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PHENOTYPIC PLASTICITY WITHIN AND AMONG GENOTYPES OF *ROSA CENTIFOLIA* FROM PAKISTAN AND THE USA

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Rosa centifolia is famous as a new strain in Pakistan for producing highly fragrant flowers around the year even at high temperatures. It is used to extract oil. Eight landraces of *Rosa centifolia* were selected from eight districts of Punjab, Pakistan to evaluate their morphological variations. Eight varieties of *Rosa centifolia* from USA were also used to evaluate their phenotypic diversity by using 24 morphological parameters. In Pakistani landraces, maximum phenotypic variation was observed between Sargodha and Sheikhupura landraces, while maximum similarity was found among the Pattoki and Faisalabad landraces. For the American varieties, the highest similarity was found among Fantin-Latour and Rosa de Meaux and the least similarity among Cabbage rose 2 and Paul Ricault varieties. Leaf size, leaf green color at first flowering, and undulation of leaf margins were positively correlated with number of petals per flower, flower diameter, and flower fragrance in Pakistani landraces. However, in the USA genotypes, only undulation of leaf margins showed negative association with flower fragrance.

Keywords: Morphological diversity, *Rosa centifolia*, landraces, phenotypic variation

INTRODUCTION

Rose is the king of flowers with great diversity in the world. Roses are used beyond ornamental purposes, such as in the food, cosmetics (Kaur *et al*., 2007) and the medicinal industry (Bown, 2002; Achuthan *et al*., 2003; Basim and Basim, 2003; Ozkan *et al*., 2004) since the dawn of civilization (Randhawa and Mukhopadyay, 1994). Nowadays, roses have proved to be the most famous garden plant and cut flower in the world (Anonymous, 2013). During 2010 cut flower of worth 35 million rupees have been exported from Pakistan (Bashir, 2010). *Rosa centifolia* is one of the four famous oil producing species of the genus Rosa (Jaskani *et al*., 2005; Tabaei-Aghdaei *et al*., 2007). It is famous in Pakistan and growers from various districts of Punjab are cultivating this species and trading its flowers and by-products. An acre of 5,000 plants can produce 5,000 kg flowers and almost 1.0 kg of oil. The oil sells at much higher value than input costs. Another advantage of this species is that it can produce flowers throughout the year as compared to other rose species which are not able to produce flowers during the hot summer conditions.

Morphological differences were observed among *Rosa centifolia* plants found in different districts of Punjab, Pakistan. This has resulted in the selection of several promising landraces in different parts of the country. These local landraces of *Rosa centifolia* with different characters are preferred by farmers. Most farmers entrust in landraces rather than in formal breeding systems (Almekinders *et al*., 1994). As landraces contain convincing phenotypic variations, can develop resistance against biotic and abiotic stresses (Eagles and Lothrop, 1994), have historical origin, noticeable identity and genetic diversity are accepted and preferred by local farmers, and are empty from formal crop improvements (Camacho Villa *et al*., 2005). Standardization of different landraces is difficult because they differ due to by environmental conditions or genetic variations (Adugna *et al*., 2006; Mahmood *et al*., 2013, 2014).

Growing of *Rosa centifolia* is not very common in Pakistan, but the demand for *Rosa centifolia* is increasing due to its use in variety of products and high returns to producers. The globalization of the worldwide economy has diminished the boundaries and magnified the competition in the world market, which has also increased the opportunities for *Rosa centifolia* production both domestically and globally. The opening up of the world flower market has also built new challenges in terms of product quality, safety, and price in order to fulfill the global competition as well as WTO standards. WTO standards have increased the competition of our product from its competitors. At a global level, morphological and genetic characterization is very important to meet the WTO standards and intellectual rights.

The present study was designed to investigate the morphological diversity among landraces of *Rosa centifolia* expressing differing useful characters in Pakistan and its different varieties used as ornamental purpose in USA.

