DOI : 10.5958/0974-0112.2022.00054.8

Indian J. Hortic. 79(4), December 2022: 387-393

Molecular characterization and relationship among wild and partially cultivated *Rosa* species

Aparna Veluru[*], K.V. Bhat[***], Tolety Janakiram[****], K. V. Prasad[**], D.V.S Raju[**], Namita, Sapna Panwar and K. P. Singh

Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi- 110012, NCT Delhi India.

ABSTRACT

Knowledge of population structure and genetic relationships within and among wild rose (*Rosa* spp.) genotypes is important to enhance its use in breeding and conservation. In the present study, we assessed the diversity among 21 wild roses belonging to 7 sections of subgenera *Rosa*, using morphological (14) and molecular markers (48). The neighbor-joining (NJ) tree clustered the species into two major and four minor clusters based on morphological similarity. Of the used SSRs, 31 SSRs were found to be polymorphic within selected genotypes. The matching co-efficient value calculated for each pair of species revealed the highest (0.91) and lowest (0.386) genetic similarity between species *Rosa brunonii* (*Synstyle*) - *R. indica major* (*Indicae*) and *R. multiflora* (*Synstyle*) - *R. slancensis* (*Caninae*). The phylogenetic chart obtained using molecular data clustered the genotypes in two major clusters. *R. bourboniana* exhibited a distant relationship with the remaining 20 genotypes and clustered separately. All the species from *Gallicanae* and most of the *Indicae* section species were clustered together and showed genetic similarities with each other. Significant variability was noticed among cultivated *R. damascene* selections. A local fragrant loose flower variant namely 'Kakinada Red' which is cultivated widely in southern peninsular India under tropical climate showed genetic nearness with Damascena cv. Ranisahiba. The Bayesian analysis conducted among genotypes showed three distinct genetic groups (ΔK=3). Concerning the genetic variability among different sections, highest and lowest variability values were observed with *Synstyle* and *Pimpinellifoliae,* respectively.

Key words: *Rosa* species, morphological markers, SSR markers, characterization, genetic diversity.

INTRODUCTION

Rose is a commercially important floricultural crop belonging to the family *Rosaceae*. Genus '*Rosa*' comprises around 150-200 species (Wissemann, 15). Wild species are primary sources for creating diversity among existing genotypes and carry valuable alleles with them. India is native to a decent number of rose species, as many as 16 species were found to grow in India in different phytogeographical zones (Rathore and Srivastava, 11). Characterization of wild genotypes and knowing their genetic relationships with other species are important for their utilization in any breeding programmes. Traditionally, germplasm identification and characterization has been done using morphological characters (Veluru *et al*., 14; Panwar *et al*., 6; Gaurav *et al*., 4; Aparna *et al*., 2) in India. However, the development of new techniques has allowed these analyses based on DNA information. Molecular markers can be useful tools to determine genetic variability. Simple Sequence Repeats (SSRs) or Microsatellite markers are ideal markers for assessing genetic variability among species and varieties. In India works have been initiated in the recent years to identify and characterize the rose germplasm belongs to different groups and to check the existing diversity among modern (Hybrid Teas and Floribundas etc.), cultivated, wild, and some of the fragrant genotypes (Aparna *et al*., 1; Gaurav *et al*., 4). But the studies focusing on wild genotypes diversity including the species from southern peninsular India has not been taken up till date. In the current study we have studied the diversity and similarity among available wild and partially cultivated native species belong to different sections of genera "*Rosa*". Information available from this study can be used to take up crosses between important wild species for inclusion of spectacular allele diversity for fragrance, color, resistance towards biotic and biotic stress and nutraceuticals.

