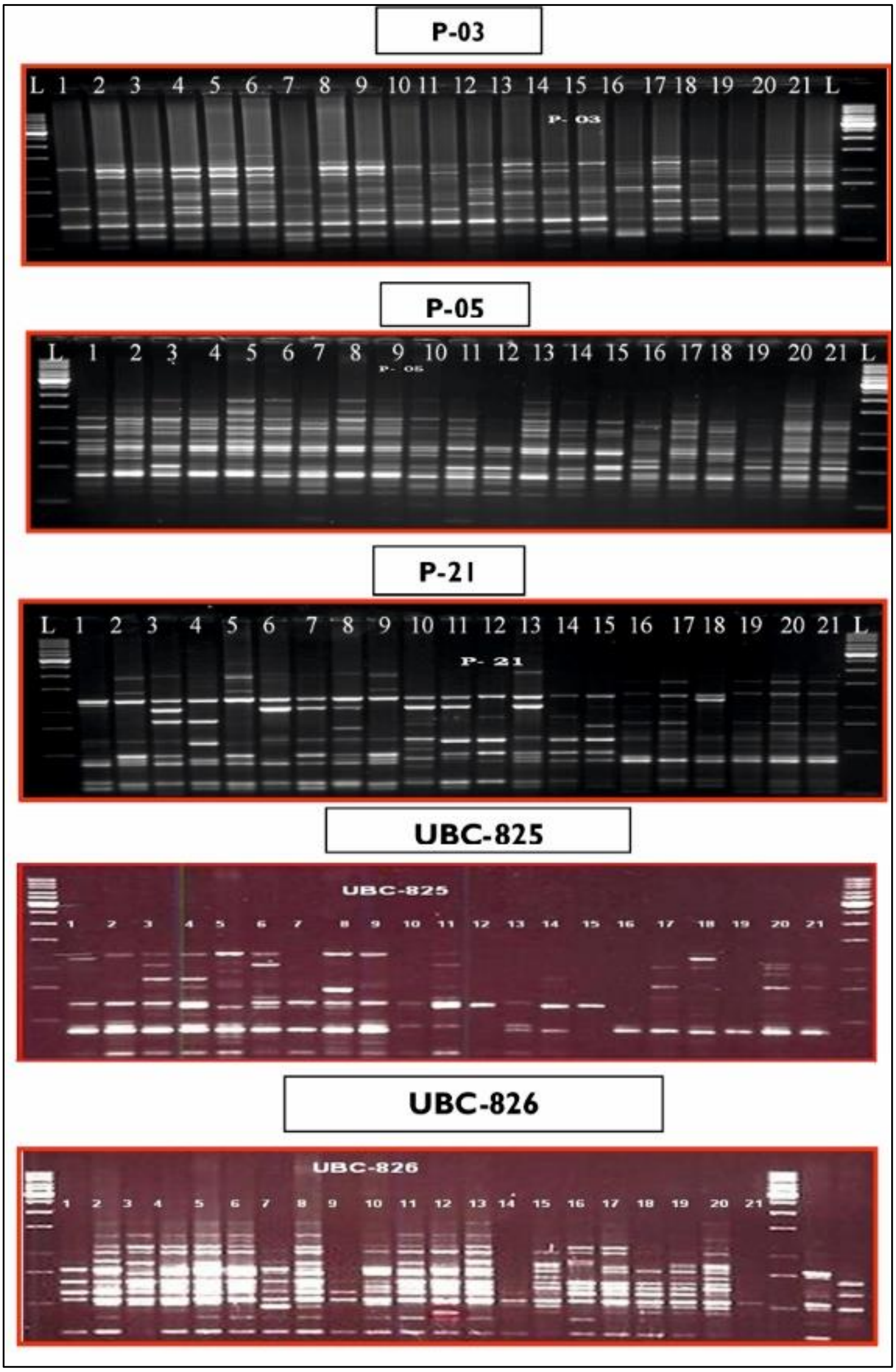


Figure 1. Amplification profile of 21 Rose genotypes obtained by Primer P-03, P-05, P-21, UBC- 825 & UBC- 826.

L- 1 KB ladder,1-Hapiness, 2-Kiss of Fire, 3-Superstar, 4-Mirnalani, 5-Lady X, 6-Eiffel Tower, 7-Avon, 8-Swarthmore, 9-Abhisarika, 10-President, 11-Delhi Prince,12-Ice Berg, 13-Pink Delight, 14-Laher, 15-Nimish, 16-Noorjahan, 17-Rani Sahiba, 18-Gruss n Tepliz, 19-Himroz, 20-Jawala, 21-Chati Gulab Rose genotypes.