MATERIALS AND METHODS

***Areas selected for morphological study*:** Punjab is heavily populated and a flourishing province of Pakistan located at 31.33° North and 74.21° East with temperatures ranging between -2° and 45°C, but can reach 53°C in summer and -5°C in winter. Many districts in Punjab have been growing *Rosa centifolia* for many years and are increasing their acreage with the passage of time. Eight districts (Sargodha, Sialkot, Lahore, Pattoki, Pakpattan, Kahror Pakka, Faisalabad and Sheikhupura) of Punjab were selected for morphological and genetic study after observing high morphological diversity among the landraces of *Rosa centifolia* in these districts (Table 1). Soil (Table 2) and water (Table 3) samples used for growing these landraces were also collected and tested by the Soil and Water Testing Laboratory, Ayub Agriculture Research Institute, Faisalabad, Pakistan.

***Arrangements of experiment*:** Plants of similar age were selected from all the above mentioned districts and undergo same type of pruning and other cultural practices. All the plants in all districts were propagated though cuttings.

Cuttings of genotypes collected from Rogue Valley Roses, Oregon, USA grown in a greenhouse at the Horticulture Department, Texas A&M University, USA (Table 4). Growing media used for these cuttings was a Metro-Mix Professional Growing Mix #900 composed of pine bark, vermiculite, Canadian sphagnum moss, peat moss, perlite and dolomitic limestone. Reverse Osmosis water was used for irrigation

Table 1. Different sites of *Rosa centifolia* collection Punjab, Pakistan

Landrace Code	Collection site	Latitude (N)	Longitude (E)	Altitude (m)	Rainfall (mm)
D1	Sargodha	32°33’	72°73’	193	441
D2	Sialkot	32°33’	74°75’	256	981
D3	Lahore	31°32’	74°75’	213	588
D4	Pattoki	31°32’	73°74’	186	413
D5	Pakpattan	30°31’	73°74’	155	256
D6	Kahror Pakka	29°30’	71°72’	123	167
D7	Faisalabad	31°32’	73°74’	184	256
D8	Sheikhupura	31°32’	74°75’	236	588

(The New Oxford Atlas for Pakistan, 2006).

Table 2. Analysis of water samples collected from different sites of Punjab, Pakistan

Landrace Code	Collection site	Electrical Conductivity (EC µs/cm)	Sodium Absorption Ratio (SAR)	Residual Sodium Carbonate (RSC)
D1	Sargodha	367	0.38	Nil
D2	Sialkot	201	0.03	0.02
D3	Lahore	1567	4.05	3.88
D4	Pattoki	976	0.35	Nil
D5	Pakpattan	707	1.83	1.20
D6	Kahror Pakka	283	0.09	Nil
D7	Faisalabad	1146	6.30	5.07
D8	Sheikhupura	384	0.89	Nil

Table 3. Analysis of soil samples collected from different sites of Punjab, Pakistan

Landrace Code	Collection site	Electrical Conductivity (EC mS/cm)	Soil pH	Organic Matter (%)	Saturation (%)
D1	Sargodha	1.99	7.7	0.36	38
D2	Sialkot	1.04	7.5	0.36	37
D3	Lahore	1.07	7.2	0.52	38
D4	Pattoki	1.89	7.5	0.47	40
D5	Pakpattan	1.16	7.4	0.26	40
D6	Kahror Pakka	1.15	7.4	0.88	42
D7	Faisalabad	1.09	7.3	0.36	40
D8	Sheikhupura	28.50	6.0	0.36	100

Table 4. Genotypes of *Rosa centifolia* from USA

Genotypes Code	Genotypes
G1	Fantin-Latour
G2	Gros Choux d' Hollande
G3	Centifolia varigata
G4	Rosa de Meaux
G5	Cabbage rose1
G6	Cabbage rose2
G7	Pompon de Bourgogne
G8	Paul Ricault

***Morphological parameters*:** At the onset of flowering, data of 24 different morphological parameters were collected from all germplasm of the eight districts of Punjab. Twenty plants were randomly selected from each district and data was collected from nine sites of a plant on parameters like plant height; plant width; young shoot anthocyanin; prickles; prickles shape of lower side; short prickles number and long prickles number; leaf green color; glossiness of upper side; leaflet cross section; leaf undulation of margin; terminal leaflet length of blade; terminal leaflet width of blade and terminal leaflet shape of base; flowering shoot number of flowers; flower type number of petals; flower diameter; view from above; side view of upper part; side view of lower part; fragrance; petal size and time of beginning of flower. All these qualitative parameters were selected and measured according to the descriptions in the plant descriptor.