MATERIALS AND METHODS

The work was carried out at ICAR-IARI and ICAR-NBPGR, New Delhi during August 2018-February 2020. A total of 21 wild roses were identified

*Corresponding author: aparna.cpcri@gmail.com
**ICAR- Directorate of Floriculture Research, Pune, 411 005, Maharashtra, India
***ICAR- National Bureau of Plant Genetic Resources, Pusa, New Delhi, 110 012, India
**** Dr Y.S.R.H.A.U, Venkataramannagudem, 534 101, West Godavari, Andhra Pradesh

for assessing genetic diversity (Table1). These species were collected and maintained at the Division of Floriculture and Landscape Architecture, ICAR-Indian Agricultural Research Institute, New Delhi, India. Selected germplasm was characterized by 14 different morphological characteristics, namely, plant growth habit, plant height (cm), young shoot: Anthocyanin colouration (present/absent), Hue of anthocyanin colouration in young shoots, prickles on the stem: present/absent, prickles: predominant colour, prickles: the shape of lower side, leaf: intensity of green colour, leaf: glossiness of upper side, leaf length (cm), leaflet: serration of margin, terminal leaflet: the shape of the base, leaflet pubescence and type of stipule.

Total genomic DNA was extracted using the CTAB method. All 21 species were analysed using 48 SSR primers (Kimura *et al*., 5; Yan *et al*., 16). Of those, 31 primers showed polymorphism. Primer sequence information, repeat motifs, annealing temperatures of the primers and sizes of amplified DNA fragments were shown in Table 2. DNA amplification was carried out in a 25 µl reaction volume containing, 1.5 µl $MgCl_2$ (25 mM), 2.5 µl Taq DNA buffer (10X), 2 µl of dNTPs (20 mM), 1µl of each forward and reverse primers (1 µM), 0.33 µl of Taq DNA polymerase (5 U/ µl) (Genei, India), 2 µl of template DNA (20 ng/µl) and 14.7 µl autoclaved distilled water. The amplification reaction was carried out at 94˚C for 6 minutes, followed by 35 cycles of denaturation at 94˚C for 1 minute, annealing at 48˚C-55˚C (based primer annealing temperature) for 1 minute and 72˚C for 2 minutes and final extension at 72˚C for 10 minutes before cooling it to 4˚C. PCR products were determined in 3% agarose using 1X TAE buffer. Amplified fragments size determined using a 100 bp DNA ladder as size standard and SSR profiles were visualized using Alpha Digi Doc Gel Documentation System.

Table 1. Details of wild rose species used in the study.

Section/ population	Genotype
Group-1 (*Caninae*)	*R. dumalis* *R. glutinosa* *R. tomentosa* *R. slancensis*
Group-2 (*Cinnamomeae*)	*R. macrophylla*
Group-3 (*Gallicanae*)	*R. damascena* cv. Himroz *R. damascena* cv. Jwala *R. damascena* cv. Rani Sahiba
Group-4 (*Indicae*)	*R. indica* × *R. nitida,* *R. indica major* *R. bourboniana* *R. chinensis viridiflora* *Rose* spp. (Rose Sherbet)
Group-5 (*Pimpinellifoliae*)	*R. lutea*
Group-6 (*Synstyle*)	*R. brunonii* *R. wichuraiana* *R moschata* *R. multiflora* Dr. Huey (Hybrid Wichurana)
Group-7 (Unknown)	*Rosa* spp. (Nepal) *Rosa* spp. (Kakinada Red)

Morphological data were subjected to interval analysis using NTSYS version 2.02. Distance coefficient values were calculated and used to construct a dendrogram using Neighbour Joining (NJ) method. For molecular characterization, bands at each polymorphic SSR marker were noted as either present (1) or absent (0) and these scores for all alleles at each locus were considered allelic phenotypes. Genetic variability parameters for wild genotypes were calculated section-wise using POPGENE 32 version 1.31. The phylogenetic dendrogram was generated with DARwin software using Nei and Li's distances (Perrier and Jacquemoud-Collet, 9). A model-based approach was used to determine the genetic clusters in studied genotypes. Population structure was determined using STRUCTURE HARVESTER [run for 20 replications of each K (2-10), a burn-in period length of 50000-75000 MCMC] which groups the individuals into clusters based on multi-locus genotypic data (Earl and VonHoldt, 3).

