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Assessment of genetic diversity and population structure of fragrant rose (*Rosa* × *hybrida*) cultivars using microsatellite markers

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ABSTRACT

Rose (*Rosa* × *hybrida* L.) is a commercially important ornamental crop which represents major share in world floriculture market and essential oil industry. In the present investigation, genetic diversity among 25 fragrant rose cultivars belonging to exotic and Indian origin was studied during 2016–17 at New Delhi. Thirty one SSR markers were used for characterization. A total of 96 alleles were identified among the genotypes with an average of 3.9 alleles per loci. Diversity among populations of different origins was analysed and it revealed that, cultivars from Indian origin exhibited higher diversity as compared to the selected cultivars from American and European origin. Effective number of alleles (N_e) and expected heterozygosity (H_e) values were more in Indian population (N_e=1.93, H_e=0.49) as compared to selected populations of American (N_e=1.45, H_e=0.45 and European (N_e=1.29, H_e=0.29) origin. Matching coefficient values ranged from 0.55– 0.86 indicated the existence of moderate variability among fragrant roses of different origin. Unweighted Pair Group Method using Arithmetic Mean (UPGMA) dendrogram clearly separated all the 25 genotypes into 7 different clusters. Cultivars, Rose Sherbet, Century Two Seedling and Brandy showed distant relationship with other cultivars and were found in three different individual clusters V, VI and VII respectively. The highest and lowest similarity values were noticed between the cultivar sets, Jawahar and Double Delight (86%) and Century Two Seedling and Brandy (55%). The molecular data generated in present investigation would be highly helpful for cultivar identification, conservation and for breeding fragrant roses.

Key words: Characterization, Genetic diversity, Fragrant roses, Population structure, SSR markers

Rose (*Rosa* × *hybrida* L.) is one of the most important commercial crops belonging to the family of Rosaceae. Genus *Rosa* contains nearly 200 species and more than 18000 cultivars (Gudin 2000), still breeding work continues in roses for search of novel flowering traits. Early rose breeders mainly focused on development of varieties suitable for gardens, exhibition and cut flower purposes suited to diversified climatic conditions. Meagre research work has been done for breeding fragrant quality rose cultivars. Most of the commercial rose cultivars lack distinct fragrance due to long selection for other quality parameters like vase life, flower colour, disease resistance (Zukar *et al*. 1998). A negative correlation between vase life and scent was also reported in some roses (Barletta 1995). Development of fragrant cultivars along with other quality parameters such as keeping quality, colour and resistance to biotic and abiotic stresses etc. through different breeding tools will improve the overall quality of roses.

Characterization and assessment of diversity are prerequisites for planning any breeding programme. Now a day's, molecular markers are widely in use for characterization and variability studies in germplasm due to some limitations with the use of morphological and biochemical markers in plants (Kim and Byrne 1994). Several molecular marker systems have been developed for effective, accurate and fast identification of roses. Various DNA markers such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter Simple Sequence Repeats (ISSR) and Simple Sequence Repeats (SSR) have been successfully used in roses for effective characterization and diversity analysis of various rose types (Esselink *et al*. 2003, Zhang *et al*. 2006, Panwar *et al*. 2015). Present study was undertaken for characterization and diversity analysis of fragrant rose cultivars of Indian and exotic origin using SSR markers.

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MATERIALS AND METHODS

Plant material: In the present study, 25 fragrant rose cultivars were maintained at the research farm of ICAR-Indian Agricultural Research Institute, New Delhi (Table 1) during 2016–17.

SSR analysis: The total genomic DNA was isolated from leaves using CTAB method (Murray and Thompson 1980) with slight modifications. A total of 48 SSR primers were selected from literature (Yan *et al.* 2005, Kimura *et al*. 2006, Jowkar *et al*. 2009) and were synthesized for analysis (Table 2). PCR reactions were performed using 25 µL reaction mixtures consisting of 2 µL of template DNA (20 ng), 0.33 µL of Taq DNA polymerase (5U/µL) (Genei, India), 1 µL of each forward and reverse primers (1 µM), 2 µL of dNTPs (20 mM), 3.33 µL Taq DNA buffer (10X) and 15.33 µL autoclaved distilled water. The PCR thermal profile was 94ºC for six minutes followed by 35 cycles of denaturation at 94ºC for one minute, annealing at 48–55ºC for one minute and 72ºC for two minutes followed by final extension at 72ºC for 10 minutes before cooling it to 10ºC. After amplification, PCR products were separated on 3% Metaphor agarose gel and amplified SSR profiles were visualized by Alpha Digi Doc Gel Documentation System.

