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

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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.

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

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 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, [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

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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.

Materials and Methods

For the present investigation, the experimental material comprised of 21 rose varieties () 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. These rose genotypes are representing 3 different classes of roses commercially cultivated in India for different purposes and are of high economic importance in flower industry. This includes the hybrid teas which are standard for cut flower roses and are popularly used in bouquet or cut floral arrangement. The second class selected is floribundas which are used in informal and formal landscape setting, hedging, trained as espaliers or topiary also, grow well in containers. Roses from damask class are popular for their high oil content, source of attar of roses used in perfumes, these are edible, used in cooking as a flavouring ingredient and are of value in traditional medicinal system.

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]. Further, crude DNA samples were purified twice with an equal volume of chloroform: isoamyl alcohol (24:1) and precipitated by using 1/10 volumes of 3 M sodium acetate (PH 5.6) and 2.5 volumes of 95 % chilled ethanol. DNA concentration was estimated by agarose gel electrophoresis (0.8 % gel) containing 0.5 µg mL-1 ethidium bromide) using known concentration (50 ng) of uncut lambda DNA as standards. The purified rose genomic DNA was diluted in sterile double distilled water to make a concentration of 20 ng µL-1 for each sample and stored at 4^0C.

Polymerase chain reaction (PCR) condition was optimized for rose DNA to obtain reproducible amplification with 24 ISSR primers. The reaction was performed in 25 µl volumes containing 20 ng µl-1 genomic DNA, 2.5 µl 10X PCR buffer, 1.25 µl of 17.5mM $MgCl_2$, 0.5 ìl of 10mM dNTPs (each of dATP, dGTP, dCTP and dTTP), 2.5 µl of 10 pmole/µl primer and 5U/µl Taq DNA Polymerase in 200 µl PCR tube. The PCR amplification conditions were: initial extended step of DNA denaturation at 94°C for 7 min, followed by 35 cycles of denaturation at 94°C for 30 sec, primer annealing at 50°C for 45 sec and elongation at 72°C for 2 min, followed by final an elongation step at 72°C for 10 min then held at 4°C till electrophoresis.

After completion of PCR amplification reactions, 2 µl of loading dye (6X) was added to each PCR tube. 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). Electrophoresis was carried out at 90V for 4-5 hours using horizontal gel electrophoresis system (Bio-Rad). A 1 kb DNA ladder (Gene Ruler, Fermentas) was run alongside the amplified products to determine the approx band size of PCR product. DNA fragments were visualized under UV light at 302 nm and photographed using a gel documentation system (Alphaimager HP, Cell Biosciences, USA).

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; 9 for missing). The discriminatory power of band was evaluated by two parameters. The resolving power (Rp) which is based on the distribution of alleles within the sampled genotypes was calculated according to Prevost and Wilkinson [30] as Rp = ?Ib where Ib (band informativeness) takes the value of 1- {2x|0.5-p|}, p being the proportion of the genotypes containing the band. The other parameter marker index (MI) as proposed by [35], is the product between diversity index (equivalent to PIC) and effective multiplex ratio (EMR), where EMR is defined as the product of the fraction of polymorphic loci and number of polymorphic loci. Genotype index (GI) by [36], indicating proportion of genotypes identified by the primer 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. `The reliability of the node of UPGMA tree was tested by bootstrap analysis using 1000 permutations.

The population structure was inferred using Structure 2.3.4 software [38]. The structure outputs were visualized using Structure Harvester from which Evanno plots were constructed [39]. An assumed admixed model with independent allele frequency and a uniform prior probability of the number of populations, K was used in structure. The relation between genetic similarity identified by ISSR markers and taxonomic distance measured by mean genetic distance and yield were analyzed using Jaccard's similarity index and average taxonomic distance was calculated by NTSYS-pc v2.1 software. Duncan's Multiple Range Test (DMRT) (P = 0.05) was used to evaluate differences among clusters for significance by using SPSS ver. 19.0 software.

