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Characterization and analysis of genetic diversity among different species of rose (*Rosa* species) using morphological and molecular markers

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ABSTRACT

The present studies were undertaken to find out genetic diversity among twenty three genotypes of rose (*Rosa* species) using morphological and Random Amplified Polymorphic DNA (RAPD) markers. Morphological characterization was done for 16 different morphological traits and genetic diversity of rose species based on morphological characters was determined using Jaccard's pair wise similarity coefficient. *R.damascena* var. Rani Sahiba and *R. moschata* showed highest diversity. Molecular characterization was carried out using 50 random amplified polymorphic DNA (RAPD) primers, out of which 26 produced 168 polymorphic fragments. Analysis of genetic similarity based on Jaccard's pair wise similarity coefficient showed that *R. moschata* and *R. tomentosa* has the highest similarity. There was good concurrence between morphological and molecular characterization with considerable diversity in *R. damascena*. *R. damascena* var. Rani Sahiba and *R. damascena* var. Himroz have shown divergence from *R. damascena* var. Jwala. The members of the Section Indicae; *R. indica* var. *odorata, R. indica major, R. chinensis viridiflora* and *R. bourboniana* were also grouped together by molecular characterization. Kakinada rose, a local variety of South India was found to cluster with *R. damascena* var. Himroz through both morphological and molecular characterization. Both morphological and molecular analysis showed a high degree of variation in the rose germplasm which is an invaluable source of genetic diversity for rose improvement.

Key words: Cluster analysis, Random Amplified Polymorphic (RAPD) DNA, *Rosa* species

Rose (*Rosa* species) occupies first position in international flower trade. Its popularity as a garden plant, cut flower and as a source of essential oil makes it very important among the ornamental plants. They have been cultivated for the last 5000 years during ancient civilization of China, Western Asia and Northern Africa (Gudin 2000). The genus *Rosa* belongs to *Rosaceae* and contains approximately 150 species (Quest-Ritson and Quest-Ritson 2003). From many of the wild species, the large number of cultivated varieties and hybrids were developed. Frequent hybridizations and allopolyploidization have occurred, which make the classification and the search for relationships between species difficult (Zhang and Gandelin 2003). The genetic diversity available within *Rosa* species is so huge and its potential for utilization in the rose improvement depends on the systematic characterization of the genetic resources and on the study of possible hybridization mechanisms. Morphological markers are the phenotypic traits of any organism and are the earliest markers used to describe the observable characters of an organism. Because each species of the genus *Rosa* has a wide and overlapping range of morphological variations that are influenced by environmental conditions, classification based on morphology alone is not adequate (Lewis 1957). Chemotaxonomic studies of roses (Mikanagi *et al*. 1993) based on the wide range of variant polyphenolic compounds and isozyme markers have also been used for rose identification and classification (Kim and Byrne 1996, Kuhns and Fretz 1978, Lee and Kim 1982, Walker and Werner 1997). However, the small number of consistently resolvable loci (Kim 1994, Kim and Byrne 1994) limits the utility of these markers. The molecular approach is more effective, because it allows direct access to the hereditary material (the genome) and makes it possible to understand the relationships between plants (Williams *et al.* 1990). The advent of molecular markers, e.g. restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), mini and microsatellites, has provided a

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different approach to identify and study genetic diversity. These markers have been used to classify plants that cannot be identified by morphological characters. RAPD markers have proven to be a powerful tool for investigating genetic variation in various plant groups. Estimates derived by those RAPD are very similar to those of other methods (AFLP and ISSR) and may be directly comparable (Nybom 2004).

The present study was carried out for systematic characterization of the rose species germplasm in India with the help of morphological and molecular markers.

MATERIALS AND METHODS

The planting material utilized for the present study consisted 23 genotypes of rose including 6 cultivars, 1 interspecific hybrid and 16 wild species (Table 1).