RESULTS AND DISCUSSION

The knowledge of existing plant diversity is necessary for the successful management of crop genetic resources. In the present study, morphological data was analysed and a dendrogram was generated using Euclidian distance values (Fig.1). All 21 species were separated into two major clusters and four subclusters. Rose species belong to different sections, [*R. indica major* (*Indicae*), *R. dumalis* (*Caninae*), *R. brunonii* (*Synstyle*), *R. macrophylla* (*Synstyle*) and *R. glutinosa*] were grouped in cluster I based on their morphological similarities. Thornless cultivar Dr Huey exhibited higher morphological similarity with species *R. slancensis* and interspecific hybrid (*R. indica* × *R. nitida*) but was distinct from ancestral species *R. wichuraiana* (Fig.1). Similar results were reported by Rai *et al*. (10) with cv. Dr. Huey and species *R. wichuraiana*. All the selections made from *R. damascena* belong to section *Gallicanae*, majority of genotypes from *Indicae* and *Synstyle* were present in cluster II. As per Rehder's classification system (1940), *R. bourboniana* is believed to be originated

Table 2. Details of polymorphic SSR markers used for study.

SSR	Primer sequences	Source	LG	T_A°C
Rh79	F: TTCTTCTTGCTCGCCATTTTGATT R:GAACGTCCACCACCACCCACTCTG	Yan *et al*. (16)	1	50
RhAB9	F:GTCAATTTGTGCATAAGCTC R:GTGAGAACAGATGAGAAATG	Yan *et al*. (16)	1	50
Rh48	F:GATAGTTTCTCTGTACCCCACCTA R:TTGACCAGCTGCAACAAAATTAGA	Yan *et al*. (16)	2	50
Rh80	F:CATGCCAAACGAAATGAGTTA R:TTATCTAAAGGGCTGCTGTAAGTT	Yan *et al*. (16)	2	50
Rh96	F:GCCGATGGATGCCCTGCTC R: AGATTCCCTGCGACATTCACATTC	Yan *et al*. (16)	2	50
Rh50	F: TGATGAAATCATCCGAGTGTCAG R: TCACTTTCATTGGAATGCCAGAAT	Yan *et al*. (16)	3	50
Rh58	F: ACAATTTAGTGCGGATAGAACAAC R: GGAAAGCCCGAAAGCGTAAGC	Yan *et al*. (16)	3	50
RhABT12	F: CAAGTTTGTCTCCTTGGACC R: CATAGATGATTATCCTAGAGCC	Yan *et al*. (16)	4	50
Rh65	F: AGTACGCCGACGCAGATCCAGTGA R: ACGGCGTTGTAGGTCGTCATTCTC	Yan *et al*. (16)	4	50
Rh78	F: AAAGAAACGCGAAATCTATGATGC R: TCTGGATGGGATTTAAAAGACAGG	Yan *et al*. (16)	4	50
Rh77	F: CAACTGAAAGGAACAAATGGATGT R: GGAATGGCTTGTAAATTTGTGATT	Yan *et al*. (16)	5	50
Rh93	F: GCTTTGCTGCATGGTTAGGTTG R: TTCTTTTTGTCGTTCTGGGATGTG	Yan *et al*. (16)	5	50
RhAB38	F: GAGGTGGTCGATTCCATGTC R: TTACCGTTCTACCTAAGTGACTAAC	Yan *et al*. (16)	5	50
Rh60	F: TCTCTTTTCACGGCCACCACT R: TGAATCCAAGGCCGTATAGTTAGA	Yan *et al*. (16)	6	50
Rh85	F: ACTTTTGGGCGTTCATCGCATTACAC R:GGCTATATGGGCTCAAGTCTAGACAA	Yan *et al*. (16)	6	50
Rh98	F: GGCCTCTAGAGTTTGGGATAGCAG R: ACGACGTCAATAACTCCATCAGTC	Yan *et al*. (16)	6	50
Rh72	F: CCAAAAGACGCAACCCTACCATAA R: TCAAAACGCATGATGCTTCCACTG	Yan *et al*. (16)	7	50
Rh73	F: GGTTAGACGGGTGGAAGAAG R: ACTGCCGATAGAAGTATTTCATCA	Yan *et al*. (16)	7	55
RhAB28	F: GCAGATGTTATTCATGTTAA R: CCAAGTATTTTAGTTTCTTC	Yan *et al*. (16)	7	55
RA013a	F: GAGGGAAAGAGATACACAAA R: GTAAGACCTTGCGTGTTCATA	Kimura *et al*. (5)	-	55
RA023b	F: CATCCTCGGTGTTGCGTTGA R: TGTCTCCAGCAACCTTTTTTTCCC	Kimura *et al*. (5)	-	50
RA043a	F: GCAACGTACTTCAATTTCCAC R: CAAGCTCAGAACTGAGACAC	Kimura *et al*. (5)	-	50