Results and discussion

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 [21,22]. 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 [23], 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.

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).

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). [24] 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, [25] also reported that ISSR markers with high genetic polymorphism are beneficial to discriminate rose cultivars. Carvalho et al. [26] 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 [26,27]. 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 [28] and by [22] 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 GI (proportion of genotypes identified by the primer) 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).

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 and P-30 (Table-3).

Table-1 : Rose genotypes representing Hybrid Tea (HT), Floribunda (FL) and Damask-Oil Contant (OC) groups.

Sr. No.	Cultivar		Pedigree and Source
1.	Happiness	(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

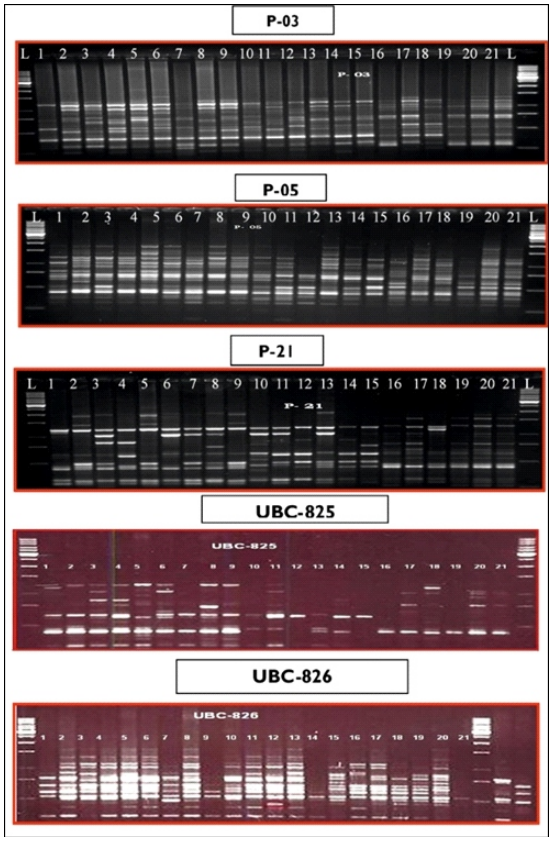

Figure-1 : Amplification profile of 21 Rose genotypes obtained by Primer P-03, P-05, P-21, UBC- 825 & UBC- 826.
L-1 KB ladder,1-Hapiness, 2-Kiss of Fire, 3-Superstar, 4-Mirnalani, 5-Lady X, 6-Eiffel Tower, 7-Avon, 8-Swarthmore, 9-Abhisarika, 10-President, 11-Delhi Prince,12-Ice Berg, 13-Pink Delight, 14-Laher, 15-Nimish, 16-Noorjahan, 17-Rani Sahiba, 18-Gruss n Tepliz, 19-Himroz, 20-Jawala, 21-Chati Gulab Rose genotypes.

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].

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).

