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RESEACH ARTICLE

Molecular Characterization of Garden Rose Germplasm using SSR Markers

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Abstract

A set of 205 garden rose germplasm consisting of 87 hybrid tea, 111 floribundas, and seven wild species were evaluated using 20 simple sequence repeat (SSR) primers. A total of 109 bands were generated from 205 rose cultivars. The average polymorphism for 205 cultivars tested with 20 SSR primers was 88%. On average,1.78 unique alleles (Na) and 1.46 expected number of alleles (Ne) were discovered. The genetic diversity analysis showed an excess of observed heterozygosity over expected. AMOVA revealed that 98% of total molecular variance occurred within groups, with only 2% occurring between groups. Principal coordinate analysis and cluster analysis were carried out to investigate the genetic relatedness of cultivars. The scatter plot did not reflect the grouping of cultivars used for analysis, indicating that the cultivars of different garden rose classes are related to each other. A dendrogram is also produced to better understand the presence of a grouping pattern and three major clusters were found. However, the clustering pattern does not correspond to the horticultural classifications that group accessions. The study suggests higher genetic similarity among the rose cultivars across different classes of garden roses and a need to broaden the genetic base. There is unexploited rose germplasm, which has the potential to broaden the overall gene pool of rose breeding programs in general. The wild species of roses used in the study showed considerably higher dissimilarity with almost all the cultivars and can be used to broaden the genetic base.

Keywords- Garden rose, Rose cultivar, Genetic diversity, Molecular marker, SSR.

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Introduction

A rose (*Rosa* sp.) is a woody perennial flowering plant cultivated for over 2,000 years. During the 1800s, aggressive breeding initiatives resulted in a huge growth in rose cultivation. Gardening, cutting flowers, house plants (pot rose), oil production, and medicinal use are the primary goals of the rose improvement programs. In the 1950s, improved breeding techniques and extensive testing of new cultivars resulted in roses with longer vase lives and higher yield quality. However, it has been stated that only a small %age of the variability has been employed in breeding programs because most breeders begin crossing with a limited number of cultivars as parents (Dutta, 2018; Byrne *et al.*, 2005). Zlesak (2006), reported that the fertility of modern roses is generally poor due to the meiotic abnormalities and the accumulation of deleterious recessive genes as a result of the long crossing history of polyploid and heterozygous parents. Hence, selecting a more diverse parent for making crosses is necessary. Genetic diversity plays an important role in developing hybrids as progenies of genetically diverse parents reveal greater heterosis compared to the progenies of genetically related parents (Dutta, 2015). Selecting genetically diverse parents for crossing needs estimation of genetic diversity, which has been successfully done in rose using various marker systems such as amplified fragment length polymorphism (AFLP)

(Baydar *et al*., 2004; Pirseyedi *et al*., 2005), random amplified polymorphic DNA (RAPD) (Jan *et al*., 1999; Kiani *et al*., 2007; Tabaei-Aghdaei *et al*., 2007; Kaul *et al*., 2009; Henuka *et al*., 2015) micRosatellite (Babaei *et al*., 2007; Kaul *et al*., 2009; Nadeem *et al*., 2014; Singh *et al*., 2017, Velluru *et al*, 2019) markers. Among molecular markers, SSRs are useful over others because of high reproducibility, a high degree of polymorphism, co-dominant inheritance, and uniform distribution in the genome. Scariot *et al*., (2006) showed that micRosatellite markers are useful in describing genetic diversity and relationships among roses. These are reserved between closely related species; they could provide a marker database for phylogenetic and evolutionary studies (Essellink 2003). SSR markers have been developed in roses by many researchers and had been used for various diversity studies in rose species and varieties (Esselink *et al*., 2003; Kimura *et al*., 2006; Scariot *et al*., 2006; Zhang *et al*., 2006; Babaei *et al*., 2007; Hibrand-Saint Oyant *et al*., 2008; Kaul *et al*., 2009; Samiei *et al*., 2009; Smulders *et al*., 2009). Division of Floriculture and Landscaping, ICAR-IARI, New Delhi, is maintaining more than 200 rose cultivars frequently used to develop new cultivars. In this study, 205 garden rose germplasm were evaluated using sSimple sequence repeat (SSR) markers to understand genetic relatedness among the cultivars using SSR markers.

