PHILIPP AGRIC SCIENTIST
Vol. 99 No. 1, 25–33
March 2016

ISSN 0031-7454

Molecular and Morphological Characterization of Rose Mutants Produced via *In Vitro* Mutagenesis

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Rose, a valuable flowering ornamental around the world, is used as cut flower, indoor potted plant, in landscape, and for the extraction of essential oil. The rose varieties *Rosa borboniana*, *Rosa centifolia* and *Rosa gruss-an-teplitz* are tall but have small flower size, which is not a desirable character as the essential oil content is dependent on flower size. To improve this trait in these rose varieties, *R. borboniana*, *R. centifolia* and *R. gruss-an-teplitz* were treated with different doses of gamma radiation and colchicine. Surviving mutant lines were evaluated for genetic diversity from their parents using morphological and simple sequence repeat (SSR) markers. Remarkable variability was noticed for different morphological traits among the putative mutants. Maximum plant height and flower diameter was found in mutants of *R. centifolia* treated with colchicine. To detect genetic variation, 14 SSR primers were selected. Clusters were made using unweighted pair-group method with an arithmetic average (UPGMA) analysis on the basis of close resemblances of these genotypes based on morphological characters and SSR markers. Both SSR and morphological markers showed more diversity among mutants produced through colchicine. This approach is useful due to its simplicity and rapidity for identification of mutant lines which will be helpful for further improvement of desired characteristics in these mutants.

Key Words: *Rosa* spp., SSR, colchicine, gamma irradiation, variation

Abbreviations: PC – principal component, PIC – polymorphism information content, SSR – simple sequence repeat, UPGMA – unweighted pair-group method with an arithmetic average

INTRODUCTION

Rose, a most valuable flowering ornamental belonging to the family *Rosaceae* and the genus *Rosa* L., is used as cut flower, indoor potted plant as well as in landscaping (Zhang et al. 2006). It contains phytochemicals with antioxidant, antibacterial, anti-carcinogenic and anti-HIV properties (Tabaei-Aghdaei et al. 2007). *Rosa centifolia*, *Rosa borboniana* and *Rosa gruss-an-teplitz* are the commonly grown rose species in Pakistan. *Rosa gruss-an-teplitz* has strong fragrance (Baig et al. 2011) and is used as fresh or dried petals in the making of wreaths and bouquets (Saeed 2005). *R. centifolia* is used for the commercial extraction of essential oil (Beales et al. 1998; Farricielli 2008). Another important rose species, *R. borboniana,* has attractive fragrance and is also used for making rose oil, rose conserve and rose water. Due to disease resistance, it is also used as a rootstock (Saeed 2005). Although these species have unique characteristics, they are tall (Baig et al. 2011) and have small flower size. However, large-sized flowers are desirable, as the essential oil content of rose is also influenced by the size of the flower and the number of petals (Hashmi 2006).

Mutation breeding can improve the existing characteristics of rose species. It can be induced through *in vitro* technique by using physical or chemical mutagens (Kumar et al. 2006). There are many types of mutagens such as ionizing radiation (X-rays, gamma rays, alpha, beta particles, neutron and proton), ultraviolet radiations and chemicals such as colchicine, EMS and oryzalin (Baig et al. 2011; Senapati and Rout 2011; Behera et al. 2012; Abu-Qaoud and Shtaya 2014). Ionizing radiations penetrate deeper into the tissues and can induce a number of different types of chemical changes. Gamma rays induce physical mutations having the advantages of uniform and high penetration, accurate dosimetry and reasonable reproducibility (Jain 2010). Mutation results in chromosomal aberrations including deletion or addition of DNA and chromosomal rearrangements by DNA translocation and inversion (Yamaguchi 2013). Colchicine is a poisonous alkaloid that is widely used for polyploidy induction as it binds to the tubulin dimers, prevents the formation of spindle fibers, hence, cell division fails (Sajjad et al. 2013). It has played an important role in genetic and phenotype diversity as well as plant evolution and breeding (Xing et al. 2011).

To fulfill the need for more efficient, accurate and fast identification tools for roses, several molecular marker systems have been used. However, simple sequence repeat (SSR) markers are considered better than other markers for the investigation of genetic variation (Eujayl et al. 2001). They are relatively simple, co-dominant, reproducible, highly polymorphic and inexpensive (Hasan et al. 2006). By using SSR markers, closely related individuals can also be discriminated efficiently (Nadeem et al. 2014). These markers can be utilized in a number of ways such as genetic diversity evaluation, genetic mapping, cultivar discrimination and identification, clarifying taxonomic relations, and breeding for desirable traits (Akond et al. 2012).

Genetic diversity can also be evaluated on the basis of morphological and agronomic characters for different crops (Liu et al. 2004). Selection owing to morphological characters is very important for many qualitative characters and is considered to be a practical way of evaluating germplasm (Nemera et al. 2006; Nadeem et al. 2014). Mutant lines of *R. borboniana*, *R. centifolia* and *R. gruss-an-teplitz* were the result of induced mutation through *in vitro* application of colchicine and gamma irradiation. As genetic variation was expected in rose genotypes, therefore, morphological and SSR markers were used to select genotypes of *Rosa* species.

MATERIALS AND METHODS

The study was conducted at the Plant Tissue Culture Laboratory, Department of Horticulture, PMAS-Arid Agriculture University Rawalpindi, Pakistan and the National Agricultural Research Centre, Islamabad, Pakistan in 2013–2014. Sixty micropropagated uniform-sized shoot tips (1 cm) of *R. centifolia*, *R. borboniana*, and *R. gruss-an-teplitz* were treated in four replications with different doses (0, 10, 20, 30, 40, 50 and 60 Gy) of gamma rays in a Co_{60} gamma radiation chamber as LD_{50} of these species was 33, 47 and 54 Gy, respectively (Baig et al. 2012).

