

RESEARCH ARTICLE

Phenotypic Characterization and Grouping of Rose (*Rosa × Hybrida* L.) Genotypes Using DUS Descriptors

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Abstract

Characterization and evaluation of native germplasm is essential for improvement, protection and conservation. The present investigation was carried out to study the phenotypic diversity of 148 rose (*Rosa × hybrida* L.) genotypes at ICAR-Indian Agricultural Research Institute, New Delhi during two consecutive years (2018-19 and 2019-20). Characteristics (48) were used to identify and group the genotypes. Phenotypic data of genotypes was collected during peak flowering season by following DUS (distinctiveness, uniformity and stability) test guidelines developed by PPV & FRA, India. The DUS characteristic profile was made of the all 148 genotypes using 48 phenological traits. Hierarchical cluster analysis was conducted for the grouping of studied genotypes. A total of eight different clusters were identified in the dendrogram with high characteristic uniformity. Cluster-I is identified with a maximum number of genotypes (109), followed by cluster-III (11), cluster- II and VIII (7 each), cluster-IV & VII (5 each), cluster-V (3) and cluster-VI (1). This information about an existing huge varietal wealth of rose will be useful for researchers, farmers, nursery men to pursue protection for their new material under PPV and FRA, New Delhi.

Keywords: Rose, Germplasm, Phenotypic diversity, Characterization, DUS.

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Introduction

Morphological characterization is the traditional approach used for the classification of existing germplasm and assessing variability. Though phenotypic traits are considered as primitive markers, they are powerful indeed to determine the taxonomic status of any life form and scientific grouping of the plant kingdom still relies on morphological traits. Genetic variability in roses was assessed by using morphological markers (Panwar *et al.*, 2010; Gaurav *et al.*, 2018; Aparna *et al.*, 2019; Aparna *et al.*, 2020). Different national and international IPR (Intellectual Property Rights) authorities regulating plant varieties and breeder's rights (UPOV, PPV&FRA, etc.) considered the phenotypic characteristics (DUS characteristics) as the basis for variety or genotype registration in order to provide protection to the material. Protection of Plant Varieties and Farmers Rights Authority (PPV&FRA), India developed DUS test guidelines considering 61 and 16 phenotypic characteristics for *Rosa* spp. (including varieties, hybrids, and wild genotypes) and *R. damascena*, respectively. In addition to identifying organisms, morphological characterization helps in studying genetics, taxonomy, evolutionary developments, measuring diversity and determining the material's economic usage, etc. Therefore, the present study was taken up with the objective of probing existing diversity within in Indian rose germplasm. In the current study, 148 rose genotypes, including wild species, were taken and were assessed for their variability using 59 DUS descriptors developed by PPV& FRA, India.

Materials and Methods

The study was carried out at division of Floriculture and Landscaping, ICAR- IARI, New Delhi, during two successive flowering seasons (February-March) of 2017-18 and 2018-19. The experimental material consisted of 148 rose genotypes (Table 1). The selected genotypes (10 plants/genotype) were maintained in the field at a spacing of 60 × 60 cm and a uniform package of practices were followed in genotypes to nullify the statistical errors. The data on 59 different morphological characters were collected from the selected genotypes (10 plants/genotype) during two consecutive years (2018 and 2019) based on PPV & FRA drafted DUS guidelines of roses (PPV & FRA, 2009) (Table 2). Qualitative and pseudo-qualitative characteristic parameters were recorded by visual assessment and quantitative parameters were measured physically under daylight conditions on ten plants per replication by excluding border rows. The observations related to color was recorded using the Royal Horticultural Society (RHS) color chart. The data recorded for all morphological characters was transformed into qualitative data by assigning the respective numerical note or rank based on state of expression of each character mentioned in Table 2. To reveal the morphological similarities and dissimilarities among characterized rose genotypes, recorded morphological data was analyzed and dendrogram was generated (using average linkage between the groups) using hierarchical cluster analysis based on Euclidian distance values with IBM SPSS statistic software (version 20).