Material and Method

Plant Material

The plant material consisted of 205 cultivars of rose germplasm (Table 1) maintained at the Division of Floriculture and Landscaping, ICAR-IARI, New Delhi. These cultivars mostly comprised Indian and exotic cultivars of rose and some of the rose species, which form the basis of the rose improvement program at ICAR-IARI, New Delhi, the pioneer institute working for the development of rose cultivars for various traits.

DNA Exwtraction and SSR Analysis

Each accession's fresh, young, and healthy leaves were harvested, fixed in liquid nitrogen, and stored at -80°C until use. The total genomic DNA was extracted using cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990) with some modifications. A total of 68 primers were screened for polymorphism and optimum amplification, of which 20 polymorphic primers (Table 2) were selected for further analysis. Each 20 µL PCR mix contained 2 µL Taq buffer, 0.5 µL $MgCl^2$, 2 µL dNTPs, 1 U *Taq* polymerase, 0.5 µL SSR primer, and 2 to 3 µL of genomic DNA. The PCR procedure was as follows: initial denaturation at 95^{o}C for 4 minutes followed by 35 cycles of denaturation at 95^{o}C for 30 seconds, primer annealing at respective annealing temperatures of primer pair for 30 seconds, and primer extension at 72^{o}C for 45 seconds was done. The final extension was carried out at 72^{o}C for 10 minutes. The PCR product was separated on 3% agarose gel, prepared in 0.5x TBE buffer stained with 4 µL ethidium bromide (Life Technology PVT Ltd, India) per 100 mL of gel. The resolved amplification products were visualized and photographed using a gel documentation system. DNA amplification was visualized as the presence and absence of a band and was compared with the DNA ladder of 500 bp to quantify the bands. Allelic data were recorded in a binary data matrix as one for presence and 0 for absence.

Data Analysis

The molecular data generated using 20 SSR markers for 205 cultivars were analyzed using Gen-Alex software ver. 6.5 to estimate genetic diversity parameters including band pattern, percent polymorphism, number of different alleles, the effective number of alleles, Shannon information index, expected heterozygosity and unbiased expected heterozygosity, and analysis of molecular variance. DARwin 6.0.21 software was used to estimate the diversity index and create a dendrogram.

Result and Discussion

Results

Using 20 SSR primers, a total of 109 bands were generated from 205 rose cultivars, ranging from 2 to 8 bands per locus. Floribunda had 108 bands, hybrid tea had 98, and wild species had 87. The band pattern is shown in Figure 1. The percent polymorphism, the effective and observed number of alleles (allelic richness), Shannon Information index, and heterozygosity parameters were calculated (Table 3). The average polymorphism for 205 cultivars tested with 20 SSR primers was 88%. On average, 1.78 unique alleles (Na) and 1.46 expected number of alleles (Ne) were discovered. The Shannon information index (I) has an average value of 0.41. The expected (He) and unbiased expected (uHe) heterozygosity were 0.269 and 0.276, respectively. The Jaccards dissimilarity index ranged from 0.15 to 0.82, with an average value of 0.51 (Figure 2). AMOVA revealed that 98% of total molecular variance occurred within groups, with only 2% occurring between groups (Figure 3 and Table 4). The principal coordinate analysis is performed, and a 2D scatter plot for PCoA is generated, as shown in Figure 4, which does not reflect the grouping pattern of cultivars used for analysis. A dendrogram is generated using the Jaccards dissimilarity index, as shown in Figure 5, to understand the presence of any grouping pattern. Cluster I had 94 cultivars, cluster II had 47, and cluster III had 64 cultivars. The grouping of cultivars is given in Table 5.

