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Assessment of Genetic Diversity among the Elite Rose (*Rosa* spp.) Accessions Using Rapd Markers

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MV, RM, MG, NMB and SV designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors RM and SG managed the analyses of the study. Author RM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Owing to its export value in flower trade elsewhere in the world, Rose is the key commercial flower crop and the area under rose cultivation is ever increasing and the end-users always prefer new color variations. Hence, evolving new cultivars with novel color characteristics is the need of the hour, for which understanding genetic variation in the available cultivars is very much needed.
Study Design: This investigation was conducted to analyze the genetic diversity of 11 elite and commonly cultivated rose accessions in South India by using randomly amplified polymorphic DNA (RAPD) markers.
Place and Duration of Study: Department of Floriculture and Landscape Architecture, Horticultural College and Research Institute, TNAU, Coimbatore.
Methodology: A total of 10 RAPD primers were employed, which was sufficient to distinguish the investigated rose cultivars.

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Results: Among the 44 PCR products produced by these markers, 39 (88.64%) were found to be polymorphic bands. The number of amplified products per RAPD primer varied from 3 to 8 with a mean of 4.4 bands per primer. The Un weighted paired group of arithmetic means (UPGMA) dendrogram distinguished the rose accessions into two major clusters suggesting that the accessions were different from each other. The genetic similarity coefficients were determined with this RAPD data, and they were ranged from 0.59 to 0.89.
Conclusion: Molecular profiling data of this study have contributed to characterize and catalogue the rose germplasm data, which will be useful to identify the diverse rose lines for further breeding program that have the potential to improve the color variations.

Keywords: Rose; genetic diversity; randomly amplified polymorphic DNA; oligomers; flower color; rose germplasm.

1. INTRODUCTION

Within the Rosaceae family, roses (Rosa spp.) are the most widely grown commercial flower crop. Grown worldwide, this woody perennial flowering plant is most common in temperate and subtropical areas of the northern hemisphere. Roses are considered the “Queen of Flowers” and come in around 200 species and 18,000 cultivars, each with unique shapes, sizes, colors, and fragrances. It has been farmed for the last 5,000 years, as evidenced by historical accounts from the ancient civilizations of China, Western Asia, and Northern Africa [1].

Roses are an important part of landscaping, and once roses are added, a garden is deemed structurally complete. In terms of the global flower trade, it is in the lead. In addition, it is one of the most significant aromatic and therapeutic crops and a valuable product in the commercial essential oil markets. Roses have an unmatched fragrance and aroma that is highly valued by the food and cosmetics sectors. Rose-flavored ready-to-serve (RTS) beverages, rose petal jam, rose flower tea, and rose wine are significant culinary items with additional value. Since they are the primary raw materials utilized in the perfume industry, rose oil, rose water, rose concrete, and rose absolute are always in high demand on the international market [2].

High yielding rose genotypes must evolve since the demand for unique color variations and the area under commercial cultivation and rose production are growing yearly. As a result, improved genotypes with high yield and distinctive color combinations must arise; this is essentially dependent on capturing the genetic diversity of roses that are currently available. Due to numerous hybridizations and allopolyploidization, many cultivated rose types and hybrids exist in nature, making it challenging to classify and identify relationships between species. The process of evolving novel cultivars would be aided by the evaluation of genetic diversity among the breeding materials and the incorporation of such information into the breeding program. The effectiveness of the selection program is contingent upon the degree of genetic variation and heritability of a trait. In addition, the genetic variety of crop species contributes to the expansion and protection of genetic resources in addition to being helpful in accurately identifying cultivars, which in turn helps to safeguard the legal rights of breeders [3].

A genetic diversity evaluation can be performed with molecular, biochemical, or morphological indicators. Nevertheless, because to their large range of feature overlap and susceptibility to environmental influences, morphological and biochemical markers are not very consistent for diversity analysis. Lewis [4] advised against analyzing genetic diversity based solely on morphological qualities because genetically closer cultivars are hard to distinguish from one another based only on morphological traits. Similarly, the isozyme markers' low ability to produce many reliably resolvable loci hindered their application in rose diversity studies.

Therefore, as molecular markers quantify genetic links more precisely than other markers, they have been recommended for a trustworthy and accurate investigation of genetic variation as well as an evaluation of the intra- and interspecific interactions. Because they guarantee unambiguous identification, they can be applied to phylogenetic relationships, genomic organization studies, transformant characterisation, germplasm characterization, and genetic diagnostics. Their ability to differentiate between phenotypically identical but strikingly near cultivars is based on variances in

their genetic variants. Moreover, they are independent of climatic conditions and can be applied at any stage of plant growth.