The rose germplasm was characterized for 16 different morphological characters, namely, plant growth habit, young shoot anthocyanin colouration, plant height (cm), young shoot hue of anthocyanin coloration, prickles, prickles: predominant colour, prickles: shape of lower side, leaf: intensity of green colour, leaf: glossiness of upper side, leaf length, leaflet serration of margin, terminal leaflet: length, terminal leaflet: breadth, terminal leaflet: shape of the base, leaflet pubescence and type of stipule.

Total genomic DNA was extracted from 0.2 gram of young and healthy leaves using C-TAB (CetylTrimethyl Ammonium Bromide) method (Doyle and Doyle 1990).

Polymerase chain reaction (PCR) condition was optimized for rose DNA to obtain reproducible amplification with RAPD. The amplified fragments were resolved on 1.2 per cent agarose (Agarose SFR™, amresco®) gel containing the nucleic acid stained with ethidium bromide using 10X TBE buffer (89 mMTris base, 89 mM Boric acid, 2mM EDTA pH 8.0). DNA fragments were visualized under UV light and photographed using gel documentation system (Alphaimager HP, Cell Biosciences, USA).

Morphological data was analyzed using multivariate "cluster analysis" with the help of NTSYS-pc Version 2.02. Jaccard's pair wise similarity coefficient was used to determine genetic diversity and 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). Similarity index values for RAPD patterns were calculated for all the possible pair wise comparisons, using Jaccard's (genetic) similarity coefficient. The resolving power (Prevost and Wilkinson 1999), polymorphism information content (PIC) (Lynch and Walsh 1998) and marker index (MI) as proposed by Powell *et al.* (1996) were also calculated. Computation for multivariate analysis was done using NTSYS-pc Version 2.02 software (Rohlf 2000) and similarity matrix was subjected to the cluster analysis of Unweighted Paired Group method using Arithmetic Averages (UPGMA) and dendrogram was constructed.

Table 1 Details of rose genotypes used in the study

Species	Source
Rosa nitida × *R. rugosa*	NBPGR, Regional Station Shimla
R.slancensis	NBPGR, Regional Station Shimla
R.indica major	NBPGR, Regional Station Shimla
R. brunonii	NBPGR, Regional Station Shimla
R. macrophylla	NBPGR, Regional Station Shimla
R. wichurana	NBPGR, Regional Station Shimla
R. moschata	NBPGR, Regional Station Shimla
R. tomentosa	NBPGR, Regional Station Shimla
R. lutea	NBPGR, Regional Station Shimla
R. rubrifolia	NBPGR, Regional Station Shimla
R. damascena var. Himroz	IARI, New Delhi
R. damascena var. Jwala	IARI, New Delhi
R. dumalis	NBPGR, Regional Station Shimla
R. chinensis viridiflora	IARI, New Delhi
Rosa spp. (Kakinada rose)	IARI, New Delhi
Dr. Huey (Hybrid Wichurana)	IARI, New Delhi
Rosa spp.(Rose Sherbet)	IARI, New Delhi
R. bourboniana	NBPGR, Regional Station Shimla
R. damascena var. Rani Sahiba	IARI, New Delhi
R. indica var. *odorata*	IARI, New Delhi
R. hybrida	IARI, New Delhi
R. glutinosa	NBPGR, Regional Station Shimla
R. multiflora	NBPGR, Regional Station Shimla

RESULTS AND DISCUSSION

Morphological characterization

Jaccard's pair wise similarity coefficient ranged from 0.15 to 0.93. *R.damascena* var. Rani Sahiba and *R. moschata* showed highest diversity with coefficient index of 0.15. *R. hybrida* and *R. bourboniana* showed the highest similarity with similarity coefficient of 0.93. Cluster analysis based on average linkage between groups divided the species into four different clusters (Fig 1). Dr. Huey was found to be the most distinct from all other rose genotypes. There is a significant diversity in the species *R. damascena*. The variety Jwala was found to fall in a different cluster, whereas the varieties Himroz and Rani Sahiba clustered together. The species *R. moschata* in contradiction to Rehder's classification was observed to be close to *R. damascena* and *R. chinensis* and dissimilar from other group members like *R. brunonii, R. multiflora* and *R. wichurana* of Section 7 (Indicae). Kakinada rose which is a local loose flower variant cultivated in South India is showing similarity with Himroz (*R. damascena*) and *R. bourboniana.*

