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Full Length Article

Genetic Plasticity among Genotypes of *Rosa centifolia* and *R. damascena* from Pakistan, USA and Iran

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Abstract

Molecular analysis of various genotypes is the key component for evaluating their true to type genotype to start a commercial business and for future breeding projects. This study was performed to investigate the genetic diversity among genotypes of *Rosa centifolia* and *R. damascena* from Pakistan, USA and Iran. Thirty-six genotype of rose were analyzed with thirteen microsatellite markers scattered throughout the genome. The exclusive analysis of twenty-four *R. Centifolia* genotypes from Pakistan by UPGMA and PCA demonstrated genetic divergence among the districts, while high level of homology within the district. On the other hand, comparison of Pakistani genotypes with USA and Iran, *R. centifolia* Faisalabad and *R. centifolia* Sargodha, were found genetically divergent to Fantin-Latour and Paul Ricault but closely related with cabbage rose 2. *R. damascena* Faisalabad was found genetically diverse from other genotypes of *R. damascena* from Pakistan and Iran, while genetically closer to Rose de Meaux, Gros Choux d'Hollande and Fantin Latour of *R. centifolia* genotypes from USA. Genetic diversity of *R. centifolia* for Pakistani genotypes showed highly conserved nature at district level. In comparison, Pakistani genotypes of *R. centifolia* have maximum genetic diversity from USA genotypes except for Cabbage rose2 which showed their common ancestor. Findings of this study will help to construct a database helpful for characterization of other rose genotypes having economic importance and can be utilized for marker assisted breeding for improvement of *R. centifolia*.

Keyword: Pakistani genotypes; PCA; *Rosa centifolia*; Simple sequence repeat; UPGMA

Introduction

Rose is a most substantial floriculture crop in the world (Senapati and Rout, 2008). It belongs to family Rosaceae, genus *Rosa* and consists of 200 species and 18000 cultivars (Gudin, 2000). It was originally cultivated in Bulgaria then distributed in Turkey (Baydar *et al.,* 2004) and is commonly grown in different parts of world including Morocco, France and Egypt (Brechbill, 2011), also grown in certain areas of Pakistan (Akhtar *et al.,* 2014). Genetic diversity plays important role in the cultivation of roses that are used for oil extraction. Genetic diversity among different Rosa species have been investigated by using genetic markers (Liorzou *et al.*, 2016) such as amplified fragment length polymorphisms (AFLP) (Pirseyedi *et al.,* 2005; Mezghani *et al*., 2015), random amplified polymorphic DNA (RAPD) (Fredrick *et al.,* 2002; Kiani *et al.,* 2008), simple sequence repeats (SSR) (Akcay *et al.,* 2014; Tan *et al.,* 2017) and inter-simple sequence repeat (ISSR) (Ogras *et al.,* 2017). Out of these SSRs are neutral, co-dominant polymorphic markers (Song *et al.*, 2011), which are more informative, easily transferable (Rajapakse, 2003) and show a high level of heterozygosity in the Rosaceae family (Esselink *et al.,* 2003).

Some rose plants have a pleasant fragrance in their petals and are used for the extraction of essential oils (Marchant *et al.,* 1996; Lu *et al.,* 2003). *R. centifolia* is the one famous among oil producing species of roses in the world (Lawrence, 1991). It has pink fragrant flowers with many petals. *R. damascena* is also an important species of rose used for oil extraction and as a garden plant (Rusanov *et al.,* 2005). This is very effective for antiseptic, antidepressant, astringent, digestive, antioxidant, antibacterial and antimicrobial activities (Achuthan *et al.,* 2003; Ozkan *et al.,* 2004).

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In Pakistan, roses are highly valued and demanding nontraditional crop, popular among small and large growers. The genotypes of *R. centifolia* preferred by the farmers are dispersed in different districts of Punjab. Pakistani rose showed differences in the plant, leaf and flower characters (Akhtar *et al.,* 2014). *R. centifolia* found in Pakistan produces flowers throughout the year included warmer summer months at which its related genotypes around the globe stop flowering. This extended flowering season is in favor of farmers to fetch more price and also ensures the regular supply of petals for oil extraction. This change in behavior of *R. centifolia* in Pakistan pushes the researcher for detailed investigations. This genotype can be used in future breeding program due to presence of this novel characteristic. Comparing the genetic similarity is efficient tool to know that whether the genotypes found in Pakistan are genetically differ from the genotypes found in another country. Therefore, there is a dire need to develop a signature that can be used before using these genotypes in a breeding program. By keeping in view this aspect this study was designed to find out the genetic diversity among its genotypes found in Pakistan and other countries and develop a signature that can be used for maintaining true to type plants for breeding.

