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Gamma irradiation of *in-vitro* proliferated cultures of rose (*Rosa hybrida*) for induction of novel mutants

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ABSTRACT

In-vitro mutations were induced in two Hybrid Tea rose (*Rosa hybrida* L.) cultivars Pusa Mohit and Raktima for induction of novel traits. Single node cuttings were used to proliferate cultures under *in-vitro* and were irradiated with different doses of γ-rays (0, 5, 10, 15, 20 or 25 Gy) using a ^{60}Co source. The γ-irradiated microshoots were then cultured aseptically on to rooting medium containing 0.5 × MS basal medium supplemented with 2.69 μM α-naphthalene acetic acid (NAA) plus 2.46 μM 6-benzylaminopurine (BAP) plus 40 g/l sucrose and 0.8% (w/v) agar-agar for rooting. Decreased root lengths, numbers of roots per shoot were observed at 25 Gy. *In-vitro* proliferated cultures and non-irradiated (control) plants were transferred to plastic pots 1 month after acclimatization under laboratory conditions and examined for their morphological traits. Marked vegetative abnormalities with respect to leaf shape, leaf lamina, fused leaves, albinism or leaves with less chlorophyll, variegated leaves with stunted growth etc. were observed between the mutated and control populations. Four new variants with altered or novel flower colour, form, shape and size were isolated and numbered as PM 1, PM 2 (from cv. Pusa Mohit) and RK 1, RK 2 (from cv. Raktima) as compared to the original cultivars. These variants were multiplied on a large-scale through micropropagation and evaluated for their stability. This study developed a mutagenesis protocol that could be used to develop novel flower colour mutants in rose.

Key words: Gamma rays, MS medium, Rose, Variants

Rose (*Rosa hybrida* L.) is an important ornamental crop, belongs to family Rosaceae with chromosome number x = n = 7 and comprises more than 150 species. Rose is mainly cultivated due to its different uses like cut flower, loose flower, bush, standard, climber, pot plant, potpourris and dry flowers. Currently, rose is the top ranking commercial flower in the international cut flower market due to its attractive flowers with longer stem length and vase-life. Rose varieties have been developed over centuries by hybridisation and natural mutation.

Breeding programmes have focused on the improvement of various characteristics to enhance ornamental value, including the colour, size, form, and longevity of rose blooms, and the reactions of rose plants to the environment. These novel commercial traits are valued and always preferred by consumers. The scope for breeding new rose cultivars with valuable new traits remains high. *In-vitro* culture methods have facilitated the use of mutation-assisted breeding techniques to improve both sexually and vegetatively propagated crops. *In-vitro* mutagenesis is a powerful tool that can be exploited to increase genetic variability in an already economically important cultivar. Induced mutation were reported to be an efficient technique to achieve the desirable characters in flowers and ornamental plants. Induced mutations have been used to generate a wide range of plants with improved resistance to abiotic and biotic stresses (Jain 2005 and Sudhir *et al.* 2006). Among the mutagens available to induce *in-vitro* mutations, physical or chemical mutagens have high potential for horticultural crops due to their ease of application and high efficiency. Among the physical mutagens, X-rays and γ-rays are the most convenient and easiest to apply and have led to altered flower colours, higher oil contents, or better quality oils in ornamental crops. *In-vitro* mutagenesis to induce novel mutants in rose and chrysanthemum has also been reported previously (Datta 1995, Arnold *et al.* 1998 and Kim *et al.* 2006). The aim of this study was to standardize an efficient protocol for *in-vitro* mutagenesis that could be used for further improvement of rose varieties.

MATERIALS AND METHODS

Shoots from 2-3 years old healthy plants of the *R. hybrida* cultivar Pusa Mohit and Raktima were obtained from the farm of the Division of Floriculture and Landscaping, IARI, New Delhi, India and were used as the