***Statistical analysis*:** Correlation between parameters was determined by Pearson's coefficient and cluster analysis was conducted by using Statistica 7 (Statsoft. Inc., 1984-2007).

RESULTS

***Cluster analysis for different morphological characters of landraces of Rosa centifolia from Pakistan*:** Diversity among different landraces of *Rosa centifolia* in Pakistan was assessed by constructing a dendrogram of the eight landraces on the bases of their morphological characters (Fig. 1). All the landraces are divided into three groups at a linkage distance of 1.0. There are two landraces in group A, namely Sheikhupura and Pakpattan. Group B is the largest group consisting of Kahror Pakka, Faisalabad, Pattoki, and Lahore. Group C consists of landraces Sialkot and Sargodha. Group B, the largest of the dendrogram, is again divided into two groups at a linkage distance of 0.6. First subgroup is made up of D6 (Kahror Pakka) and other with Faisalabad, Pattoki and Lahore landraces. Landrace of Faisalabad is closest to Pattoki and at maximum distance of Sialkot.

***Principle component analysis for different morphological characters of landraces of Rosa centifolia*:** The results of different characters of landraces of *Rosa centifolia* are explained by a plot in Fig. 2. Principle component analysis showed 35.74% and 34.29% of total variation was due to factor 1 and factor 2, respectively. All landraces of *Rosa centifolia* collected from different the districts are in the same group. The landraces are distributed into groups in the plot, which are in accordance with the results of cluster analysis in Fig. 2.

***Correlation between morphological parameters of Rosa centifolia landraces from Pakistan*:** Nine important morphological parameters were selected to estimate their correlation (Table 4). Number of small prickles was negatively correlated with the number long prickle, leaf size, number of prickles on flower pedicel, number of flowers, number of petals per flower, flower fragrance (-0.314, -0.087, -0.090, -0.087, -0.040, and -0.027, respectively). Number of small prickles was positively correlated with all other parameters. Longer prickles number was positively correlated with all other parameters except number of prickles on flower pedicel. The character leaf size also has negative association with number of prickles on flower pedicel, number of flowers, and number of petals per flower and a positive correlation with rest of parameters included in the study. Leaf green color and undulation of leaf margin showed positive and strong association with all parameters. Negative association of number of prickles on flower pedicel

Table 4. Correlation between morphological parameters of *Rosa centifolia* landraces from Pakistan

	Short prickle No.	Long prickle No.	Leaf size	Leaf green color	Leaflet undulation of margin	Flower pedicel No. of prickle	No. of petals	Flower diameter	Fragrance
Short prickle No.	1.000								
Long prickle No.	-0.314	1.000							
Leaf size	-0.087	0.009	1.000						
Leaf green color	0.178	0.028	0.348	1.000					
Leaflet undulation of margin	0.177	0.031	-0.150	0.421	1.000				
Flower pedicel No. of prickle	-0.090	-0.009	-0.134	0.292	0.464	1.000			
No. of petals	-0.040	0.166	0.541	0.247	0.091	-0.034	1.000		
Flower diameter	0.156	0.131	0.369	0.611	0.470	0.215	0.468	1.000	
Fragrance	-0.027	0.070	0.541	0.288	0.164	-0.014	0.780	0.521	1.000

Table 5. Correlation between morphological parameters of *Rosa centifolia* landraces from USA

Parameters	Short prickle No.	Long prickle No.	Leaf size	Leaf green color	Leaflet undulation of margin	Flower pedicel No. of prickle	No. of petals	Flower diameter	Fragrance
Short prickle No.	1.000								
Long prickle No.	0.404	1.000							
Leaf size	0.298	-0.193	1.000						
Leaf green color	0.086	-0.371	0.455	1.000					
Leaflet undulation of margin	-0.071	-0.410	0.756	0.401	1.000				
Flower pedicel No. of prickle	0.795	0.404	-0.027	-0.374	-0.071	1.000			
No. of petals	0.324	0.093	0.342	0.072	0.422	0.324	1.000		
Flower diameter	0.444	-0.023	0.746	0.571	0.688	0.181	0.706	1.000	
Fragrance	0.082	-0.259	0.156	0.460	-0.137	-0.312	0.180	0.347	1.000