All the primers analyzed in the present study showed a high degree of polymorphism among the selected rose genotypes regardless of the repeat size of the 24 ISSR primers have ranged from 60.00% to 100.00%. Primers, P-01, P-02, P-14, P-25, UBC-824 and UBC-825 showed the highest values of percentage of polymorphism (100.00%) followed by UBC-826 (93.33%), UBC-848 (93.33%) and UBC-847 (92.85%). While the lowest percentage of polymorphism was observed in primer P-22 (**Table 2**). Talas Oğraş et al. [23] reported a high percentage of polymorphism (99.52%) in a study conducted to determine the genetic diversity among nineteen rose genotypes using a molecular fingerprinting method based on fifteen ISSR markers. In another study, Panwar et al. [24] also reported that ISSR markers with high genetic polymorphism are beneficial to discriminate rose cultivars. Carvalho et al. [25] also reported a high percentage of polymorphism (93.7%) with dinucleotide repeats using 9 ISSR primers on 33 distant rose genotypes. Mostly, high polymorphism is revealed by primers anchored either at the 3' or 5' end with dinucleotide repeats [25,26]. The reason for high percentage of polymorphism can be attributed to the heterozygous nature of the polyploidy genome structure of rose species. A positive correlation in percent polymorphism was observed between ISSR Primers with CA and AC repeat motif when compared to the primers with AG motifs that showed low percentages of polymorphism. These findings were contrary to the result reported in the case of Gerbera by Bhatia et al. [27] and by Namita et al. [28], in marigold where CA motifs showed low polymorphism.

Two approaches were adopted for the efficient utilization of ISSR markers in the identification of rose cultivars. In one method, an effort was made to identify the most efficient primer(s) that could individually or in combination distinguish all cultivars. For this purpose, two parameters were calculated, Resolving Power (Rp) and Marker Index (MI). These Markers showed variation in the percentage of polymorphism, band informativeness (Ib), which is a measure of closeness of a band to be present in 50% of the genotypes under assessment and resolving power (Rp) which is the sum of Ib values of all the bands amplified by a primer [29]. The average Ib ranged from 0.15 to 0.60 while Rp ranged from 2.19 to 9.52. The highest average value Ib (0.60) was shown by P- 02 while P- 03 revealed the highest value of Rp (9.523) as shown in **Table 2**. The primers with high Rp were examined for their ability to uniquely distinguish all 21 genotypes. Primer P-02 has the highest discrimination power i.e., the genotype index (GI) of 1.00 followed by 0.90 with primers P- 05, P- 21, UBC- 825, and UBC- 826. However, the primer P- 17 distinguished the least proportion of genotypes (GI of 0.23).

Table 2. Banding pattern and polymorphism exhibited by 24 ISSR primers in 21 Rose cultivars