R. glutinosa, R. tomentosa and *R. dumalis* of section Caninae clustered together in cluster 3 along with *R. indica, R. multiflora* and Rose Sherbet, which is in contradiction to Rehders classification. *R. bourboniana* originated before 1819 through hybridization between *R. chinensis* and *R. damascena* as per Rehder's system (1940). By crossing this hybrid with the different roses of Gallicae group, the hybrid bourbon roses have originated and these crossed with *R. chinensis* and its varieties have produced the Hybrid Perpetuals or Remontant roses. The Hybrid Perpetuals crossed with Tea roses have given birth to Hybrid Teas (*R.*

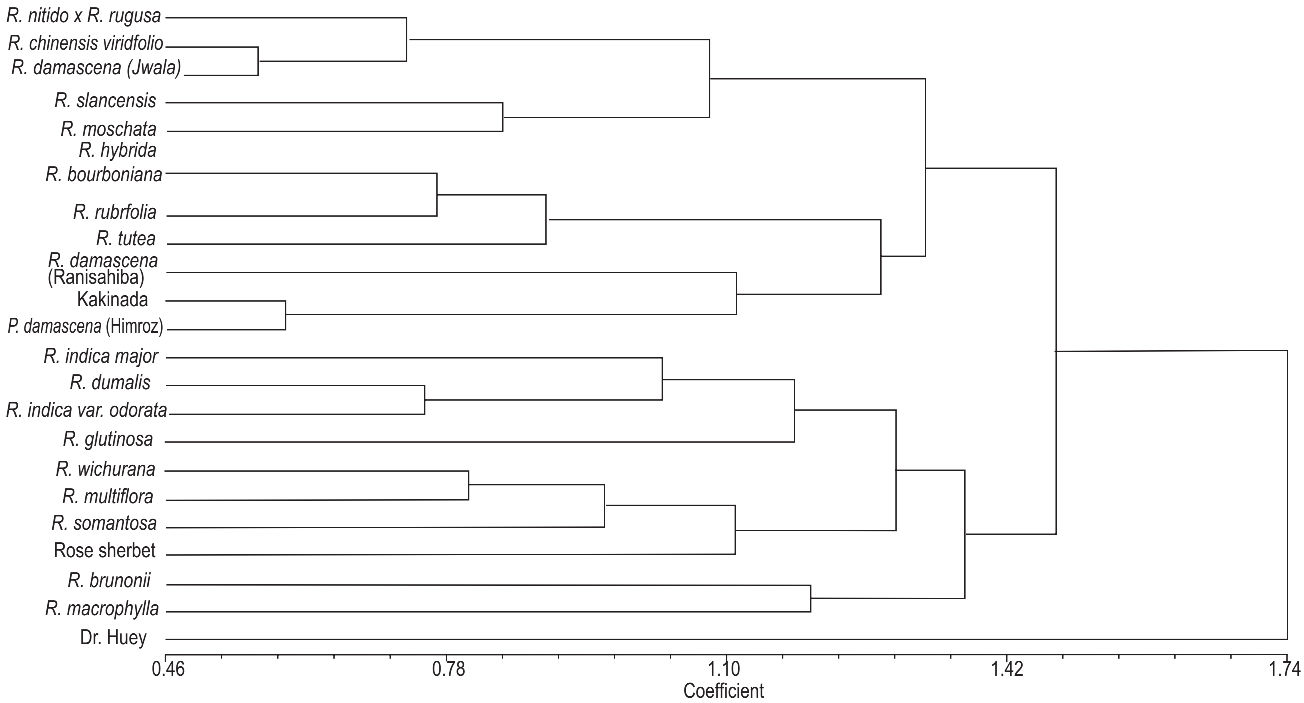

Fig 1 Dendrogram based on the similarity index values of rose species using morphological markers.

hybrida). The highest similarity between *R. hybrida* and *R. bourboniana* is indicating preponderance of *R. bourboniana* blood in modern roses. *R. brunonii* (Himalayan musk rose) and *R. macrophylla*, both originating from India, showed high similarity and clustered together in the same group. Dr. Huey, which is a Hybrid Wichurana, was observed to show a lot of variation from its ancestor *R. wichurana* and the rest of the species. This is the only one thornless rose used in the present study.