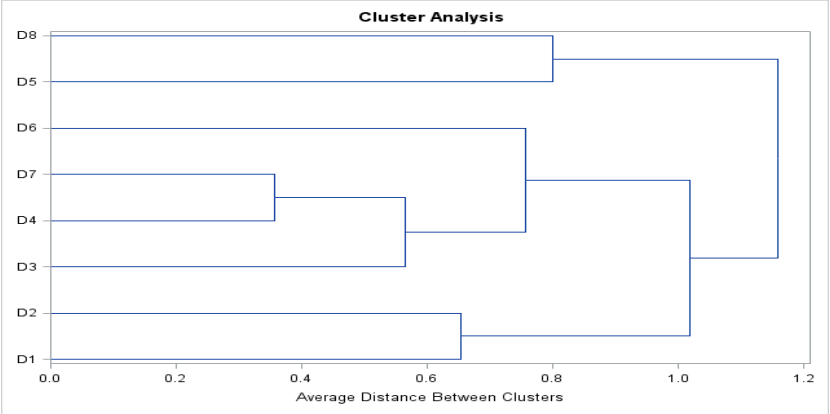

Figure 1. Cluster analysis of landraces of *Rosa centifolia* from Pakistan

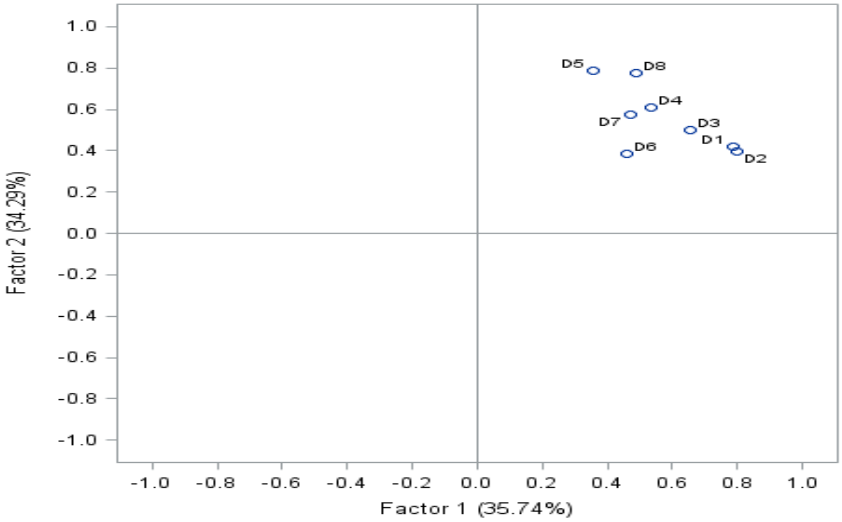

Figure 2. Principle component analysis of landraces of *Rosa centifolia* from Pakistan

was found with all of the parameters except for flower diameter. Flower number, petal number, and flower diameter showed positive correlation with rest of parameters. Flower fragrance was positively correlated with all parameters except number of small prickles and number of prickles on flower pedicel.

***Cluster analysis for different morphological characters of different varieties of Rosa centifolia from the USA*:** Cluster analysis Fig. 3 showed the diversity among eight different

varieties of *Rosa centifolia* grown in the USA. This diversity was determined through 26 morphological characters. In the dendrogram all eight varieties of centifolia are differentiated into three main groups at the linkage distance of 1.00. Among these three groups group A and C are the shortest groups containing only genotypes Fantin-Latour and Rosa de Meaux, respectively. These two varieties are maximum apart and variant from each other. Group B is the second largest group in the dendrogram, containing the remaining 6 varieties and again divided into 4 subgroups at a linkage distance of 0.76. First three subgroup of group B are made by *Centifolia varigata*, Cabbage rose1 and Pompon de Bourgogne, respectively. The fourth subgroup combines Paul Ricault, Cabbage rose 2 and Gros Choux d' Hollande together.