Results and Discussion

Vegetative Characteristics

Plant growths, anthocyanin coloration, prickle and leaf characteristics

All vegetative traits used for characterization of germplasm and the number of genotypes identified for each trait was mentioned in Table 2. The genotypes noticed a considerable amount of variability for almost all the parameters. The type of growth observed among the genotypes was shrub, bed, and climber and majority of the germplasm were found with shrub type growth behavior. While the growth habit of studied genotypes varied from upright to spreading. Maximum genotypes were identified with semi-upright and intermediate growth behavior. Plant height in different genotypes varied from tall (>150 cm) to short (<60 cm). The growth type, habit and height of the plants collectively decide the architecture of plants, which is indirectly responsible for gathering resources from the environment. Most commercial varieties belonging to the hybrid tea group were found with a shrub- upright/semi-upright/intermediate with medium- tall plant height. Whereas floribundas had the characteristic feature of low growing

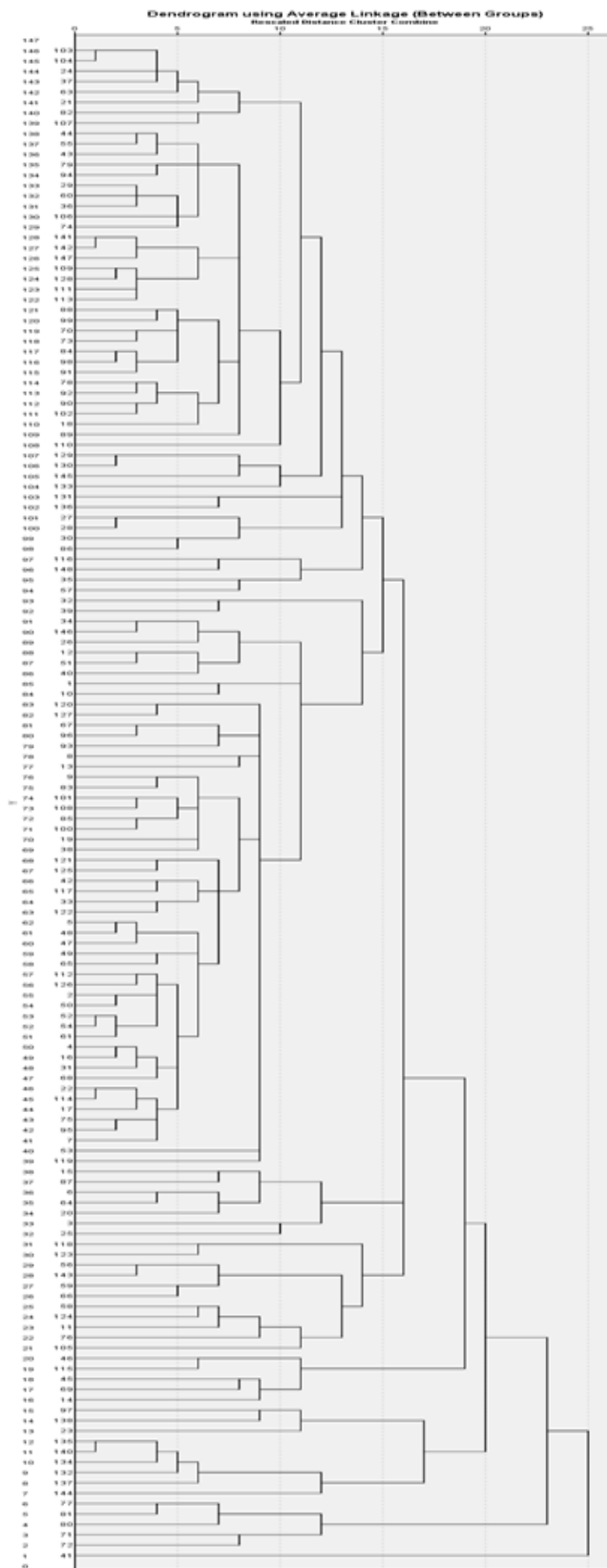


Figure 1: Hierarchical cluster analysis dendrogram of rose genotypes based on morphological data using average linkage (between groups) (Names of the rose genotypes with sequence mentioned in Table)

