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IN VITRO IMPROVEMENT OF CHARACTERS IN *ROSA SPP.* THROUGH COLCHICINE

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ABSTRACT

Flower size and color variation are the main attributes in the floriculture industry that affect the consumers demand. *Rosa gruss an teplitz* and *Rosa centifolia* are the fragrant rose species known for their wonderful aesthetic beauty. However, they are limited in use due to their small sized flowers and no color variegation. In order to improve characters in rose species, shoot tips taken from micropropagated plants were incubated for three different periods of 5, 7 and 11 days in MS medium containing different levels (0,100, 150, 300 and 450 mg l^{-1}) of colchicine. *Rosa gruss an teplitz* and *Rosa centifolia* yielded maximum plant height (15.12 and 17.23cm), number of nodes (4.80 and 6.93), internodal distance (3.15 and 2.48 cm), leaf area (5.26 and 10.50 cm^2) and flower size (6.24 and 5.78cm) respectively at colchicine level of 450 mg l^{-1} with incubation period of 11days. Colchicine also induced variegation and variation in flowers of *Rosa gruss an teplitz*. Genetic variation was analyzed and dendrogram was developed to assess the relationship with parent material. The findings of this work add to the ongoing efforts to improve the characters through induction of mutation in rose flowers.

Key words: *Rosa gruss an teplitz, Rosa centifolia*, colchicine, RAPD, variation

INTRODUCTION

Rose, the most important commercial flower crop (Horn, 1992), is used not only for its wonderful aesthetic beauty but also for its potential market value as cut flowers and potted plants in many countries of the world (Roy *et al*., 2004). Its expensive essential oil (Baydar and Baydar, 2005) is used in the perfume and pharmaceutical industries (Gudin, 2000). In Pakistan commonly grown rose species i.e *Rosa gruss an teplitz* and *Rosa centifolia* have a strong fragrance and blooms in small clusters. However, these are limited in use due to their small sized flowers and no color variegation. Economic return from cut flowers is dependent on flower quality (Kaul *et al*., 2011), therefore floriculture market is avidly searching for novelties by increasing the diversity within the genus (Heywood, 2003).

Breeding of rose is concentrated on the improvement of its ornamental value, which include color, size, form and keeping quality of the bloom (Broertjes and Van Harten, 1988). Therefore, mutation inducted by physical as well as chemical mutagen is used for genetic variation to develop new variants of better characteristics as described by Wongpiyasatid *et al*. (2000) and Arulbalachandran *et al*. (2009). Chemicals are used in many ornamental plants (Horn, 2002) to produce new genotypes with variation in color and size of flowers (Notsuka *et al.,* 2000). Among the different chemical mutagens (Novak, 1990), colchicine has been successfully used in different ornamental crops (Thao *et al*., 2003). It is a poisonous alkaloid that is widely used for polyploidy induction (Ade and Rai, 2010). It has played an important role in genetic and phenotypic diversity as well as plant evolution and breeding (Xing *et al*., 2011). Genome doubling helps in selection, variation in duplicated genes and attainment of novel function (Pickett and Meeks-Wagner, 1995). Induced polyploids have rapid variation in genome, recombination sequence elimination, sequence rearrangements and changes in DNA methylation (Osborn *et al*., 2003). These variations have direct impact on the structure of genome and evolution (Adams and Wendel, 2005).

Rosa gruss-an-teplitz and *Rosa centifolia* are fragrant rose species having novel characteristics but their proper exploitation to fulfil the recent demands of consumers is not in practice due to flowers of small size with no color variegation. These limitations urge the need of mutant breeding approaches to induce genetic variation in the vegetative and floral characteristics of these species to enhance their commercial value.

MATERIALS AND METHODS

Shoot tips of uniform size (1 cm) taken from the micropropagated *Rosa gruss-an-teplitz* and *Rosa centifolia* were incubated for 5, 7 and 11 days in MS medium containing different level of colchicine (0,100, 150, 300 and 450 mg l^{-1}). Explants after incubation in this media were inserted in MS medium (macro, micro elements and vitamins) supplemented with IBA (0.01 mgl^{-1}), GA_3 (0.4 mg l^{-1}), and BAP (1.0 mg l^{-1}), sucrose (30 g l^{-1}) and agar (7 g l^{-1}). The pH of the medium was adjusted at 5.8. Treated shoot tips were kept at 24°C in

growth room under light for a photoperiod of 16 hour (Philips TL 40 W Fluorescent tubes).