Materials and Methods

Plant Material

For an exclusive study of the rose diversity of Pakistani genotype, twenty-four *R. Centifolia* genotypes were collected from Sialkot, Sargodha, Faisalabad, Kahror Pakka (district Lodhran), Sheikhupura, Lahore, Pattoki (district Kasur), Pakpattan and were authenticated by plant taxonomist at the University of Agriculture, Faisalabad, Pakistan. For comparative analysis, eight genotypes of *R. centifolia* from the USA were obtained from Rogue Valley Roses nursery, Oregon State, the USA in the Horticulture Department, Texas A&M University, Texas, USA along with two *R. centifolia* genotype, Faisalabad and Sargodha districts of Punjab, Pakistan. Four genotypes of *R. damascena* comprised of two from Pakistan (Faisalabad and Pattoki districts of Punjab province) and two from Iran (Isfahan and East Azerbaijan districts) were used as shown in Table 1.

DNA Extraction

Young leaves were used for DNA extraction from all selected genotypes by using the CTAB method as described by Doyle and Doyle (1990). DNA quantification of all samples was done by using Hoefer DQ 300 fluorometer and 1% agarose gel. All the DNA samples were diluted to 10 ng/μL by using nuclease free water and stored at 4°C for use in PCR.

SSR Markers

Thirteen microsatellite primers were selected based on their polymorphism as reported by Oyant *et al.* (2008) and Kiani *et al.* (2010). The selected SSR markers were diluted to prepared at 10 μM. The sequence, expected band size and annealing temperature were provided in Table 2.

PCR Amplification

PCR reactions were performed by Eppendorf PCR machine with total volume of 10 μL, which included 8.0 μL Go Taq Master Mix (New England BioLabs, Inc.), 0.5 + 0.5 μL forward and reverse primers, 1 μL DNA. PCR reaction was performed by using the following cycle: Initial denaturation at 94°C for 5 min, denaturation at 94°C for 90 sec, annealing at 50 to 56°C for 30 sec depending upon primer, extension at 72°C for 60 sec, the step two to four were repeated for 35 cycles and final extension was done at 72°C for 5 min and finally store at 4°C.

Gel Electrophoresis

PCR products supplemented with 3 μL loading dye were loaded on the 1% agarose gel and run at 155 mA and 81 volts for 45 min to 60 min. The gel was visualized under UV transilluminator and photographed.

Data Analysis

Data was recorded and put in the Microsoft Excel from gels as A, B, C up to N depends upon the number of alleles as depicted by SSR markers started from lowest to highest respectively. The data recorded were analyzed for four statistical parameters: major allele frequencies, allele number, genetic diversity, and polymorphism information content by putting data into Power Marker V3.25 software package (Liu and Muse, 2005) (http// www.powermarker.net). The output file of power marker for allele frequency was used as input file for NTSYS-PC version 2.2 (Numerical Taxonomy and Multivariate Analysis System, Exeter Software) used for Unweighted pair-group method and principal component analysis by using similarity coefficient Dice (Nei and Li, 1979) and Jaccard respectively.

Results

Genetic Diversity Analysis of *R. centifolia* from Pakistan

All the primers except RW22B6 produced scorable bands and displayed polymorphism among genotypes. RW22B6 was therefore excluded for rest of the analysis. The accessions exhibited 29 alleles among 24 genotypes from Pakistan with an average of 2.417 alleles per locus. In Pakistan accessions major allele frequency was observed between 0.417 to 0.75 with an average of 0.628. The genetic

Table 1: Roses genotypes used for SSR analysis

Collection site	Name of species/Cultivar	Province/Country
Faisalabad1	*R. centifolia*	Punjab/Pakistan
Faisalabad2	*R. centifolia*	Punjab/Pakistan
Faisalabad3	*R. centifolia*	Punjab/Pakistan
Sargodha1	*R. centifolia*	Punjab/Pakistan
Sargodha2	*R. centifolia*	Punjab/Pakistan
Sargodha3	*R. centifolia*	Punjab/Pakistan
Sialkot1	*R. centifolia*	Punjab/Pakistan
Sialkot2	*R. centifolia*	Punjab/Pakistan
Sialkot3	*R. centifolia*	Punjab/Pakistan
Lahore1	*R. centifolia*	Punjab/Pakistan
Lahore2	*R. centifolia*	Punjab/Pakistan
Lahore3	*R. centifolia*	Punjab/Pakistan
Pattoki1	*R. centifolia*	Punjab/Pakistan
Pattoki2	*R. centifolia*	Punjab/Pakistan
Pattoki3	*R. centifolia*	Punjab/Pakistan
Pakpattan1	*R. centifolia*	Punjab/Pakistan
Pakpattan2	*R. centifolia*	Punjab/Pakistan
Pakpattan3	*R. centifolia*	Punjab/Pakistan
Kahror pakka1	*R. centifolia*	Punjab/Pakistan
Kahror pakka2	*R. centifolia*	Punjab/Pakistan
Kahror pakka3	*R. centifolia*	Punjab/Pakistan
Sheikhupura1	*R. centifolia*	Punjab/Pakistan
Sheikhupura2	*R. centifolia*	Punjab/Pakistan
Sheikhupura3	*R. centifolia*	Punjab/Pakistan
Rogue Valley Roses	*R. centifolia* (Fantin-Latour)	Oregon/USA
Rogue Valley Roses	*R. centifolia* (Gros Choux d' Hollande)	Oregon/USA
Rogue Valley Roses	*R. centifolia* (Centifolia variegata)	Oregon/USA
Rogue Valley Roses	*R. centifolia* (Rosa de Meaux)	Oregon/USA
Rogue Valley Roses	*R. centifolia* (Cabbage rose1)	Oregon/USA
Rogue Valley Roses	*R. centifolia* (Cabbage rose2)	Oregon/USA
Rogue Valley Roses	*R. centifolia* (Pompon de Bourgogne	Oregon/USA
RogueValleyRoses	*R. centifolia* (Paul Ricault)	Oregon/USA
Faisalabad	*R. damascena*	Punjab/Pakistan
Pattoki	*R. damascena*	Punjab/Pakistan
Osco	*R. damascena*	East Azerbaijan/Iran
Kashan	*R. damascena*	Isfahan/Iran