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source of explants. Single-node cuttings were proliferated under *in-vitro* conditions following a standard protocol (Bala *et al.* 2010 and Bala *et al.* 2013) developed before γ-irradiation treatment. *In-vitro* proliferated cultures of both rose cultivars were exposed to different doses of γ-rays (5, 10, 15, 20 or 25 Gy) using the ^{60}Co source at the Nuclear Research Laboratory, IARI, New Delhi. Cultures were maintained at 25° ± 1°C under cool-white fluorescent lamps with a 16 hr photoperiod at 47 µmol/m^2/s. After elongation, shoots were transferred, individually, to 250 ml conical glass flasks containing 0.5 × MS basal medium supplemented with 2.69 µM α-naphthalene acetic acid (NAA) plus 2.46 µM 6-benzylaminopurine (BAP) plus 40 g/l sucrose and 0.8% (w/v) agar-agar (Qualigens) for rooting in both cultivars. After successful rooting, rooted plantlets were removed from the flasks and washed thoroughly using autoclaved doubled-distilled water to remove all traces of adhering 0.8% (w/v) agar-agar. The roots were then dipped in 0.1% (w/v) carbendazim (BASF, Mumbai, India) for 30 sec. and transferred to 500 ml glass jars with polypropylene lids and filled with a 1:3 (v/v) mix of vermiculite: agro-peat (Argosy, New Delhi, India), moistened with 25% (v/w) MS basal liquid medium for acclimatisation.

The observations were recorded on days to root initiation, percentage root induction, number of roots per shoot, root length, percent survival, plant height, number of branches per plant, number of leaves per plant, internodal length, spine density per 5 cm, leaf colour, leaf variations, days to flowering, number of flowers per plant, number of petals per flower, length of flower bud, length of flower stalk, flower diameter and flower colour variations. Data were analyzed based on a simple, factorial completely randomised design (FCRD). Percentage data were subjected to arc sine √% transformation before carrying out ANOVA.

RESULTS AND DISCUSSION

At rooting stage, untreated shoots had the best rooting ability and the highest mean number of roots per shoot within the shortest time period, with the maximum mean root length. The maximum delay in rooting, minimum rooting percentage, and lowest number of roots per shoot, with the shortest roots, were recorded following the 25 Gy treatment. Each treatment showed significant differences from the others. Amongst the genotypes, the best rooting ability and the highest mean number of roots per shoot within the shortest time period, with the maximum mean root length was recorded for cultivar Raktima as compared to rooting ability and mean number of roots per shoot within the time period, with mean root length in cultivar Pusa Mohit. The interaction of γ rays treatments and genotypes was also found significant.

The best rooting ability and the maximum mean number of roots with shortest time period for root initiation, and the highest number of root length was registered in cv. Raktima in untreated plants. The maximum days for root initiation with rooting ability, and mean number of roots per shoot with least mean root length was observed with the 25 Gy treatment in cultivar Pusa Mohit (Table 1). Good rooting ability in untreated control, as compared to all γ-ray-treated proliferated cultures could be due to perturbations of the endogenous hormone balance, or the action of phytohormones synthesized and transported from the shoot tip to their site of action, or by the exogenous

Table 1 Effect of gamma irradiation on rooting in Hybrid tea rose cv. Pusa Mohit and Raktima

Dose –ray dose (Gy)	Days to root initiation			Root induction (%)			Number of roots per shoot			Root length (cm)		
	Pusa Mohit	Raktima	Mean	Pusa Mohit	Raktima	Mean	Pusa Mohit	Raktima	Mean	Pusa Mohit	Raktima	Mean
Control	14.82	11.39	13.10	87.68† (69.46)*	95.96 (78.50)	91.82 (73.98)	3.09	5.40	4.24	3.48	5.52	4.50
5	17.01	15.45	16.23	69.21 (56.28)	90.44 (72.06)	79.82 (64.17)	2.91	5.26	4.08	3.30	5.33	4.31
10	20.36	18.27	19.31	67.55 (55.28)	83.73 (66.41)	75.64 (60.85)	2.50	4.53	3.52	3.53	4.34	3.93
15	23.26	21.25	22.25	55.65 (48.28)	77.13 (61.40)	66.39 (54.84)	2.43	4.18	3.31	2.53	3.66	3.10
20	28.26	25.66	26.96	54.25 (47.42)	74.22 (59.47)	64.23 (53.44)	2.28	3.34	2.81	2.16	3.26	2.71
25	32.40	29.04	30.72	45.92 (42.64)	65.27 (53.89)	55.59 (48.26)	1.67	2.27	1.97	1.33	2.76	2.05
Mean	22.69	20.17		63.38 (53.22)	81.12 (65.29)		2.48	4.16		2.72	4.15	
(P<0.05)												
Treatment (T)		0.26			2.09			0.18			0.15	
Genotype (G)		0.48			3.63			0.32			0.26	
Interaction (T × G)		1.09			5.13			0.46			0.37	

*Arc sine √% transformed data.