Primer sequences (F: forward and R: reverse), Literature Source for Primers, Band sizes of each amplified DNA fragment, LG: linkage group, T_A: Annealing temperature

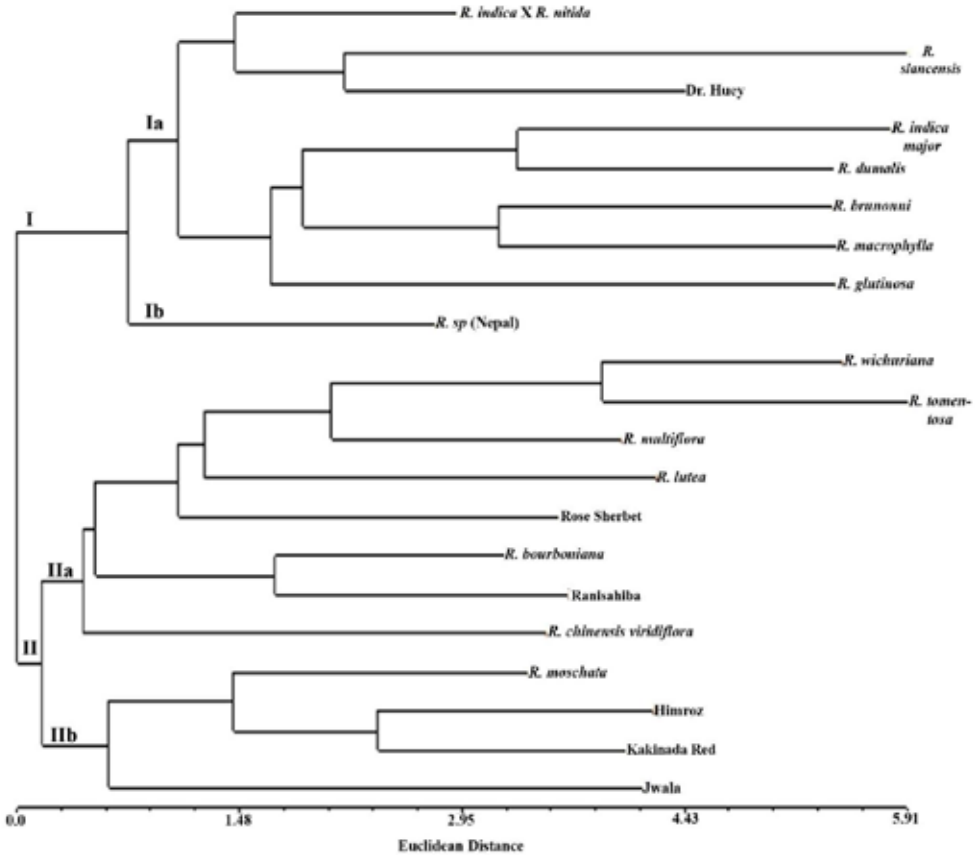

Fig. 1. Neighbor joining tree for the rose species based on variations for morphological characters

from a natural cross between *R. chinensis* and *R. damascene*, while this hybrid is again crossed with *Gallicanae* section members, Hybrid bourbon roses have originated. The presence of *R. bourboniana* along with *R. chinensis* and other *Gallicanae* members in the same cluster strengthens the above system of rose classification and explains the role of these species in the origin of today's modern roses. Kakinada Red, which is a fragrant loose flower cultivar, grown in southern states of India exhibited more similarity with fragrant damask cultivars Himroz and Jwala and Synstyle member *R. moschata* in subcluster II_a.