diversity ranged from 0.375 for RW32D19 and RW55C6 to 0.653 for RW10M24. The PIC value observed for accessions varies from 0.305 to 0.579 was an average of 0.398. The RW10M24 showed the highest PIC value (Table 3).

Phylogenetic Analysis

An unweighted paired - group of Arithmetic Mean (UPGMA) analysis of Dice similarity distributed all genotypes of *R. centifolia* into four groups at 0.68 similarity coefficient (Fig. 1). Group-A (G-A) contained all three genotypes of Kahror Pakka, closer to the Sargodha genotypes of G-B which composed of all three genotypes of district Sargodha and 1st and 2nd genotypes of district Sialkot. G-C was the largest group with all genotypes of districts Faisalabad, Sheikhupura and Lahore with the 3rd genotype of district Sialkot. All three genotypes of the district Pakpattan and Pattoki were in the separate G-D. G-B was further divided into two groups at similarity coefficient of 0.78, one of them contained three genotypes of Sargodha and other occupy first two genotypes of district Sialkot (Fig. 1). At same 0.78 values of similarity coefficient G-C also divided into two group first of them

Table 2: Selected thirteen SSR Primers (Hibrand-Saint Oyant*et al.,* 2008; Kiani *et al.,* 2010**)** along with primer sequence expected size and annealing temperature

S.No	Primer code	Primer sequence	Expected size (bp)	Annealing Temp.°C
1	RW3	F: GCCATCACTAACGCCACTAAA	418	56
	K19	R: GCGTCGTTCGCTTTGTTT		
2	RW3	F: CTGGCTGGTTCTCTTTCTG	125	54
	N19	R:ATGGGTCGTCGTCGATATG		
3	RW1	F: ATCATGTGCAGTCTCCTGGT	145	55
	4H21	R:AATTGTGGGCTGGAAATATG		
4	RW1	F: CAGGTAATTTGCGGATGAAG	216	58
	717	R: GATCCGCCGTTTCCAGT		
5	RW1	F: CCCGAGAAAGAGACAGTAAA	250	54
	8N19	R: ATCGAGAGAGACACCGACTC		
6	RW2	F: AGAGAATTGAAAAGGGCAAG	150	53
	2A3	R: GAGCAAGCAAGACACTGTAA		
7	RW2	F: ACAGTGAGTTGTTCGCTTCT	158	54
	2B6	R: TTCATTGCTAGGAAGCAGTA		
8	RW3	F: GAAGTCCAGAGCCAATTCCA	540	54
	2D19	R: AGGGTCCTCATCCACCACTT		
9	RW5	F: GTGGATTTTCAGAGATACGC	265	50
	5C6	R: TCACAGACAGGACCACCTAT		
10	RW5	F: GATCCGTTTAAGTAACCTTT	201	51
	5D22	R: CCACAAGGATTCTGATTTAT		
11	H22C	F: TCATAACCAACCATCTCCATCA	228	51
	O1	R: AGGATTTCACCCAGAACACG		
12	H24D	F: CCTCCTCAGCTTTCCTCCTT	168	55
	11	R: CAGCAACCATCTCTTCGTGA		
13	RW1	F: TTAATCCAAGGTCAAAGCTG	252	53
	0M24	R: TCTCTTTCCCTCCTCACTCT		

was made up of three genotypes of Faisalabad along with 3rd genotype of Sargodha district. A second group was contained all three genotypes of district Lahore which also closer to Pakpattan genotypes of G-D. Genetic divergence was observed among the districts, while more genetic relatedness within the districts. Genotypes of district Pattoki were genetically more dissimilar with genotypes of district Kahror Pakka of group-A. Genotypes from Faisalabad district were genetically more similar to Sheikhupura and Sialkot genotypes but genetically diverse from genotypes of Kahror Pakka and Pattoki of group A and D, respectively (Fig. 1).