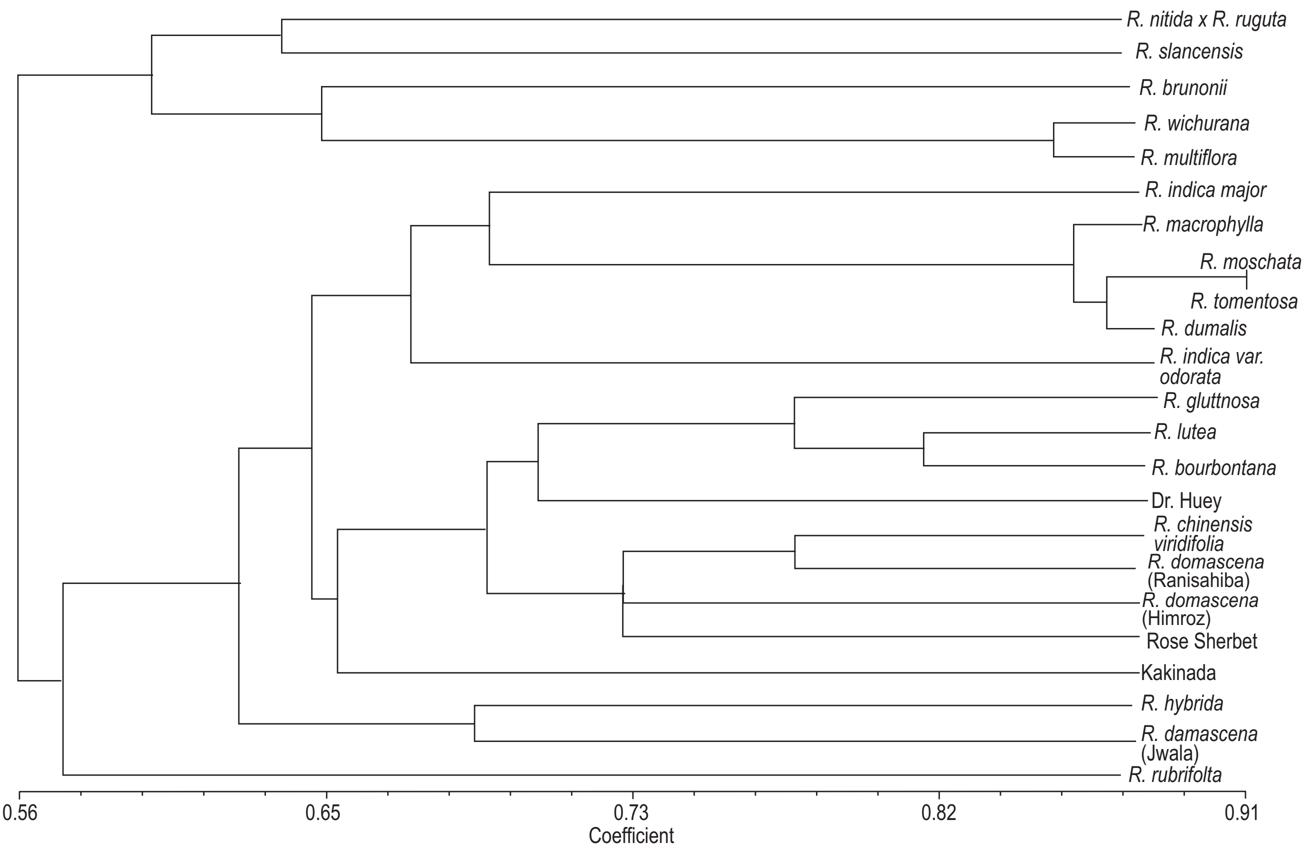

Fig 2 Dendrogram based on the similarity index values of rose species using RAPD markers