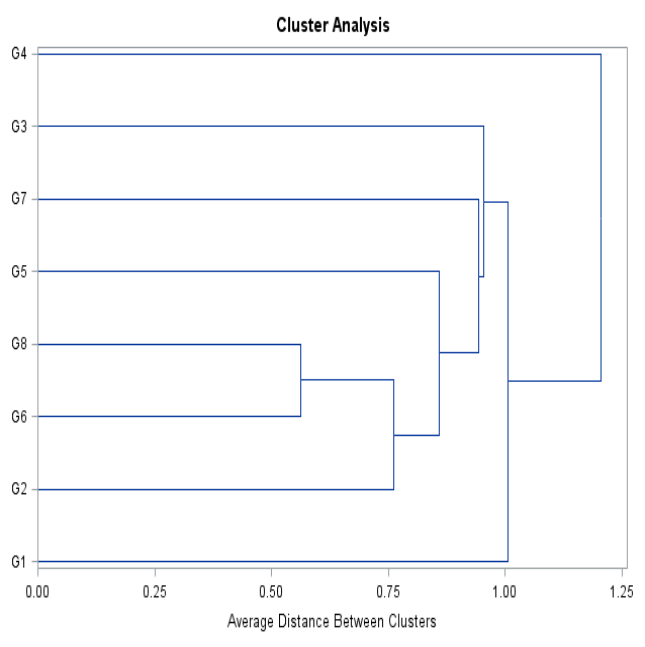

Figure 3. Cluster analysis of different varieties of *Rosa centifolia* from USA

***Principle component analysis for different morphological characters of different varieties of Rosa centifolia from the USA*:** Principle component analysis was conducted on various morphological parameters of different varieties of centifolia found in the USA and a total variation of 47.78% and 39.75% was documented by factor 1 and factor 2, respectively (Fig. 4). All genotypes were grouped in one block in of plot. All different types of centifolia were divided into three groups. Two most variant types, G1 and G6 are in two separate groups, while all the remaining ones found in the same group. These results of principle component analysis are according to the preceding results of results of cluster analysis.

***Correlation between morphological parameters of Rosa centifolia varieties from the USA*:** Eleven important morphological parameters were selected for studying correlation among them (Table 5). There was a negative correlation of number of small prickles with undulation of leaf margin (-0.071) but a positive one with all other characters. Longer prickles number had negative association with all characters except for the numbers of prickles on flower pedicel and with petal number. Leaf size was positively correlated with all parameters except number of prickles on flower pedicel. Leaf green color was negatively correlated with flower pedicel number of prickles. Undulation of leaf margin had negative correlation with number of prickles on flower pedicel and flower fragrance but positive with petal number and flower diameter while flower pedicel number of prickles had only negative association with fragrance. Petal number and flower diameter were positively associated with all parameters.

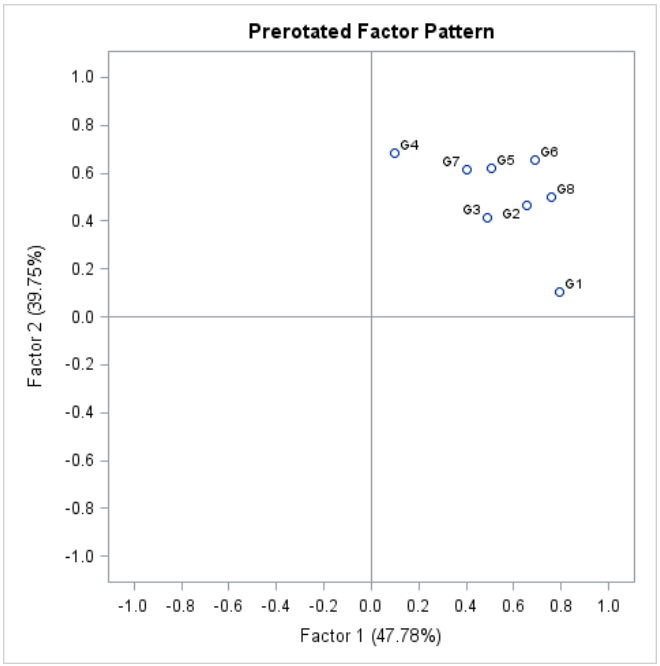

Figure 4. Principle component analysis of different varieties of *Rosa centifolia* from USA