After Six weeks of culture, the excised shoots from micro propagated clumps were transferred to *in vitro* rooting medium (1/2 MS macro, micro elements and vitamins) which was fortified with sucrose (20 g l^{-1}) and IBA (0.50 mg l^{-1}) as described by Baig *et al.* (2012). Rooted micro shoots after six weeks of culturing were transplanted to pots and acclimatized under controlled glass house conditions. Data regarding different morphological and floral characteristics were noted at different stages. Putative mutants were analyzed through RAPD for any typical variation. For this purpose DNA was extracted from young tender leaves of putative rose mutants by the method described by Dellaporta *et al*. (1983) amended by Weising *et al.* (1995). OPS and OPV decamer random primers (Operon Technologies Inc., Alameda, California, USA) were employed to find genetic variation. Amplification was performed in 25 µL volumes having 2 µL primers, 3 µL genomic DNA of rose, 1 unit Taq DNA polymerase, 6µL 10X PCR buffer and 8 µL distilled water. DNA amplification was done with a thermo cycler programmed for initial pre-denaturation at 94 °C for 5 min. 40 cycles at 94°C (1 min), 38 °C (1 min), 72 °C (2 min) and incubation period at 72 °C for 5 min. After amplification, Gel Electrophoresis was carried out on agarose gel (1%) in 0.5 x Tris-borate-EDTA buffer and using Ethidium Bromide stain (Sambrook *et al.*, 1989). Gel was visualized under UV light and photographs were taken by the Gel Documentation apparatus. RAPD bands were counted as a binary variable presence (1) and absence (0) of each of the primer accession combinations.

The experiment was laid out following CRD design with four replications. The data was analyzed by two-way analysis of variance and the means were compared by Duncan's Multiple Range Test at probability level of 5% (Steel *et al*., 1997).

RESULTS AND DISCUSSION

The work was aimed to induce mutation in *Rosa gruss an teplitz* and *Rosa centifolia* through the colchicine application. Statistical analysis depicted that colchicine (450 mg l^{-1}) with incubation period of 11 days had a stimulative effect on most traits. It showed a declining trend in survival percentage with the increase in the colchicine concentration and incubation period. At shoot proliferation stage minimum survival percentage was found in *Rosa gruss an teplitz* (82.88%) and *Rosa centifolia* (82.50%) when incubated for 11 days in a medium fortified with colchicine @ 450 mg l^{-1} (Table 1 & 2). Similarly at acclimatization stage 52.50 and 63.75% plants of *Rosa gruss an teplitz* and *Rosa centifolia respectively* survived in a medium with 450 mg l^{-1} and incubation period of 11 days. According to Thao *et al*. (2003) and Addink (2002) high colchicine concentration and incubation period reduced survival percentage of the shoot tips. With colchicine treatment similar results have also been found in *Phlox subulata* (Zhang *et al*. 2008) and *Gerbera jamesonii* (Gantait *et al*., 2011).

A declining trend regarding number of shoots was recorded with increase in colchicine level and incubation period (Table 1). *Rosa gruss an teplitz* produced less number of shoots at 450 mg l^{-1} of colchicine incubated for 5 (1.20), 7 (1.00) and 11 days (1.20) (Table 1). However, colchicine produced more number of shoots (1.45, 1.25 and 1.30) when shoot tips were treated with 300 mg l^{-1} for a period of 5, 7 and 11 days. Number of shoots in *Rosa centifolia* were decreased upto 1.22 in a medium treated with colchicine @ 450 mg l^{-1} as compared to control (2.81) for a period of 11 days (Table 2). Kadota and Niimi (2002) found inferior shoot proliferation rate subjected to colchicine treatment in comparison to control individuals. These results are also in conformity with Hewawasam *et al.* (2004) who reported that colchicine treated plants of crossandra decreased number of shoots with increase in concentration.

Percentage of plants showing leaf abnormalities was maximum (13.33 and 20.00 %) in *Rosa gruss an teplitz* and *Rosa centifolia* respectively when plants were incubated for a period of 11days in the medium having 450 mg l^{-1} of colchicine as illustrated in Table 1 & 2. No abnormality was found in untreated plants of these species. Occurrence of leaf abnormalities in the colchicine treated plants might be due to chromosomal aberrations (Datta, 1997). Colchicine has been known to cause abnormality in growth and morphology, rearrangements or loss of chromosomes and gene mutation (Wan *et al*., 1989).

Culture rooting percentage, number of roots and root length was significantly decreased with increase in incubation period and colchicine concentration (Table 1 & 2). Minimum rooting percentage (82.50 & 52.50%), number of roots (4.35 & 2.75) and root length (0.88 and 1.05 cm) was observed in *Rosa gruss an teplitz* and *Rosa centifolia* respectively in the medium incubated for 11 days with colchicine (450 mg l^{-1}). Similarly plants of *Rosa gruss an teplitz* and *Rosa centifolia* incubated for a period of 11 days in medium fortified with colchicine (300 mg l^{-1}) resulted in less rooting percentage (92.50 &61.2), number of roots (6.16 &1.62) and root length (1.34&1.16 cm) as compared to other incubation periods. The most probable reason for this outcome could be the necessary endogenous hormone balance required for rooting in untreated shoots that could have been maintained in the hormone free medium. Increase in mutagen rate postponed root development resulting in poor rooting percentage (Hewawasam *et al.*, 2001).