Table 1: List of characterized rose genotypes

Population	Rose genotypes
Modern rose genotypes (128)	Abhisarika, Anurag, Arjun, Arka Parimala, Aruna, Ashwini, Bhim, Century Two seedling, Chambe-di-Kali, Chitra, Dil-Ki-Rani, Dr. B.P. Pal, Dr. Benjamin Pal, Dr. Bharat Ram, Dr. M.S. Randhawa, Dr. R.R. Pal, Dulhan, Eiffel tower × Queen Elizabeth, Ganga, Golden Afternoon, Haseena, Homage, Jawani, Lalima, Lal Makhmal, Madhosh, Maharani, Mother Teresa, Mridula, Mrinalini, Mrs. K. B. Sharma, Nayika, Nehru Centenary, Nurjahan, Pink Montezuma, Preyasi, Priyadharshini, Pusa Ajay, Pusa Arun, Pusa Bahadur, Pusa Garima, Pusa Mansij, Pusa Mohit, Pusa Priya, Pusa Sonara, Raja Ram Mohan Roy, Raja Surendra Singh of Nalagarh, Rajkumari, Raktagandha, Raktima, Ranjana, Ratnaar, Sahasradhara, Shanti Pal, Shreyasi, Sir. C. V. Raman, Soma, Sugandha, Surabhi, Surekha, Surkhab, Jawahar, Shiloz Mukherjee, Indian Princess, Pusa Gaurav, Pusa Shatabdi, Akash Sundari, Delhi White Powder Puff, Delhi Pink Powder Puff, Anitha, Arunima, Banjaran, Chingari, Deepak, Delhi Brightness, Delhi Princess, Dr. S. S. Bhatnagar, Himangini, Jantar Mantar, Krishna, Lahar, Loree, Madhura, Manmatha, Manasi, Mohini, Navneet, Neelambari, Prema, Punchu, Pusa Abhishek, Pusa Barahmasi, Pusa Komal, Pusa Pitambar, Rupali, Sabnam, Sadabahar, Shola, Sindhoor, Suchitra, Surdas, Suryakiran, Suryodaya, Tarang, Pusa Virangana, Pusa Manhar, Pusa Urmil, Pusa Muskan, Rose Sherbet, Velvet Fragrance, Perfume de French Comete, Papa Meilland, Melody Perfume, Elle, Karen Blixon, Christian Dior, Bonne Nuit, Sweet Afton, Oklahoma, Midas Touch, Memorial Day, Fragrant Plum, Fragrant Lace, Eiffel Tower, Double Delight, Century Two, Brandy, Blue Moon
Wild genotypes (20)	<i>R. dumalis</i> , <i>R. glutinosa</i> , <i>R. tomentosa</i> , <i>R. macrophylla</i> , <i>R. damascena</i> var. Himroz, <i>R. damascena</i> var. Jwala, <i>R. damascena</i> var. Rani Sahiba, <i>R. indica</i> × <i>R. nitida</i> , <i>R. indica</i> major, <i>R. bourboniana</i> , <i>R. chinensis</i> <i>viridiflora</i> , <i>R. lutea</i> , <i>R. brunonni</i> , <i>R. wichuriana</i> , <i>R. moschata</i> , <i>R. multiflora</i> , Dr. Huey (Hybrid <i>Wichuriana</i>), <i>R. slancensis</i> , <i>Rosa</i> spp. (Nepal), <i>Rosa</i> spp. (Kakinada rose)

Table 2: Number of genotypes identified for each characteristic state in the studied rose germplasm