Moreover, explants were separately treated with different concentrations of autoclaved aqueous colchicine solutions (0, 100, 300, 500, 700, 900 and 1100 mg L^{-1}). Treated explants were transferred to shoot proliferation medium (MS macro, microelements and vitamins) supplemented with 0.01 mg L^{-1} indole butyric acid (IBA), 0.4 mg L^{-1} gibberellic acid (GA_3), 1.0 mg L^{-1} benzylaminopurine (BAP), 30 g L^{-1} sucrose and 7 g L^{-1} agar. Treated explants were placed for a 16 h photoperiod of white light in a growth room at 24 ºC. Shoot tips were micropropagated for one culture cycle and then to induce *in vitro* rooting, transferred to the rooting medium (½ MS macro and microelements) supplemented with MS vitamins, 20 g L^{-1} sucrose and 0.50 mg L^{-1} IBA (Baig et al. 2011). After 6 wk, rooted micro-shoots were acclimatized under controlled glasshouse conditions. After acclimatization, surviving plants of *R. centifolia, R. gruss-an-teplitz* and *R. borboniana* treated with gamma rays [(20 and 30 Gy), (40 and 50 Gy) and (50 and 60 Gy), respectively] and colchicine (900 and 1100 mg L^{-1}) were selected to find variation.

The phenotypic characters, *i.e.,* plant height, number of shoots, number of leaves per leaflet, leaf shape, leaf size, flower color, flower size, number of flowers per plant, number of petals and number of sepals of mutants of *R. borboniana*, *R. centifolia* and *R. gruss-an-teplitz* were recorded for evaluation of diversity from their parent plants.

Putative mutants of these species were analyzed for genomic variation from their parent plants. For this purpose, fresh juvenile leaves were used for the extraction of DNA by following the cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1987). Gel electrophoresis was done for the examination of qualification and quantification of DNA. 1% Agarose (1%) in Tris borate EDTA (TBE) buffer pre-stained with 0.5 µg mL^{-1} ethidium bromide was used for gel electrophoresis, run for 1 h at 50 V, U.V. trans-illuminator for visualization of DNA. DNA was stored at a storage temperature of 4 ºC.

Fourteen SSR primers (Table 1) were selected for SSR analysis (Zhang et al. 2006; Hibrand et al. 2008). The reaction mixture (20 µL) contained DNA template 20 ng of each forward and reverse primers, 25 mM each dNTPs, 0.2 Units Taq polymerase, and 1 x PCR assay buffer (50 mM KCl, 10 mm Tris-HCl, 1.5 mM $MgCl_2$ and pH 9.0). Amplification of DNA was done in a thermal cycler and programming was done as follows: 94 ºC for 4 min followed by 35 cycles for 1 min at 94 ºC, then 55 ºC for 1 min, 72 ºC for 2 min and the final cycle for 7 min at 72 ºC. The PCR product was visualized by gel electrophoresis in 3% metaphor agarose gel and size determination was done by using DNA ladder of 100 bp (Kaul et al. 2009). They were run and visualized by using Alpha Digi Doc Gel Documentation and image system.

Data taken in the form of presence (1) or absence (0) of bands for all 36 genotypes were analyzed by using NTSYS-pc v. 2.2 (Numerical Taxonomy and Multivariate Analysis System, Exeter Software), and cluster analysis was performed by using unweighted pair-group method with an arithmetic average (UPGMA) (Rohlf 2005). Morphological data were analyzed for mean, variance and standard deviation. Quantitative data were analyzed by cluster and Principal component analysis (Sneath and Sokal 1973) by using the software NTSYS-pc v.2.2 and "Statistica". The polymorphism information content (PIC) values of SSR markers were evaluated by the equation $PIC = 1 - n\,(Pi)^2$, where P is the proportion of the number of alleles in genotype and n is the total number of alleles in a primer (Moyib et al. 2007).

RESULTS

Thirty-six putative mutants of *R. gruss-an-teplitz, R. centifolia* and *R. borboniana* were evaluated for genetic diversity. A total of 37 bands were detected by seven SSR primers with an average of 5.28 bands per primer.

Table 1. Primer description and amplification information of rose species.

Primer	Primers Forward and Reverse	Band Size (bp)	No. of Loci Amplified	Total Bands Amplified	PIC Value
Rw3K19	GCCATCACTAACGCCACTAA GCGTCGTTCGCTTTGTTT	75	1	36	0
Rw10J19	GCGAGTTGACGACGAGTT GGGTGGGCTTCCTTAGTTA	100-300	2	10	0.99
Rw10M24	TTAATCCAAGGTCAAAGCTG TCTCTTTCCCTCCTCACTCT	0	0	0	0
Rw3N19	CTGGCTGGTTCTCTTTCTG ATGGGTCGTCGTCGATATG	0	0	0	0
Rw14H21	ATCATGTGCAGTCTCCTGGT AATTGTGGGCTGGAAATATG	0	0	0	0
Rw1717	CAGGTAATTTGCGGATGAAG GATCCGCCGTTTCCAGT	100-200	2	8	0.96
Rw22A3	AGAGAATTGAAAAGGGCAAG GAGCAAGCAAGACACTGTAA	200	1	2	0.99
Rw18N19	CCCGAGAAAGAGACAGTAAA ATCGAGAGAGACACCGACTC	0	0	0	0
Rw22B6	ACAGTGAGTTGTTCGCTTCT TTCATTGCTAGGAAGCAGTA	100	1	36	0
Rw32D19	GAAGTCCAGAGCCAATTCCA AGGGTCCTCATCCACCACTT	100	1	2	0.99
Rw55C6	GTGGATTTTCAGAGATACGC TCACAGACAGGACCACCTAT	0	0	0	0
Rw55D22	GATCCGTTTAAGTAACCTTT CCACAAGGATTCTGATTTAT	100-200	2	7	0.97
H22C01	TCATAACCAACCATCTCCAT AGGATTTCACCCAGAACACG	100-300	2	7	0.97
H24D11	CCTCCTCAGCTTTCCTCCTT CAGCAACCATCTCTTCGTGA	100	1	1	0.99

PIC – polymorphism information content

The number of alleles varied from 1 to 2 in some primers which gave information on the homozygosity and heterozygosity of traits. Six SSR primers (Rw10J19, Rw1717, Rw22A3, Rw22B6, Rw32D19, and Rw55D22) belong to the *Rw* series as developed by Zhang et al. (2006), while the other two primers were from the H series developed by Hibrand et al. (2008). Rw55D22, Rw1717, Rw10J19 and H22C01 not only provided information on the size of allele, presence, absence or both but also gave information on the homozygosity and heterozygosity of the mutants. The product size of the markers ranged from 75 to 300 bp. Polymorphism information content (PIC) value was assessed on the basis of the marker locus amplified, which altered considerably from 0.96 to 0.99 (Table 1).