S.No	**Primer**	**Sequence**	**No. of Bands Amplified**	**No. of Polymorphic Bands**	**% of Polymorphism**	**Size range (bp)**	**Rp**	**Marker Index (MI)**					**GI**
								Ib(av)	**Di(av)**	**EMR**	**$MI_{(Ib)}$**	**$MI_{(Di)}$**	
1	P- 01	$5'(CA)_6RY3'$	10	10	100.00	1200- 275	5.60	0.44	0.24	10.00	4.40	2.40	0.80
2	P- 02	$5'(CA)_6RG3'$	14	14	100.00	1250- 250	5.58	0.60	0.17	14.00	8.40	2.38	1.00
3	P- 03	$5'(CA)_6R3'$	16	14	87.50	1400- 280	9.52	0.27	0.18	12.25	3.30	2.20	0.80
4	P- 05	$5'(AGC)_4Y3'$	15	13	86.66	1350- 450	7.33	0.37	0.16	11.26	4.16	1.80	0.90
5	P- 14	$5'(AC)_8YT3'$	10	10	100.00	1100- 275	5.81	0.41	0.27	10.00	4.10	2.70	0.71
6	P- 15	$5'BDB(CA)_73'$	11	9	81.81	1010- 300	4.86	0.37	0.20	7.30	2.70	1.46	0.90
7	P- 17	$5'VHV(GT)_73'$	8	6	75.00	900- 350	2.19	0.47	0.37	4.50	2.11	1.66	0.23
8	P- 18	$5'HVH(TG)_73'$	7	6	85.71	740- 275	3.50	0.35	0.20	5.14	1.79	1.02	0.35
9	P- 21	$5'(AC)_8T3'$	17	15	88.23	1800- 280	6.76	0.48	0.14	13.20	6.33	1.84	0.90
10	P- 22	$5'(AG)_8T3'$	10	6	60.00	800- 400	3.60	0.24	0.28	3.60	0.86	1.00	0.70
11	P- 23	$5'(AG)_8C3'$	12	8	66.66	800- 270	4.50	0.29	0.32	5.33	1.54	1.70	0.75
12	P- 25	$5'(GA)_8C3'$	10	10	100.00	900 -260	5.33	0.46	0.45	10.00	4.60	4.50	0.47
13	P- 30	$5'(AC)_8YA3'$	10	9	90.00	1100- 255	3.52	0.54	0.42	8.10	4.37	3.40	0.38
14	UBC-807	$5'(AG)_8T3'$	12	9	75.00	770- 375	4.30	0.39	0.48	6.75	2.63	3.24	0.70
15	UBC-810	$5'(GA)_8T3'$	11	8	72.72	990- 300	4.10	0.35	0.40	5.81	2.03	2.32	0.52
16	UBC-812	$5'(GA)_8A3'$	9	7	77.77	1250- 300	3.90	0.34	0.15	5.44	1.84	0.81	0.50
17	UBC-814	$5'(CT)_8A3'$	9	7	77.77	1250- 350	5.62	0.15	0.18	5.44	0.81	0.97	0.52
18	UBC-824	$5'(TC)_8G3'$	9	9	100.00	1600- 600	5.16	0.42	0.31	9.00	3.78	2.79	0.63
19	UBC-825	$5'(AC)_8T3'$	17	17	100.00	1200- 250	8.29	0.51	0.11	17.00	8.67	1.87	0.90
20	UBC-826	$5'(AC)_8C3'$	15	14	93.33	1050- 350	7.33	0.44	0.25	13.00	5.72	3.25	0.90
21	UBC-827	$5'(AC)_8G3'$	8	7	87.50	850- 300	4.20	0.35	0.15	6.12	2.14	0.91	0.60
22	UBC-847	$5'(CA)_8RC3'$	14	13	92.85	1500- 255	7.43	0.39	0.14	12.07	4.70	1.68	0.90
23	UBC-848	$5'(CA)_8RG3'$	15	14	93.33	1400- 255	7.70	0.42	0.26	13.00	5.46	3.38	0.70
24	UBC-896	$5'AGGTCGCGGCCGC(N)_6ATG3'$	11	9	81.81	1400- 300	4.70	0.39	0.24	7.36	2.87	1.76	0.70
		TOTAL	**280**	**244**	-----	-----	**130.8**	**9.44**	**6.07**	**215.7**	**89.31**	**51.04**	**16.46**
		AVERAGE	**11.66**	**10.16**	**86.84**		**5.45**	**0.39**	**0.25**	**8.98**	**3.72**	**2.12**	**0.68**

Marker indices based on band informativeness (Ib) and Average Diversity Index (DI (av)) were determined. Marker Index calculated based on band informativeness was found to be higher (3.72) than the MI calculated based on DI (2.12). The highest and lowest MI (ib) and MI (di) were obtained in primers UBC- 825 (8.53), UBC- 814 (0.81) and P- 25 (4.5), UBC- 812 (0.81) primers respectively (**Table 2**). In the second method, distinctive bands were sited across all primers that individually identified a particular accession. Five cultivars could be identified by the presence or absence of a single marker. In one cultivar, Happiness more than one unique band could be detected in primers P- 17 & P- 30 (**Table 3**).