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,

Table-1 : 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'$(CA)_6$RY3'	10	10	100.00	1200-275	5.60	0.44	0.24	10.00	4.40	2.40	0.80
2.	P-02	5'$(CA)_6$RG3'	14	14	100.00	1250-250	5.58	0.60	0.17	14.00	8.40	2.38	1.00
3.	P-03	5'$(CA)_6$R3'	16	14	87.50	1400-280	9.52	0.27	0.18	12.25	3.30	2.20	0.80
4.	P-05	5'$(AGC)_4$Y3'	15	13	86.66	1350-450	7.33	0.37	0.16	11.26	4.16	1.80	0.90
5.	P-14	5'$(AC)_8$YT3'	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'$(AC)_8$T3'	17	15	88.23	1800-280	6.76	0.48	0.14	13.20	6.33	1.84	0.90
10.	P-22	5'$(AG)_8$T3'	10	6	60.00	800-400	3.60	0.24	0.28	3.60	0.86	1.00	0.70
11.	P-23	5'$(AG)_8$C3'	12	8	66.66	800-270	4.50	0.29	0.32	5.33	1.54	1.70	0.75
12.	P-25	5'$(GA)_8$C3'	10	10	100.00	900-260	5.33	0.46	0.45	10.00	4.60	4.50	0.47
13.	P-30	5'$(AC)_8$YA3'	10	9	90.00	1100-255	3.52	0.54	0.42	8.10	4.37	3.40	0.38
14.	UBC-807	5'$(AG)_8$T3'	12	9	75.00	770-375	4.30	0.39	0.48	6.75	2.63	3.24	0.70
15.	UBC-810	5'$(GA)_8$T3'	11	8	72.72	990-300	4.10	0.35	0.40	5.81	2.03	2.32	0.52
16.	UBC-812	5'$(GA)_8$A3'	9	7	77.77	1250-300	3.90	0.34	0.15	5.44	1.84	0.81	0.50
17.	UBC-814	5'$(CT)_8$A3'	9	7	77.77	1250-350	5.62	0.15	0.18	5.44	0.81	0.97	0.52
18.	UBC-824	5'$(TC)_8$G3'	9	9	100.00	1600-600	5.16	0.42	0.31	9.00	3.78	2.79	0.63
19.	UBC-825	5'$(AC)_8$T3'	17	17	100.00	1200-250	8.29	0.51	0.11	17.00	8.67	1.87	0.90
20.	UBC-826	5'$(AC)_8$C3'	15	14	93.33	1050-350	7.33	0.44	0.25	13.00	5.72	3.25	0.90
21.	UBC-827	5'$(AC)_8$G3'	8	7	87.50	850-300	4.20	0.35	0.15	6.12	2.14	0.91	0.60
22.	UBC-847	5'$(CA)_8$RC3'	14	13	92.85	1500-255	7.43	0.39	0.14	12.07	4.70	1.68	0.90
23.	UBC-848	5'$(CA)_8$RG3'	15	14	93.33	1400-255	7.70	0.42	0.26	13.00	5.46	3.38	0.70
24.	UBC-896	5'AGGTCGCGGC CGC$(N)_6$ATG3'	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

Table-2 : Cultivars and clones identified by presence or absence of specific markers.

Sr. No.	Primer	Cultivar	Marker sequence' band size	
			Present	Absent
1.	P-14	President	–	5'$(AC)_8$YT3'515
2.	P-17	Happiness	–	5'VHV(GT)73'490
3.	P-30	Happiness	–	5'$(AC)_8$YA3'300
4.	P-30	Happiness	–	5'$(AC)_8$YA3'350
5.	UBC-825	Swarthmore	5'$(AC)_8$T3'730	–
6.	UBC-826	Avon	5'$(AC)_8$C3'480	–
7.	UBC-847	Jawala	5'$(CA)_8$RC3'1500	–

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.

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.

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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	0.508	0.518																		
0.444	0.68	0.571	0.639																	
0.481	0.644	0.58	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											
0.417	0.475	0.445	0.529	0.495	0.54	0.506	0.481	0.445	0.474	0.517										
0.437	0.68	0.58	0.621	0.648	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	0.482	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	0.369	0.359	0.341	0.277	0.349	0.299	0.304	0.356	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	0.583	0.477	0.376			
0.326	0.405	0.408	0.421	0.453	0.396	0.352	0.357	0.333	0.358	0.378	0.371	0.432	0.329	0.409	0.517	0.549	0.403	0.705		
0.327	0.36	0.399	0.326	0.386	0.339	0.321	0.31	0.308	0.327	0.333	0.331	0.391	0.343	0.306	0.486	0.539	0.421	0.701	0.618	0.467