Principal Component Analysis

The result depicted by PCA was in harmony with the UPGMA. The genotypes under study were grouped into four, named as G-A to G-D. The G-A comprised of genotypes form Kahror Pakka while the G-B consist of genotypes from district Sargodha and two genotypes of district Sialkot. The genotypes from districts Faisalabad, Sheikhupura and Lahore along with 3rd genotype of district Sialkot comprised the G-C, the largest one in terms of genotypes contains. Pakpattan and Pattoki genotypes gathered into a separate group G-D. This group showed little bit more divergence than the rest of the genotypes (Fig. 2).

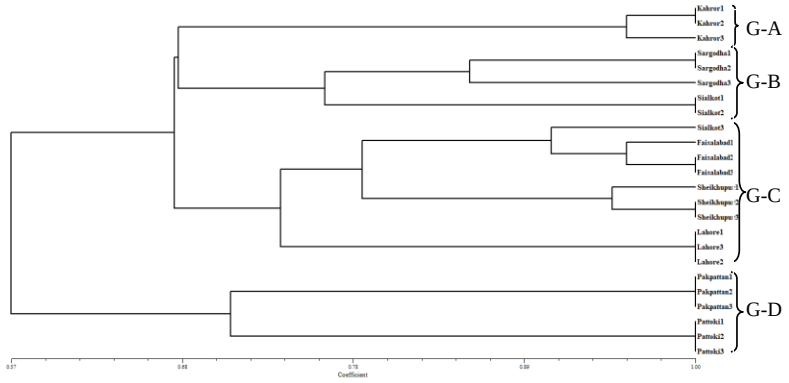

Fig. 1: Dendrogram of microsatellite diversity among twenty genotypes of *R. centifolia* from Pakistan

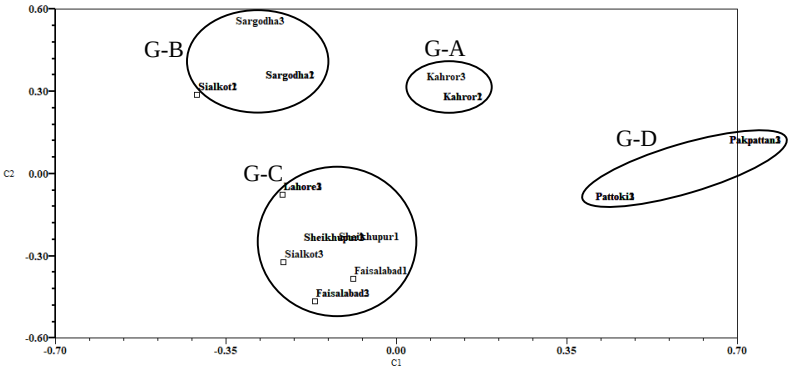

Fig. 2: PCA based on microsatellite variation among twenty genotypes of *R. centifolia* from Pakistan

Table 3: Represent statistical parameters of SSR markers for Pakistani genotypes

Marker	Major Allele Frequency	Allele No	Gene Diversity	PIC
RW3K19	0.625	2.000	0.469	0.359
RW3N19	0.500	3.000	0.625	0.555
RW14H21	0.583	3.000	0.569	0.505
RW1717	0.625	2.000	0.469	0.359
RW18N19	0.708	2.000	0.413	0.328
RW22A3	0.583	2.000	0.486	0.368
RW32D19	0.750	2.000	0.375	0.305
RW55C6	0.750	2.000	0.375	0.305
RW55D22	0.708	3.000	0.434	0.369
H22CO1	0.750	3.000	0.406	0.371
H24D11	0.542	2.000	0.497	0.373
RW10M24	0.417	3.000	0.653	0.579
Mean	0.628	2.417	0.481	0.398

DNA Fingerprinting

The selected genotypes from Pakistan depicted that have divergence among district. Therefore, we are able to develop a signature that can be used to differentiate genotypes from eight districts. The RW3N19 can be used to differentiate genotypes from Lahore and Pattoki and the rest of the districts (Table 4).

The RW14H21 produce two alleles that were able to draw a line between Sargodha, Pakpattan, Kahror Pakka and Pattoki from Faisalabad, Lahore, Sialkot, and Sheikhupura. The RW55D22 resulted in production of three alleles used to separate Pakpattan and Lahore from the genotypes than other districts. The genotypes from districts Sheikhupura depicted a unique allele for H22CO1.

Genetic Analysis of *R. centifolia* and *R. damascena* from Pakistan, USA and Iran

The SSR primers under analysis resulted in scorable bands and displayed polymorphism among genotypes. Selected fourteen genotypes from three countries produced 31 alleles with an average of 2.385 alleles per locus. Major allele frequency ranged from 0.929 for RW3K19 to 0.429 for RW3N19 with an average of 0.709. The highest genetic diversity was 0.643 while the lowest was 0.133 with a mean of 0.3943. The highest PIC value was 0.567 for RW3N19, on the other hand lowest was 0.124 depicted by RW3K19 with an average of 0.333 (Table 5).