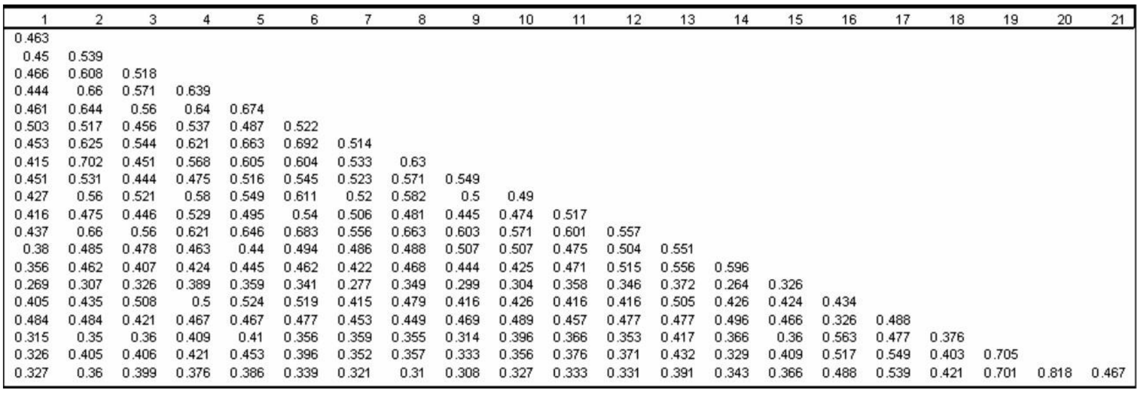
Table 3. Cultivars and clones identified by presence or absence of specific markers

Sr. No.	Primer	Cultivar	Marker	Marker
			Present	Absent
1	P- 14	President	_	5‟(AC)8YT3‟515
2	P- 17	Happiness	_	5‟(CA)6RY3‟490
3	P- 30	Happiness	_	5‟(AC)8YA3‟300
4	P- 30	Happiness	_	5‟(AC)8YA3‟350
5	UBC-825	Swarthmore	5‟(AC)8T3‟730	_
6	UBC-826	Avon	5‟(AC)8g3‟480	_
7	UBC-847	Jawala	5‟(CA)8RCg3‟1500	_

In the present study, the resolving power of primers correlates with polymorphism as primer P- 22 with lowest percent polymorphic bands had lowest Rp and GI. Similarly, primer UBC-825 with the highest percent polymorphic bands reported maximum GI but second-highest Rp. Such low correspondence of polymorphism detected by a primer and proportion of genotypes discriminated by it has been observed in earlier studies of Prevost and Wilkinson [30].

2.2. Genetic variation among rose genotypes

The binary data matrix generated by the amplified fragments of the 21 rose individuals in the ISSR-PCR analyses was used for computing Jaccard's similarity indices. From Jaccard's similarity analysis it was deduced that the values observed in the similarity matrix ranged from 0.264 between Noorjahan and Laher to 0.818 between Chaiti Gulab and Jawala. The minimum similarity was observed between genotype Noorjahan and Laher (0.264) followed by Noorjahan and Happiness (0.269) as shown reported in **Figure 2**. Thus, these genotypes are said to be distantly related and the cross between them may prove beneficial maybe because both of the genotypes belong to different species i.e., *hybrida* and *damascena* as well as the different type of varieties i.e., Hybrid Tea, Floribunda and Oil containing cultivars (Damask Roses).



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
0.463																				
0.45	0.539																			
0.466	0.608	0.518																		
0.444	0.66	0.571	0.639																	
0.461	0.644	0.56	0.64	0.674																
0.503	0.517	0.456	0.537	0.487	0.522															
0.453	0.625	0.544	0.621	0.663	0.692	0.514														
0.415	0.702	0.451	0.568	0.605	0.604	0.533	0.63													
0.451	0.531	0.444	0.475	0.516	0.545	0.523	0.571	0.549												
0.427	0.56	0.521	0.58	0.549	0.611	0.52	0.582	0.5	0.49											
0.416	0.475	0.446	0.529	0.495	0.54	0.506	0.481	0.445	0.474	0.517										
0.437	0.66	0.56	0.621	0.646	0.683	0.556	0.663	0.603	0.571	0.601	0.557									
0.38	0.485	0.478	0.463	0.44	0.494	0.486	0.488	0.507	0.507	0.475	0.504	0.551								
0.356	0.462	0.407	0.424	0.445	0.462	0.422	0.468	0.444	0.425	0.471	0.515	0.556	0.596							
0.269	0.307	0.326	0.389	0.359	0.341	0.277	0.349	0.299	0.304	0.358	0.346	0.372	0.264	0.326						
0.405	0.435	0.508	0.5	0.524	0.519	0.415	0.479	0.416	0.426	0.416	0.416	0.505	0.426	0.424	0.434					
0.484	0.484	0.421	0.467	0.467	0.477	0.453	0.449	0.469	0.489	0.457	0.477	0.477	0.496	0.466	0.326	0.488				
0.315	0.35	0.36	0.409	0.41	0.356	0.359	0.355	0.314	0.396	0.366	0.353	0.417	0.366	0.36	0.563	0.477	0.376			
0.326	0.405	0.406	0.421	0.453	0.396	0.352	0.357	0.333	0.356	0.376	0.371	0.432	0.329	0.409	0.517	0.549	0.403	0.705		
0.327	0.36	0.399	0.376	0.386	0.339	0.321	0.31	0.308	0.327	0.333	0.331	0.391	0.343	0.366	0.488	0.539	0.421	0.701	0.818	0.467