***Cluster analysis for different morphological characters of different varieties of Rosa centifolia from Pakistan and USA*:** After separate analysis of genotypes of *Rosa centifolia* from Pakistan and USA, a cluster analysis was made to determine morphological relationships among all genotypes together (Fig. 5). All genotypes were divided into 3 groups at a linkage distance of 1.13. In first group, only Rosa de Meaux genotype was present. The second group was made up of Cabbage rose1 and Pompon de Bourgogne genotypes. The third group was the largest which was further divided

into two sub groups, one made up of Fantin-Latour and the other subgroup containing all other remaining genotype of USA and Pakistani landraces.

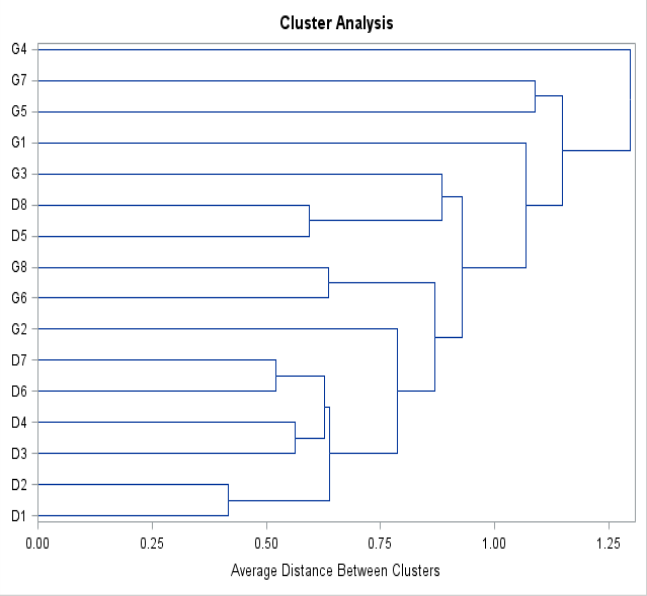

Figure 5. Cluster analysis of 8 landraces of *Rosa centifolia* from Pakistan and 8 varieties from USA

DISCUSSION

Interaction of plants with their environment and their phenotypic plasticity allows for better adjustment and survival in a climate (Bradshaw, 1965; McNaughton *et al.*, 1974). Diversity in ecology supports the genetic variations and survival of a variety of plants (Nilsson, 1972). Morphological differences can be used to differentiate closely related species and to study variations within or among species (Nybom *et al*., 1996, 1997). Morphologically, *Rosa centifolia* from Sargodha and Sialkot districts were identical. Similar attributes were found with Pakpattan and Sheikhupura landraces and with Faisalabad and Pattoki landraces. Maximum morphological diversity was observed between Sargodha and Sheikhupura. Riaz *et al*. (2007) reported a higher morphological similarity level among the landraces of *Rosa webbiana* and *R. brunonii* found in the hilly areas of Pakistan.

Soil is ultimate storage of water and effective nutrients necessary for healthy plant growth. If soil is not properly drained, it can affect roots and nutrient availability to plants (Karlik *et al*., 2003). Nutrients affect the quality and growth of rose plants (Savvas, 2002). Flower, production also depends on adequate nutrient supply. Water and soil samples of these sites carry differences which may be develop morphological variations in *Rosa centifolia* growing in the different sites of Punjab. Younis *et al*. (2006) studied the effect of rainfall and temperature on the yield of four Rosa species (*Rosa damascena, Rosa centifolia, Rosa borboniana* and Rosa 'Gruss an Teplitz') and found positive correlation among rainfall and temperature. Griffing and Langridge (1963) documented more phenotypic stability of *Arabidopsis thaliana* with a range of temperature. Edwards and Richardson (2004) noticed the effect of climate on different types of organisms found in various climates.

Different number of small and large prickles found in roses which are also called thorns (Rosu *et al*., 1995). The number of prickles decreases from bottom to top (Andre, 2003) and is controlled by one dominant gene (Debener, 1999; Debener, 2003; Rajapakse *et al*., 2001). Number of short thorns adversely affected leaf size, petal number and flower fragrance but the effect of long thorns does not correlate with these findings.