S. No.	Characteristic	State and No of genotypes	S. No.	Characteristic	State and No of genotypes
1.	Plant growth type	Bed (34), Shrub (110), Climber (4)	25.	Density of petals	Very loose (18), Loose (37), Medium (37), Dense (74)
2.	Plant growth character	Up-right (53), Semi-upright (68), Intermediate (18), Moderately spreading (9), Strongly spreading (0)	26.	Flower shape	Round (110), Irregularly round (12), Star shaped (26)
3.	Plant height	Very short (0), Short (14), Medium (80), Tall (45), Very tall (9)	27.	Flower side view of the upper part	Flat (51), Flatted convex (97), Convex (0)
4.	Young shoot anthocyanin coloration	Present (143), Absent (5)	28.	Flower side view of the lower part	Concave (39), Flat (50), Flatted Convex (37), Convex (22)
5.	Young shoot-intensity of anthocyanin coloration	Very weak (18), Weak (27), Medium (41), Strong (43), Very Strong (13)	29.	Flower fragrance	Absent (22), Weak (48), Medium (37), Strong (41)
6.	Stem-Number of prickles	Absent (6), Few (18), Medium (58), Many (64)	30.	Sepal extensions	Absent (7), Few (89), Medium (41), Many (10)
7.	Leaf Size	Small (7), Medium (89), Large (52)	31.	Reflexing of the petals	Absent (13), Present (134)
8.	Leaf Intensity of green Color on upper side	Very light (0), Light (3), Medium (51), Dark (74), Very dark (20)	32.	Petal shape	Elliptic (3), Obovate (115), Rounded (29)
9.	Leaf anthocyanin Coloration	Absent (129), Present (19)	33.	Petal incisions	Absent (44), Weak (59), Medium (16), Strong (29)
10.	Leaf glossiness on upper side	Absent (14), Weak (42), Medium (58), Strong (38)	34.	Petal reflexing of margin	Absent (16), Weak (37), Medium (57), Strong (37)
11.	Leaflet undulation of margin:	Absent (8), Weak (62), Medium (53), Strong (24)	35.	Petal undulation of margin	Absent (14), Weak (38), Medium (61), Strong (35)
12.	Leaflet serration of margin	Absent (0), Fine (19), Medium (51), Dense (78)	36.	Petal length	Very short (0), Short (9), Medium (94), Long (38), Very long (7)
13.	Terminal leaflet: shape of the blade	Narrow elliptic (14), Medium elliptic (48), Ovate (64), Circular (22)	37.	Petal width	Very short (0), Short (9), Medium (82), Long (47), Very long (9)

14.	Terminal leaflet: Shape of the base of the blade	Acute (27), Obtuse (88) Rounded (32), Cordate (1)	38.	Petal: Number of Colors on inner side	One (134), Two (11), More than two (2)
15.	Terminal leaflet: shape of the apex of the blade	Acuminate (94), Acute (54), Obtuse (0), Round (0)	39.	Varieties with one Color on inner side of the petal	Lighter towards the base (25), Uniform (127), Lighter towards the top (2)
16.	Flowering shoot: Flowering shoot laterals	Present (68), Absent (80)	40.	Petal: Spot at base of inner side	Absent (7), Present (141)
17.	Flowering shoot: Number of flowering laterals	Few (20), Medium (25), Many (23)	41.	Size of the spot at base of inner side	Small (83), Medium (29), Large (29)
18.	Flowering shoot: Number of flowers (varieties with no flowering laterals)	Few (58), Medium (11), Many (10)	42.	Petal: Spot at the base of outer side	Absent (7), Present (141)
19.	Flowering shoot: number of flowers per lateral (varieties with flowering laterals)	Few (18), Medium (25), Many (27)	43.	Size of the spot at base of outer side	Small (83), Medium (23), Large (24)
20.	Flower bud: shape of longitudinal section	Elliptic (10), Round (13), Ovate (88), Broad ovate (24)	44.	Outer stamen predominant Color of the filament	White (1), Green (3), Light yellow (20), Medium yellow (67), Orange (24), Pink (26), Red (4), Brown red (0), Purple (1)
21.	Flower type	Single (9), Semi-double (56), Double (86)	45.	Seed vessel size	Very small (12), Small (71), Medium (60), Large (3), Very large (1)
22.	Flower color group	White or near white (16), White blend (1), Green (1), Yellow (4), Yellow blend (3), Orange (7), Orange blend (4), Pink (67), Pink blend (7), Red (24), Red blend (1), Red Purple (1), Purple (2), Violet blend (2), Brown blend (0), Multi Colored (2), Mauve (2), Apricot blend (6)	46.	Hip: Shape of longitudinal section	Funnel shaped (32), Picture shaped (112), Pear shaped (3)
23.	Flower diameter	Small (33), Medium (44), Large (71)	47.	Flower: Length of pedicel	Short (54), Medium (61), Long (33)
24.	Flower color of the center	Green (0), Yellow (13), Orange (10), Pink (8), Red (4), Purple (2)	48.	Venation of petals	Absent (35), Weak (50), Medium (36), Strong (27)