After acclimatization significant increase in plant height (15.12 & 17.23 cm), number of nodes (4.80 and 6.93), internodal distance (3.15 and 2.48 cm), leaf area (5.26 & 10.50 cm^2) (Table 3 & 4) and flower size (6.24 & 5.78cm) (Table 5 & 6) was depicted in *Rosa gruss an teplitz* and *Rosa centifolia* respectively at colchicine level of 450 mg l^{-1} with incubation period of 11days (Table 3 & 4). However, colchicine @300 mg l^{-1} gave comparatively less plant height (14.56 & 14.63 cm), number of nodes (4.62 & 6.73), internodal distance (3.15& 2.17cm), leaf area (5.24 & 8.69cm) and flower size (6.15 & 5.74 cm) in *Rosa gruss an teplitz* and *Rosa centifolia* respectively with same incubation period of 11 days. This increase might be due to induction of polyploidy by the application of colchicine. These cells have bigger size with larger cell volume oftenly grow into thicker tissues, ensuing large plant organs (Rauf *et al*., 2006). A study on Arabidopsis tetraploid plants clearly indicated increase in ploidy level having a better effect on size of cell (Breuer *et al*., 2007).

We evaluated several additional factors, which might contribute to color variance such as *L** (Lightness), a* (+ redness – green), b* (+ yellow – blue), chroma and hue angle. All the above-mentioned parameters were measured using a chromameter to record the variation in floral color of rose species as given in Table 5 & 6. Statistical analysis of data regarding color components showed that increase in both colchicine concentration and incubation period decreased the value of *L** , *b** and hue angle. Plants incubated in colchicine (450 mg $L^{-1)}$ depicted value of *L** (22.28 and 43.01), *b** (3.80 and -4.36) and hue angle (4.03 and -6.67) in flower of *Rosa gruss an teplitz* and *Rosa centifolia* respectively. However, value of *a** and chroma showed an inclining trend with increase in colchicine level and incubation period. Flower color *a** (54.44 and 37.63) and chroma (54.57 and 37.88) was maximum in a medium fortified with colchicine (450 mg L^{-1}) for a period of 11 days. However, minimum value of *a** and chroma was found in untreated plants. This variation may be due to change in ploidy level as colchicine induces ploidy (Osborn *et al*., 2003). Doubling of mitotic chromosome in cyclamen (Takamura and Miyajima, 1996) and carnation (Yamaguchi, 1989) caused deeper flower color. Polyploidy in orchids produces desirable characteristics like giantism (an increase in floral piece) and intensification in flower coloring (Watrous and Wimber, 1988).

Variegation was not found in treated plant population of *Rosa centifolia* but plants of *Rosa gruss an teplitz* treated with colchicine @450 mg l^{-1} produced variegated flowers (Figure 1). Three plants had pink flowers with pink and red petals in same flower. However, in one flower there were pink margins. Moreover, one plant had brighter flowers as compared to control. According to De Schepper *et al*. (2001) pigments in the cell vacuole generates color pattern, depending on the polyploidy level. During successive cell division, the mutated cells compete normal surrounding cells for survival. If these mutated cells survive in diplotic selection, they are expressed in plants (Datta *et al*., 2005).

Detection of the induced genetic variation has a pivotal role in recording the mutant plants behavior towards colchicine. RAPD technique is used for the easy evaluation of mutant's genetic variation and is a potential mean for the rapid mutants selection with huge genetic variation (Teng *et al*., 2008). In this study 40 primers of S and V series were tested, out of which twelve primers amplified the DNA of genotypes. These primers were used to amplify the genotypes of rose species. Maximum numbers of bands (9) were noted in GM7 and GM8 among the mutants of *Rosa gruss an teplitz* while among the genotypes of *Rosa centifolia* RC and CM9 yielded maximum bands (10).

In OPS series four primers amplified DNA of rose mutants of *Rosa gruss an teplitz* and *Rosa centifolia*. Out of these primers OPS-18 amplified highest number of alleles (14). In OPV series eight primers were able to amplify the DNA. OPV-8 (Figure 2 & 3) amplified maximum number of alleles (14) than other primers. Total amplified bands were 335 and 344 in genotypes of *Rosa gruss an teplitz* and *Rosa centifolia* respectively (Table 7).

Dendrogram was constructed on the basis of the computed genetic similarity (Nei and Li's genetic coefficient). Cluster analysis resulted in the grouping of thirteen *Rosa gruss an teplitz* genotypes into two main clusters. Main clusters were named as "A" and "B" (Figure 4). Cluster "A" consists of five genotypes which are labeled as GM5, GM6, GM7, GM8 and GM9. These genotypes were quite diverse from other genotypes but the similarities in them were very low. Cluster "B" was further subdivided into two subcluster B1 and B2. Subcluster B1 and B2 each consisted of four genotypes of *Rosa gruss an teplitz*.