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

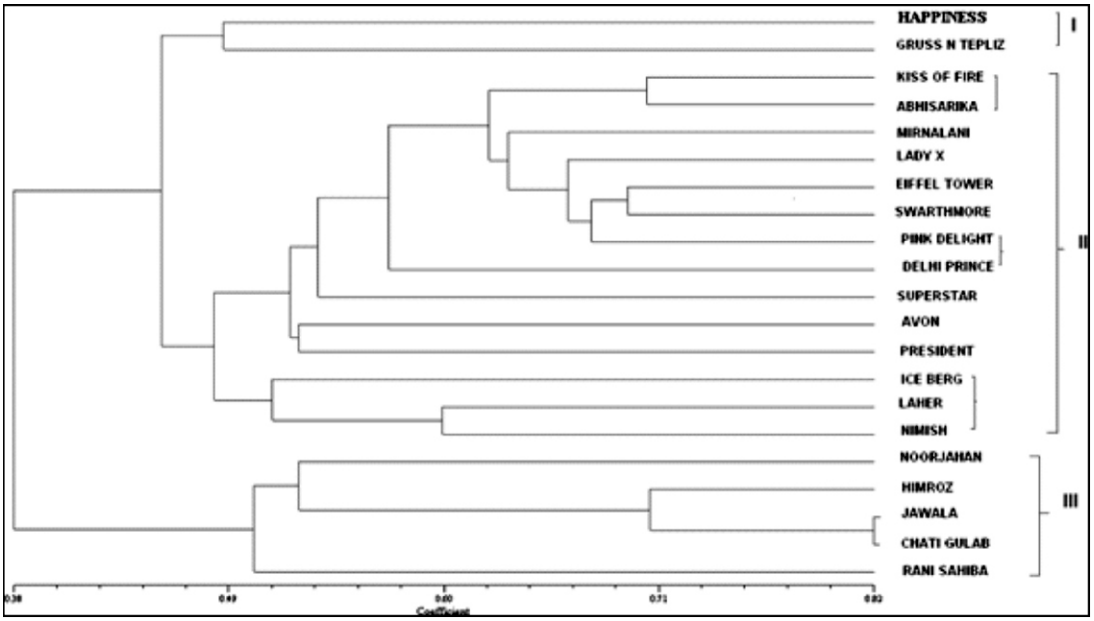

Figure-3 : UPGMA clustering pattern revealed by 21 Rose genotypes using ISSR markers.

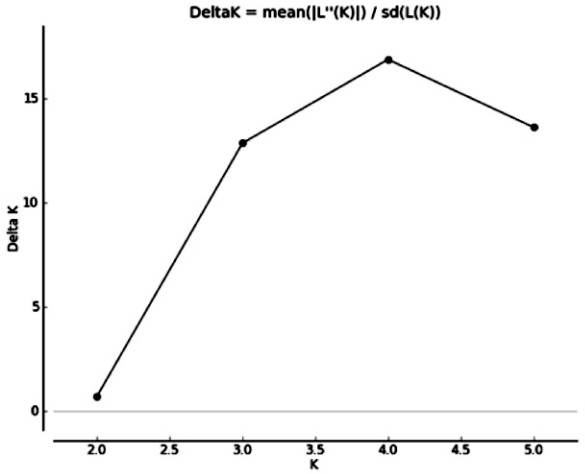

Figure-4 : Highest K value for the rose genotype at K = 3.

the mutant of Kiss of fire so it has paired into a separate sub-cluster in Cluster-II as even discussed by [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 [22]. It surfaces as an effective and reliable alternative to the conventional methods which are based on morphological markers.

Population Structure analysis : The population structure of the rose genotypes was estimated using

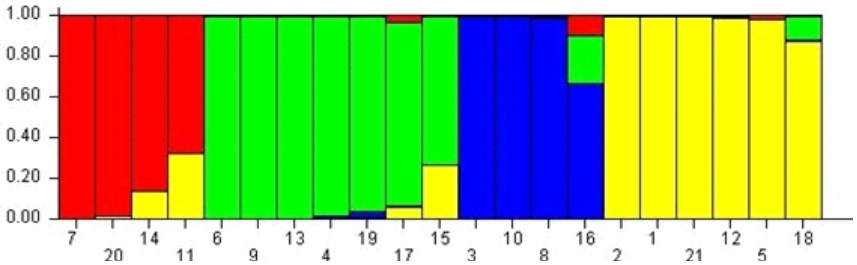

Figure-5: Model based population structure plot of 21 rose germplasms with K = 4, using polymorphic ISSR markers.