Plant leaves are also the source of variation estimation and are being considered to distinguish between the species (Olsson *et al*., 2000; Mclellan, 2000) as well as vegetatively propagated plant (Persson and Gustavsson, 2001). Leaf size positively affected the number of petals per flower, flower diameter and fragrance, which are very influential parameters for rose plant yield. Nawaz *et al*. (2011) found environmentally important anatomical variations in the leaves of *Rosa damascena* plants collected from different sites. Leaf green color at time of first flowering had supportive influence on all morphological parameters but flower diameter had strong positive correlation with it. Number of petals per flower had strong positive effect on the flower diameter and fragrance. Debener (2003) and Rajapakse *et al*. (2001) described the effect of environment and genes on the production of number of petals per flower. Fragrance is also positively associated by flower diameter. In roses, the number of petals, flower diameter, and fragrance are the main product liked by farmers and used to make different products like rose jam, oil etc. Farooq *et al*. (2013) noticed significant correlation between flower diameter and number of petals with oil content. Positive correlation among number of petals, flower diameter and fragrance showed the linkage of these traits with one common gene or some linked genes.

Among different varieties of *Rosa centifolia* present in the USA, Rosa de Meaux showed maximum morphological diversity compared to the others. Fantin-Latour also showed morphological plasticity while Cabbage rose 2 and Paul Ricault appeared highly similar in morphological features.

Crespel *et al*. (2002) observed that diversity among the density of prickles is controlled by various QTL. Number of short prickles was positively correlated with all characters but had negative association with undulation of leaf margins only. Leaf size affected positively the number of petals, flower diameter, and fragrance. Leaf green color at the time of first flowering also had positive relationship with all parameters except number of prickles on flower pedicel.

Undulation of leaf margins and number of prickles on flower pedicel both had positive relationship with number of petals and flower diameter but adversely affected the flower fragrance for oil extraction, a significant parameter for selection of rose plants. Number of petals, flower diameter and flower fragrance are important characteristics of *Rosa centifolia*. Variation in morphological data collected from Pakistan and the USA may be due to differences in genetic makeup or environment. Riaz *et al*. (2011) found 83% similarity of *Rosa webbiana* through morphological markers and 72% similarity of *Rosa brunonii* through genetic markers during his diversity study of four wild Rosa species from hilly areas of Pakistan. Younis *et al*. (2006) documented that *Rosa centifolia* found in Pakistan is a different strain because it showed recurrent flowering at high temperature.

All genotypes of *Rosa centifolia* from Pakistan and the USA produced double flowers with many petals. In roses, this character is controlled by one dominant gene (Debener, 1999, 2003; Crespel *et al*., 2002). Recurrent blooming as a prominent and important character was observed in all landraces of *Rosa centifolia* from Pakistan, while all genotypes from the USA were non-recurrent. De Vries and Dubois (1978) and Semeniuk (1971) described that the character of recurrent blooming present in many roses is controlled by recessive alleles. *Rosa centifolia* found in Pakistan can produce fragrant flowers at high temperature (Younis *et al*., 2006) but different genotypes of *Rosa centifolia* found in the USA are used for ornamental purposes and to produce flowers at high temperature.

Pakpattan and Sheikhupura landraces of Pakistan were morphologically more close to the Centifolia varigata genotypes of *Rosa centifolia* from the USA. Gros Choux d' Hollande, Cabbage rose 2 and Paul Ricault genotypes of USA were phenotypically closer to the Pakistani landraces, which showed a common source of origin.

***Conclusion*:** Present research work has explained the morphological diversity among landraces of *Rosa centifolia* found in Pakistan and the USA. Maximum diversity was observed between landraces collected from Sargodha and Sheikhupura of Pakistan and Fantin-Latour and Rosa de Meaux of the USA. Gros Choux d' Hollande, Cabbage rose 2 and Paul Ricault genotypes of USA were morphologically more similar with Pakistani landraces. Additional investigation is needed to determine QTLs are producing recurrent flowering at high temperature in *Rosa centifolia* of Pakistan.

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