Discussion

The genetic relatedness of the accessions was revealed by molecular characterization using SSR markers. AMOVA found

Table 1: Rose germplasm used in the study

S.No.	*Classification*	*Cultivars*	*No. of cultivars*
I	Floribunda	Akash sundari, American hero, Andora, Anita, Anne Elizabeth, Aruna, Arunima, Atago, Ave maria, Bacardi, Banjaran, Black Chanakya, Black lady, Brides dream, Bronze star, Camara, Camaray, Charleston, Cherry parfait, Chingari, Christion Dior, Deepak, Delhi brightness, Delhi princess, Dil ki rani, Double knock out, Dr. Benjamin pal, Dr. Hue, Ico, Edmond rose, Else Polson, Emma Harkness, Flamenco, Gemini, Granada, Himalayan wonder, Hoot and howler, Iceberg, Imperator, Ingel horst, Jaimbra, Jantar mantar, Jogan, June bride, Krishna, Kungfu, Lahar, Legar field, Liebehour, Loree, Louisiana, Madhura, Magic central, Manas, Manasi, Manmatha, Marcel pangle, Mist, Moon drop, Mrs. K B Sharma, Navneet, Neelambari, Oh la la, Paraben nandjee, Pearl Polson, Pinata, Pink knock down, Pink parfait, Prema, Punchu, Pusa Ajay, Pusa Baramasi, Pusa Garima, Pusa Komal, Pusa Manhar, Pusa Manjis, Pusa Muskaan, Pusa Pitambar, Pusa Shatabdi, Pusa Urmil, Queen Elizabeth, Raja S. S. of Nalagharh, S S Bhatnagar, Sadabahar, Sea pearl, Shabnam, Shahastradhara, Shailaj Mukharjee, Shanti pal, Sheer delight, Skati metal, Stainless steel, Suchitra, Surabhi, Surdas, Suryakiran, Suryoday, Table Mountain, Tequila, Tarang, Taichung sunset, Tipus flame, Twin balad, Zine borner, Zizi	111
II	Hybrid tea	Abhisharika, American heritage, Angelique, Arjun, Ashwini, Balat spirit, Barbara bush, Bharat ram, Bheem, Borolina, Brandy, Kardinal, Carmousine, Chambe di kali, Command performance, Dr. B. P. Pal, Dr. M S Randhawa, Dulhan. Folk lore, Folklore drakshi, Ganga, Grand gala, Grand Mughal, Gulzar, Haseena, Hollywood, Homaze, Imperetice farah, Jacaranda, Jawani, Kiss of fire, Konrad honki, Lady X, Lalima, Lynn Anderson, Madam Delbert, Madhosh, Melody perfume, Montreal, Mother Teresa, Mridula, Nagabelle, Nayika, Nehru centenary, Olympiad, Parody of England, Pasadina, Priyadarshini, Pusa Abhishek, Pusa Arun, Pusa bahadur, Pusa Gaurav, Pusa Mohit, Pusa Priya, Pusa Sonara, Pusa Veerangana, Raja Ram Mohan Rai, Raktgandha, Scarlet Elizabeth, Soma, Sonia Meiland, Surekha, Surkhab, White Elizabeth, Woods of Windsor, Yankee doddle	87
III	Wild species	*R. indica* var. major, *R. macrophylla, R. multiflora, R. slancensis, R. tomentosa, R. wichuriana, R. dumalis*	7