As the advancement in molecular markers develops, new molecular marker technologies such as Restriction Fragment Length Polymorphisms (RFLPs), Amplified Fragment Length Polymorphism (AFLP), Randomly Amplified Polymorphic DNA (RAPD), mini and microsatellite probes, Simple Sequence Repeat (SSR), Inter Simple Sequence Repeat (ISSR), Single sequence polymorphism (SNP), Start Codon Targeted polymorphism (SCoT) have been used for identification. After exploiting several molecular markers for rose cultivars in many laboratories, RAPDs were found to be the best and simple and can be used for identification, certification, and patent protection of roses. Utility of RAPDs in rose were documented in several studies [5,6,7,8,9] owing to the following advantages: no requirement for DNA probes and sequence information for the design of specific primers, higher frequency of polymorphism, rapidity, technical simplicity, requirement of a few nanograms of DNA and low cost assay procedure [10].

However, there is little genetic diversity data available for the locally grown rose types in Tamil Nadu, India, which prevents them from being further improved genetically. Therefore, the goal of the current study was to use RAPD to assess and analyze the genetic diversity among the 11 elite rose accessions that are primarily grown in South India. In addition to providing a germplasm base for upcoming rose breeding initiatives, the research results will be helpful in selecting the varied parents for more genetic improvement.

2. MATERIALS AND METHODS

2.1 Plant Materials

A total of 11 accessions of Rosa sp. (Table 1) including 5 IIHR varieties and 6 local types that were collected and maintained at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India were used for the present investigation.

2.2 RAPD Analysis

Genomic DNA was isolated from the young leaf samples using modified CTAB (Cetyl Trimethyl Ammonium Bromide) method [11]. The extracted DNA was confirmed for its integrity using 0.8% agarose gel and visualized by ethidium bromide staining under UV transilluminator. Ten different decamer oligonucleotides derived from Operon series (OP) sequences were used in this study (Table 2). Polymerase Chain Reaction (PCR) was performed in a 10 µl reaction mixture containing 1 µl of 2.5 µmol/L primer, 1 µl of DNA template, 5 µl of 1X PCR Mastermix

Table 1. Details of rose accessions used in this study

ACC. No	Name of the accessions	Location of collection				Flower color
		Collection site	Latitude (°N)	Longitude (°E)	Altitude (m)	
1	Arka Parimala	IIHR, Bengaluru	13.134891	77.496005	862	Strong purplish red
2	Arka Pride	IIHR, Bengaluru	13.134891	77.496005	862	Vivid reddish orange
3	Arka Savi	IIHR, Bengaluru	13.134891	77.496005	862	Vivid purplish red
4	Arka Sinchana	IIHR, Bengaluru	13.134891	77.496005	862	Vivid red
5	Arka Swadesh	IIHR, Bengaluru	13.134891	77.496005	862	Vivid red
6	Seven Days Rose	Coimbatore	10.969899	76.956024	988.46	Pinkish white
7	Scent Pink	Coimbatore	10.969899	76.956024	988.46	Vivid purplish pink
8	Mirabel Red	Hosur	12.728469	77.856160	848.86	Vivid reddish orange
9	Roman Red	Hosur	12.482216	77.662249	988.46	Strong red
10	Roman Yellow	Hosur	12.482216	77.662249	988.46	Brilliant greenish yellow
11	Mookuthi Yellow	Hosur	12.728469	77.856160	848.86	Light greenish yellow

(SmartPRIME Mastermix 2X) and 3 µl of double distilled water. For DNA amplification, Proflex - PCR Thermal cycler was employed with the following PCR Profile: initial denaturation at 95°C for 90s, followed by 35 cycles of 40s at 94°C, 60s at 37°C, 90s at 72°C, and final extension of 5 min at 72°C. PCR amplified products were subjected to electrophoresis using 3% agarose gels along with 100-bp DNA ladder (Puregene DNA ladder 3X) and visualized by ethidium bromide staining under UV trans-illuminator.

Table 2. List of RAPD Primers used in this study

S.No	Name of Primer	Primer sequence (5'- 3')
1	OPC 05	GATGACCGCC
2	OPC 06	GAACGGACTC
3	OPD 02	GGACCCAACC
4	OPE 03	CCAGATGCAC
5	OPF 07	CCGATATCCC
6	OPT 05	GGGTTTGGCA
7	OPT 07	GGCAGGCTGT
8	OPT 17	CCAACGTCGT
9	OPT 20	GACCAATGCC
10	OPS 11	TGCTCTGCCC

2.3 Data Analysis

The size of the amplified products was measured by comparing with the known size of DNA ladder. RAPD fragments were scored as 1 for presence and 0 for absence of band in the agarose gel and if there was any ambiguity, it was scored as missing data. The discriminatory power of bands was evaluated by polymorphic information content (PIC) and Marker index (MI) as proposed by Powell et al. [12]. PIC was calculated by using GeneCalc by following the default procedure (https://gene-calc.pl/pic). MI was calculated using the following formula:

