



Mutagenic-sensitivity and variability induction in garden rose (*Rosa × hybrida*)

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Received: 08 March 2024; Accepted: 10 June 2025

ABSTRACT

Mutation breeding is one of the most desirable breeding methods to enhance genetic variability and to improve economically important traits in ornamental crops. An induced mutation leading to altered phenotypes after a permanent heritable change in the structure of the genetic material is now established as a time saving and inexpensive approach for flower crop improvement. Gamma (γ)-irradiation induces changes in plant growth and flowering traits of flower crop plants. The present study was carried out during 2020–2022 at ICAR-Indian Agricultural Research Institute, New Delhi to induce the variability in rose (*Rosa × hybrida* L.) using gamma rays under *in vivo* conditions. Semi-hardwood cuttings of cv. Pusa Virangana were treated with gamma rays at different doses and it was observed that 100% cuttings were dried beyond 40 Gy doses of gamma rays. Gamma rays adversely affect plant growth (days to bud sprouting, plant height and internodal length. Maximum primary branches (6.0/plant) and plant spread (55.50 cm) were observed in 20 Gy treatment, however, leaf characters, viz. leaflet length and width and compound leaf area showed random effects. Leaflet apex margin found undulated type at 30 Gy comparisons to the other treatments and control. Flowering traits i.e. maximum flower stalk length (6.83 cm), number of flowers/plant (9.00), flower diameter (7.67 cm), number of petals/flower (30.00), petal size and single flower weight (5.09 g) were observed at 20 Gy of gamma rays. Two mutants were isolated from mutation population of cv. Pusa Virangana at 30 Gy (PVM-1) and 20 Gy (PVM-2) doses in *in vivo* mutagenesis. PVM-1 has bicolour, medium size, red purple group flower. PVM-2 has single colour, large size, red purple group flower, have greater number of petals and compact flower. Based on survival percentage after 30, 60 and 90 days of planting of cuttings, the lethal dose 50 (LD₅₀) for gamma irradiation was determined using probit analysis values, which were 27.9, 16.2 and 11.2, respectively.

Keywords: Gamma rays, *In vivo* mutagenesis, Pusa Virangana, Physical mutagens, Rose

Rose (*Rosa × hybrida* L.) is an important perennial flower crop in the world including more than 30,000 cultivars and 150 spp. (Yadav *et al.* 2023). It is grown as flowering landscape shrub, cut and loose flower, potted plant, source of perfume and vitamin C and use in the pharmaceutical and nutraceutical industry (Hussain and Khan 2004, Namita *et al.* 2015). A wide range of variability has been created in the genus *Rosa* due to considerable advancement in rose breeding. Unfortunately, only a small portion of this variability has been used in the present breeding of rose in India due to limited gene pool of any species (Mol *et al.* 1989), incompatibility in genotypes and differences in ploidy level between putative parents (Lowe *et al.* 1993) and polygenic traits like uniform growth and synchronous flowering. Thus, the enhancement of existing variability for trait of interest becomes a prerequisite for present rose breeding.

As the demand for novel and high-quality cultivars is increasing, cross breeding alone can no longer meet this demand. Approximately 3,300 mutants have been registered in the International Atomic Energy Agency (IAEA) Mutant Variety Database/ Food and Agriculture Organization (FAO) indicating the widespread use of mutagenesis in modern plant breeding. About 75% of all mutant varieties registered were created through γ - irradiation (FAO/IAEA 2023). Hence, mutagenesis has also been extensively used for the development of new lines/cultivars with improved traits in vegetatively propagated ornamental plants (Kumar *et al.* 2006). Induced mutations using gamma rays are considered to be more suitable for obtaining mutants with less radiation damage (Yamaguchi *et al.* 2010) and have been successfully applied to study the induced mutagenicity in roses. Mutagen dose, mutation rate and the genetic constitution of the genotypes used greatly influence the efficiency of mutation. Lethal dose 50 (LD₅₀) is considered the dose resulting in high mutation frequency, while maintaining the biological damages at tolerable levels. Therefore, optimization of radiation dose is foremost for successful mutation breeding

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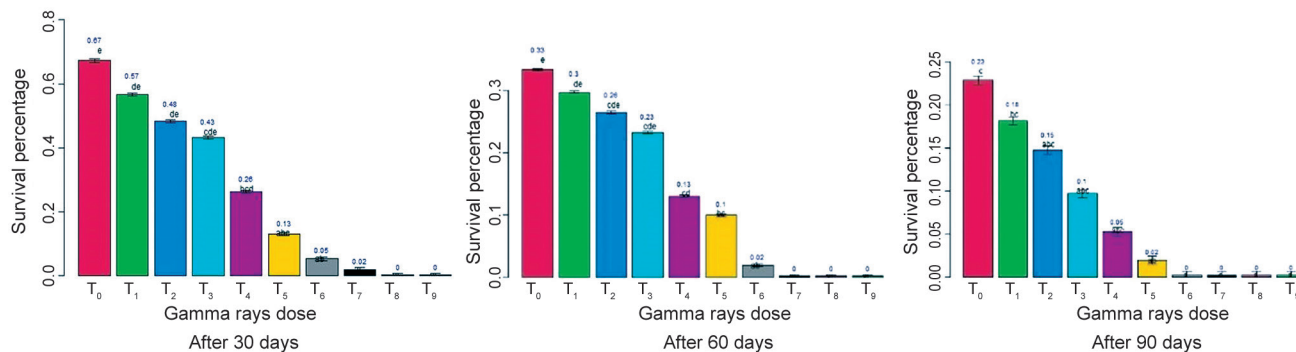