Genetic relationship among the 36 genotypes was computed by UPGMA method and a dendrogram was constructed on the basis of similarity (Fig. 1). Clusters A and F each have four genotypes while C consisted of five genotypes at 0.79, 0.80 and 0.78 similarity, respectively. In cluster A, genotypes M14, M15 and M16 were developed by using colchicine dose of 900 mg L^{-1} while in cluster C, M18 and M19 were developed by using colchicine dose of 1100 mg L^{-1}. In cluster F, genotypes M3 and M27 were the result of gamma irradiation doses of 50 and 40 Gy while M5 and M20 in cluster C belong to gamma irradiation doses of 60 and 20, respectively. In clusters B and D, genotypes M22 and M24 were obtained by gamma irradiation dose of 30 Gy while M21 and M29 were obtained by 20 and 50 Gy of gamma irradiation. In cluster G, genotypes M7, M8, M9, M31, M32 and M10,

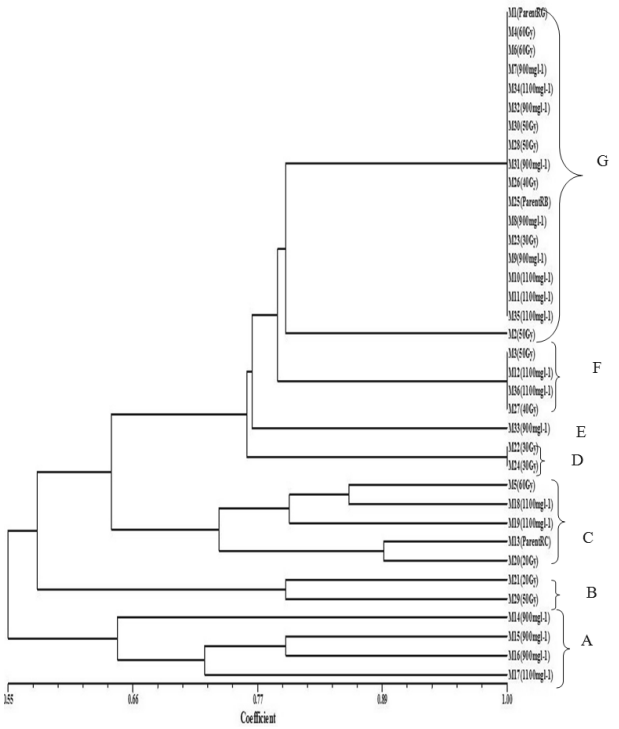

Fig. 1. Simple sequence repeat (SSR) based genetic relationship between mutants of *Rosa gruss-an-teplitz*, *Rosa centifolia* and *Rosa borboniana* using unweighted pair-group method with an arithmetic average (UPGMA) cluster analysis.

M11, M34, M35 in this cluster belong to the group of mutants produced by colchicine doses of 900 and 1100 mg L^{-1}. Hence, these genotypes had similar genetic makeup, which was further confirmed by clustering closely. Genotype M33 formed no association with others and was positioned independently in the dendrogram. This mutant was generated by 900 mg L^{-1} colchicine application. The mutants in clusters A, B, D, E and F were genetically different from their parents.

The level of genetic similarity was computed among 36 putative mutants obtained from *Rosa* species. Pair-wise estimates of genetic similarity index ranged from 0.33 to 1.00 (Supplementary Table 1). Minimum similarity (0.33) was observed between M 21(20 Gy) and M 17 (1100 mg L^{-1}) while maximum similarity (1.00) was observed in many genotypes. Close resemblance (0.86) was found between M 18 (1100 mg L^{-1}) and M 5 (60 Gy).

Maximum plant height was recorded in putative mutants obtained through colchicine application in *R. centifolia* (35 cm), *R. gruss-an-teplitz* (47.5 cm) and *R. borboniana* (35 mm) (Table 2). The highest number of shoots was also found in putative mutants obtained through colchicine. Number of leaves were found to be maximum (5) in mutants of *R. borboniana* and *R. centifolia* produced from both mutagens. However, the number of leaves remained the same (3) in *R. gruss-an-teplitz*. Maximum flower size (4.70 cm), number of flowers per plant (20) and number of petals were found in mutants obtained through colchicine application at the rate of 900 mg L^{-1} (Fig. 2). All three species showed non-significant results in the number of sepals (5).

Morphological traits solely cannot give the true impression of genetic makeup, thus SSR primers were essential in screening the genotypic relationships among these mutants. Some mutants were assessed as unique by combination of molecular markers and morphological traits. Mutant M3 showed less plant height while M36 displayed more plant height and less leaf size. M21 and M29 depicted more number of flowers. M14 showed more plant height, number of shoots, flower size, number of petals and number of flowers. These mutants assessed by SSR markers were also found to be genetically different from their parents (Fig. 1).