MI= [PIC × (Number of polymorphic bands ÷Total number of bands)] × Number of polymorphic bands

The binary matrix, constructed using the RAPD scoring data, was used to calculate a genetic distance matrix using Dice's coefficient. The dendrogram was constructed using the software NTSYS-pc ((Numerical Taxonomy and Multivariate Analysis System, version 2.2) based on the Un weighted pair group method with arithmetic mean algorithm (UPGMA) generated using the SAHN module in the NTSYS pc 2.2 software and the resulted dendrogram was analysed further to identify the genetic similarity among the investigated rose accessions.

3. RESULTS AND DISCUSSION

A total of 44 different PCR products were generated from the investigated rose accessions using the 10 RAPD primers (Table 3). Out of which, 39 (88.64%) were found to be polymorphic and remaining five (11.36%) were monomorphic bands. The number of amplified fragments per primer was varied from 3 to 8 with a mean of 4.4 bands per primer. Maximum number of bands (8) was produced by primer OPC 06 while the minimum number of bands (3)

Table 3. Details of banding pattern and discriminative statistics obtained with RAPD markers

Primers	Mono-morphic bands per primer	Poly-morphic bands per primer	Total amplified bands per primer	% Polymorphic bands per primer	Range of molecular weight of bands (bp)	PIC*	MI$
OPC 05	1	3	4	75	150-700	0.489	1.1
OPC 06	0	8	8	100	200-800	0.5	4
OPD 02	0	3	3	100	200-500	0.5	1.5
OPE 03	0	5	5	100	150-600	0.5	2.5
OPF 07	1	4	5	80	200-1200	0.493	1.577
OPT 05	0	5	5	100	200-700	0.5	0.842
OPT 07	2	3	5	60	200-800	0.468	1.5
OPT 17	0	3	3	100	300-600	0.5	0.64
OPT 20	1	2	3	66.67	200-300	0.48	1.5
OPS 11	0	3	3	100	450-800	0.5	1.5
Total	5	39	44	881.67	-	4.93	16.66
Mean	0.5	3.9	4.4	88.17	-	0.49	1.67
Range	0 – 2	3 - 8	3 – 8	60 - 100	150 – 1200	0.468-0.5	0.64-1.5

**PIC, Polymorphic information content; $MI, Marker index*

was produced by primers OPD 02, OPT 17, OPT 20, OPS 11. The molecular weight of the RAPD markers generated in this study was ranged from 150 to 1200 bp (Table 3). Primer GLD-20 produced the maximum number of bands (10) and primer GLC-02 produced the most minimal number of bands (2). In the study conducted by Azeem et al. [13], primers GLA-03, GLA-05, GLA-07, GLA-10, GLC-02, GLC-06, GLC-08, GLC-10, OPG-11, and OPE-19 created 100% polymorphic bands, but primer GLB-11 produced only 42.85% polymorphic bands. These primers yielded the highest polymorphism.

Cluster analysis was accomplished using the binary matrix data obtained from presence or absence of RAPD fragments and dendrogram was drawn. The dendrogram generated in this study distinguished the investigated rose accessions into two major groups: A and B. Group A comprised Arka parimala, Arka savi, Mirabel Red, Mookuthi yellow, Roman red, Roman yellow with two subclusters. Group B consisted of Arka pride, Seven days rose, Arka sinchana, Arka swadesh, Scent pink with two subclusters (Fig. 1). Similarity coefficient values based on Dice's coefficient ranges from 0.59 to 0.89.

The knowledge on genetic variation and inter and intra-specific relationship is essential for devising sound strategies to maintain any germplasm conservations. In the investigation reported here, RAPD markers were used to examine the relationship among the 11 elite rose accessions collected from different locations. RAPD markers have the potential for identification of clusters and characterization of genetic variation within the closed related cultivars. In this study, even though it employed 10 RAPD primers, they recorded a mean of 88.63 % polymorphism among the investigated rose accessions. Such result is in agreement with previous studies on rose genotypes, which have also revealed higher level of polymorphisms (for example, 98.5% among the rose cultivars [14] and 87.5% among Indian rose cultivars and fragrant roses [15]. Morphological and genetic study of the Kakinada rose revealed that it clusters with *R. damascena* var. Himroz, a local variation from South India [16]. A great degree of variation was found in the rose germplasm according to both morphological and molecular analyses, making it a priceless source of genetic diversity for improving roses.

Genetic distance was shown to be quite significant among the rose species, according to Korkmaz and Dogan [17]. The morphological characteristics, phytogeographical locations, and evolutionary relationships of the Rosa species were shown to be closely related genetically.