Figure 5 presented dendrogrm of thirteen genotypes of *Rosa centifolia* and was divided into two cluster "A" and "B". Cluster "A" consisted of two genotypes CM11 and CM12 of *Rosa centifolia.* Cluster "B" was further divided into four subclusters B1, B2, B3 and B4. Subcluster B1 consisted of CM5 and CM6 genotypes which were diverse from other subclusters but quite similar to each other. Subcluster B4 contained three genotypes which were labeled as RC, CM1 and CM2. The pair-wise estimation of genetic resemblance exhibited that most accessions that included interspecific derivatives depicted a high degree of genetic resemblance at the molecular level following RAPD assay (Dwivedi *et al*., 2001).

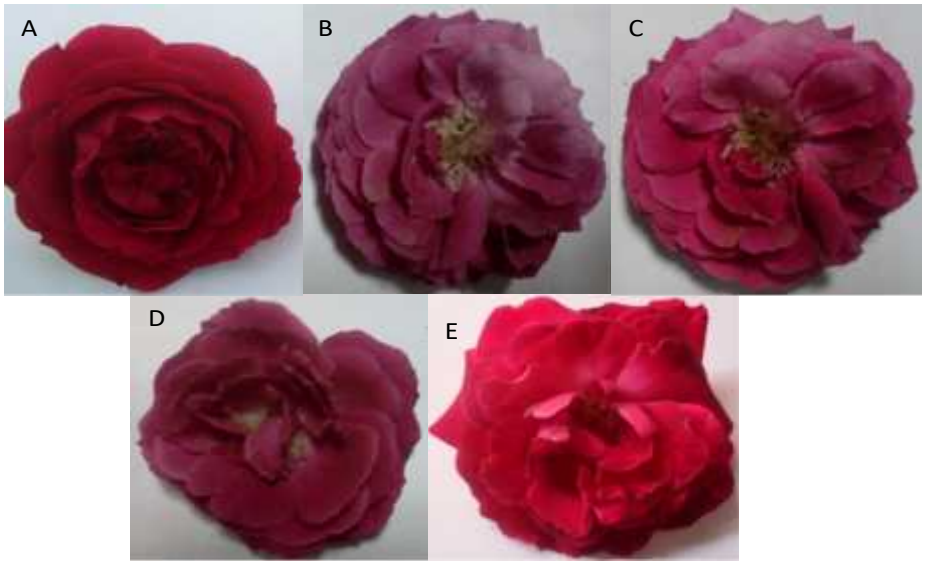

Fig. 1. Variegation in flower color (B, C, D & E) of *Rosa gruss an teplitz* incubated for 11 days in colchicine @ 450 mg l^{-1} as compared to control (A)

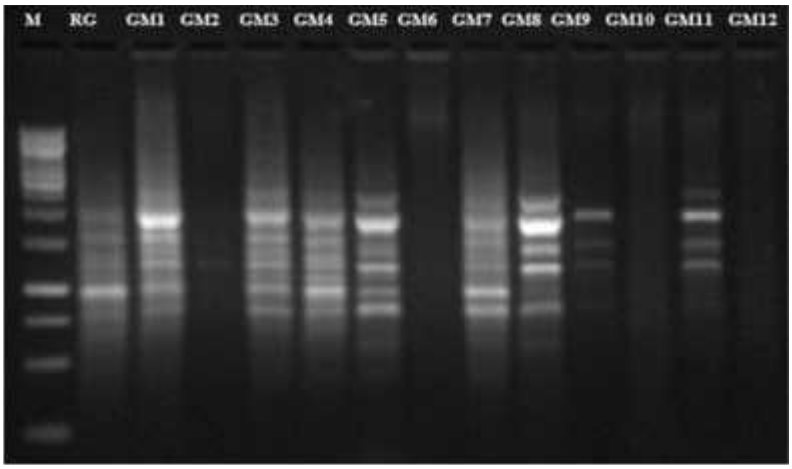

Fig. 2. Agarose gel showing RAPD banding pattern produced with primer OPV-8 (RG (*Rosa gruss an teplitz*), GM1, GM2,….GM12 (Mutants obtained from *Rosa gruss an teplitz*)

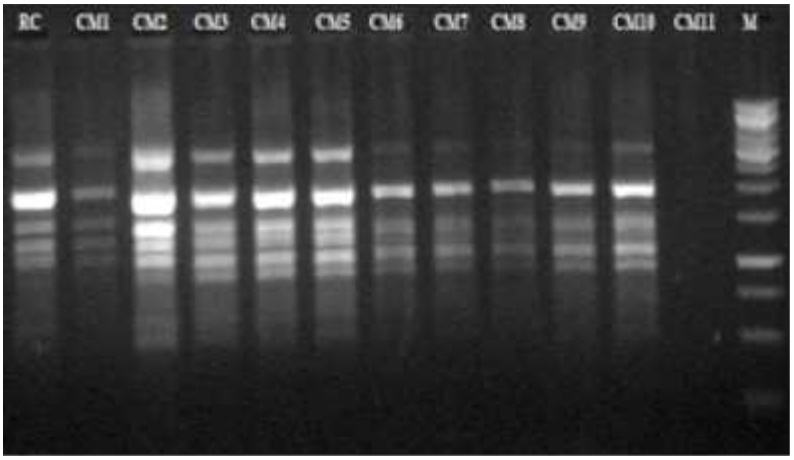

Fig. 3. Agarose gel showing RAPD banding pattern produced with primer OPV-8 (RC (*Rosa centifolia*), CM1, CM2,…….CM11 (Mutants obtained from *Rosa centifolia*)