Data analysis: For SSR data, the bands at each

Table 1 Fragrant Rose cultivars used for characterization and their grouping based on origin and genic variation statistic parameters for three groups

Cultivar	Descendance	Population group
Velvet fragrance	Deep Secret × Fragrant Cloud	Group-I
Perfume de French Comete	*Not known	Group-I
Papa Meilland	Chrysler Imperial × Charles Mallerin	Group-I
Bonne Nuit	*Not known	Group-I
Elle	Purple Splendour × (Chicago Peace × Parador)	Group-I
Melody Perfumee	Dioressence × Stephen's Big Purple	Group-I
Christian Dior	(Independence × Happiness) × (Peace × Happiness)	Group-I
Blue Moon	Sterling Silver × Seedling	Group-I
Karen Blixen	*Not know	Group-I
Century Two Seedling	Open pollinated seedling from cv. Century Two	Group-II
Sugandha	*Not known	Group-II
Rose Sherbet	A seedling of "Gruss an Teplitz"	Group-II
Raktima	Pink Parfait × Sugandha	Group-II
Jawahar	Sweet Afton × Delhi Princess	Group-II
Haseena	You Ki San × Balinese	Group-II
Sweet Afton	(Charlotte Armstrong × Signora) × (Alice Stern × Ondine)	Group-III
Oklahoma	Chrysler Imperial × Charles Mallerin	Group-III
Midas Touch	Brandy × Friesensohne	Group-III
Memorial Day	Blueberry Hill × New Zealand	Group-III
Fragrant Plum	Shocking Blue × (Blue Nile × Ivory Tower × Angel Face)	Group-III
Fragrant Lace	Unnamed Seedling × Cherry Jubilee	Group-III
Eiffel Tower	First Love × (Charlotte Armstrong × Signora)	Group-III
Double Delight	Granada × Garden Party	Group-III
Century Two	Charlotte Armstrong × Duet	Group-III
Brandy	First Prize × Golden Wave	Group-III

Population Group		*Na*	*Ne*	*I*	*Ho*	*He*	*h*	*Number of Polymorphic loci*	*Percentage of polymorphism*
Group-1(Europe)	Mean	1.29	1.29	0.20	0.29	0.29	0.14	9	29.03 %
	SD	0.46	0.46	0.31	0.46	0.46	0.23		
Group-II(India)	Mean	2.38	1.93	0.69	0.50	0.49	0.43	29	93.55 %
	SD	0.66	0.56	0.30	0.36	0.21	0.18		
Group-III (USA)	Mean	1.45	1.45	0.31	0.45	0.45	0.22	14	51.61 %
	SD	0.50	0.50	0.35	0.50	0.50	0.25		

Na, Observed number of alleles; Ne, Effective number of alleles; I, Shannon's Information index; Ho, observed Heterozygosity; He, Expected Heterozygosity; h- Nei's gene diversity