The diversity among different populations based on calculated genetic parameters (Table 3) was concerned, a relatively higher amount of diversity was noticed in *Indicae* and *Synstyle* populations with higher heterozygosity (H_e, h, and H_o) values and the lowest variability was found in *Pimpinellifoliae* and *Cinnamomea*e populations. Levels of polymorphism were observed among populations ranging from 35.8% (*Pimpinellifoliae*) to 100% (*Gallicanae*, *Indicae* and *Synstyle*). Panwar *et al*. (8) noticed 94% polymorphism with ISSR markers in Indian rose cultivars. Markers used in the present study were more useful for identifying the genotypes belonging to *Gallicanae*, *Indicae* and *Synstyle* sections of sub-genus *Rosa*.

The pair-wise matching coefficient values ranging from 0.55 to 0.92 indicated the occurrence of a considerable amount of diversity in studied species. A phylogenetic dendrogram was generated using marker data, and two major clusters were identified, major cluster I consist of all genotypes except *R. bourboniana* (Fig. 2). Cluster I was further divided into two subclusters, sub-cluster I consist of 18 genotypes and subcluster II consists of two genotypes i. e. *R. damascena* cv. Jwala and *R. chinensis viridiflora*. Subcluster I is further divided into three minor clusters consisting of 10, 3 and 5 genotypes each. Interspecific hybrid (*R. indica* × *R. nitida*) showed higher similarity to indigenous species *R. macrophylla* and *R. slancensis* and these three genotypes were grouped with *R. brunonii* (Himalayan musk rose), *R. indica major* and *R. dumalis* within a minor cluster I. The studies also reveal that *R. moschata* (*Synstyle*) and *R. glutinosa* (*Caninae*) belonged to different sections with more morphological dissimilarity and

had greater resemblance in their genetic makeup. Yellow colour progenitor for modern rose i. e. *R. lutea* and Nepal *Rose* spp. (Nepal) had comparable genetic makeup and were clustered together with *R. moschata* and *R. glutinosa* (Fig.3). All *Caninae* section genotypes except *R. tomentosa* were found together in one minor cluster. Members of the *Indicae* section, *R. chinensis viridiflora*, *R. bourboniana* and

Table 3. Summary of genetic variation statistics parameters for rose populations (section-wise).

Groups		N_a	N_e	I	H_o	H_e	h	Polymorphic loci	% of polymorphic loci
Caninae	Mean	2.58	2.25	0.82	0.45	0.61	0.51	30	96.77
	SD	0.76	0.71	0.31	0.30	0.19	0.16		
Cinnamomeae	Mean	1.45	1.45	0.31	0.45	0.45	0.22	14	45.16
	SD	0.50	0.50	0.35	0.50	0.50	0.25		
Gallicanae	Mean	2.51	2.18	0.80	0.55	0.61	0.51	31	100
	SD	0.62	0.55	0.24	0.35	0.14	0.12		
Indicae	Mean	2.77	2.22	0.85	0.52	0.58	0.52	31	100
	SD	0.71	0.55	0.23	0.33	0.12	0.11		
Pimpinellifoliae	Mean	1.35	1.35	0.24	0.35	0.35	0.17	11	35.48
	SD	0.48	0.48	0.33	0.48	0.48	0.24		
Synstyle	Mean	2.74	2.24	0.85	0.52	0.58	0.52	31	100
	SD	0.81	0.59	0.26	0.32	0.13	0.12		
Unknown	Mean	2.32	1.98	0.69	0.39	0.52	0.43	27	87.1
	SD	0.79	0.68	0.35	0.34	0.24	0.20		

N_a- Observed number of alleles, N_e: Effective number of alleles, I: Shannon's Information index H_o: Observed Heterozygosity, H_e: Expected Heterozygosity, h- Nei's genetic diversity