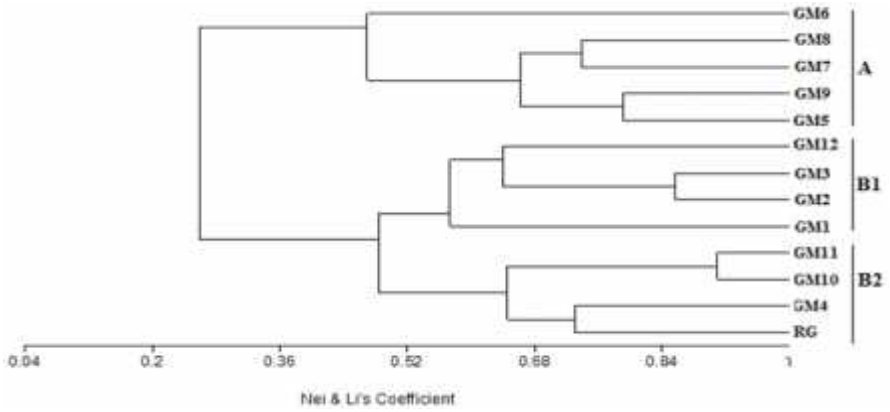

Fig. 4. RAPD based genetic relationship between mutants of *Rosa gruss an teplitz* obtained through colchicine supplemented medium (RG (*Rosa gruss anteplitz*),GM1, GM2,…….GM12 (Mutants obtained from *Rosa gruss an teplitz*)

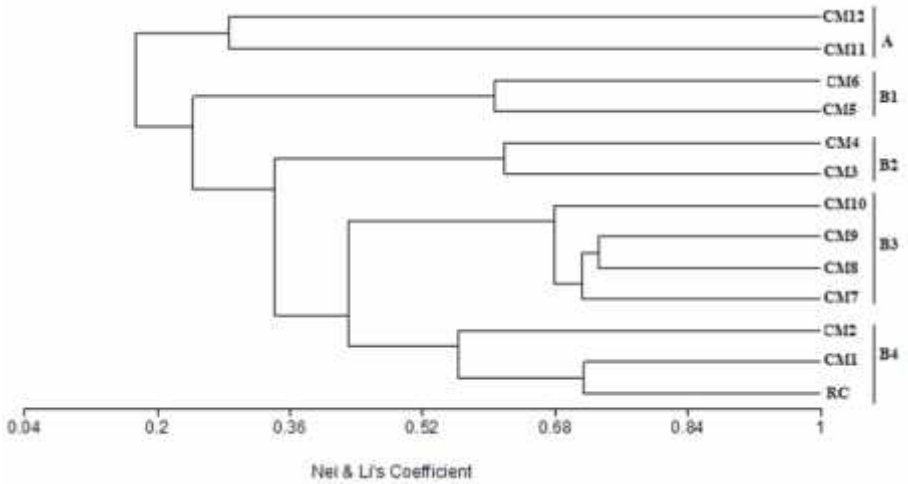

Fig. 5. RAPD based genetic relationship between mutants of *Rosa centifolia* obtained through colchicine supplemented medium (RC (*Rosa centifolia*), CM1,CM2,…….CM12 (Mutants obtained from *Rosa centifolia*).

Table 1. Effect of colchicine supplemented medium on different traits of *Rosa gruss an teplitz*

Colchicine (mg l^{-1})	Survival %age			%age of plants showing leaf abnormalities			Number of shoots			Rooting %age			Number of roots			Root length (cm)		
	Incubation period (Days)			Incubation period (Days)			Incubation period (Days)			Incubation period (Days)			Incubation period (Days)			Incubation period (Days)		
	5	7	11	5	7	11	5	7	11	5	7	11	5	7	11	5	7	11
T_0 (0)	100 a	100 a	100 a	0.00 i	0.00 i	0.00 i	1.82 a	1.85 a	1.80 a	100 a	100 a	100 a	8.82 a	8.95 a	8.57 ab	1.89 a	1.88 a	1.88 a
T_1 (100)	100 a	100 a	100 a	0.00 i	3.30 h	10.00 c	1.70 a	1.40 bc	1.25 cd	100 a	100 a	100 a	8.50 ab	8.50 ab	8.47 ab	1.87 a	1.82 a	1.62 b
T_2 (150)	100 a	100 a	98.75 a	5.00 g	5.00 g	11.64 b	1.72 a	1.32 bcd	1.21 d	1000 a	100 a	98.75 a	8.20 ab	8.16 ab	8.15 ab	1.63 b	1.59 b	1.60 b
T_3 (300)	100 a	90.13 c	88.38 c	5.62 fg	6.33 ef	11.65 b	1.45 b	1.25 cd	1.30 bcd	100 a	95.00 b	92.50 c	7.50 bc	6.40 cd	6.16 d	1.45 bc	1.37 cd	1.34 cd
T_4 (450)	95.25 b	85.75 d	82.88 e	6.56 e	8.50 d	13.33 a	1.20 d	1.00 e	1.20 d	93.69 bc	84.16 d	82.50 d	6.91 cd	4.28 e	4.35 e	1.20 de	1.12 e	0.88 f
LSD $_{0.05}$	2.29			0.767			0.153			2.482			1.169			0.192		
Means within a column followed by different letters are significantly different at $p < 0.05$																		