Table 2 SSR markers used for characterization of fragrant rose cultivars

SSR marker	Primer sequences	Size (bp)	LG	TA(ºC)	H
Rh79	F: TTCTTCTTGCTCGCCATTTTGATT R:GAACGTCCACCACCACCCACTCTG	135, 142, 149, 160	1	50	0.37
RhAB9	F:GTCAATTTGTGCATAAGCTC R:GTGAGAACAGATGAGAAATG	101, 116, 124	1	50	0.19
Rh48	F:GATAGTTTCTCTGTACCCCACCTA R:TTGACCAGCTGCAACAAAATTAGA	93, 107, 117, 122, 144	2	50	0.24
Rh80	F:CATGCCAAACGAAATGAGTTA R:TTATCTAAAGGGCTGCTGTAAGTT	134, 148	2	50	0.35
Rh96	F:GCCGATGGATGCCCTGCTC R: AGATTCCCTGCGACATTCACATTC	267, 276, 294	2	50	0.14
Rh50	F: TGATGAAATCATCCGAGTGTCAG R: TCACTTTCATTGGAATGCCAGAAT	240, 303, 340	3	50	0.35
Rh58	F: ACAATTTAGTGCGGATAGAACAAC R: GGAAAGCCCGAAAGCGTAAGC	214, 238, 258, 286	3	50	0.5
RhABT12	F: CAAGTTTGTCTCCTTGGACC R: CATAGATGATTATCCTAGAGCC	156, 174, 200	4	50	0.23
Rh65	F: AGTACGCCGACGCAGATCCAGTGA R: ACGGCGTTGTAGGTCGTCATTCTC	128, 140, 160	4	50	0.54
Rh78	F: AAAGAAACGCGAAATCTATGATGC R: TCTGGATGGGATTTAAAAGACAGG	216, 296, 336	4	50	0.25
Rh77	F: CAACTGAAAGGAACAAATGGATGT R: GGAATGGCTTGTAAATTTGTGATT	246, 262, 280, 309	5	50	0.49
Rh93	F: GCTTTGCTGCATGGTTAGGTTG R: TTCTTTTTGTCGTTCTGGGATGTG	244, 275	5	50	0.35
RhAB38	F: GAGGTGGTCGATTCCATGTC R: TTACCGTTCTACCTAAGTGACTAAC	120, 139, 173	5	50	0.53
Rh60	F: TCTCTTTTCACGGCCACCACT R: TGAATCCAAGGCCGTATAGTTAGA	258, 285, 300	6	50	0.28
Rh85	F: ACTTTTGGGCGTTCATCGCATTACAC R:GGCTATATGGGCTCAAGTCTAGACAA	203, 217	6	50	0.34
Rh98	F: GGCCTCTAGAGTTTGGGATAGCAG R: ACGACGTCAATAACTCCATCAGTC	160, 221	6	50	0.29
Rh72	F: CCAAAAGACGCAACCCTACCATAA R: TCAAAACGCATGATGCTTCCACTG	254, 285, 310	7	50	0.41
Rh73	F: GGTTAGACGGGTGGAAGAAG R: ACTGCCGATAGAAGTATTTCATCA	150, 175, 205	7	55	0.40
RhAB28	F: GCAGATGTTATTCATGTTAA R: CCAAGTATTTTAGTTTCTTC	164, 180, 190	n. d*	55	0.29
ABR II/ Rpu 4	F: CTTGTTCAAAGGGTTCTCTG R: CACCTAAATGATGCTTTTCC	274, 312	n. d*	50	0.38
ABRII/Rpu 7	F: GAAACCCAGTTATTGATGCCTGGA R: CCAGATTATTGATGCCTGAT	213, 220	n. d*	50	0.19
ABRII/Rpu 10	F: AAGATTGGTGTTGGGTGTTA R: CTCGTTCTGGTTCTGTCTTC	115, 122	n. d*	50	0.09
ABR II/RPU 11	F: AGTTGGACCTGTTTTCTTCA R: AGCACGACGACGAGTTTC	205, 220, 236, 248	n. d*	57	0.41
ABRII/RPU12	F: GAAGAAGAACGACTGAGAGC R: GAGCAGAGAATTTCCATTTG	103, 109, 117, 131	n. d*	50	0.47

Contd.

Table 2 (*Concluded*)

SSR marker	Primer sequences	Size (bp)	LG	TA(ºC)	H
ABRII/RPU 14	F: AGCACTTACAGGCTCATCAT R: CCTCCAAGTCAAGTTCTACG	206, 216, 222, 230	n. d*	50	0.23
ABRII/RPU32	F: TAGAGCTATTTTCGATTCGG R: GGGTGACACAGAGAGAGAGA	238,2 65	n. d*	50	0.12
ABRII/RPU33	F: TCTCTCTCTGTGTCACCCTC R: CGTCTCCCTCTTCTTCTTCT	150, 180	n. d*	50	0.45
ABRII/Rpu 36	F: TAGTTGAGAGCTCGGAGAAG R: GAAGTTACAAGACGAAACCG	235, 255	n. d*	50	0.13
RA013a	F: GAGGGAAAGAGATACACAAA R: GTAAGACCTTGCGTGTTCATA	145, 155	n. d*	55	0.13
RA023b	F: CATCCTCGGTGTTGCGTTGA R: TGTCTCCAGCAACCTTTTTTTCCC	166, 174, 194	n. d*	50	0.26
RA043a	F: GCAACGTACTTCAATTTCCAC R: CAAGCTCAGAACTGAGACAC	123,133, 149, 171	n. d*	50	0.48