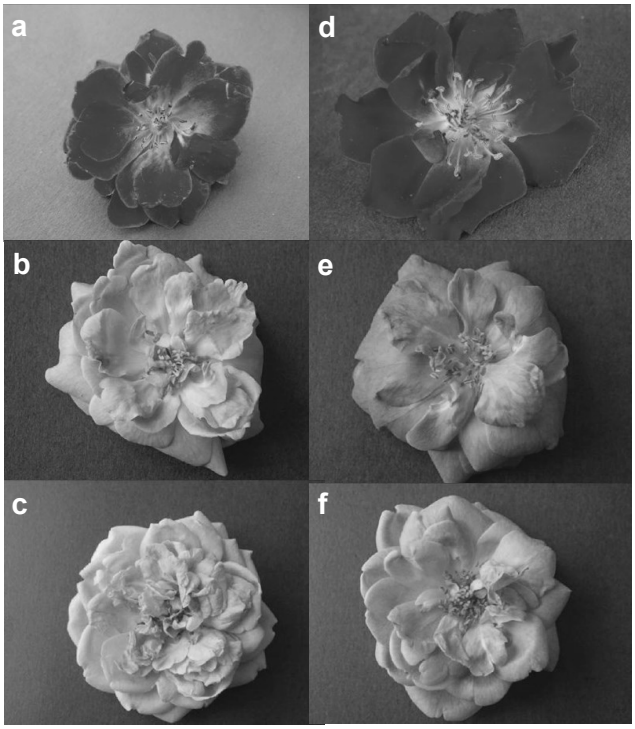

Fig. 2. Flower color and number of petals in parents of *Rosa gruss-an-teplitz* (a), *Rosa borboniana* (b), *Rosa centifolia* (c) compared with mutants (d), (e) and (f), respectively.

Table 2. Morphological characteristics of mutants and parents of rose species obtained through gamma rays and colchicine mutagens.

Morphological Traits	Parents			Gamma Ray Mutants						Colchicine Mutants (mg L^{-1})					
	Rosa borboniana	*Rosa centifolia*	*Rosa gruss-an-teplitz*	*Rosa borboniana*		*Rosa centifolia*		*Rosa gruss-an-teplitz*		*Rosa borboniana*		*Rosa centifolia*		*Rosa gruss-an-teplitz*	
				40 Gy	50 Gy	20 Gy	30 Gy	50 Gy	60 Gy	900 mg L^{-1}	1100 mg L^{-1}	900 mg L^{-1}	1100 mg L^{-1}	900 mg L^{-1}	1100 mg L^{-1}
Plant height (cm)	32	26	20.5	27-30	28-34	28-33	30-34	19-20	10.7-24.5	19.5-31	29-35	26-47	34-55	18-47.5	18-25.5
No. of shoots	6	5	5	4-5	3-5	4-5	3-5	3-5	4-5	3-6	4-5	5-7	4-5	3-7	2-3
Leaf margins	Dentate	Dentate	Dentate	Dentate	Dentate	Dentate	Dentate	Dentate	Dentate	Dentate	Dentate	Dentate	Dentate	Dentate	Dentate
No. of leaves	3	3	3	3	3-5	3-5	3	3	3	3-5	3-5	3-5	3-5	3	3
Leaf size (cm)	3.0	2.7	2.7	2.3-2.9	2.1-3.4	2.5-2.8	2.1-3.4	2.3-2.5	2.3-3.9	1.9-3.0	1.8-3.3	2.4-3.8	1.9-2.5	1.9-3.8	2.1-3.1
Flower color	Light pink	Whitish pink	Dark pink	Whitish pink-Reddish pink	Whitish pink	Whitish pink	Whitish pink-Reddish pink	Whitish pink-pinkish red	Whitish pink	Whitish pink	Whitish pink-Reddish pink	Whitish pink-reddish pink	Whitish pink-reddish pink	Whitish pink-pinkish red	Whitish pink-Reddish pink
Flower diameter (cm)	3.0	3.5	3.8	3.0-4.0	2.4-3.3	2.4-2.9	2.5-3.3	1.2-2.0	3.4-3.6	2.7-3.4	3.1-3.7	3.0-4.7	2.8-3.8	1.3-4.0	1.4-2.2
No. of flowers per plant	2	3	4	2-4	3-7	3-7	3-5	2	1-3	3-10	3-5	3-20	2-4	1-10	2-6
No. of petals	20	25	15	20-25	20-25	20-25	20-25	15-20	15-25	20-25	20-25	20-35	20-25	15-25	15-20
No. of sepals	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Euclidean dissimilarity coefficient matrix was computed based on their morphological data in terms of both quantitative and qualitative traits. Mutants M35 and M13 had the lowest dissimilarity index (1.0), while maximum dissimilarity was noticed in M14 and M10 (9.9) (Supplementary Table 2). Genotypes were classified into 5 clusters having close resemblances in morphological characters of genotypes. The mutants present in clusters B, C, D and E have variation in morphological traits compared with the parents. Cluster A had 15 genotypes among which M13 and M35 had close resemblance at a dissimilarity index of 1.1 (Fig. 3). Another major cluster C had 9 genotypes among which M24 and M27, and M21 and M29 had close resemblances with each other with dissimilarity indices of 1.2 and 1.0, respectively. Cluster D had 8 genotypes and cluster B had 2 genotypes, M30 and M31, having dissimilarity index of 2.5. Cluster E also consisted of 2 genotypes, M8 and M14, with close resemblance showing dissimilarity of 4.7. Application of colchicine to induce mutation among these species revealed better results compared with gamma irradiation.

First three principal components (PCs) accounted for 76.30% variability among 36 parental and mutated genotypes of *Rosa* species (Table 3). PC1 presented 48.08% total variation in morphological characters. Plant height, number of shoots, number of leaves per leaflet, leaf size, flower size, number of petals per flower and number of flowers contributed negatively to PC1. The three principal components were plotted to observe the relationships between mutated genotypes of *R*. species (Fig. 4 and 5). PC1 showed a separation between *R. centifolia* and *R. gruss-an-teplitz*, which was based on various morphological differences. Various genotypes had a wider spread across PC2.