Table 2 Effect of gamma irradiation on growth parameters under *ex-vitro* conditions

Dose –ray dose (Gy)	Survival (%)			Plant height (cm)			Number of branches/plant			Number of leaves/plant		
	Pusa Mohit	Raktima	Mean	Pusa Mohit	Raktima	Mean	Pusa Mohit	Raktima	Mean	Pusa Mohit	Raktima	Mean
Control	83.63† (66.14)*	93.33 (75.07)	88.48 (70.60)	13.80	16.70	15.08	3.20	3.53	3.36	32.20	40.40	36.30
5	82.13 (65.03)	91.45 (73.05)	86.79 (69.04)	12.93	15.53	14.23	3.03	3.51	3.27	29.93	35.70	32.81
10	74.10 (59.42)	84.64 (66.93)	79.37 (63.17)	11.03	13.50	12.26	2.60	3.26	2.93	26.73	32.70	29.71
15	64.14 (53.20)	63.67 (52.95)	63.90 (53.07)	8.56	12.46	10.51	2.40	2.73	2.56	18.80	26.40	22.60
20	51.67 (45.94)	52.41 (46.37)	52.04 (46.15)	7.33	11.60	9.46	2.13	2.56	2.35	19.19	21.76	20.48
25	43.98 (41.91)	47.71 (43.67)	45.84 (42.79)	6.63	8.59	7.61	2.00	2.46	2.23	13.10	17.90	15.50
Mean	66.61	72.20 (55.27)	(59.67)	10.05	13.01		2.56	3.01		23.32	29.14	
(P<0.05)												
Treatment (T)		0.84			0.42			0.14			1.17	
Genotype (G)		1.46			0.73			0.24			2.03	
Interaction (T × G)		2.07			1.03			0.35			2.88	

*Arc sine √% transformed data.

supply of synthetic auxin(s). The inhibition of rooting following higher doses of γ-irradiation supports the results obtained by others (Singh *et al.* 1999 and Kumar 2002).

There was a significant reduction in survival percentages, plant heights, numbers of branches, numbers of leaves per plant, internodal length and spine density on plantlets regenerated from nodal explants treated at higher doses of γ-ays, even after acclimatisation under *ex-vitro* conditions (Table 2). Amongst the genotypes, the highest rate of *ex-vitro* plant survival, mean plant height, number of branches per plant, number of leaves per plant was recorded in the cultivar Raktima as compared to cultivar Pusa Mohit. The interaction effect of the two factors, i.e. gamma rays and genotypes was also found significant. The highest number of spines per 5 cm of stem was counted 30 days after transfer to field from the plants obtained from control (8.8) whereas, the minimum prickles were recoreded in the 25 Gy treatment. In cv. Pusa Mohit spineless plants were obtained in all the treatments as to the original cultivar.

Gamma irradiation and/or variable temperatures, low humidity, or high light intensity, might have contributed to the reduction in survival percentages when plantlets were transferred to *ex-vitro* environments. An inhibitory effect of γ-radiation on rose plant height was noticed, as reported previously (Ferol 1996, Banerjee and Datta 2002 and Senapati and Rout 2008). The decline in vegetative growth parameters may have been due to chromosome damage, inhibition of mitosis (Gray 1956), or the selective killing of meristematic cells which then produced fewer progeny cells (Hagberg and Nymb 1954). Inhibition of mitosis induces physiological changes, while inhibition of auxin biosynthesis leads to reduced growth and the inability of γ-irradiated cells to absorb available nutrients. With respect to morphological variations, all leaves were normal, with a dark-green colour, in control (untreated) plants and in plants from treated *in-vitro* proliferated cultures at lower doses of γ-rays (5, or 10 Gy), while light green-coloured leaves were observed at higher γ-ray doses (15,or 20 Gy). Morphological abnormalities in the foliage, which included changes in leaf shape and size, serrated margins, and lower chlorophyll contents were induced at higher doses of γ-rays (Table 3). The highest leaf variations was registered at 25 Gy gamma rays in cv. Pusa Mohit followed by in cv. Raktima with same dose of γ ray.