Phylogenetic Analysis

According to the results presented in the Fig. 3, the genotypes were divided into 5 clusters at the similarity coefficient of 0.81. Cluster-A (C-A) was further divided into two subclusters at a similarity coefficient of 0.87. One consists of genotype Fantin-Latour, Gros Choux d'Hollande and Rose de Meaux and another one *R. damascena*

Table 4: Markers selected for DNA fingerprinting among accessions of Pakistan

Markers	Differentiate accessions of column 2 from column 3	
RW3N19	Lahore, Pattoki	Kahror, Pakpattan, Sargodha, Sialkot, Faisalabad, Sheikhupura
RW14H21	Sargodha	Lahore, Pattoki, Kahror, Pakpattan, Sialkot, Faisalabad, Sheikhupura
RW55C6	Lahore, Pattoki	Kahror, Pakpattan, Sargodha, Sialkot, Faisalabad, Sheikhupura
H22CO1	Sheikhupura,	Lahore, Pattoki, Kahror, Pakpattan, Sialkot, Faisalabad, Sargodha
RW10M24	Kahror, Sialkot, Sargodha	Lahore, Pattoki, Pakpattan, Faisalabad, Sheikhupura,

Table 5: Represent statistical parameters of SSR markers for *R. centifolia* and *R. damascena* from Pakistan, USA and Iran

Marker	Major Allele Frequency	Allele No	Gene Diversity	PIC
RW3K19	0.929	2.000	0.133	0.124
RW3N19	0.429	3.000	0.643	0.567
RW14H21	0.643	3.000	0.500	0.427
RW1717	0.643	3.000	0.520	0.464
RW18N19	0.714	2.000	0.408	0.325
RW22A3	0.500	2.000	0.500	0.375
RW22B6	0.786	2.000	0.337	0.280
RW32D19	0.857	2.000	0.245	0.215
RW55C6	0.571	2.000	0.490	0.370
RW55D22	0.786	3.000	0.357	0.325
H22CO1	0.857	2.000	0.245	0.215
H24D11	0.643	3.000	0.500	0.427
RW10M24	0.857	2.000	0.245	0.215
Mean	0.709	2.385	0.394	0.333

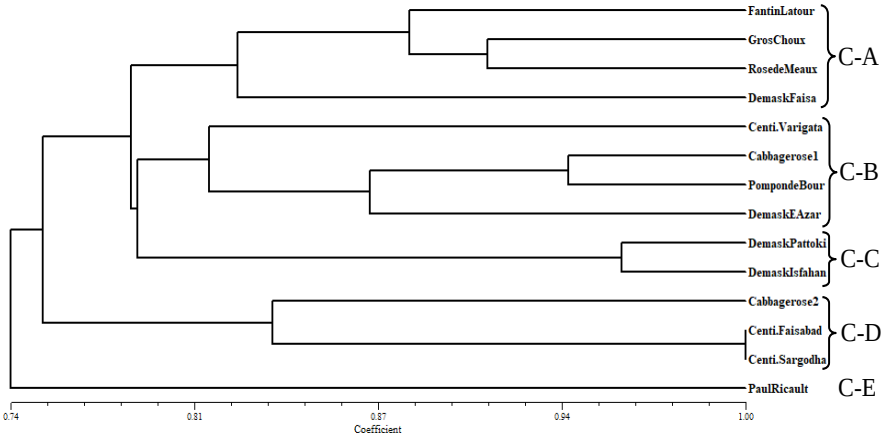

Fig. 3: Dendrogram of microsatellite diversity among genotypes of *R. centifolia* and *R. demascena* from Pakistan, USA and Iran

Faisalabad. C-B was made up of two subclusters. One of them contained genotype centifolia variegated, while another contained genotypes of Cabbage rose1, Pompon de Bourgogne, *R. damascena* East Azerbaijan. *R. damascena* Pattoki and *R. damascena* Isfahan were combined in C-C. C-D was made up of genotypes Cabbage rose 2, *R. centifolia* Faisalabad and *R. centifolia* Sargodha (Fig. 3). Genotype Paul Ricault was alone in the C-E. *R. damascena* Faisalabad was genetically dissimilar from other three genotypes of *R. damascena*. *R. damascena* Pattoki and *R. damascena* Isfahan were in the same group which showed genetic relatedness among them. Two genotypes of *R. centifolia* from Pakistan was closer to *R. damascena* Isfahan, *R. damascena* Pattoki and *R. damascena* East Azerbaijan of C-C and C-B respectively than *R. damascena* Faisalabad of C-A.