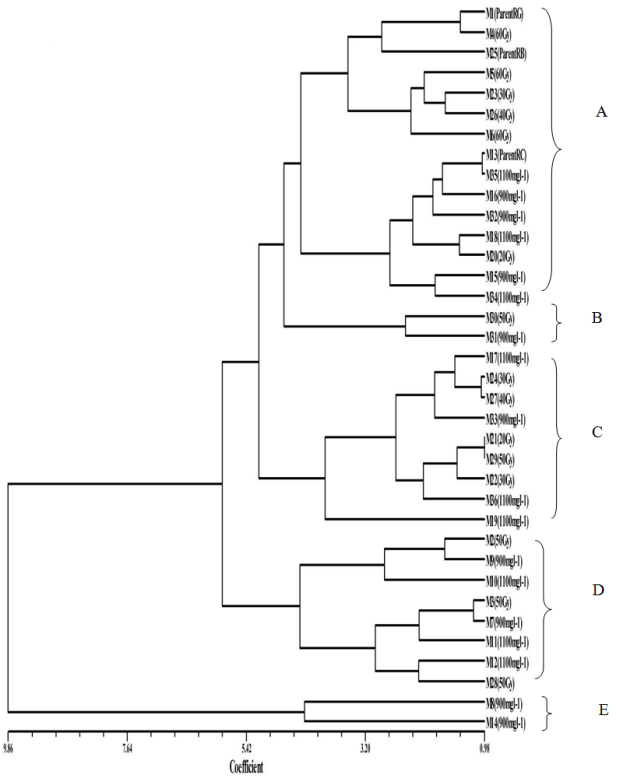

Fig. 3. Relationship among mutants of *Rosa gruss-an -teplitz*, *Rosa centifolia* and *Rosa borboniana* on the basis of agromorphological traits.

Table 3. Variation among rose species accounted for first three principal components.

Traits	PC1	PC2	PC3
Eigenvalue	3.37	1.13	0.85
Cumulative Eigenvalue	3.37	4.49	5.34
Percent variance	48.08	16.12	12.10
Cumulative variance	48.08	64.20	76.30
Eigenvectors			
Plant height (cm)	-0.399	-0.048	-0.505
No. of shoots	-0.425	0.128	0.402
No. of leaves/leaflet	-0.077	0.860	-0.268
Leaf size (cm)	-0.359	0.114	0.526
Flower size (cm)	-0.451	0.200	0.090
No. of petals/flower	-0.392	-0.180	-0.476
No. of flowers	-0.410	-0.397	0.021

DISCUSSION

SSR analysis confirmed considerable genetic variation induced by colchicine solution and gamma rays, among the mutants of three distinct genetic backgrounds. Genotypes of *R. centifolia* showed distant relationships with all genotypes and more diversity was revealed in this species. Moreover, colchicine treatments generated more distinct mutants independently located in the dendrogram. *R. centifolia* and *R. gruss-an-teplitz* are closely related because of common ancestral origin (Farooq et al. 2013). Variability caused by mutagens might be dependent on genotype. Clusters demonstrated genomic relatedness, which might be due to common background (Aras et al. 2005). The low genetic diversity can be interpreted by narrow genetic base (Akond et al. 2012). These differences may be due to evolution caused by mutations (Thiyagu et al. 2011).

Although selection on the basis of morphological characteristics is the most primitive method, it is still effective (Nemera et al. 2006). It was anticipated that gamma irradiation and colchicine application are oppositely related to all the parameters. With the increase in gamma irradiation doses, quantitative traits decreased, while they significantly increased by increasing the colchicine doses. Improper nutrient transportation, less hormone synthesis, metabolic disorders and decrease in the rate of cell division may be the reason behind reduced growth of mutants produced through gamma irradiation. Reduction in growth is the result of chromosomal and non-chromosomal damage because of radiation effects (Hewawasam et al. 2004). On the other hand, colchicine increases polyploidy level. As polyploidy level rises, DNA content also rises, which results in increased metabolic processes of cells, helping to increase growth (Leiva-Neto et al. 2004).

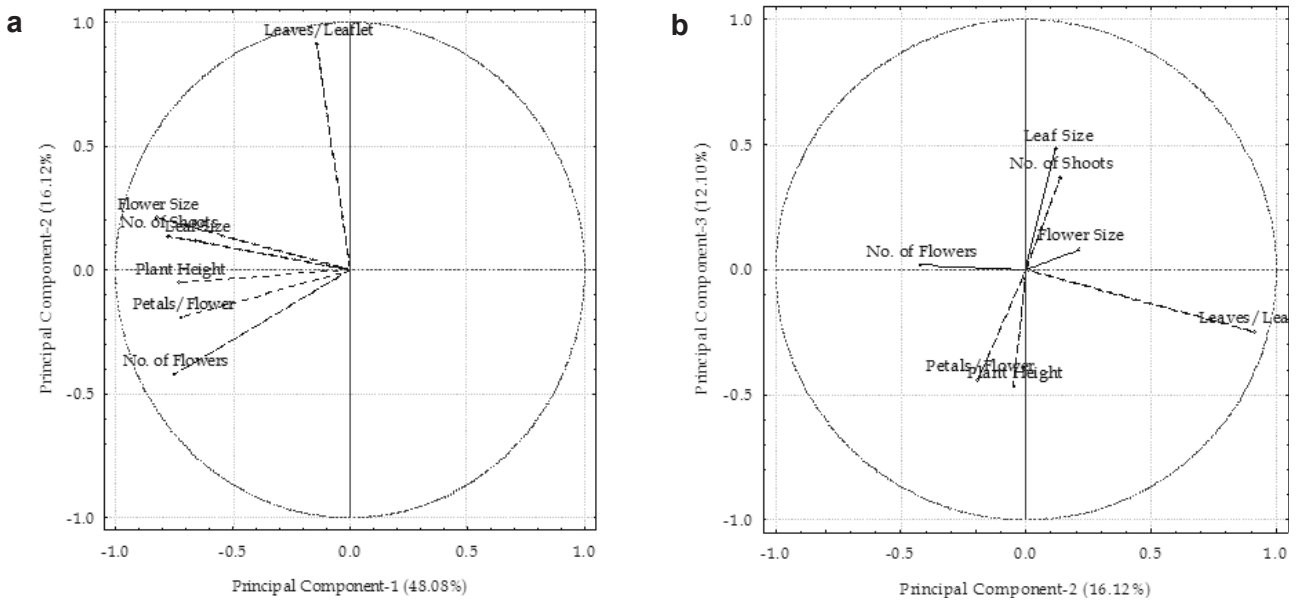

Fig. 4. Contribution of agro-morphological traits in principal components 1 & 2 (a) and 2 & 3 (b).