A morphological variation in leaves is a primary effect of mutagens. Variations such as, yellowish or light-green leaves, narrow leaves with small leaflets, wider leaves, lanceolate leaves, serrated margins, chlorophyll-deficient leaves, leaflets fused together, and unequal development of laminae from γ-irradiated nodal explants have been reported in rose and other ornamental crops (Gupta and Datta 1982 and Singh and Sharma 1993).

The stimulatory effect of gamma irradiation at 5 and 10 Gy doses of gamma rays was observed with the minimum days of flowering at 5 Gy significantly followed by 10 Gy. The maximum delay in flowering was observed after the 25 Gy treatment. Among the two genotypes the minimum days to flowering were registered in cv. Raktima which was significantly lower than cv. Pusa Mohit . The interaction of treatment and genotypes was estimated to be highly significant with minimum days for flowering after 5 Gy followed by 10 Gy dose of gamma rays in cv. Raktima. The other charcters like length of flower stalk, flower diameter, length of flower buds were also affected by gamma

Table 3 Effect of gamma irradiation on internodal length, spine density and leaf colour variations

Dose–ray dose (Gy)	Internodal length (cm)			Spine density/5 cm			Leaf colour			Leaf variations (%)		
	Pusa Mohit	Raktima	Mean	Pusa Mohit	Raktima	Mean	Pusa Mohit	Raktima	Mean	Pusa Mohit	Raktima	Mean
Control	3.33	4.10	3.71	8.80			Dark green	Dark green	Dark green	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
5	3.16	3.89	3.53	8.18			Dark green	Dark green	Dark green	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
10	3.09	3.71	3.40	7.44			Dark green	Dark green	Dark green	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
15	2.10	3.54	2.82	7.02			Green	Green	Green	6.90 (15.23)	6.58 (14.86)	6.26 (14.49)
20	2.02	3.09	2.56	5.18			Green	Green	Green	7.00 (15.34)	6.73 (15.04)	6.46 (14.74)
25	1.61	2.45	2.03	6.37			Light green	Light green	Light green	8.10 (16.15)	7.36 (15.53)	6.63 (14.92)
Mean	2.55	3.46		7.16						3.44 (10.63)	3.22 (10.31)	
(P<0.05)												
Treatment (T)		0.07									0.10	
Genotype (G)		0.13									0.17	
Interaction (T × G)	0.19									0.24		

*Arc sine √% transformed data.

irradiations in both cultivars (Table 4). Treatment with higher doses delayed flowering and other flowering characters depending on the dose. This may be an indirect effect of γ-rays through delayed sprouting and slower growth, or may be due to the deleterious effects of γ-irradiation on plant growth hormones such as auxins and gibberellins, or the induction of photoinsensitivity following irradiation. A delay in flowering in various γ-irradiated ornamental crops has been reported by others (Datta and Banerjee 1995). The probable cause of the reductions in flower diameter, the length of the flower bud, and the number of petals might have been the reduced size of petals and/or abnormalities and variations in floral characters due to hindered development by irradiation. Similar results have been reported in rose, hibiscus, gladiolus, portulaca, and carnation (Walther 1986 and Reddy and Bhalla 1994).

The effect of different doses of gamma rays was seen on both the cultivars, i.e. Pusa Mohit and Raktima in terms of induction of flower colour variations over the control (Table 5).The maximum number of flowers with variations in flower colour were registered with 25 Gy dose of gamma rays .Whereas, plants under control and treated with 5, 10 and 15 Gy dose produced normal flowers comparable to the original variety in control. Irrespective of treatments, the two genotypes differed significantly with regard to induction of flower colour variations. The maximum flower colour variations were registered in cv. Pusa Mohit as compared to cv. Raktima.

Table 4 Effect of gamma irradiation on flowering parameters in Hybrid tea rose cv. Pusa Mohit and Raktima