Table 2. Effect of colchicine supplemented medium on different traits of *Rosa centifolia*

Colchicine (mg l^{-1})	Survival %age			%age of plants showing leaf abnormalities			Number of shoots			Rooting %age			Number of roots			Root length (cm)		
	Incubation period (Days)			Incubation period (Days)			Incubation period (Days)			Incubation period (Days)			Incubation period (Days)			Incubation period (Days)		
	5	7	11	5	7	11	5	7	11	5	7	11	5	7	11	5	7	11
T_0 (0)	100^{a}	100^{a}	100^{a}	0.00^{h}	0.00^{h}	0.00^{h}	2.87^{a}	2.75^{ab}	2.81^{ab}	100^{a}	100^{a}	100^{a}	5.70^{d}	6.07^{cd}	5.80^{d}	1.99^{a}	1.99^{a}	1.98^{a}
T_1 (100)	98.75^{ab}	97.50^{abc}	96.25^{abcd}	0.00^{h}	0.00^{h}	5.00^{e}	2.55^{bc}	2.37^{cd}	2.32^{cd}	100^{a}	95.00^{b}	81.25^{c}	6.75^{bc}	4.45^{e}	3.81^{ef}	1.87^{ab}	1.86^{ab}	1.87^{ab}
T_2 (150)	98.75^{ab}	96.25^{abcd}	93.75^{cde}	0.00^{h}	5.00^{e}	11.63^{c}	2.25^{cd}	2.07^{de}	1.90^{e}	100^{a}	83.75^{c}	63.91^{ef}	7.95^{a}	5.65^{d}	3.57^{fg}	1.68^{bc}	1.49^{cd}	1.38^{de}
T_3 (300)	97.50^{abc}	95.00^{bcd}	92.63^{de}	1.33^{g}	1.33^{g}	18.15^{b}	1.50^{f}	1.35^{f}	1.45^{f}	96.25^{b}	73.75^{d}	61.25^{f}	7.40^{ab}	4.10^{ef}	1.62	1.24^{ef}	1.19^{ef}	1.16^{f}
T_4 (450)	96.25^{abcd}	90.00^{e}	82.50^{f}	2.50^{f}	8.30^{d}	20.00^{a}	1.30^{f}	1.35^{f}	1.22^{f}	81.25^{c}	66.25^{e}	52.5^{g}	5.3^{d}	2.90^{gh}	2.75^{h}	1.15^{f}	1.11^{f}	1.05^{f}
LSD $_{0.05}$	4.029			0.951			0.305			3.0609			0.8194			0.2148		

Means within a column followed by different letters are significantly different at $p < 0.05$

Table 3. Effect of colchicine supplemented medium on different traits of *Rosa gruss an teplitz* after acclimatization

Colchicine (mg L^{-1})	Survival %age			Plant height (cm)			Number of nodes			Internodal distance (cm)			Leaf area (cm^2)		
	I.P*. (Days)			I.P. (Days)			I.P. (Days)			I.P. (Days)			I.P. (Days)		
	5	**7**	**11**	**5**	**7**	**11**	**5**	**7**	**11**	**5**	**7**	**11**	**5**	**7**	**11**
T_0 (0)	94.62^{ab}	96.25^{a}	95.00^{ab}	8.81^{def}	8.75^{def}	8.66^{ef}	4.145^{e}	4.13^{e}	4.14^{e}	2.12^{bcd}	2.11^{bcd}	2.09^{bcd}	4.35^{abc}	4.31^{abc}	4.35^{abc}
T_1 (100)	86.43^{cd}	88.91^{bc}	82.50^{de}	8.48^{f}	8.52^{f}	8.73^{def}	4.192^{e}	4.20^{e}	4.22^{de}	2.02^{d}	2.02^{cd}	2.06^{bcd}	4.300^{abc}	4.00^{bc}	5.41^{a}
T_2 (150)	80.00^{e}	87.55^{cd}	72.25^{fg}	9.29^{cdef}	8.79^{def}	10.33^{bc}	4.26^{de}	4.38^{cd}	4.51^{bc}	2.18^{bcd}	2.00^{d}	2.29^{bc}	4.75^{abc}	4.85^{abc}	3.80^{c}
T_3 (300)	78.50^{ef}	66.00^{gh}	63.50^{h}	9.80^{bcd}	9.65^{bcde}	14.56^{a}	4.57^{b}	4.59^{b}	4.62^{b}	2.14^{bcd}	2.09^{bcd}	3.15^{a}	5.203^{a}	5.17^{a}	5.24^{a}
T_4 (450)	78.18^{ef}	65.85^{h}	52.50^{i}	10.75^{b}	10.29^{bc}	15.12^{a}	4.65^{ab}	4.66^{ab}	4.80^{a}	2.31^{b}	2.20^{bcd}	3.15^{a}	5.11^{ab}	5.10^{a}	5.26^{a}
LSD $_{0.05}$	6.366			1.117			0.164			0.266			1.135		