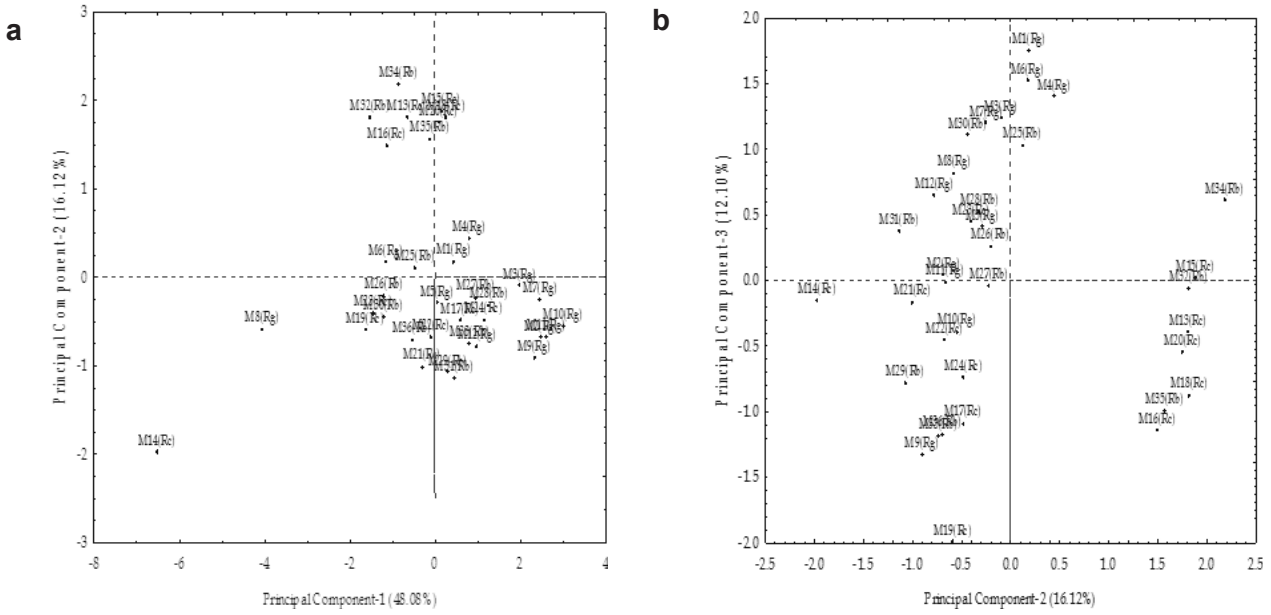

Fig. 5. Scatter diagram of PC1 & PC2 (a) and PC2 & PC3 (b) on 10 agro-morphological traits of rose species.

This study revealed genetic diversity that was confirmed by SSR markers in mutants of *R. borboniana*, *R. centifolia* and *R. gruss-an-teplitz*. The mutants M14, M8 and M29, M33 of *R. centifolia*, *R. gruss-an-teplitz* and *R. borboniana,* respectively, developed through colchicine dosage of 900 mg L^{-1} may be utilized for commercial purpose due to large flower size and more number of petals. The mutant M5 of *R. gruss-an-teplitz* obtained by gamma irradiation (60 Gy) may be used to increase the essential oil content due to large flower size.

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Supplementary Table 1. Coefficient of similarity among the putative genotypes of rose species.