Dose –ray dose (Gy)	Days to flowering			Number of flowers/plant			Number of petals/flower			Length of flower bud (cm)		
	Pusa Mohit	Raktima	Mean	Pusa Mohit	Raktima	Mean	Pusa Mohit	Raktima	Mean	Pusa Mohit	Raktima	Mean
Control	55.36	42.56	48.96	3.10	3.76	3.43	42.66	25.76	34.21	1.63	1.83	1.73
5	51.96	41.88	46.92	3.10	3.66	3.38	38.93	24.00	31.46	1.60	1.80	1.70
10	54.69	42.05	48.37	2.53	3.06	2.80	24.60	21.36	22.98	1.60	1.56	1.58
15	62.98	55.24	59.11	2.33	2.73	2.53	24.42	18.59	21.51	0.80	1.30	1.05
20	62.52	55.33	58.93	2.30	2.70	2.50	20.13	12.53	16.33	0.83	1.16	1.00
25	65.91	61.91	63.91	1.23	1.66	1.45	15.16	9.63	12.40	0.86	1.06	0.96
Mean	58.90	49.83		2.43	2.93		27.65	18.64		1.22	1.45	
(P<0.05)												
Treatment (T)		1.79			0.14			0.91			0.06	
Genotype (G)		3.10			0.25			1.59			0.11	
Interaction (T×G)	4.39	0.35			2.25			0.16				

Table 5 Effect of gamma irradiation on length of flower stalk, flower diameter and flower colour variations

Dose –ray dose (Gy)	Length of flower stalk (cm)			Flower diameter (cm)			Flower colour variations (%)		
	Pusa Mohit	Raktima	Mean	Pusa Mohit	Raktima	Mean	Pusa Mohit	Raktima	Mean
Control	16.51	19.61	18.06	6.33	8.63	7.48	0.00† (0.00)*	0.00 (0.00)	0.00(0.00)
5	16.38	19.13	17.76	6.23	8.53	7.38	0.00(0.00)	0.00 (0.00)	0.00(0.00)
10	15.61	18.23	16.92	5.83	8.46	7.14	0.00 (0.00)	0.00 (0.00)	0.00(0.00)
15	15.15	16.80	15.98	5.23	7.51	6.37	0.00(0.00)	0.00 (0.00)	0.00(0.00)
20	14.89	15.52	15.20	5.56	7.50	6.53	8.43(16.88)	7.59 (15.89)	8.01(16.38)
25	12.55	13.55	13.05	5.16	7.03	6.10	9.96(18.34)	9.76 (18.15)	9.86(18.24)
Mean	15.18	17.14		5.72	7.94		3.06(10.01)	2.89 (9.67)	
(Pd<0.05)									
Treatment (T)		0.81			0.16			0.20	
Genotype (G)		1.41			0.28			0.35	
Interaction (T × G)		1.99			0.40			0.50	

*Arc sine √% transformed data.

The variants developed from variety Pusa Mohit were coded: PM-1 induced at 20 Gy gamma ray dose with frequency at 8.4%, pure homozygote (solid mutant) producing compact flowers with pink coloured petals (red-purple 62-B) as compared to red colour in original cv. Pusa Mohit (control); PM-2 having flower colour, shape, form, size and number of petals completely distinct from original cultivar. The colour of flower buds of variant was purplish (red-purple 65-A) derived 25 Gy dose of gamma rays with mutation frequency of 9.9%. Flowers were of small size, with unfurled outer petals and compact at the center. Whereas, variants developed from variety Raktima were coded: RK-1 induced at 20 Gy gamma irradiation dose with mutation frequency 7.6%, compact flowers having light pink petals (red purple 62-C) with stripes (white N155-B) as compared red in original cv. Raktima (control); RK-2 induced at 25 Gy gamma irradiation dose with frequency of 9.8%, fully opened large flowers with dark red coloured petals (red purple-58-B) along with yellow orange midribs (yellow orange 20-D) as compared to pure red in original cv. Raktima (control). Gamma-irradiation induced changes in flower colour, form, petal shape and other modifications of floral parts. Radiation-induced floral colour changes may be due to chromosomal aberrations, changes in chromosome number, genetic mutations, rearrangements of different histogenic layers, and/or altered biochemical pathways. The latter can increase and/or decrease the concentrations of one or more pigments by inhibiting their synthesis, or synthesising new pigments. The induction of colour mutations after γ-irradiation agrees with previous results in ornamental crops such as chrysanthemum, carnation, and gladiolus (Singh *et al.* 2000, Nonomura *et al.* 2001, Dao *et al.* 2006, Kim *et al.* 2006, Yamaguchi *et al.* 2008).

A protocol for *in-vitro*-mutagenesis of hybrid rose has been established using γ-rays and may be used in future breeding programmes. The present study revealed that γ-irradiation of *in-vitro* proliferated of two hybrid tea rose cultivars at 15, 20, or 25 Gy was effective at inducing novel flower colour variants.

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