Figure 2. ISSR Jaccard's Similarity Co-efficient Matrices based on ISSR Data

With UPGMA cluster analysis based on ISSR markers, the 21 rose cultivars are grouped in three major clusters (**Figure 3**). Cultivars Happiness and Gruss n Tepliz formed Cluster- I. Cultivars Kiss of fire, Abhisarika, Mrinalini, Lady X, Eiffel Tower, Swarthmore, and Pink Delight, Delhi prince, Superstar, Avon, President, Iceberg, Laher, and Nimish forms Cluster- II. Cluster-III comprised varieties, Noorjahan, Himroz, Jawala, Chaiti Gulab, and Rani Sahiba.

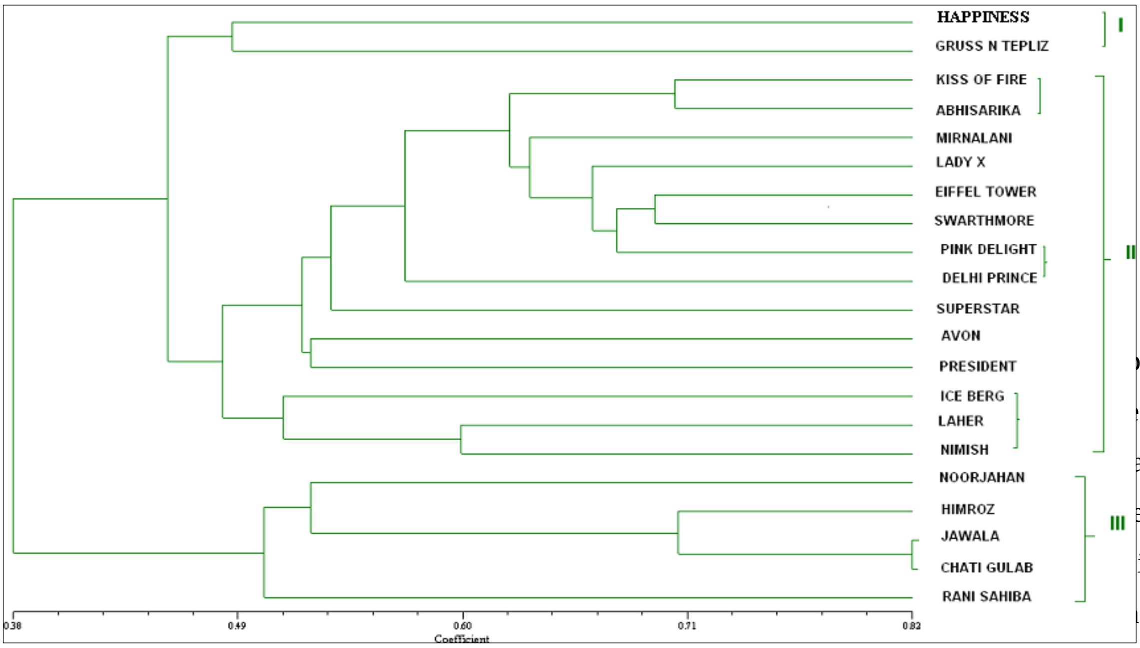


Figure 3. UPGMA clustering pattern revealed by 21 Rose genotypes using ISSR markers

The clustering of genotypes may be due to various reasons, first and foremost is the genotype and their parentage, the second reason may be the phenotypical difference (or) similarity between the cultivars including inflorescence type, leaf size, and type of flower color. In Cluster- I cultivar Gruss n Tepliz which belongs to species *Rosa damascena* has branched off from its main cluster (Cluster- III) and is grouped with cultivar Happiness. This might be because all the other oil content varieties that grouped belong to species *Rosa damascene* and are of Indian (*desi*) origin whereas, Gruss n Tepliz is an exotic cultivar. All the 14 varieties that have pooled in Cluster- II are grouped possibly because all the genotypes in this cluster belong to species *Rosa hybrida*, as well as all of them, are Hybrid Tea varieties and Floribunda varieties. As Abhisarika is the mutant of Kiss of fire so it has paired into a separate sub-cluster in Cluster- II as even discussed by Sasikumar et al. [31] Markedly, all the genotypes that belong to Damask roses (*R. damascena*) have grouped in Cluster- III as they represent the same species and have distinctly formed a separate cluster with an exception of cultivar Gruss n Tepliz which have out grouped with cultivar Happiness in Cluster- I [5]. The above results and discussion showed ISSR profiling is an effective tool for evaluating genetic variation and thus would be useful for differentiation of elite breeding lines and varieties [28]. It surfaces as an effective and reliable alternative to the conventional methods which are based on morphological markers.