Means within a column followed by different letters are significantly different at $p < 0.05$

Table 4. Effect of colchicine supplemented medium on *Rosa centifolia* after acclimatization

Colchicine (mg L^{-1})	Survival %age			Plant height (cm)			Number of nodes			Internodal distance (cm)			Leaf area (cm^2)		
	I.P*. (Days)			I.P. (Days)			I.P. (Days)			I.P. (Days)			I.P. (Days)		
	5	**7**	**11**	**5**	**7**	**11**	**5**	**7**	**11**	**5**	**7**	**11**	**5**	**7**	**11**
T_0 (0)	98.75^{a}	96.56^{b}	96.25^{b}	10.76^{g}	10.91^{fg}	10.77^{g}	6.02^{h}	6.05^{h}	6.03^{h}	1.78^{ef}	1.80^{ef}	1.78^{ef}	5.69^{f}	5.64^{f}	5.61^{f}
T_1 (100)	90.90^{c}	89.58^{cd}	78.26^{f}	10.35^{g}	10.10^{g}	9.72^{g}	6.085^{gh}	6.14^{gh}	6.23^{fg}	1.70^{fg}	1.64^{fg}	1.56^{g}	5.49^{f}	5.76^{f}	5.74^{f}
T_2 (150)	90.00^{cd}	86.11^{e}	72.22^{g}	12.63^{de}	13.37^{bcd}	13.87^{bcd}	6.235^{fg}	6.35^{ef}	6.41^{de}	2.02^{bcd}	2.10^{bc}	2.16^{b}	7.93^{e}	8.01^{de}	8.14^{de}
T_3 (300)	88.80^{d}	84.23^{e}	65.62^{i}	12.68^{de}	12.06^{ef}	14.63^{b}	6.52^{de}	6.57^{cd}	6.73^{bc}	1.94^{cde}	1.83^{def}	2.17^{b}	8.34^{de}	8.69^{cd}	8.69^{cd}
T_4 (450)	65.62^{i}	69.06^{h}	63.75^{i}	13.11^{cde}	13.98^{bc}	17.23^{a}	6.74^{bc}	6.78^{ab}	6.93^{a}	1.94^{cde}	2.06^{bc}	2.48^{a}	9.22^{bc}	9.50^{b}	10.50^{a}
LSD $_{0.05}$	2.048			1.262			0.173			0.205			0.747		

Means within a column followed by different letters are significantly different at $p < 0.05$

Table 5. Effect of colchicine supplemented medium on color and size of flowers of *Rosa gruss an teplitz*

Colchicine (mg L^{-1})	Flower color															Flower Size (cm)		
	*L**			*a**			*b**			*c**			*h°*					
	I.P*. (Days)			I.P. (Days)			I.P. (Days)			I.P. (Days)			I.P. (Days)			I.P. (Days)		
T_0 (0)	36.71^{a}	36.71^{a}	36.71^{a}	39.85^{d}	39.85^{d}	39.85^{d}	7.605^{a}	7.60^{a}	7.60^{a}	40.58^{d}	40.58^{d}	40.58^{d}	10.76^{a}	10.76^{a}	10.76^{a}	4.71^{c}	4.71^{c}	4.71^{c}
T_1 (100)	36.95^{a}	36.80^{a}	35.49^{a}	40.46^{d}	39.99^{d}	41.98^{cd}	7.00^{ab}	6.25^{abc}	6.29^{abc}	41.08^{d}	40.52^{d}	42.47^{cd}	9.91^{a}	9.25^{ab}	8.59^{abc}	4.97^{c}	4.99^{c}	5.37^{abc}
T_2 (150)	35.29^{a}	34.25^{abc}	33.22^{abc}	40.71^{d}	41.31^{cd}	42.02^{cd}	6.12^{abc}	6.00^{abc}	5.84^{abc}	41.17^{d}	41.75^{cd}	42.43^{cd}	8.50^{abc}	8.40^{abcd}	7.98^{abcd}	5.00^{c}	5.23^{abc}	5.58^{abc}
T_3 (300)	34.79^{ab}	32.01^{abc}	28.42^{c}	41.89^{cd}	44.37^{bcd}	49.62^{ab}	6.47^{abc}	5.22^{bcd}	4.84^{cd}	42.46^{cd}	44.69^{bcd}	49.86^{ab}	8.82^{abc}	6.69^{bcde}	5.59^{de}	5.12^{c}	5.36^{abc}	6.15^{ab}
T_4 (450)	32.23^{abc}	28.95^{bc}	22.28^{d}	40.12^{d}	47.32^{bc}	54.44^{a}	6.10^{abc}	5.19^{bcd}	3.80^{d}	40.62^{d}	47.61^{bc}	54.57^{a}	8.63^{abc}	6.33^{cde}	4.03^{e}	5.09^{c}	5.22^{bc}	6.24^{a}
LSD $_{0.05}$	5.848			6.224			1.996			6.195			2.878			1.014		