habit, most of these types had bed type of growth with intermediate/spreading/moderately spreading growing habit. While climbing growth type was observed in very few genotypes, most of which were wild except cultivar Dr. Huey.

Anthocyanin coloration on young shoots and leaves and its hue is another important adaptive feature of roses. Presence of anthocyanin pigment is highly correlated with resistance to various biotic (fungi, herbivores) and abiotic factors (cold, excess radiation both visible as well as UV) in plants (Karageorgou and Manetas, 2006). Nearly, 95% of genotypes found with anthocyanin coloration on young shoots and leaves except for few (Lalima, Nehru Centenary, Pusa Bahadur, Sabnam, *R. lutea*) (Figure 2). Of 143 pigmented varieties, 13, 43 and 41 genotypes had very strong, strong, and medium pigment intensity on young shoots. The genotypes with more color intensity might carry some amount of tolerance against abiotic stress factors. Similarly, prickles provide defense against number of predators, at the

same time provide support to the plant. The genotypes with presence of prickles greatly varied in their color as well as in shape. The prickle variability present in the genotypes can be used for breeding genotypes with herbivore tolerance (Gaurav *et al.*, 2018). But in modern rose breeding prickle-free character is preferred over prickle character for ease of harvesting and maintenance. Genotypes identified with prickle-free characters were Pusa Mohit, Delhi White Powder Puff, Pusa Komal, Rupali and *R. lutea*.

Leaf size, intensity of green color on the leaves, leaf glossiness, and undulation, serration of leaf margin and shape of the leaflet were observed. The variability between the wild and cultivated types was observed for leaf size, leaflet size, and number. In modern cultivars leaves were found with medium to large leaf size with maximum 5 leaflets, whereas in wild types leaf size was comparatively small, and the number of leaflets varied from 5 to 9. Likewise, intensity of green color and glossiness of the leaves also



Figure 2: Rose genotypes devoid of anthocyanin pigmentation of young shoots.



Figure 3: Rose genotypes having more than one petal Color.

varied among modern roses, but in wild genotypes, glossiness and green color is absent in most of them. The differences noticed for leaf characters among wild and cultivated types might be due to the involvement of artificial



Figure 4: Rose genotypes identified with more number of flowering laterals

selection of genotypes for attractive leaf characteristics over the generations. Many of the characterized genotypes were found with medium to large size (10–16 cm). While few genotypes, such as Dil-ki-Rani, Delhi White Powder Puff, Arunima, Mansi, *R. slancensis*, *R. lutea*, Dr. Huey were observed with small leaf and leaflet sizes. Genotypes were identified with fine medium and dense leaf serration. Instead of acting as a mere marker, leaf serration is found to be an important adaptable trait for plants growing in temperate regions. Leaves with teethed margins have better transpiration and photosynthetic abilities than leaves with smooth margins (Steve Nix, 2018). Leaf undulation is another marker for studying phylogeny. The trait improves the contact between cells which allows efficient signaling, increases epidermal integrity, helps in leaf flex and alleviates the stress caused by their own growth (Sotiriou *et al.*, 2018). More than 94% of the characterized roses were found with varying degrees of undulation.