Mutants	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20	M21	M22	M23	M24	M25	M26	M27	M28	M29	M30	M31	M32	M33	M34	M35	M36
M1	1.00																																			
M2	0.80	1.00																																		
M3	0.80	0.67	1.00																																	
M4	1.00	0.80	0.80	1.00																																
M5	0.67	0.57	0.57	0.67	1.00																															
M6	1.00	0.80	0.80	1.00	0.67	1.00																														
M7	1.00	0.80	0.80	1.00	0.67	1.00	1.00																													
M8	1.00	0.80	0.80	1.00	0.67	1.00	1.00	1.00																												
M9	1.00	0.80	0.80	1.00	0.67	1.00	1.00	1.00	1.00																											
M10	1.00	0.80	0.80	1.00	0.67	1.00	1.00	1.00	1.00	1.00																										
M11	1.00	0.80	0.80	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00																									
M12	0.80	0.67	1.00	0.80	0.57	0.80	0.80	0.80	0.80	0.80	0.80	1.00																								
M13	0.57	0.50	0.50	0.57	0.67	0.57	0.57	0.57	0.57	0.57	0.57	0.50	1.00																							
M14	0.57	0.50	0.50	0.57	0.44	0.57	0.57	0.57	0.57	0.57	0.57	0.50	0.80	1.00																						
M15	0.57	0.50	0.50	0.57	0.44	0.57	0.57	0.57	0.57	0.57	0.57	0.50	0.60	0.80	1.00																					
M16	0.57	0.75	0.50	0.57	0.44	0.57	0.57	0.57	0.57	0.57	0.57	0.50	0.40	0.60	0.80	1.00																				
M17	0.50	0.44	0.67	0.50	0.40	0.50	0.50	0.50	0.50	0.50	0.50	0.67	0.36	0.55	0.73	0.73	1.00																			
M18	0.80	0.67	0.67	0.80	0.86	0.80	0.80	0.80	0.80	0.80	0.80	0.67	0.75	0.50	0.50	0.50	0.44	1.00																		
M19	0.67	0.57	0.57	0.67	0.75	0.67	0.67	0.67	0.67	0.67	0.67	0.57	0.67	0.67	0.67	0.67	0.60	0.86	1.00																	
M20	0.67	0.57	0.57	0.67	0.75	0.67	0.67	0.67	0.67	0.67	0.67	0.57	0.89	0.67	0.67	0.44	0.40	0.86	0.75	1.00																
M21	0.50	0.44	0.44	0.50	0.60	0.50	0.50	0.50	0.50	0.50	0.50	0.44	0.73	0.55	0.55	0.36	0.33	0.67	0.60	0.80	1.00															
M22	0.80	0.67	0.67	0.80	0.57	0.80	0.80	0.80	0.80	0.80	0.80	0.67	0.50	0.50	0.75	0.75	0.67	0.67	0.57	0.57	0.44	1.00														
M23	1.00	0.80	0.80	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	0.80	0.57	0.57	0.57	0.57	0.50	0.80	0.67	0.67	0.50	0.80	1.00													
M24	0.80	0.67	0.67	0.80	0.57	0.80	0.80	0.80	0.80	0.80	0.80	0.67	0.50	0.50	0.75	0.75	0.67	0.67	0.57	0.57	0.44	1.00	0.80	1.00												
M25	1.00	0.80	0.80	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	0.80	0.57	0.57	0.57	0.57	0.50	0.80	0.67	0.67	0.50	0.80	1.00	0.80	1.00											
M26	1.00	0.80	0.80	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	0.80	0.57	0.57	0.57	0.57	0.50	0.80	0.67	0.67	0.50	0.80	1.00	0.80	1.00	1.00										
M2	0.80	0.67	1.00	0.80	0.57	0.80	0.80	0.80	0.80	0.80	0.80	1.00	0.50	0.50	0.50	0.50	0.67	0.67	0.57	0.57	0.44	0.67	0.80	0.67	0.80	0.80	1.00									
M28	1.00	0.80	0.80	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	0.80	0.57	0.57	0.57	0.57	0.50	0.80	0.67	0.67	0.50	0.80	1.00	0.80	1.00	1.00	0.80	1.00								
M29	0.67	0.57	0.57	0.67	0.50	0.67	0.67	0.67	0.67	0.67	0.67	0.57	0.67	0.67	0.67	0.44	0.40	0.57	0.50	0.75	0.80	0.57	0.67	0.57	0.67	0.67	0.57	0.67	1.00							
M30	1.00	0.80	0.80	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	0.80	0.57	0.57	0.57	0.57	0.50	0.80	0.67	0.67	0.50	0.80	1.00	0.80	1.00	1.00	0.80	1.00	0.67	1.00						
M31	1.00	0.80	0.80	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	0.80	0.57	0.57	0.57	0.57	0.50	0.80	0.67	0.67	0.50	0.80	1.00	0.80	1.00	1.00	0.80	1.00	0.67	1.00	1.00					
M32	1.00	0.80	0.80	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	0.80	0.57	0.57	0.57	0.57	0.50	0.80	0.67	0.67	0.50	0.80	1.00	0.80	1.00	1.00	0.80	1.00	0.67	1.00	1.00	1.00				
M33	0.80	0.67	0.67	0.80	0.57	0.80	0.80	0.80	0.80	0.80	0.80	0.67	0.50	0.50	0.50	0.50	0.44	0.67	0.57	0.57	0.67	0.67	0.80	0.67	0.80	0.80	0.67	0.80	0.57	0.80	0.80	0.80	1.00			
M34	1.00	0.80	0.80	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	0.80	0.57	0.57	0.57	0.57	0.50	0.80	0.67	0.67	0.50	0.80	1.00	0.80	1.00	1.00	0.80	1.00	0.67	1.00	1.00	1.00	0.80	1.00		
M35	1.00	0.80	0.80	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	0.80	0.57	0.57	0.57	0.57	0.50	0.80	0.67	0.67	0.50	0.80	1.00	0.80	1.00	1.00	0.80	1.00	0.67	1.00	1.00	1.00	0.80	1.00	1.00	
M36	0.80	0.67	1.00	0.80	0.57	0.80	0.80	0.80	0.80	0.80	0.80	1.00	0.50	0.50	0.50	0.50	0.67	0.67	0.57	0.57	0.44	0.67	0.80	0.67	0.80	0.80	1.00	0.80	0.57	0.80	0.80	0.80	0.67	0.80	0.80	1.00

Supplementary Table 2. Euclidean distances among pairs of rose species.