Means within a column followed by different letters are significantly different at $p < 0.05$

Table 6. Effect of colchicine supplemented medium on color and size of flowers of *Rosa centifolia*

Colchicine (mg L^{-1})	Flower color															Flower Size (cm)		
	*L**			*a**			*b**			*c**			*h°*					
	I.P*. (Days)			I.P. (Days)			I.P. (Days)			I.P. (Days)			I.P. (Days)			I.P. (Days)		
	5	**7**	**11**	**5**	**7**	**11**	**5**	**7**	**11**	**5**	**7**	**11**	**5**	**7**	**11**	**5**	**7**	**11**
T_0 (0)	67.38^{a}	67.38^{a}	67.38^{a}	27.67^{c}	27.67^{c}	27.67^{c}	-7.50^{d}	-7.50^{d}	-7.50^{d}	28.72^{c}	28.72^{c}	28.72^{c}	-15.52^{d}	-15.52^{d}	-15.52^{d}	5.11^{c}	5.11^{c}	5.11^{c}
T_1 (100)	65.83^{ab}	65.90^{ab}	63.70^{ab}	31.28^{bc}	32.35^{bc}	31.72^{bc}	-6.10^{bcd}	-6.04^{bc}	-6.13^{cd}	31.89^{bc}	32.94^{bc}	32.31^{bc}	-11.22^{c}	-10.74^{bc}	-10.95^{bc}	5.26^{abc}	5.20^{abc}	5.16^{bc}
T_2 (150)	64.08^{ab}	62.16^{abc}	60.87^{abc}	31.97^{bc}	32.34^{bc}	32.52^{bc}	-6.23^{cd}	-6.081^{bcd}	-5.71^{abc}	32.58^{bc}	32.90^{bc}	33.02^{abc}	-10.99^{bc}	-10.61^{bc}	-10.02^{abc}	5.28^{abc}	5.26^{abc}	5.38^{abc}
T_3 (300)	61.28^{abc}	58.49^{abc}	52.00^{bcd}	30.48^{bc}	32.21^{bc}	33.39^{ab}	-4.84^{abc}	-5.64^{abc}	-4.94^{abc}	30.89^{bc}	32.70^{bc}	33.76^{ab}	-8.89^{abc}	-9.89^{abc}	-8.44^{abc}	5.46^{abc}	5.50^{abc}	5.74^{ab}
T_4 (450)	50.60^{bcd}	47.35^{cd}	43.01^{d}	32.85^{ab}	34.77^{ab}	37.63^{a}	-4.84^{abc}	-4.66ab	-4.36^{a}	33.25^{abc}	35.09^{ab}	37.88^{a}	-8.80^{abc}	-7.72^{ab}	-6.67^{a}	5.55^{abc}	5.73^{ab}	5.78^{a}
LSD $_{0.05}$	15.37			5.078			1.451			4.940			3.470			0.595		

Means within a column followed by different letters are significantly different at $p < 0.05$

Table 7. List of the amplifiable primers for mutant detection in Rose species

Sr. No.	Primer Name	Sequence (5'-3')	Amplified Bands in mutants of *Rosa gruss an teplitz*	Amplified Bands in mutants of *Rosa centifolia*
1	OPS-03	CAGAGGTCCC	14	28
2	OPS-05	TTTGGGGCCT	20	31
3	OPS-11	AGTCGGGTGG	10	7
4	OPS-18	CTGGCGAACT	21	51
5	OPV-01	TGACGCATGG	34	27
6	OPV-03	CTCCCTGCAA	9	8
7	OPV-06	ACGCCCAGGT	79	22
8	OPV-08	GGACGGCGTT	26	43
9	OPV-10	GGACCTGCTG	38	30
10	OPV-12	ACCCCCCACT	35	54
11	OPV-15	CAGTGCCGGT	38	32
12	OPV-20	CAGCATGGTC	11	11

Conclusion: Mutation was induced successfully in rose species through application of colchicine and effectively demonstrated the behavior of the various concentrations and exposure time of colchicine. Explants of *Rosa gruss an teplitz* and *Rosa centifolia* incubated in colchicine (450 mg l^{-1}) medium for a period of 11days gave better performance to induce the variation in morphological and floral characteristics. This study adds to the ongoing efforts to obtain large size color variegated flowers for horticultural purposes.

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