Flowering Characteristics

Flowering shoot and flowering shoot laterals

The modern grouping system of roses indirectly depends on flower bearing nature of the plant along with flower type

and plant growth behavior. Hybrid Tea varieties had mainly upright growth habit with around 1 to 2 m plant height and bears mostly single, well-shaped flowers with high spiraling centers. Floribundas, another class, produce abundant clusters of small flowers on its flowering stems. Majority of hybrid tea genotypes perfectly fit into its characteristic nature with 'single flowering shoot or absence of flowering laterals', while few hybrid tea cultivars varied slightly with this character by bearing few or medium number of laterals on their main flowering shoots. Although these varieties were identified with flowering laterals, the flower and plant growth behaviour characteristics match with the hybrid tea group. Likewise, almost all floribunda varieties observed in the study were found with varying number of flowering laterals (Figure 4) on their flowering shoots except nine cultivars. However, these varieties were grouped under the category of floribundas due to their lower plant growth habit and flower characteristic resemblance with floribunda types. Whereas in wild species, flower laterals were present in all apart from Kakinada rose and *R. indica major*.

Flower bud shape, flower type, size, flower color, petal density and fragrance

Flower bud shapes in majority of genotypes were ovate and broadly ovate. Very few genotypes had elliptic and round bud shape. Petal number decides the flower type in roses. Usually, wild rose species have a 'simple flower' with typically five petals per flower, while in modern roses, flowers are found to be double type consisting of >10 petals per flower (Panwar *et al.*, 2012). Among wild genotypes single flowers were found in *R. slancensis*, *R. brunonni*, *R. macrophylla*, *R. wichuriana*, *R. moschata*, *R. multiflora*, *R. glutinosa*, *Rosa* spp. (Nepal species) and *R. tomentosa* and remaining few genotypes were identified with semi-double and double flowers. The semi double wild genotypes present in the study were *R. indica* × *R. nitida*, *R. indica major*, *R. dumalis*, *R. lutea*, *R. bourboniana* and Dr. Huey. Whereas in modern roses, 86 double, 53 semi-double and 9 single type flowers were identified. Flower color is foremost important feature of roses. Around, 16 different flower color groups (as per DUS characteristic groups) were identified in the study (Table 2 and Figure 3). Flower size in roses varies from 1.25 (miniatures) to 17.5 cm (Hybrid Teas). Diameter of the flower depends upon size of the flower organs, especially petals. The flower size varies from 3.5 to 12 cm among the studied genotypes.

Petal density depends upon petal number, arrangement and flower size. Majority of the genotypes with double flowers were identified under the dense petal group. Likewise, flower type with inward petal arrangements such as blooms with quartered, cupped, globular, rosette and high centered blooms with many petals showed more petal density than exerted flat bloom shapes. The shape of rose blooms depends upon the size of the petals (inner

and outer whorl of the flower), petal undulation, serration, and refluxing of petal margins. In different roses, the bloom shape varies from round, irregular and star. Among the studied genotypes 74% of the flowers possess round flower shape, while 17 and 8% flowers possess star and irregular flower shape. The star shaped flowers were found with strong petal reflexion. Likewise, serration and undulation were more common in genotypes with round and irregularly round flowers. In bloom types such as cup, pompon and in globular shaped flowers petal reflexion is comparatively poor or absent as compared to the flowers with flat, rosette and quartered shape. Flower fragrance is another superior economic trait. The quality, quantity, and composition of the hundreds of volatile molecules decide the scent profile (Prasad *et al.*, 2006; Aparna *et al.*, 2019). A great amount of diversity is noticed for fragrance trait in studied roses. In this study, around 41 highly fragrant, 37 medium and 48 weak fragrance types were identified and 22 were found to be non-fragrant.

Petal basal spot, venation, filament color, sepal extensions, seed vessel and petal venation