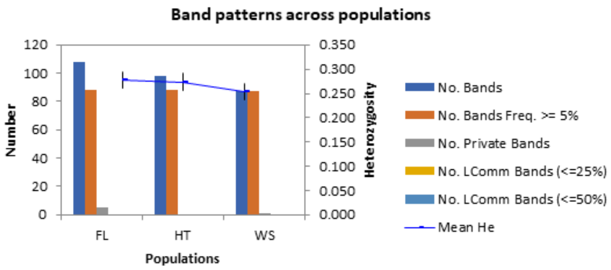

Figure 1: Band patterns across populations

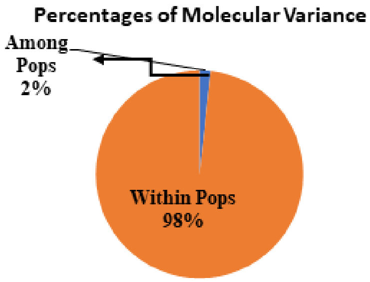

Figure 3: Percentage of molecular variance

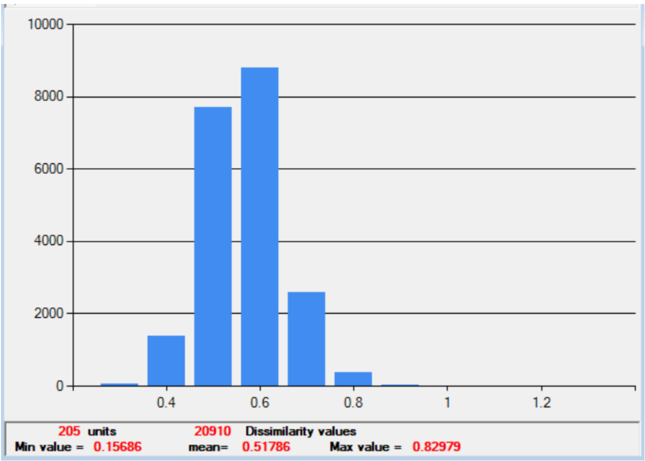

Figure 2: Distribution of Jaccard's dissimilarity coefficients

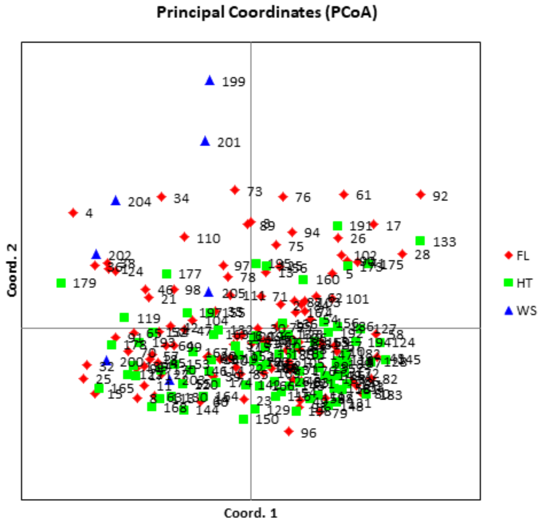

Figure 4: Principal coordinate analysis of 205 rose germplasm based on SSR markers