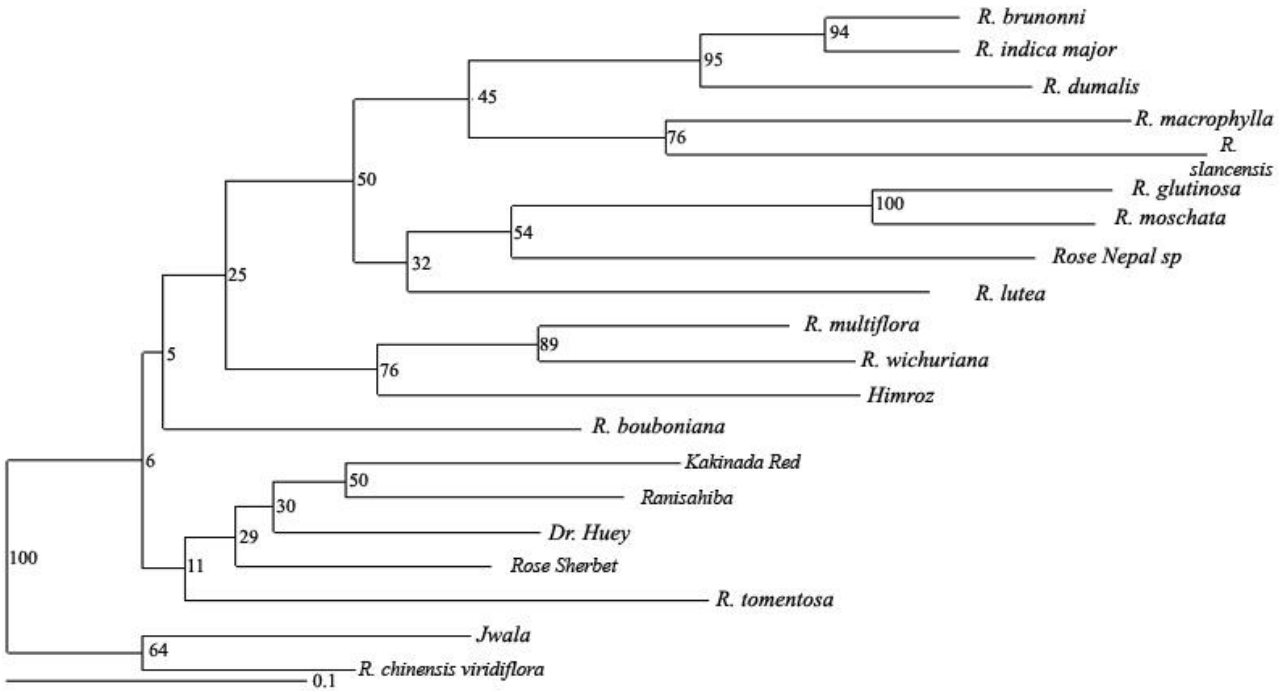

Fig. 2. Neighbor-joining tree based on SSR data for the rose species generated using Darwin software based on genetic distance. Bootstrap values based on 10000 resamplings indicated on branches in percentages, 50% or higher values indicate strong statistical support

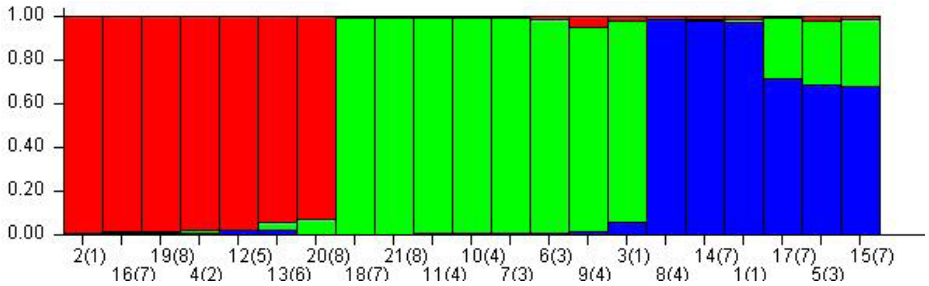

Fig. 3. Population structure of the *Rosa* species arranged based on inferred ancestry (k=3), Colors represent the different subgroups and each bar represents an individual species

cultivar Rose Sherbet fell into the different clusters and exhibited a deviation from the basic botanical classification system of rose developed by Rehder (12). However, the other member of section *Indicae* i.e., *R. indica major* (Bengal Rose hybrid) clustered separately (minor cluster I) and revealed maximum similarity with *R. brunonii.*