Principal Component Analysis

The clustered pattern was in close agreement with the UPGMA analysis. The genotype under study gathered into five clusters (C-A to C-E). Out of which C-A and C-B consist of four genotypes, while C-C two, C-D three and C-E contains only one genotype. The Paul Ricault showed highly diverge pedigree than the rest of the genotypes (Fig. 4).

Discussion

Genetic diversity assessment is one of the key factors for crop improvement in case of agronomic crop and has economic importance as well in case of the horticultural crop. The desire for highly true to type plants are increasing with the awareness among the growers about the economic

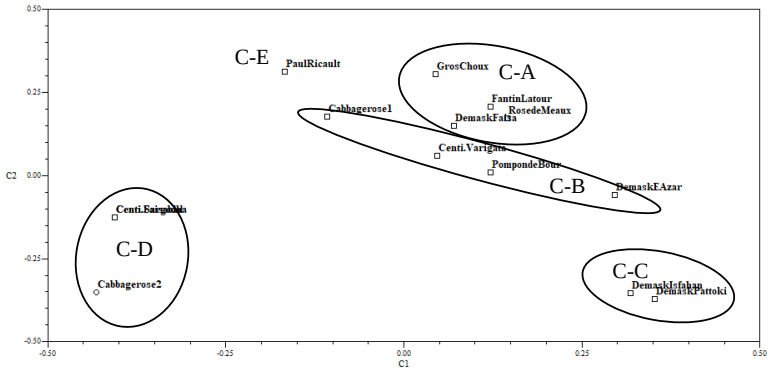

Fig. 4: PCA based on microsatellite variation among genotypes of *R. centifolia* and *R damascena* from Pakistan, USA and Iran

benefits. Pakistan rose genotypes are little exploited for genetic relationship within Pakistan and other countries based on the molecular analysis (Farooq *et al.,* 2013). In this study, 13 microsatellite markers were used to determine the genetic diversity among 24 genotypes of *R. centifolia* found in 8 districts of Punjab, Pakistan. The summary statistics results demonstrated that an average number of alleles were lower than reported by Soules (2009), Samiei *et al*. (2010) and Tan *et al*. (2017) by utilizing Old Chinese garden roses, Iranian rose species and Rosa accessions from China respectively. The genetic diversity for the genotype under analysis ranged from 0.375 to 0.653. Tan *et al*. (2017) observed a genetic diversity of ~0.22–0.99 which demonstrated a relatively higher level of genetic variation than our results. The possible reason could be the difference in the number of accessions studied, species of rose and marker selection *i.e*., mono-, di-, tri- repeats, along with the genotype understudies are mostly propagated by cuttings.

All genotypes were divided into 4 groups based upon phylogenetic analysis by using UPGMA method, which shows the genetic variation and divides genotypes into groups in accordance with rose history (Wylie, 1954; Maia and Venard, 1976). All the genotypes from one district were in same groups exhibit closed ancestry within the district. Oil bearing roses are mostly propagated by vegetative means (Roberts and Schum, 2003). Stem cutting is one of the most common method of propagation in all these districts of Punjab authenticated by our results by proving that the rose genotypes under analysis in a district are progenies from a common ancestor. Cock *et al*. (2008) also noticed in *R. canina* and *R corymbifera* that genotypes from the same locality reflect a high level of homogeneity as compared to different localities. The genetic difference between the collection sites indicates variability among the centifolia clones used commercially in Pakistan by phylogenetic analysis and PCA. Samiei *et al*. (2010) on exploring genetic architect of wild Iranian rose species also reported that the accessions within a site were more closely related than those from different sites by using 10 SSR markers. Kiani *et al*. (2008) experienced a higher level of genetic diversity in *R. damascena* collected from different provinces of Iran as compared to the same province.

Genetic analysis of *R. centifolia* and *R. damascena* from Pakistan, USA and Iran resulted in a close relationship between *R. damascena* Isfahan and *R. damascena* Pattoki. These findings are supported by the previous finding of Farooq *et al*. (2013). *R. damascena* Isfahan was also genetically closer to *R. damascena* East Azerbaijan according to the previous reports Kiani *et al*. (2010) and Farooq *et al*. (2013). These three *R. damascena* genotypes were also reflected genetic similarity with genotypes of Pompon de Bourgogne, Cabbage rose 1 and *Centifolia variegata* of the same group and with Cabbage rose 2 of the next group. This close lineage between *R. damascena* from Pakistan, USA and Iran showed their evolution from a common ancestor and then their distribution by invader into the different part of the world.

R. damascena Faisalabad was in a separate group from other *R. damascena* genotypes and had genetically more relation with Rose de Meaux and Gros Choux d'Hollande which showed relatively genetic relatedness among them. The genetic distance between *R. damascena* Faisalabad and other damask genotypes under analysis are in harmony with the previous finding of Farooq *et al*. (2013). Other investigation on genetic diversity of *R. damascena* and *R. centifolia* resulted in an agreement on diverse lineage (Mohapatra and Rout, 2005; Babaei *et al.,* 2007; Kiani *et al.,* 2010). Farooq *et al*. (2013) observed the genetic diversity among *R. damascena* and other garden roses from Pakistan and compare them with genotypes from Iran and USA by using 10 simple sequence repeat (SSR) markers and concluded that Pakistani genotypes were closer to Iran demonstrating their close parentage.