(Primer name, Primer sequences, F, forward; R, reverse), sizes of amplified DNA fragments, LG, linkage group; TA, annealing temperature; h, Neis heterozygosity value), n.d*, not determined.

polymorphic marker were noted as either present (1) or absent (0) and molecular weight of the bands was estimated by using 100 bp DNA ladder as size standard. Various genic variation statistical parameters for three population groups were computed using POPGENE 32 version 1.31. A dendrogram was generated by Unweighted Pair Group Method using Arithmetic Mean (UPGMA) using matching coefficients values. The multi-locus SSR data generated for fragrant rose genotypes was analysed using STRUCTURE programme (Earl and VonHoldt 2012). The programme assigns the individual genotypes to distinct populations. To get the optimal number of genetic clusters (best K) Evanno *et al*. (2005) method was used. The STRUCTURE programme runs for 20 replications of each k (2–9). The burn in period consists of 5×10^5 iterations and 7.5×10^5 Markov Chain Monte Carlo (MCMC) replications. The ΔK value obtained was *L(K)*=7, based on this information individuals were assigned to seven distinct genetic groups.

RESULTS AND DISCUSSION

The present study was done for characterization and diversity analysis of 25 highly fragrant rose cultivars with desirable flower and plant characteristics in order to select the potential parental lines for initiation of breeding programmes for scented quality roses. Out of 48 SSR markers used, 31 markers exhibited polymorphism. A total of 92 alleles were detected and the average number of alleles per loci (2.9) detected in the study was slightly comparable with the results obtained by Smulders *et al*. (2009). Number of alleles obtained per locus and type of alleles present in sets of population are important signs for rich allelic diversity which decides the probable utility of genotype in future breeding programs. Nei's heterozygosity value (h) of markers is an indirect measure of allelic diversity tested among the fragrant genotypes, which was found to be comparatively high in tested SSRs (Table 2). Samiei *et al*. (2010) and Nadeem *et al*. (2014) also reported higher heterozygosity values of SSRs ranging from 0.67–0.96 and 0.5–1 in different rose genotypes. In the present study, SSR markers such as, Rh65, RhAB38, Rh58, Rh77, RA043a, ABRII/RPU12, ABRII/RPU33 revealed higher diversity values ($h\geq0.45$) and were more informative and suitable for characterization of fragrant rose germplasm.

Genic variation statistical parameters were computed for three different populations of European, Indian and American origin (Table 1). Diversity among different populations were concerned, the highest number of alleles and heterozygosity values were found in fragrant cultivars of Indian origin followed by European and American origin cultivars, suggesting lower diversity among selected introduced cultivars of exotic origin (Table 1). Most of the fragrant genotypes of Indian origin selected in the study were derived from the crosses between native germplasm and exotic types in order to incorporate fragrant genes and other quality parameters in adopted cultivars (Table 1) this might be reason for slightly higher variability among the selected genotypes of Indian origin than exotic populations. Whereas, absence of much variability among the cultivated fragrant varieties of European and American origin could be due to the use of uniform or less variable parental material while development of these fragrant genotypes. To assess the genetic relationships among fragrant cultivars, matching coefficient values were calculated. Pairwise estimates of matching coefficient values ranged from 0.55–0.86 with an average of 0.71 indicating moderate diversity among selected cultivars. Highest and lowest similarity values were found in cultivar sets, Jawahar and Double Delight (0.86) and Century Two Seedling and Brandy (0.55). Similarly Akond *et al*. (2012) noticed average similarity coefficient values of 0.66 (0.47–0.98) and 0.73 (0.57–0.83) in domesticated miniature roses and hybrid lines using SSRs. Panwar *et al*. (2015) also reported an average similarity coefficient of 0.67

(0.44–0.91) in *Rosa* × *hybrida* cultivars and these values were more or less similar in range with matching coefficient value of present experiment (0.55-0.86) explaining the moderate or low amount of genetic variability among the cultivated fragrant roses of different origins. Most of the cultivars in the present study belongs to modern rose group Hybrid Teas and narrow genetic variability among the modern rose cultivars was also reported by DeVries and Dubois (1996). The lower variability among the fragrant cultivars of different origin might be due to the involvement of several common parents in their genetic makeup during their development. In the present investigation itself we have observed several common cultivars used as a parent material for development of many other fragrant lines. For example, cv. Brandy was fragrant cultivar of American origin selected for characterization in the present study was also as a parent for another selected American origin cultivar Midas Touch. Likewise cv. Charlotte Armstrong was a common parent for three selected cultivars Sweet Afton, Eiffel Tower and Century Two. Cultivar Signora was one of the common parents of cultivars Sweet Afton and Eiffel Tower and Sweet Afton also acted as a parent for cv. Jawahar. European and American cultivars Oklahoma and Papa Meilland shared a genetic material from common parent Charles Mallerin. The Indian cultivars such as, Rose Sherbet, Century Two Seedling and Haseena also developed from introduced exotic lines of European and Asian origin, suggested presence of common share of genetic material among fragrant cultivated varieties in the world.