It has also been noticed in the present study that maximum polymorphism (100% polymorphic bands) was generated by RAPD primers OPC 06, OPD 02, OPE 03, OPT 05 and OPS 11 whereas the primer OPT 07 generated only 60% polymorphic bands (Table 3). Such relatively higher level of identified polymorphism may reflect the diverse genetic pool of investigated

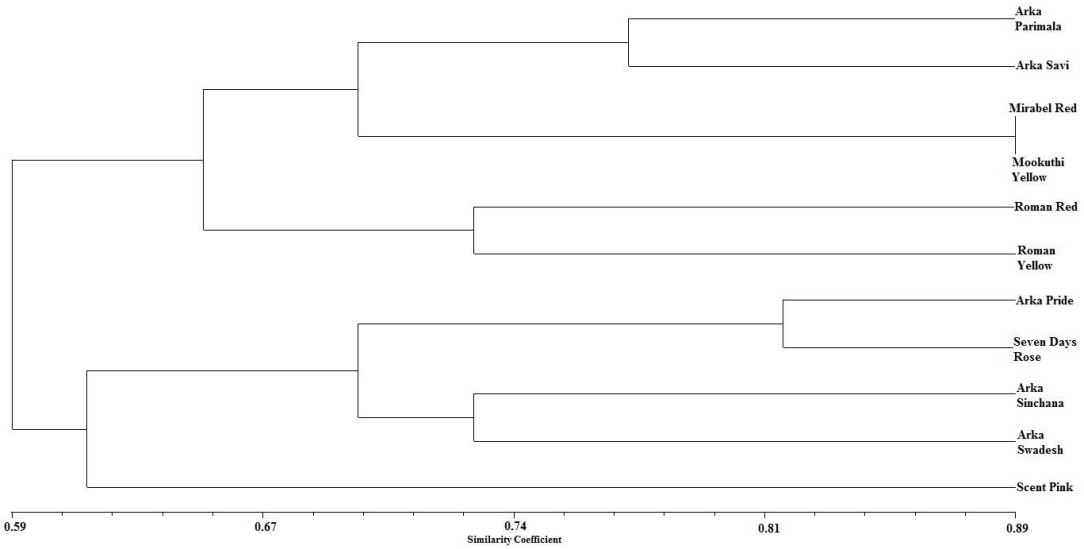

Fig. 1. The genetic diversity among the rose accessions investigated in this study based on 44 RAPD fragments generated by 10 RAPD primers

Rosa spp., and similar results have also been reported using RAPDs in other species [18]. The average polymorphism information content for the DAMD, CDDP, and SCoT markers was 0.36, 0.37, and 0.36, respectively, according to the data, suggesting that the marker types under study were comparable in terms of evaluating diversity. While DAMD marker data classified the variations into three clusters, cluster analysis utilizing SCoT and CDDP separated the varieties into four unique clusters [19].

The dendrogram constructed in this study using molecular data clearly separated the 11 rose accessions, which widely cultivated in South India, into distinct clusters based on their genetic similarities and dissimilarities. The obtained dendrogram was similar to the studies in rose by Mohapatra & Rout [20] who have also employed 10 RAPD primers and classified 34 rose cultivars into 9 clusters. Though, there is a study [21] which reported no genetic variations among rose cultivars even after employing two marker classes *viz.*, AFLP and microsatellite markers (the poor polymorphism rate produced such marker classes might be due to their origination from the conserved regions of the investigated genomes), majority of the reports [20,22] clearly demonstrated that molecular markers can be effectively employed to distinguish the rose cultivars, as shown in this study. A comparison of the difference between the linkage groups suggested that linkage group 5 might include significant QTLs related to winter hardiness. Of the several types of garden roses, linkage group 2 is the most differentiated, while linkage group 6 has the most genetic variety as reported in the study conducted by Vukosavljev et al. [23] in garden rose. Cluster analysis and main coordinate analysis supported the genetic links found by STRUCTURE analysis, according to the study carried out by Mostafavi et al. [24]. There was no correlation between the patterns of grouping and the place of origin. The accessions were not genetically varied as predicted, according to both URP and SCoT molecular markers, raising the likelihood that gene flow took place between populations.

4. CONCLUSION

Molecular characterization of genotypic variations distinguished the investigated rose accessions into different clusters. Such information will be useful for future breeding programs. For example, to design a productive and effective hybridization program, parental genotypes should be selected from different clusters to provide maximum genetic variation for evolution of novel hybrids. This study has revealed that Arka Parimala and Scent Pink were the most distantly related and hence they would be the ideal candidate parental lines for the hybridization program. Thus, this investigation concluded that RAPD is a simple method to document the genetic diversity in rose and would be useful to design productive breeding programs.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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