3.

4. Materials and Methods

For the present investigation, the experimental material comprised of 21 rose varieties (Table 1) which were collected from Rose Germplasm Block at the Model Floriculture Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, and molecular assessment work was carried out at National Research Centre for DNA Fingerprinting at National Bureau of Plant Genetic Resources (NBPGR), New Delhi.

Fresh leaves of 21 genotypes were stored at -20ºC until DNA isolation. Total genomic DNA was extracted from 5 gram of young and healthy leaves using C-TAB (CetylTrimethyl Ammonium Bromide) method [32–34]. Polymerase chain reaction (PCR) condition was optimized for rose DNA to obtain reproducible amplification with 24 ISSR primers. The amplified fragments were resolved on 1.4 percent agarose (Agarose SFRTM, amresco®) gel containing the nucleic acid stained with ethidium bromide (10 mg/ml) using 10X TBE buffer (89 mM Tris base, 89 mM Boric acid, 2mM EDTA pH 8.0). DNA fragments were visualized under UV light and photographed using a gel documentation system (Alphaimager HP, Cell Biosciences, USA). Jaccard’s pair-wise similarity coefficient was used to determine genetic diversity and the relationship of rose species. For molecular data, the scoring of band profiles for each genotype was carried out in a binary mode (1 indicating its presence: 0 indicating its absence). The resolving power (Rp) was calculated according to Prevost and Wilkinson [30], marker index (MI) as proposed by Powell et al. [35], genotype index (GI) by McGregor et al. [36], were also calculated. Computation for multivariate analysis was done using NTSYS-pc Version 2.02 software [37] and a similarity matrix was subjected to the cluster analysis of the Unweighted Paired Group Method using Arithmetic Averages (UPGMA) and a dendrogram was constructed.

5. Conclusion

ISSR primer P- 01, P- 02, P- 14, P- 25, UBC- 824, and UBC- 825 showed the highest values of percentage of polymorphism so these markers may be used for diversity analysis. Primer P- 03 revealed the highest value of Rp (9.523) proves to be the best to uniquely distinguish all 21 genotypes. The minimum similarity was observed between genotype Noorjahan and Laher. These genotypes are said to be distantly related and the cross between them may prove beneficial. Cultivar Happiness may be used to develop SCAR (Sequenced characterized amplified region) markers for this particular genotype as more than one unique band could be detected in primers P- 17 & P- 30. Conversion of specific ISSR segments into SCAR markers can be of help in improving the value of these markers for the identification of rose genotypes. Genetic variability observed among these genotypes would be useful for selecting parents in the crop improvement program, it will also aid in the development of promising varieties that meet the demand of ornamental, medicinal and aromatic industry.