Diversity among all 25 fragrant roses of was studied. UPGMA dendrogram separated the cultivars into seven different clusters. In this clustering, cultivars were not

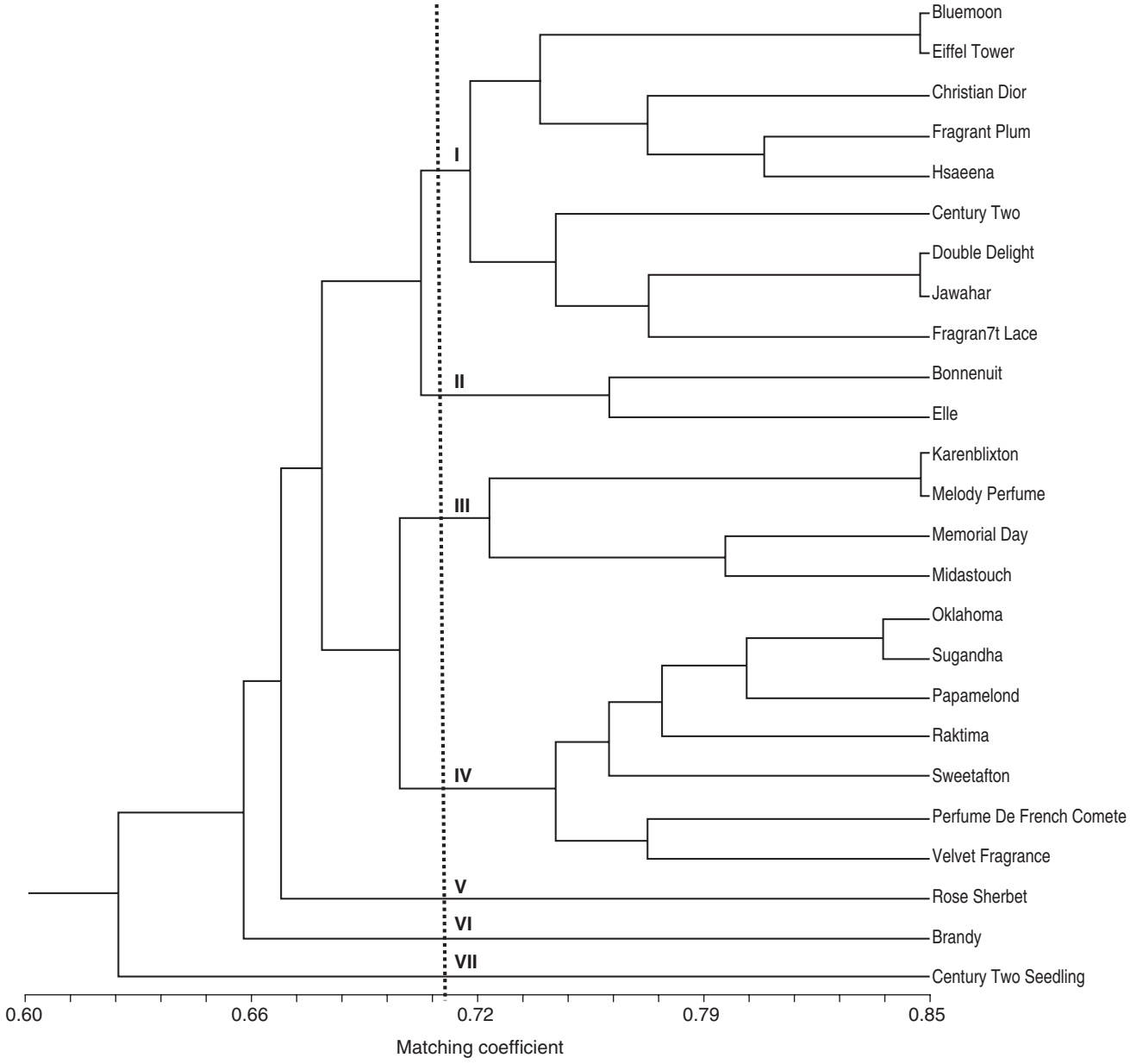

Fig 1 UPGMA dendrogram of 25 fragrant rose cultivars based on SSR data.

