ASSESSMENT OF GENETIC DIVERSITY AMONG THE ELITE ROSE (ROSA SPP.,) ACCESSIONS USING RAPD MARKERS

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 colour variations. Hence, evolving new cultivars with novel ~~colour~~ 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: Totally 10 RAPD primers were employed, which was sufficient to distinguish the investigated rose cultivars.

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 unweighted 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 ~~colour~~ variations.

Comment [C1]: Color

Comment [C2]: Un weighted

Keywords: Rose; genetic diversity; Randomly Amplified Polymorphic DNA, Oligomers; flower colour; rose germplasm

1. INTRODUCTION

Rose (*Rosa* spp.,), belongs to the family Rosaceae, is the most popular economic flower crop. It is a woody perennial flowering plant grown all over the world, especially in sub-tropical and temperate regions of the northern hemisphere. It is regarded as the “Queen of Flowers” and there are around 200 species and about 18,000 cultivars of roses with exquisite shape, sizes, ~~colour~~ and fragrance. It has been cultivated since ancient civilization of China, Western Asia and Northern Africa and historical evidence have shown their uses for the last 5000 years (Gudin, 2000).

Roses are immensely important for landscaping and every garden is considered as complete in its structure, after augmenting with roses. It occupies the first position in the international flower trade. Besides, it is one of the most important medicinal and aromatic crops and represents a major commodity in the commercial markets of the essential oil industry. The flavor ~~flavour~~ and fragrance of rose is unmatched and greatly utilized in the food and cosmetic industry. Rose petal jam, rose floral tea, rose ready to serve (RTS) drinks and rose wine are the important value-added food

products. Rose oil, rose water, rose concrete and rose absolute are always having great demand in global market as they are the basic raw materials that are used in perfume industries (Venkatesha et al., 2022).

As the commercial cultivation and area under rose production and demand for novel colour variation is increasing year after year, evolving of high yielding rose genotypes is necessary. Consequently, there is a need to evolve better genotypes with high yield and unique ~~colour~~ combinations, which fundamentally require to capture the available rose genetic diversity. Large number of cultivated rose varieties and hybrids are existed in nature due to frequent hybridizations and allopolyploidization which make the classification and the search for relationships between species difficult. For any breeding improvement program, evaluation of genetic diversity among the breeding materials and incorporation of such information into the breeding program would enhance the process of evolving novel cultivars and efficiency of selection program depends on the magnitude of genetic variation and heritability of a trait. Furthermore, genetic diversity of crop species is also useful for the conservation and broadening of genetic resources besides its utility in accurate cultivar identification, which intern help to protect the legal rights of breeders (Veluru et al., 2020).

The assessment of genetic diversity can be done using morphological, biochemical and/or molecular markers. However, morphological, and biochemical markers are not much consistent for diversity analysis since they are influenced by the environmental factors and have wide range of overlaps in many characteristics. And, genetically closer cultivars are difficult to identify with their morphological characteristics thus Lewis (1957) recommended to avoid studying genetic diversity based on the morphological characters alone. Similarly, the small number of consistently resolvable loci generated by the isozyme markers limited its use in rose diversity analysis.

Therefore, for reliable and accurate analysis of genetic variation and to evaluate the intra- and inter specific relationships, molecular markers have been recommended as they measure the genetic relationships more precisely than other markers. They ensure the unequivocal identification and hence can be used for germplasm characterization, genetic diagnostics, characterization of transformants, study of genome organization and phylogenetic relationship. They can distinguish even remarkably close, phenotypically similar cultivars, based on the differences in their genomic variations. Furthermore, they can be used at any stage of plant development and are not influenced by environmental factors.

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 (Torres et al., 1993; Debener et al., 1996; Walker & Werner, 1997; Atienza et al., 2005; Caliskan&Agaoglu, 2009) 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 (Williams et al., 1993).

However, the genetic diversity information available with the regionally employed rose varieties at Tamil Nadu, India is meagre, and it hampers their further genetic improvement. Hence, the objective of the present investigation was to study and compare the genetic diversity among the 11 elite accessions of rose that are predominantly cultivated in South India using RAPD. The findings of the research will be useful for choosing the diverse parents for further genetic improvement besides forming a germplasm base for future rose breeding programmes.

2. MATERIAL AND METHODS

2.1 Plant materials

Totally 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 (CetylTrimethyl Ammonium Bromide) method (Doyle & Doyle, 1987). 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 (SmartPRIMEMastermix 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.

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., 1996. PIC was calculated by using GeneCalc by following the default procedure (https://gene-calc.pl/pic). MI was calculated using the following formula:

$$MI = [PIC \times (Number\ of\ polymorphic\ bands \div Total\ number\ of\ bands)] \times Number\ of\ polymorphic\ bands$$

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 unweighted 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

Totally 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) 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).

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 Arkaparimala, Arkasavi, Mirabel Red, Mookuthi yellow, Roman red, Roman yellow with two subclusters. Group B consisted of Arka pride, Seven days rose, Arkasinchana, Arkaswadesh, Scent pink with two subclusters (Figure 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 (Rai et al., 2015) and 87.5% among Indian rose cultivars and fragrant roses (Panwar et al., 2015).

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 Rosa spp., and similar results have also been reported using RAPDs in other species (Monte-Corvo et al., 2000).

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 (2005) who have also employed 10 RAPD primers and classified 34 rose cultivars into 9 clusters. Though, there is a study (Baydar et al., 2004) 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 (Mohapatra& Rout, 2005; Babaei et al., 2007) clearly demonstrated that molecular markers can be effectively employed to distinguish the rose cultivars, as shown in this study.

Table 1. Details of rose accessions used in this study

ACC. No	Name of the accessions	Location of collection				Flower colour
		Collection site	Latitude (°N)	Longitude (°E)	Altitude (m)	
1	ArkaParimala	IIHR, Bengaluru	13.134891	77.496005	862	Strong purplish red
2	Arka Pride	IIHR, Bengaluru	13.134891	77.496005	862	Vivid reddish orange
3	ArkaSavi	IIHR, Bengaluru	13.134891	77.496005	862	Vivid purplish red
4	ArkaSinchana	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

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

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**

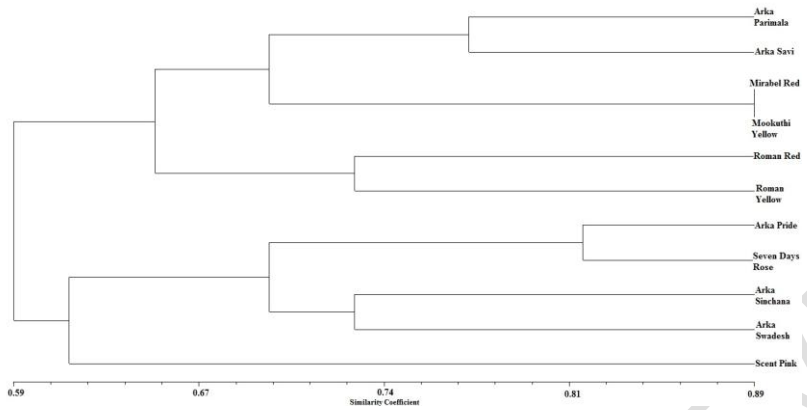

Fig. 1.The genetic diversity among the rose accessions investigated in this study based on 44 RAPD fragments generated by 10 RAPD primers

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 ArkaParimala 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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