6. References

1. Sindhu, S.S.; Saha, T.N. Research Highlights and Business Opportunities in Floriculture. Available online: https://www.cabdirect.org/cabdirect/abstract/20143114681.
2. Senapati, S.K.; Rout, G.R. Study of Culture Conditions for Improved Micropropagation of Hybrid Rose. *Horticultural Science* **2008**, *35*, 27–34, doi:10.17221/650-hortsci.
3. Wang, J.; Guterman, I.; Davydov, O.; Shalit, M.; Ovadis, M.; Adam, Z.; Piestun, D.; Vainstein, A.; Weiss, D.; Menda, N.; et al. Rose Scent: Genomics Approach to Discovering Novel Floral Fragrance-Related Genes. *Plant Cell* **2002**, *14*, 2325–2338.
4. Gustavsson, L.-Å. *Roses for Nordic Gardens: [D. 1]*; Nature and culture, 1998; ISBN 91-27-02861-5.
5. Kaur, N.; Sharma, R.K.; Sharma, M.; Singh, V.; Ahuja, P.S. Molecular Evaluation and Micropropagation of Field Selected Elites of R. Damascena. *Gen. Appl. Plant Physiol* **2007**, *33*, 171–186.
6. Uggla, M. Domestication of Wild Roses for Fruit Production, The Swedish University of Agricultural Sciences, 2004.
7. Rajeshbabu, P.; Gopalakrishnan, M.; Janarthanan, B.; Sekar, T. An Efficient and Rapid Regeneration Protocol for Micropropagation of Rosa Bourboniana from Nodal Explants. *International Journal of Current Biotechnology* **2014**, *2*, 24–29.
8. Nilsson, O. Flora of Turkey and the East Aegean Islands. In; Rosa. In: Davis PH, E., Ed.; Edinburgh University Press Edinburgh, 1997; pp. 106–128.
9. Phillips, R.; Rix, M. *Roses*; Random House, 1988;
10. Krussmann, G. *The Complete Book of Roses (German)*; Timber Press: Portland, 1981; ISBN 978-0917304644.
11. Quest, R.C.; Quest, R.B. No Title. *The American Rose Society Encyclopedia of Roses* 2003, 44.

12. Kim, Y.; Byrne, D.H. 365 Biosystematical Classification of Genus Rosa Using Isozyme Polymorphisms. *HortScience* **1994**, *29*, 483c–4483, doi:10.21273/HORTSCI.29.5.483c.
13. Rajapakse, S.; Byrne, D.H.; Zhang, L.; Anderson, N.; Arumuganathan, K.; Ballard, R.E. Two Genetic Linkage Maps of Tetraploid Roses. *Theoretical and Applied Genetics* **2001**, *103*, 575–583, doi:10.1007/PL00002912.
14. De Vries, D.P.; Dubois, L.A.M. Rose Breeding: Past, Present, Prospects. *Acta Horticulturae* **1996**, *424*, 241–248, doi:10.17660/ActaHortic.1996.424.43.
15. Gudin, S. Rose: Genetics and Breeding. In *Plant Breeding Reviews*; 2010; pp. 159–189.
16. Raymond, O.; Gouzy, J.; Just, J.; Badouin, H.; Verdenaud, M.; Lemainque, A.; Vergne, P.; Moja, S.; Choisne, N.; Pont, C.; et al. The Rosa Genome Provides New Insights into the Domestication of Modern Roses. *Nature Genetics* **2018**, *50*, 772–777, doi:10.1038/s41588-018-0110-3.
17. Esselink, G.D.; Smulders, M.J.M.; Vosman, B. Identification of Cut Rose (Rosa Hybrida) and Rootstock Varieties Using Robust Sequence Tagged Microsatellite Site Markers. *Theoretical and Applied Genetics* **2003**, *106*, 277–286, doi:10.1007/s00122-002-1122-y.
18. Jan, C.H.; Byrne, D.H.; Manhart, J.; Wilson, H. Rose Germplasm Analysis with RAPD Markers. *HortScience* **1999**, *34*, 341–345, doi:10.21273/hortsci.34.2.341.
19. Qiu, X.; Zhang, H.; Wang, Q.; Jian, H.; Yan, H.; Zhang, T.; Wang, J.; Tang, K. Phylogenetic Relationships of Wild Roses in China Based on NrDNA and MatK Data. *Scientia Horticulturae* **2012**, *140*, 45–51, doi:10.1016/j.scienta.2012.03.014.
20. Machkour-M'Rabet, S.; Henaut, Y.; Dor, A.; Perez-Lachaud, G.; Pelissier, C.; Gers, C.; Legal, L. ISSR (Inter Simple Sequence Repeats) as Molecular Markers to Study Genetic Diversity in Tarantulas (Araneae, Mygalomorphae). *Journal of Arachnology* **2009**, *37*, 10–14, doi:10.1636/A08-27.1.
21. Zietkiewicz, E.; Rafalski, A.; Labuda, D. Genome Fingerprinting by Simple Sequence Repeat (SSR)-Anchored Polymerase Chain Reaction Amplification. *Genomics* **1994**, *20*, 176–183, doi:10.1006/geno.1994.1151.
22. Matsumoto, S.; Fukui, H. Identification of Rose Cultivars and Clonal Plants by Random Amplified Polymorphic DNA. *Scientia Horticulturae* **1996**, *67*, 49–54, doi:10.1016/S0304-4238(96)00951-X.
23. Talas Oğraş, T.; Koban Baştanlar, E.; Karakaş Metin, Ö.; Kandemir, İ.; Özçelik, H. Assessment of Genetic Diversity of Rose Genotypes Using ISSR Markers. *Turkish Journal of Botany* **2017**, *41*, 347–355, doi:10.3906/bot-1608-32.
24. Panwar, S.; Singh, K.P.; Sonah, H.; Deshmukh, R.K.; Prasad, K. v; Sharma, T.R. Molecular Fingerprinting and Assessment of Genetic Diversity in Rose (Rosa × Hybrida). *Indian Journal of Biotechnology* **2015**, *14*, 518–524.
25. Carvalho, A.; Lima-Brito, J.; Maçãs, B.; Guedes-Pinto, H. Genetic Diversity and Variation Among Botanical Varieties of Old Portuguese Wheat Cultivars Revealed by ISSR Assays. *Biochemical Genetics* **2009**, *47*, 276–294, doi:10.1007/s10528-009-9227-5.
26. Joshi, S.P.; Gupta, V.S.; Aggarwal, R.K.; Ranjekar, P.K.; Brar, D.S. Genetic Diversity and Phylogenetic Relationship as Revealed by Inter Simple Sequence Repeat (ISSR) Polymorphism in the Genus Oryza. *Theoretical and Applied Genetics* **2000**, *100*, 1311–1320, doi:10.1007/s001220051440.