The rose species *Rosa moschata* (Musk rose) was usually confused with *R. brunonii* (Himalayan musk rose), a closely related tall stout climber from Himalayan ranges that bears white musky fragrant flowers in the late spring season. In this study, these species were differentiated based on morphological and molecular markers (Fig.1 and Fig. 3). Fragrant rose cultivars with high potential for loose flower production like Rose Sherbet, Kakinada Red and Ranisahiba were grouped within one minor cluster. Though three damascena selections i.e. Himroz, Jwala and Ranisahiba were said to be derived from the ancestor species *R. damascena*, a significant amount of variability was noticed with three of them and were found in three different clusters; selection 'Jwala' was shown to have higher resemblance with green rose *R. chinensis viridiflora*. Selection 'Himroz' was found to have maximum genetic similarity with Synstyle species *R. wichuraiana* and *R. multiflora* whereas Ranisahiba exhibited maximum genetic nearness with 'Kakinada Red', a local variety collected from peninsular India which is grown widely for loose flower production. Cultivars, Rose Sherbet (seeding of Gruss an Teplitz) and Dr Huey (Ethel × Gruss an Teplitz) found together minor cluster III, revealed maximum genetic similarity (matching coefficient 0.81) with each other and this similarity might be due to their common parent "Gruss an Teplitz". The Bourbon rose (*R. bourboniana*), a progeny derived from the natural cross between *R. chinensis* and *R. damascena* (Stuart,13) was found to be clustered with its related ancestral species *R. chinensis viridiflora* and *R. damascena* cv. Jwala.

The genetic architecture of various germplasm lines can be estimated by assessing the STRUCTURE of the populations using molecular data. Structure analysis divided the 21 wild rose genotypes into three population subgroups (ΔK=3) (Fig.3). Species grown widely for the production of loose flowers and aromatic compounds in India (Jwala, Ranisahiba, Rose sherbet, Kakinada Red, Dr Huey, Bourbon rose) were structured in population subgroup I along with species *R. tomentosa and R. chinensis viridiflora*, representing the share of genetic background from species *R. tomentosa, R. chinensis* and *R. bourboniana* in damask rose cultivars. Population subgroup II consists of floribunda ancestral species (*R. wichuraiana*, *R. multiflora*), Himalayan musk rose *R. brunonii*, *R. indica major* and *R. dumalis* along with fragrant cultivar Himroz. Damask rose cv. Himroz was varied from other cultivars Jwala and Ranisahiba and showed higher genetic similarity with *R. multiflora* and *R. wichuriana* species, almost similar results were also noticed with a phylogenetic dendrogram (Fig. 3). Rose species distributed in higher ranges of Himalayas (*R. moschata*, *R. macrophylla*) and Kashmir (*R. lutea*) along with interspecific hybrid (*R. indica* × *R. nitida*) and species *R. glutinosa*, *R. slancensis* were grouped separately in population subgroup III and this was also evident from its morphological data of the respective genotypes (Fig.1). The variability noticed among wild roses in the study could be utilized for future breeding programmes.

AUTHORS' CONTRIBUTION

Conceptualization of research (JT&KVP); Designing of the experiments (AV, KVB&KVP); Contribution of experimental materials (DVSR, N & SP); Execution of field/lab experiments and data collection (AV& KPS); Analysis of data and interpretation (KVB&AV) PKA), Preparation of the manuscript (AV&KVB).

DECLARATION

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The author (AV) is thankful to the Indian Council of Agricultural Research for providing fellowship and facilities during research work and thanks the Head, ICAR-NBPGR Regional station, Shimla (H.P) for sharing germplasm.

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Received : October, 2021; Revised : October, 2022
Accepted : November, 2022