Table 2: List of selected SSR primers used in the study

S. No.	*Marker*	*Forward (5'→3')* *Reverse (5'→3')*	*Expected Band Size (bp)*	*Ta (°C)*	*Reference*
1	RH93	F-GCTTTGCTGCATGGTTAGGTTG R-TTCTTTTTGTCGTTCTGGGATGTG	251, 273, 275	54	Yan *et al*., 2005
2	RH78	F-AAAGAAACGCGAAATCTATGATGC R-TCTGGATGGGATTTAAAAGACAGG	216, 250	51	
3	RH50	F-TGATGAAATCATCCGAGTGTCAG R-TCACTTTCATTGGAATGCCAGAAT	336, 339, 340, 343	54	
4	RH58	F-ACAATTTAGTGCGGATAGAACAAC R-GGAAAGCCCGAAAGCGTAAGC	248, 252, 254, 269	54	
5	Rhb510	F-AAACGATAGGTGAATCTGTGGGT R-CACTCAACCTTGTCCACTCCTAAT	159, 161	54	
6	RH73	F-GGTTAGACGGGTGGAAGAAG R-ACTGCCGATAGAAGTATTTCATCA	160, 162, 172	54	
7	RW54n22	F-CTCAACTTCCCCGCCTTATC R-CTCGGCAGCTCCACTATCTC	169	55	Oyant *et al*., 2008
8	Cl3881	F-GACAACGACCACACCACTTG R-CCAAAGCAACATTGTCAAAAGA	241	55	
9	RW23F13	F-TGCATTCATCCCTCTCACTG R-TCAAATGCATGCTGAAAGGA	172	59	
10	H2F12	F-TGGCCAACCTCTCTCTGTCT R-TCCCAGCTTCGCTTTGTTAT	247	55	
11	RW25J16	F-TGGACCTTCCCTTTGTTTCC R-GCTTGCCCACATATTGTTGA	177	50	
12	H22C01	F-TCATAACCAACCATCTCCATCA R-AGGATTTCACCCAGAACACG	228	55	
13	H22EO4	F-GACATCACCACCACCACAAG R-AACCAAGGTTTCCAGTTCCA	241	55	
14	CL2980	F-CCCTATTCGATTTCGAGTGC R-ACTTGGCTCGACGGATACAC	234	55	
15	H10D03	F-CAATTCAAAACCACCGCTCT R- CGCAGAGTCAACGAACCATA	226	55	
16	H23017	F-ACACCAAGCAAACCAAAACC R-AGCACGAAAACCGAGAGAGA	218	55	
17	RW35C24	F-GGCGAATCGAGATTCAGAGA R-GGATTAGCCCAAGTCCAGGT	247	55	
18	RW52D4	F-GGCAGTTGCTGTGCAGTG R-TTGTGCCGACTCAAAATCAA	174	55	
19	RW62D8	F-CCTGACTCTAACCTCGTGCT R-TCCATCATCAACTTCGTCTG	123	56	
20	RW15D15	F-CGGCTAGCAATCAGTGACAA R-GGTCTTCCCTAATGCCCAAT	203	55	

that within-population variation is greater, with only 2% variation between populations. PCoA and cluster analysis are used to investigate the genetic relatedness of crops. The scatter plot did not exhibit a distinct grouping pattern, indicating that the cultivars are related to each other. The genetic relatedness of cultivars could be attributed to their open outcrossing nature, which results in free gene flow among populations, contributing to more genetic variety within groups (Akond *et al*. 2012; DeVries and Dubois 1996). Hybrid tea cultivars were the first group of modern rose cultivars derived from crosses of hybrid perpetuals (derived from *R. chinensis, R. gallica*, and *R. centifolia*) and tea roses (originated from *R. chinensis* and *R. gigantea*) whereas other modern rose class 'Floribundas' was developed from the crosses of hybrid teas and polyantha roses (latter developed from species *R. multiflora* and *R. wichuriana*) (Marriott 2003) which explains the relatedness between floribunda and hybrid tea cultivars. The addition of genetic material from the polyantha group of roses in floribundas could be attributed to slightly higher heterozygosity within this

Table 3: Summary statistics of genetic diversity

Pop		*%P*	*N*	*Na*	*Ne*	*I*	*He*	*uHe*
Floribunda (Group 1)	Mean	99.08	111.0	1.982	1.472	0.424	0.279	0.280
	SE		0.00	0.018	0.034	0.022	0.017	0.017
Hybrid tea (Group 2)	Mean	89.91	87.00	1.798	1.468	0.414	0.274	0.276
	SE		0.00	0.058	0.035	0.024	0.018	0.018
Wild species (Pop3)	Mean	76.15	7.00	1.560	1.428	0.384	0.254	0.273
	SE		0.00	0.078	0.035	0.025	0.018	0.019
Total	Mean	88.38	68.33	1.780	1.456	0.407	0.269	0.276
	SE	6.66	2.46	0.034	0.020	0.014	0.010	0.010