Based on presence and absence of basal spot genotypes were grouped into two. Basal spot was absent in very few varieties. In majority of the genotypes color of basal spot is yellow. color of the basal spot was indistinguishable if color of the petal is more intense. Likewise, flowers with yellow secondary color distribution on petal base (Banjaran, Chingari) were not seen with petal spot. Similarly, genotypes had flowers with uniform color distribution across petal with minute petal attachment (Himangini, Loree and Fragrant Lace) were not seen with petal spot. Strong, medium to weak petal venation was observed in 18 and 58% of genotypes, while clear venation was not seen in 28%. Flowers having intense color and petal thickness were seen with unclear venation. Color of the filament is concerned, nearly 73% of the studied genotypes were found with yellow filament color. But in few genotypes, filament color was slightly influenced by petal color. Certain degree of similarity was observed with outer stamen filament color with petal color in genotypes such as Aruna, Dr. Bharatram, Ashwini, century two seedling, Dil-ki-Rani etc. filament colors of these genotypes varied with dark colors like orange, pink, red and purple. But in few genotypes (Oklahoma, *R. damascena* cv. Ranisahiba, *R. lutea*, Fragrant Lace), the demarcation of filaments was prominent with petals and in these types color of the filaments was varied from white to green. Based on sepal extensions genotypes were categorized into four groups (Table 2). Five categories of seed vessels (very small, small, medium, large and very large) were observed in characterized germplasm. The majority (88%) fall in the category of small and medium. Very large hips were observed in varieties 'M. S. Randhawa and Dr. Benjamin Pal. The shape of the hip in majority (75%) types were pitcher,

followed by funnel shape in 21% germplasm and very few genotypes (2%) Chambe-di-kali, Sadabahar and Chingari were found with pear shaped seed vessels. Length of the flower pedicel and its strength along with flowering stem decides the commercial use and quality of flowers. Almost all the genotypes present in hybrid tea group were found with long to medium pedicel length, whereas most of floribundas and wild roses were found with small pedicel length.

Cluster analysis among rose genotypes based on observed characteristics

Hierarchical cluster analysis separated the genotypes into eight different clusters (Figure 1). Cluster-I was found with majority of the characterized genotypes (109); which was followed by cluster-III (11), cluster-II and VIII (7 each), cluster-IV & VII (5 each), cluster-V (3) and cluster-VI (1). Within clusters, similarities were observed among the genotypes irrespective of its group (HTs/Floribundas), origin (Exotic/Indian) and habitat (wild/domesticated). For example, cluster-I was found with majority of the hybrid teas, floribundas along with 11 wild species. Cluster-II was found with cultivars Arjun, Ashwini, Dr. M.S. Randhawa, Golden Afternoon, Madhosh, Jawani, and Neelambari. Among these varieties morphological uniformity was observed for around 9 traits. Cluster-III was identified with cultivars, Dil-ki-Rani, Sir C. V. Raman, Sugandha, Surabhi, Akash Sundari, Dr. S. S. Bhatnagar, Tarang, Fragrant Lace, Midas Touch, Oklahoma, *R. bourboniana*. For these genotypes, complete trait uniformity observed for around 6 qualitative traits. Similarly, 12 traits were uniform for genotypes present in cluster-IV.

Genotypes Lalima, Sabnam, *R. lutea* were found together in cluster V showed homogeneity for 17 DUS characteristics. Cultivar 'Pusa Mansij' is a multi-Colored Hybrid Tea, found alone in cluster-VI, exhibited morphological dissimilarity maximum genotypes with its unique traits like presence of more than two Colors on petals, absence of prickle on immature shoots, absence of petal reflexing, absence of petal margin reflexion, etc. Five floribunda varieties Banjaran, Chingari, Loree, Himangini and Lahar were grouped together in major cluster-VII. Among this, high uniformity was observed for majority of qualitative and pseudo-qualitative characters along with few quantitative characters such as leaf size, flower type, size of the basal spots. Cluster-VIII was found with all six wild genotypes *R. wichuriana*, *R. moschata*, *R. multiflora*, *R. glutinosa*, *Rosa* spp. (Nepal species) and *R. chinensis viridiflora* among these species complete morphological similarity was observed

for maximum number of quantitative features (flower diameter, sepal extensions, seed vessel size, length of the flower pedicel, petal undulation and reflexing) along with qualitative and pseudo-qualitative traits.

The present study revealed the vast variability within characterized rose genotypes, including wild species for various vegetative and flowering traits. Characterized data of different traits could be used as reference collection for the identification of rose varieties. The information on different traits also helpful for future improvement programmes for developing more competent types. The given information also helps the breeders and nurserymen seek the protection of their new material under PPV and FRA, New Delhi.

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