Conclusion

Genetic diversity was observed among the districts while more genetic closeness was noticed within the districts. The *R. centifolia* grown in Pakistan have a close genetic relationship with Cabbage rose2 and genetic divergence with Fantin-Latour and Paul Ricault of *R. centifolia* found in the USA. The markers documented in the current study of value

to construct a database helpful for characterization of other rose genotypes having economic importance. The database will be further utilized for marker assisted breeding for improvement of *R. centifolia* for a desirable trait by utilizing a diverse germplasm from the USA such as Fantin-Latour and Paul Ricault.

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References

Achuthan, C.R., B.H. Babu and J. Padikkala, 2003. Antioxidant and hepatoprotective effects of *Rosa damascena*. *Pharm. Biol*., 41: 357–361

Akcay, U.C., G. Ozkan, B. San, O. Dolgun, A. Dagdelen and D.B. Konuskan, 2014. Genetic stability in a predominating Turkish olive cultivar, Gemlik, assessed by RAPD, microsatellite and AFLP marker systems. *Turk. J. Bot*., 38: 430–438

Akhtar, G., M.A. Khan, M.J. Jaskani and M. Ashfaq, 2014. Phenotypic plasticity within and among genotypes of *Rosa centifolia* from Pakistan and the USA. *Pak. J. Agric. Sci*., 5: 609–616

Babaei, A., S.R. Tabaei-Aghdaei, M. Khosh-Khui, R. Omidbaigi, M.R. Naghavi, G.D. Esselink and M.J.M. Smulders, 2007. Microsatellite analysis of Damask rose accessions from various regions in Iran reveals multiple genotypes. *BMC Plant Biol*., 10: 7–12

Baydar, N.G., H. Baydar and T. Debener, 2004. Analysis of genetic relationships among *Rosa damascena* plants grown in Turkey by using AFLP and microsatellite markers. *J. Biotechnol*., 111: 263–267

Brechbill, G.O., 2011. *Arranging Fine Perfume Compositions-the Rose*, p: 53. Fragrance Book Inc. New Jersy, USA

Cock, K.D., K.V. Mijnsbrugge, P. Breyne, E.V. Bockstaele and J.V. Slycken, 2008. Morphological and AFLP based Differentiation within the Taxonomical Complex Section Caninae (subgenus *Rosa*). *Ann. Bot*., 102: 685–697

Doyle, J.J. and J.L. Doyle, 1990. Isolation of plant DNA from fresh tissue. *Focus*, 12: 13–15

Esselink, G.D., M.J.M. Smulders and B. Vosman, 2003. Identification of cut-rose (*Rosa hybrida*) and rootstock varieties using robust Sequence Tagged Microsatellite markers. *Theor. Appl. Genet*., 106: 277–286

Farooq, A., M. Kiani, M.A. Khan, A. Riaz, A.A. Khan, N. Anderson and D.H. Byrne, 2013. Microsatellite Analysis of *Rosa damascena* from Pakistan and Iran. *Hortic. Environ. Biotechnol*., 54: 141–147

Fredrick, C., A. Waganer and N. Morvillo, 2002. Randomly amplified polymorphic DNA (RAPD) analysis of musk rose (*Rosa moschata*). *Pro. Fla. State. Hortic. Soc*., 115: 117–119

Gudin, S., 2000. Rose: Genetics and breeding. *Plant Breed. Rev*., 171: 159–190

Kiani, M., Z. Zamani, A. Khalighi, M.R.F. Moghadam and D.H. Byrne, 2010. Microsatellite analysis of Iranian Damask rose (*Rosa damascena* Mill.) germplasm. *Plant Breed.*, 129: 551–559

Kiani. M., Z. Zamani, A. Khalighi, R. Fatahi and D.H. Byrne, 2008. Wide genetic diversity of *Rosa damascena* Mill. Germplasm in Iran as revealed by RAPD analysis. *Sci. Hortic*., 115: 386–392

Lawrence, B.M., 1991. Progress in essential oil, rose oil and extracts Perfume. *Flavor*, 16: 43–77

Liorzou, M., A. Pernet, S. Li, A. Chastellier, T. Thouroude, G. Michel, V. Malecot, S. Gaillard, C. Briee, F. Foucher, C. Oghina-Pavie, J. Clotault and A. Grapin, 2016. Nineteenth century French rose Rosa spp.) germplasm shows a shift over time from a european to an asian genetic background. *J. Exp. Bot*., 67: 4711–4725

Liu, K. and S.V. Muse, 2005. Power marker: An integrated analysis environment for genetic marker analysis. *Bioinformatics*, 21: 2128–2129