Table 4: Summary of AMOVA Table

Source	*df*	*SS*	*MS*	*Est. Var.*	*%*
Among Pops	2	60.000	30.000	0.270	2
Within Pops	202	3118.283	15.437	15.437	98
Total	204	3178.283		15.707	100

group as compared to the hybrid teas. Similarly, Azeem *et al*. (2012) and Rusanov *et al*. (2005) reported lower genetic variability of cultivated roses using RAPD and SSR markers. Akond *et al.* (2012) using SSRs also observed higher similarity and narrow genetic base of modern cultivars (Miniature roses and hybrid breeding lines) as compared to their wild relatives. Smulders *et al.* (2009) also used 11 mic*Rosa*tellite markers to characterize 734 hybrid tea cultivars and found that they had relatively close genetic links.

A dendrogram is also produced to better understand the presence of a grouping pattern. Three major clusters were found. The first cluster consists primarily of Indian-bred rose cultivars and a few wild species. The second cluster consists primarily of exotic varieties, whereas the third cluster has both Indian and exotic cultivars. These classifications do not correspond to the horticultural classifications used to group the accessions. Some investigations on garden rose varieties have also revealed a similar pattern (Vainstein *et al*., 1993; Ben-Meir and Vainstein, 1994; Debner *et al.,* 1996; Panwar *et al*., 2006; Rai *et al*., 2015; Velluru *et al.* 2019). Debner *et al.* (1996) discovered that rose cultivars did not cluster according to the groups to which their parents belonged. The hybrid tea and the floribunda groups share the highest genetic similarity. Similar results were also reported by

Table 5: Clustering of cultivars based on SSR data

Cluster No.	*Cultivars*	*No. of cultivars*
Cluster I	Oh lala, Navneet, June Bride, Haseena, Pusa Muskan, Pusa Gaurav, Mother Teresa, Grand Mughal, Sugandha, Thaichun Sunset, Memorial Day, Lalima, Pusa Mahak, Montezuma, Pusa Sonara, Pusa Shatabdi, Kiss of Fire, Surekha, Arjun, Soma, Shashtradhara, Tajmahal, Pusa Garima, Manas, Folklore, Sheer Delight, Yankee doodle, Borolina, Imteretice Farah, Dil Ki Rani, Chambe di Kali, Angelique, Taqilla, Pinata, Anita, Black Lady, Blue moon, Camara, Gemini, Ingel horst, Magic central, Hollywood, Tarang, Pusa komal, Camaray, Jaimbra, Pusa Manhar, Madhura, Pusa Ajay, Pusa Veerangana, Pusa Mohit, Jawahar, Anurag, Krishna, Pusa Pitamber, Aruna, Raktagandha, Bharat Ram, Dr B P pal, Surdas, Dr Benjamin Pal, Raja Ram Mohan Rai, Raja S S of Nalagarh, Ranjana, Pusa Arun, Iceberg, Nehru Centenary, Dulhan, Delhi Brightness, Jawani, Pusa Manjis, Homaze, Pusa Bahadur, Pusa Abhishek, Dr M S Randhwa, Pusa Urmil, Jantar Mantar, Mrs. K. B. Sharma, Baccardi, Pink Parfeit, Lahar, Twin Balad, Folklore Drakshi, Brandy, Jadis, Balat Spirit, Century II, White Elizabeth, NoorJanah(HT), Legarfield, Kardinal, Ganga, Pearl Polson, Delhi Princess	94
Cluster II	Atago, Midas Touch, Double Knockout, Moon Drop, Karen Blixen, Konred Honki, Christian Dior, Lynn Anderson, Nagabelle, Carmousine, Andora, Madam Delbert, Bewitched, *Rosa salascensis,* Jwala, *R. tomentosa, R. macrophylla*, Mist, Shola, Table Mountain, Suchitra, Marcel pangel, Grandgala, Granada, Parady of England, Lousiana, Melody perfume, Leibehour, Ico, American Hero, Cherry Parfeit, Ave Maria, Lady X, Paraben Nandjee, Sonia Meiland, Barbara Bush, Anne Elizabeth, Pasadina, Impereter, Sea Pearl, Brides Dream, Superstar, Command performance, Montreal, Jogan, Maid of Honour, Velvet Fragrance	47
Cluster III	Black Chanakya, Akash Sundari, Emma Harkness, Shantipal, Jaccaranda, Scarlet Elizabeth, Noorjahan (D), *R. dumalis*, Pink knockdown, Manmatha, Ashwini, Shailaize Mukherjee, Nayika, Banjaran, Okhlahoma, Bonne nuit, Pusa Aradhan, Skati metal, Olympiad, Surkhab, Stainless steel, American Heritage, Zine borner,Kungfoo, Bronze Star, Edmond rose, Zizi, Bheem, Woods of Windsor,Tipus Flame, Neelambari, Priyadarshini, Surabhi, Pusa Priya, Deepak, Raktima, Flamenco, Charleston, Else polson, Ranisahiba, Loree, Arunima, Sadabahar, Punchu, Pusa Barahmasi, Rose sherbet, Manasi, Madhosh, Gulzar, S S Bhatnagar, Himalayan Wonder, Abhisarika, Suryoday, Suryakiran, Shabnam, Prema, Mridula, Chingari, *R. multiflora*, Dr. Hue, *R. wichuriana*, Queen Elizabeth, Hoot and howler, R. indica var major	64
	Total	205