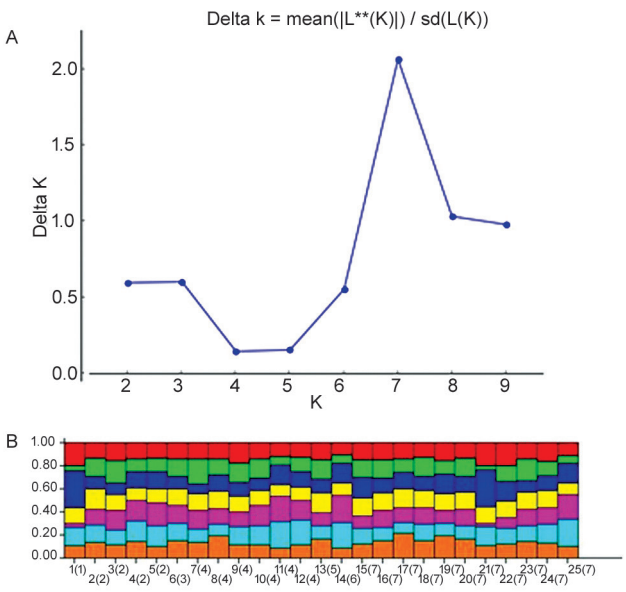

Fig 2 (a) Biplot of deltaK and k values indicating the correct K value to be 7 based on structure analysis; (b) Bar diagram for 25 accessions arranged based on inferred ancestry at K=7. Color code for each cultivar bar indicate the allelic affiliation with respect to the groups.

separated on the basis of their respective breeding place or geographic origin. Moreover, higher genetic similarities were noticed between the cultivars of different geographic origins (Fig 1). Cultivars, Blue Moon and Eiffel Tower; Double Delight and Jawahar of subclusters I_a and I_b were showing, 85.7% and 86.2% similarity with each other were suspected to have involvement of common parentage in their pedigree. Cultivars Bonne Nuit and Elle displayed 76% genetic similarity with each other and both were separated into cluster II. Cultivars, Karen Blixen and Melody Perfume were present in a single cluster III and found to have 82% genetic similarity with each other also evidenced by their similar morphological features. Cultivars Oklahoma and Papa Meilland derived from similar parentage (Chrysler Imperial × Charles Mallerin) were present in the same subcluster IV_a also reported 81.7% comparable genetic makeup. Another fragrant Indian cultivar, Sugandha of unknown origin was reported to have higher genetic similarity (84.5%) with Papa Meilland (derived from similar parentage) also identified in same subcluster IV_a. Cultivar, Raktima derived from a cross between Pink Parfait × Sugandha shared 81.5% similarity with parent Raktima was also identified in the same subcluster (IV_a). Higher variability was noticed in open pollinated varieties Rose Sherbet and Century Two Seedling which were selections from open pollinated population of cultivars, Gruss an Teplitz and Century Two respectively.

Population structures of fragrant cultivars were also studied using Bayesian model approach. Estimated membership fractions were ranged from K=2 to K=9. The highest *ad hoc* measure of ΔK, based on second order rate of change of the likelihood distribution was also noticed at ΔK=7 suggesting 7 different subgroups. Population structure of 25 fragrant rose genotypes was derived using software STRUCTURE HARVESTER (Fig 2). This model based approach has capacity to use genetic information to decide the population membership of individual genotypes without presuming predefined populations. They assign either individuals or a certain portion of their genome to number of groups based on multilocus genotypic data. (Population structure of 25 fragrant cultivars was represented in Fig 2. Jiang and Jang (2018) also studied the population structure of roses in order to understand the genetic relationship among them and to verify the genetic drift among the population of different origin. The result obtained from the structure studies related to the genotype structuring (subpopulations) was comparable with UPGMA and PCA analysis. Present study revealed a considerable amount of genetic variability among 25 fragrant rose genotypes belongs to Indian and exotic origins. Information generated in the study can be utilized for breeding fragrant quality roses.

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