Lu, L., C. Gu, C. Li, C. Alexander, B. Bartholomew, A.R. Brach, D.E. Boufford, H. Ikeda, H. Ohba and R.S.A. Robertson, 2003. *In: Flora of China*, Vol. 9, pp: 46–434. Science Press, Beijing, and Missouri Botanical Garden Press, St. LouisMaia, N. and P. Venard, 1976. Cytotaxonomie du genre *Rosa* et origine des rosierscultivés. *In*: *Travaux Sur le Rosier De Serre*, pp: 7–20. FNPH (ed.). INRA Editions, Paris, France

Marchant, R., M.R. Davey, J.A. Lucas and J.B. Power, 1996. Somatic embryogenesis and plant regeneration in Floribunda rose (*Rosa hybrida* L.) cvs. trumpeter and glad tidings. *Plant Sci*., 120: 95–105

Mezghani, N., O.S. Debbabi, S. Rouz and M. Medini, 2015. AFLP markers for the assessment of genetic variability in rose (*Rosa gallica* L.) cultivars in Tunisia. *Intl. J. Adv. Res*., 3: 35–42

Mohapatra, A. and G.R. Rout, 2005. Identification and analysis of genetic variation among rose cultivars using random amplified polymorphic DNA. *Zeits. Naturforsch. C*, 60: 611–617

Nei, M. and H.W. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. U.S.A.*, 76: 5269–5273

Ogras, T.T., E.K. Bastanlar, O.K. Metin, I. Kandemir and H. Ozçeli, 2017. Assessment of genetic diversity of rose genotypes using ISSR markers. *Turk. J. Bot*., 41: 347–355

Oyant, L.H.S., L. Crespel, S. Rajapakse, L. Zhang and F. Foucher, 2008. Genetic linkage maps of rose constructed with new microsatellite markers and locating QTL controlling flowering traits. *Tree Genet. Genom.*, 4: 11–23

Ozkan, G., O. Sagdic, N.G. Baydar and H. Baydar, 2004. Antioxidant and antibacterial activities of *Rosa damascena* flower extracts. *Food Sci. Technol. Intl*., 10: 277–281

Pirseyedi, S.M., M. Mardi, S. Davazdahemami, J.M. Kermani and S.A. Mohammadi, 2005. Analysis of the genetic diversity between 12 Iranian Damask rose (*Rosa damascena* Mill.) using amplified fragment length polymorphism markers. *Iran J. Biotechnol*., 3: 225–230

Rajapakse, S., 2003. Genetics molecular markers. *In: Encyclopedia of Rose Science*, Vol. 1, pp: 334–341. Roberts, A.V., T. Debener and S. Gudin (eds.). Elsevier Academic Press, Oxford, UK

Roberts, A.V. and A. Schum, 2003. Cell, Tissue and Organ culture (Micropropagation) *In: Encyclopedia of Rose Science*, pp: 1: 57–63. Roberts, A.V., T. Debener and S. Gudin (eds.). Elsevier Academic Press, Amsterdam, The Netherlands

Rusanov. K., N. Kovacheva, B. Vosman, L. Zhang, S. Rajapakse, A. Atanassov and I. Atanassov, 2005. Microsatellite analysis of *Rosa damascena* Mill. accessions reveals genetic similarity between genotypes used for rose oil production and old Damask rose varieties. *Theor. Appl. Genet*., 111: 804–809

Samiei, L., R. Naderi, A. Khalighi, A.A. Shahnejat-Bushehri, V. Mozaffarian, G.D. Esselink, S.K. Osaloo and M.J.M. Smulders, 2010. Genetic diversity and genetic similarities between Iranian rose Species. *J. Hortic. Sci. Biotechnol*., 85: 231–237

Senapati, S.K. and G.R. Rout, 2008. Study of culture conditions for improved micropropagation of hybrid rose. *Hortic. Sci*., 35: 27–34

Song, Y., F. Liu, Z. Zhu, L. Tan, Y. Fu and C. Sun, 2011. Construction of a simple sequence repeat marker-based genetic linkage map in the autotetraploid forage grass *Dactylus glomerata* L. *Grassl. Sci*., 57: 158–167

Soules, V.A., 2009. Analysis of genetic diversity and relationships in the China Rose group, Texas A & M University, *M.S. Dissertation*. Available at: http://oaktrust.library.tamu.edu/bitstream/handle/1969.1/ETD-TAMU-2009-12-7450/SOULES-THESIS.pdf?sequence=2

Tan, J., J. Wang, L. Luo, C. Yu, T. Xu, Y. Wu, T. Cheng, J. Wang, H. Pan and Q. Zhang, 2017. Genetic relationships and evolution of old Chinese garden roses based on SSRs and chromosome diversity. *Sci. Rep*., 7: 15437

Wylie, A.P., 1954. The history of garden roses. *J. Roy. Hortic. Soc*., 79: 555–571

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