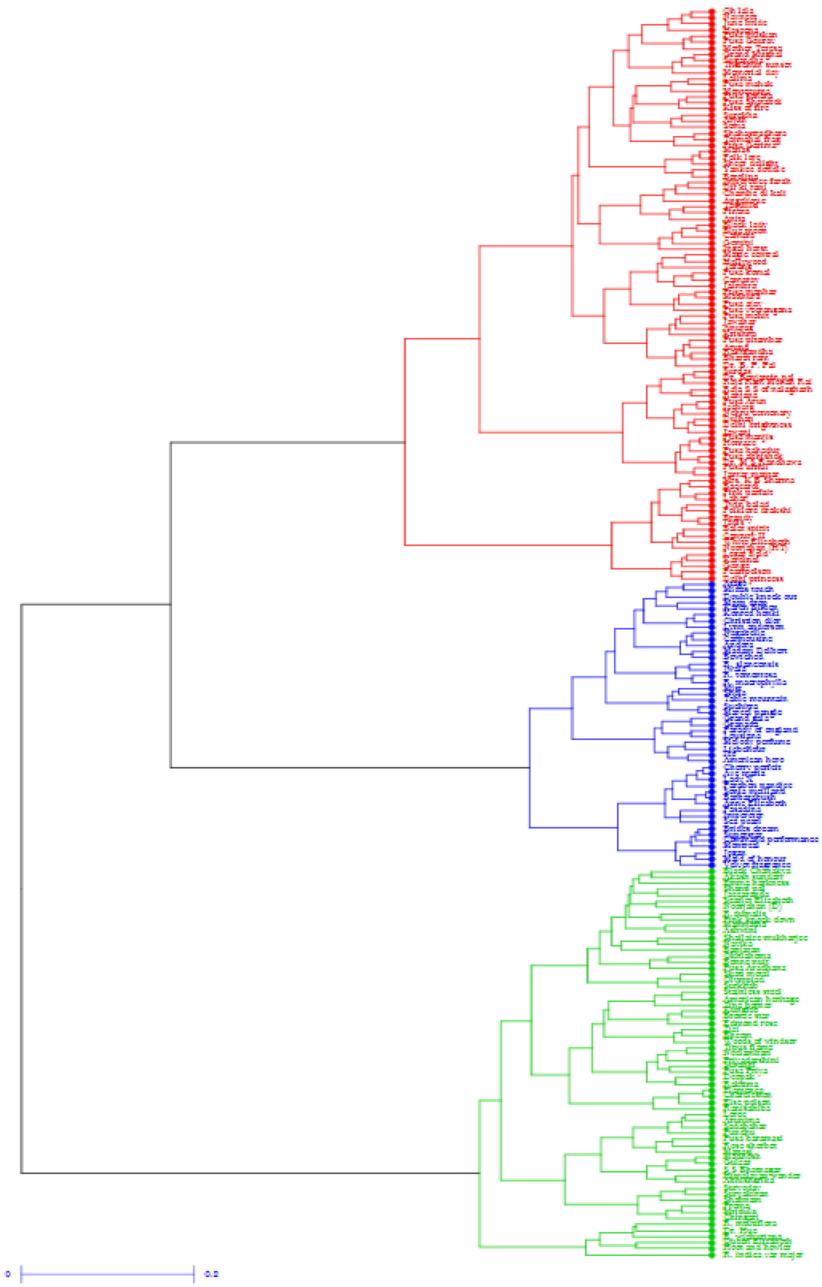

Figure 5: Clustering of cultivars

Vainstein *et al.* (1993). The lack of clear distinction between different groups may be due to the complex hybrid nature of rose cultivars. The majority of the cultivars used in the present study belong to modern rose classes (Hybrid Teas and Floribundas).

The polymorphism observed can be attributed to its polyploidy and heterozygous nature. Roses are highly heterozygous and show a wide range of variability in the color and form of rose flowers in the seedlings obtained (Dutta 2018). However, the basic material for the development of new cultivars is limited. Interspecific hybridization in roses (*Rosa* spp.) has generated thousands of cultivars with commercial value, but only eight to ten species are commonly represented in the pedigrees of modern cultivars even though there are hundreds of rose species reported (Bryne 2005). Modern classes of rose hybrids (Hybrid Teas, Floribundas, and Grandifloras, and Miniatures) are also products of such hybridizations. The majority of the rose species are products of the natural hybridization of one or more hybrids.

The study suggests genetic similarity among the rose cultivars across different horticultural classes, indicating the decreasing genetic base of hybrid roses. There is unexploited rose germplasm, which has the potential to broaden the overall gene pool of rose breeding programs in general (Akond *et al,* 2012). The most diverse cultivars should be used as parents to exploit the heterosis. The wild rose species