Mutants	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20	M21	M22	M23	M24	M25	M26	M27	M28	M29	M30	M31	M32	M33	M34	M35	M36
M1	0.0																																			
M2	4.3	0.0																																		
M3	2.6	3.8	0.0																																	
M4	1.4	4.7	2.1	0.0																																
M5	2.8	4.4	3.3	2.7	0.0																															
M6	3.5	5.4	4.3	3.4	2.1	0.0																														
M7	3.1	3.4	1.2	2.8	3.4	4.4	0.0																													
M8	5.5	6.8	7.0	6.1	5.8	5.0	7.3	0.0																												
M9	5.0	1.7	4.4	5.2	4.5	5.8	4.3	7.0	0.0																											
M10	3.9	2.2	3.4	4.0	4.4	5.7	3.0	7.3	2.8	0.0																										
M11	3.8	3.1	2.2	3.5	3.1	4.6	1.6	7.6	3.4	2.7	0.0																									
M12	3.0	2.9	3.0	3.3	2.5	3.4	2.5	5.8	3.7	2.9	2.4	0.0																								
M13	3.6	5.3	4.0	3.4	2.6	3.3	4.3	5.7	5.2	5.2	4.2	3.9	0.0																							
M14	7.9	9.4	9.1	8.4	7.2	6.7	9.4	4.3	9.4	9.9	9.4	7.7	7.2	0.0																						
M15	3.1	3.9	3.7	3.5	3.6	4.2	4.0	5.3	4.2	4.1	4.2	3.5	2.4	7.8	0.0																					
M16	3.8	5.1	4.5	3.9	3.3	4.1	4.9	4.9	4.9	5.1	4.7	4.1	1.6	6.6	2.1	0.0																				
M17	3.3	4.5	3.1	2.7	3.1	4.4	3.5	6.1	4.4	3.6	3.3	3.5	3.5	7.9	3.8	3.3	0.0																			
M18	3.6	5.0	3.3	3.2	3.5	4.4	3.8	6.2	4.9	4.5	3.9	4.0	2.0	8.1	2.4	2.1	2.6	0.0																		
M19	4.8	5.8	5.6	4.7	4.3	4.7	5.8	4.5	5.5	5.3	5.6	4.8	4.4	6.4	4.7	3.6	3.0	4.1	0.0																	
M20	3.5	4.8	3.6	3.2	3.1	3.7	3.6	5.9	5.0	4.3	3.7	3.3	1.8	7.8	2.4	2.2	3.0	1.4	4.1	0.0																
M21	3.4	4.3	3.0	3.3	2.2	3.3	3.3	5.3	4.3	4.4	3.1	2.7	3.0	6.5	3.6	3.1	2.6	3.2	4.0	3.2	0.0															
M22	3.2	3.6	3.1	3.2	2.3	3.4	3.4	4.9	3.4	3.9	3.1	2.8	3.0	6.9	3.0	2.8	2.3	3.0	3.4	3.1	1.4	0.0														
M23	3.4	5.1	4.0	3.3	2.1	1.7	4.2	4.3	5.4	5.2	4.3	3.1	2.9	5.8	3.8	3.2	3.3	3.6	3.4	3.1	2.2	2.4	0.0													
M24	2.9	4.1	2.8	2.5	2.4	4.1	3.0	6.5	4.1	3.0	2.5	2.7	3.4	8.2	3.7	3.5	1.3	3.0	3.7	2.9	2.6	2.5	3.3	0.0												
M25	2.6	3.9	3.4	2.9	3.3	3.2	3.9	3.9	4.3	4.3	4.3	3.3	3.6	7.1	2.7	3.4	3.4	3.6	3.9	3.6	3.2	2.3	2.8	3.7	0.0											
M26	2.7	4.7	4.0	2.9	1.9	2.4	4.4	4.1	4.7	4.8	4.3	3.2	2.7	6.1	3.2	2.7	3.0	3.5	3.2	3.3	2.5	2.0	1.7	3.0	2.2	0.0										
M2	2.4	4.0	2.1	1.8	1.9	3.4	2.5	6.1	4.1	3.3	2.4	2.6	3.0	8.0	3.3	3.3	1.5	2.7	3.9	2.7	2.2	2.0	2.8	1.0	3.0	2.5	0.0									
M28	2.2	2.4	2.7	2.8	2.7	4.0	2.9	5.8	2.8	2.6	2.8	2.2	3.7	8.2	2.7	3.6	3.2	3.7	4.7	3.6	3.0	2.3	3.7	2.7	2.6	2.8	2.3	0.0								
M29	3.6	4.0	3.0	3.4	2.2	3.7	3.2	5.8	3.8	3.8	2.5	2.5	3.1	7.1	3.7	3.2	2.1	3.1	3.8	3.1	1.0	1.5	2.6	2.0	3.5	2.7	1.9	2.9	0.0							
M30	2.8	5.7	3.4	2.9	3.4	3.9	4.3	4.9	5.9	5.6	4.7	4.2	3.5	6.3	3.9	3.5	3.3	3.6	4.5	3.9	2.5	2.9	3.0	3.8	3.1	2.8	3.1	3.8	3.2	0.0						
M31	3.1	4.7	2.8	3.4	3.2	4.7	3.4	6.3	4.8	4.4	3.3	3.3	3.9	7.4	4.0	3.9	3.1	3.8	5.2	4.0	2.1	2.9	3.9	3.0	4.1	3.6	2.7	3.1	2.3	2.5	0.0					
M32	3.9	5.7	4.4	3.8	3.2	3.2	4.8	5.0	5.8	5.9	4.9	4.3	1.2	6.5	2.7	1.8	3.9	2.3	4.3	2.2	3.1	3.1	2.7	4.1	3.4	2.8	3.5	4.2	3.5	3.2	4.2	0.0				
M33	3.6	4.0	3.3	3.3	2.0	3.8	3.3	6.4	3.7	3.5	2.5	2.7	3.2	7.8	3.8	3.4	1.9	3.2	3.6	3.0	2.2	2.1	3.0	1.3	3.8	2.8	1.5	2.8	1.4	4.0	3.1	3.9	0.0			
M34	3.1	5.0	4.2	3.2	3.2	3.1	4.4	4.9	5.5	5.0	4.7	3.6	2.0	7.2	1.9	2.2	4.0	2.6	4.4	1.9	3.8	3.5	3.0	3.9	2.9	2.8	3.4	3.6	4.0	3.8	4.5	1.9	4.0	0.0		
M35	3.8	4.8	3.9	3.6	2.6	3.6	4.1	6.1	4.8	4.7	3.7	3.5	1.0	7.5	2.5	1.8	3.2	1.8	4.2	1.4	2.9	2.9	3.1	2.9	3.9	3.0	2.7	3.6	2.8	4.0	3.8	1.9	2.7	2.3	0.0	
M36	3.6	5.2	3.8	3.3	2.9	4.2	4.4	5.6	4.7	4.8	4.0	4.0	3.2	6.8	3.9	2.8	1.7	3.0	3.1	3.5	2.1	2.0	3.0	2.3	3.5	2.5	2.2	3.5	2.1	2.6	2.8	3.4	2.2	4.1	3.1	0.0