27. Bhatia, R.; Singh, K.P.; Jhang, T.; Sharma, T.R. Assessment of Clonal Fidelity of Micropropagated Gerbera Plants by ISSR Markers. *Scientia Horticulturae* **2009**, *119*, 208–211, doi:10.1016/j.scienta.2008.07.024.
28. Namita; Panwar, S.; Sonah, H.; Singh, K.P.; Sharma, T.R. Genetic Diversity Analysis of Marigold (Tagetes Sp) Genotypes Using RAPD and ISSR Markers. *Indian Journal of Agricultural Sciences* **2013**, *83*, 484–490.
29. Guasmi, F.; Belhaj, K.; Triki, T.; Drine, S.; Ali, S. ben; Mohamed, M. ben; Bousorra, F. Molecular Comparison of Some Variety of Apple (Malus Domestica L .) " Douce Djerba " by ISSR and RAPD Markers. *International Journal of Biology Research* **2016**, *1*, 36–40.
30. Prevost, A.; Wilkinson, M.J. A New System of Comparing PCR Primers Applied to ISSR Fingerprinting of Potato Cultivars. *Theoretical and Applied Genetics* **1999**, *98*, 107–112, doi:10.1007/s001220051046.
31. Sasikumar, K.; Singh, S.; Mohapatra, T.; Prasad, K. Characterization of Rose Cultivars and Species by DNA Fingerprinting. *Indian Journal of Horticulture* **2007**, *64*, 435–438.
32. Murray, M.G.; Thompson, W.F. Rapid Isolation of High Molecular Weight Plant DNA. *Nucleic Acids Research* **1980**, *8*, 4321–4326, doi:10.1093/nar/8.19.4321.
33. Doyle, J.J.; Doyle, J.L. Isolation of Plant DNA from Fesh Tissue. *Focus* **1990**, *12*, 13–15.
34. Saghai-Maroof, M.A.; Soliman, K.M.; Jorgensen, R.A.; Allard, R.W. Ribosomal DNA Spacer-Length Polymorphisms in Barley: Mendelian Inheritance, Chromosomal Location, and Population Dynamics. *Proceedings of the National Academy of Sciences* **1984**, *81*, 8014–8018, doi:10.1073/pnas.81.24.8014.
35. Powell, W.; Morgante, M.; Andre, C.; Hanafey, M.; Vogel, J.; Tingey, S.; Rafalski, A. The Comparison of RFLP, RAPD, AFLP and SSR (Microsatellite) Markers for Germplasm Analysis. *Molecular Breeding* 1996, *2*, 225–238.
36. McGregor, C.E.; Lambert, C.A.; Greyling, M.M.; Louw, J.H.; Warnich, L. A Comparative Assessment of DNA Fingerprinting Techniques (RAPD, ISSR, AFLP and SSR) in Tetraploid Potato (Solanum Tuberosum L.) Germplasm. *Euphytica* **2000**, *113*, 135–144, doi:10.1023/A:1003925620546.
37. Rohlf, F.J. NTSYS-Pc: Numerical Taxonomy and Multivariate Analysis System. *The American Statistician* 2000, *41*.