showed higher dissimilarity with almost all the cultivars and can be used to broaden the genetic base. The dissimilarity coefficient for Queen Elizabeth and Surkhab had a maximum (82%) and cultivar Suchitra and Barbara Bush (15%) had a minimum dissimilarity coefficient. The cultivars Queen Elizabeth and rose species *Rosa indica* var. major, *Rosa macrophylla*, and *Rosa multiflora* are found to show higher dissimilarity coefficients ranging from 50 to 80% with almost all the cultivars and can be used as parents in the crossing program.

Conclusion

The study suggests genetic similarity among the rose cultivars across different horticultural classes, indicating the decreasing genetic base of hybrid roses. There is unexploited rose germplasm, which has the potential to broaden the overall gene pool of rose breeding programs in general (Akond *et al*., 2012). The most diverse cultivars should be used as parents to exploit the heterosis. The wild rose species showed higher dissimilarity with almost all the cultivars and can be used to broaden the genetic base. The dissimilarity coefficient for Queen Elizabeth and Surkhab had a maximum (82%) and cultivar Suchitra and Barbara Bush (15%) had a minimum dissimilarity coefficient. The cultivars Queen Elizabeth and rose species *Rosa indica* var. *major*, *Rosa macrophylla*, and *Rosa multiflora* are found to show higher dissimilarity coefficients ranging from 50 to 80% with almost all the cultivars and can be used as parents in the crossing program.

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