Table 2 Details of banding pattern and discriminative statistics of RAPD markers

Primers	Sequence (52 -32)	Total bands	Polymorphic bands	Per cent polymorphism	PIC	RP	MI
OPR-08	CCCGTTGCCT	10	10	100	0.29	4.53	0.026
OPR- 09	TGAGCACGAG	8	8	100	0.25	2.44	0.031
OPR-10	CCATTCCCCA	6	6	100	0.42	3.92	0.069
OPH-13	GACGCCACAC	4	4	100	0.26	1.48	0.064
OPN-11	TCGCCGCAAA	3	2	66.6	0.21	0.79	0.068
OPJ-17	ACGCCAGTTC	6	6	100	0.29	2.35	0.048
OPR-13	GGACGACAAG	6	6	100	0.41	3.67	0.068
OPN-16	AAGCGACCTG	9	9	100	0.42	5.57	0.046
OPO-02	ACGTAGCGTC	6	6	100	0.43	4	0.071
OPC-07	GTCCCGACGA	5	5	100	0.24	1.48	0.047
OPA-02	TGCCGAGCTG	8	8	100	0.39	4.35	0.048
OPD-01	ACCGCGAAGG	9	9	100	0.45	5.74	0.049
OPR-06	GTCTACGGCA	5	5	100	0.58	3.39	0.115
OPR-01	TGCGGGTCCT	6	6	100	0.31	2.86	0.051
OPR-02	CACAGCTGCC	6	6	100	0.39	3.14	0.066
OPB-13	TTCCCCCGCT	10	10	100	0.51	7.05	0.051
OPR-17	CCGTACGTAG	4	4	100	0.37	1.92	0.092
OPC-19	GTTGCCAGCC	4	4	100	0.36	1.74	0.088
OPB-12	CCTTGACGCA	6	6	100	0.38	2.34	0.064
OPD-11	AGCGCCATTG	11	11	100	0.38	6.08	0.035
OPG-14	GGATGAGACC	5	5	100	0.47	3.13	0.093
OPR-04	CCCGTAGCAC	7	7	100	0.32	1.82	0.045
OPR-12	ACAGGTGCGT	6	6	100	0.47	4.26	0.078
OPR-03	ACACAGAGGG	4	4	100	0.44	2.34	0.10
OPY-17	GACGTGGTGA	4	4	100	0.32	2	0.080
OPAA-02	GAGACCAGAC	11	11	100	0.44	7.04	0.040
		169	168	98.54	9.8	89.43	1.633
Mean		6.5			0.38	3.4	0.062

PIC: Polymorphic Information Content, RP: Resolution power of primer; MI: Marker Index

Molecular characterization

Out of the total 50 RAPD primers screened, a set of 26 primers were found to produce polymorphic and reproducible fragments, which were used for further analysis. PCR amplification of the DNA isolated from 23 genotypes yielded a total of 169 amplified products, of which 168 were polymorphic and only one, was monomorphic (Table 2). The total number of amplified DNA fragments varied between 3 and 11 with an average of seven bands per primer.

The polymorphism percentage ranged from 66.6% to as high as 100% for the other 25 primers. The polymorphic information content (PIC) ranged from 0.21 to 0.58 with an average of 0.38. PCR amplified fragments resolved on 1.2 per cent agarose gel.

Genetic diversity and relationship based on RAPD markers

Genetic similarity between the rose species was determined on the basis of Jaccard's pairwise similarity coefficient. *R. moschata* and *R. tomentosa* showed highest similarity while *R. lutea* and *R. nitida* × *R. rugosa* showed the highest diversity.