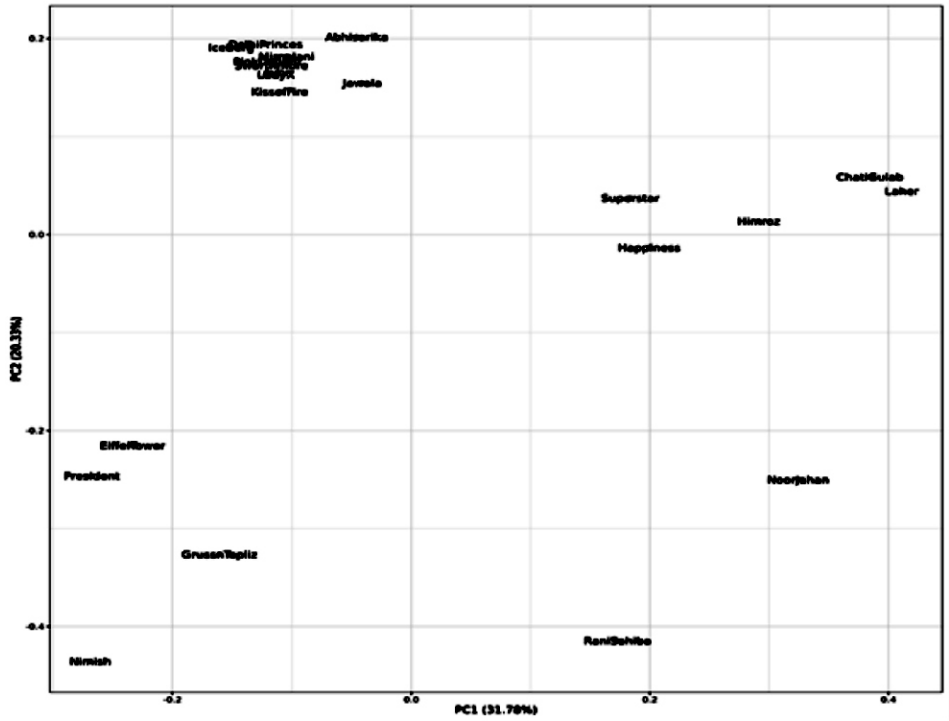

Figure 6 : Principal Co-ordinate Analysis (PCoA) of rose germplasm lines using ISSR data.

structure v2.3.3c software based on markers. The optimum K value was determined by using Structure Harvester, where the highest peak was observed at delta K = 4 suggesting four genetically distinct clusters (Figure-4). The number of sub populations (K) was identified based on maximum likelihood and delta K (dK) values, with into four subgroups.

Using a membership probability threshold of 1.0, 4 genotypes were assigned to subgroup (SG) 1, 7 genotypes to SG 2, 4 genotypes were assigned to subgroup 3 and 6 genotypes were assigned to subgroup 4. The admixture plots for mean values obtained from independent runs for K = 4 are presented in Figure 5 and Supplementary Table S1. The relationship between subgroups derived from STRUCTURE explained that SG 1 to SG 4 comprised of distinct types (Figure-5).

Principle Co-ordinate analysis : Principle Co-ordinate analysis (PCA) based on origin formed four major population groups. Group I included Noorajan and Rani sahiba, Group II included Eiffel Tower, Gruss n Tepliz, Nimish, President. Third group III consisted of Happiness, Superstar, Chati Gulab, Himroz, Laher and Group IV bhisarika, Delhi Prince, Ice Berg, Avon, Mirnalani, Pink Delight, Swarthmore, Lady X, Kiss of Fire, Jawala (Figure-6).

Conclusions

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.

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