Based on UPGMA (Unweighted Pair Group Method with Arithmetic Mean), *Rose* species were broadly divided into 4 main clusters. Cluster 1 consists of *R. nitida* × *R. rugosa* and *R. slancensis.* Cluster 2 consists of *R. brunonii, R. wichurana* and *R. multiflora.* Cluster 3 consists of the larger clusters (15 species) which includes *R. indica major, R. macrophylla, R. moschata, R. tomentosa, R. dumalis, R. indica* var. *odorata, R. glutinosa, R. lutea, R. bourboniana,* Dr. Huey, *R. chinensis viridiflora, R. damascena* var. Rani Sahiba, *R. damascena* var. Himroz, Rose Sherbet and Kakinada Rose. Cluster 4 contains *R. hybrida* and *R. damascena* var. Jwala. *R. rubrifolia* did not formed group with any other species (Fig 2).

The members of Synstylae section, *R. brunonii, R. multiflora* and *R. wichurana* fell in the same cluster 2 which is in agreement with Rehder's classification and was also reported earlier by Moreno *et al.* (1996). However in contradiction *R. moschata* of the same section has shown divergence from these and fell in cluster 3. *R. moschata* was found to show high similarity with *R. tomentosa* of section Caninae. *R. tomentosa, R. glutinosa* and *R. dumalis* of Section Caninae, fell in the same cluster 3 showing strong concurrence with Rehder's classification. However *R. rubrifolia* of the same section has shown strong divergence from the species of the same section and other species. *R. rubrifolia* was separate from all the species. *R. macrophylla* and *R. moschata* orginiating from India were observed to have high similarity; however *R. brunonii*, another Indian originated species fell in a separate cluster 3. The member of the Section Indicae, *R. indica* var. *odorata, R. indica major, R. chinensis viridiflora* and *R. bourboniana* fell in the same cluster. *R. hybrida* fell in the same cluster along with *R. damascena* var. Himroz, which is expected as per the classification by Rehder (Wissemann 2003). However the other varieties of *R. damascena* (Jwala and Rani Sahiba) fell in a separate cluster from Himroz. *R. damascena* is a highly diverse species.

Varieties with fragrance and potential for loose flower cultivation like *R. damascena* (Jawala and Rani Sahiba), *R. bourboniana*, Rose Sherbet and Kakinada rose fell in the same cluster. One interesting feature is Rose Sherbet a variety produced as a seedling of 'Gruss an Teplitz' which has been classified variously according to authors as Hybrid China or Bourbon rose is showing closeness to *R. chinensis viridiflora*. Rose Sherbet is a repeat flowering variety confirming the role of *R. chinensis* in its parentage. Dr. Huey, which is a Hybrid Wichurana, has shown a lot of variation from its ancestor *R. wichurana*. *R. nitida*×*R. rugosa* and *R. slancensis* fell in the same cluster both with morphological and molecular characterization.

Characterization by both means revealed considerable diversity in *R. damascena*. *R. damascena* var. Rani Sahiba and *R. damascena* var. Himroz fell in the same cluster and have shown divergence from *R. damascena* var. Jwala in morphological and molecular characterization. Kakinada rose a local variety of South India, the origin of which is not resolved till now, has shown closeness with *R. damascena* var. Himroz by morphological and molecular characterization. Both morphological and molecular analysis showed a high degree of variation among analyzed germplasm indicating an important source of genetic diversity that can be used in the crop improvement like *R. lutea* for source of yellow colour, *R. bourboniana*, *R. damascena* for source of fragrance, Dr. Huey (Hybrid Wichurana) for source for thornlessness, *R. indica* var. *odorata* for source for powdery mildew resistance, *R chinensis* for recurrent flowering, *R. moschata* for musky fragrance, *R. glutinosa* for dwarfness.

CONCLUSION

There exists significant diversity between different species of rose characterized in the present study, which will form an important basis for selection of variability to be used in future rose improvement. It will serve as a reference material in future studies aimed at utilization of these valuable germplasm in rose improvement. Both morphological and RAPD markers were able to group *Rosa* species according to their origin and determine genetic similarities between them. RAPD has been found to be a reliable and a relatively simple procedure to study genetic relationships among *Rosa* species, which can be useful in current rose breeding programs, allowing the identification of new cultivars as well as the assessment of the genetic similarity among different genotypes.

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