Fig.1 Effect of gamma rays on survival percentage of rose cuttings at different intervals. Treatment details are given under Materials and Methods.

programme of rose. Hence, present study was carried out to determine the LD_{50} of rose cv. ‘Pusa Virangana’ and to estimate the variability induced in the irradiated population by different doses of gamma rays, as the first step in mutation breeding program for rose.

MATERIALS AND METHODS

Plant material: The present study was carried out during 2020–2022 at ICAR-Indian Agricultural Research Institute, New Delhi. Planting materials used for the study were semi hardwood cuttings of rose cv. ‘Pusa Virangana’ which was developed by ICAR-Indian Agricultural Research Institute, New Delhi. It belongs to floribunda type, having dark red flower suitable for garden display purpose.

Irradiation of rose cuttings and planting: Semi hardwood cuttings of size 15 cm from one-year old established rose plant free from pest and diseases were excised and irradiated using a radioisotope ^{60}Co (Cobalt 60) sources at the Gamma Chamber Facility, ICAR-Indian Agricultural Research Institute, New Delhi. The treatments consisted of nine doses of gamma radiation 20 Gy (T_1), 30 Gy (T_2), 40 Gy (T_3), 50 Gy (T_4), 60 Gy (T_5), 70 Gy (T_6), 80 Gy (T_7), 90 Gy (T_8) and 100 Gy (T_9) along untreated cuttings as control T_0 . The experiment was laid out in a randomized block design (RBD) with three replications for each treatment. Each replication consisted of 30 cuttings of rose cv. ‘Pusa Virangana’. After irradiation, treated and untreated cuttings were quickly dipped in 1500 ppm IBA and planted in well prepared field. The cultural operations like weeding, irrigation and management of insect pest and disease were performed well in time as recommended by ICAR- Indian Agricultural Research Institute, New Delhi.

Data collection: Data was recorded on vegetative characters like days to sprouting of axillary bud (days), per cent survival of cuttings (%), days taken to first bud initiation (days), plant height (cm), plant spread (cm), internodal length (cm), number of primary branches (Nos.), leaflet length (cm), leaflet width (cm), compound leaf area (cm^2), prickles density (dense, medium, spars), leaf *i.e.* flower stalk length (cm), flower diameter (cm), number of petals/flower (nos.), single flower weight (g) and flower colour (RHS colour chart).

Statistical analysis: The lethal dose 50 (LD_{50}) for gamma irradiation was determined on the basis of survival

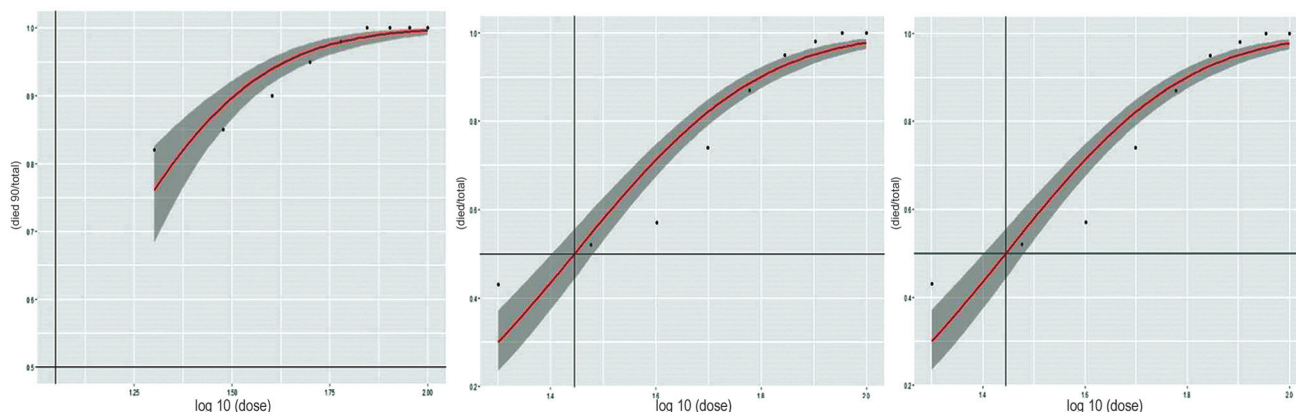
percentage after 30, 60 and 90 days of planting (DAP) of cuttings using probit analysis (Finney 1978). Per cent data was subjected to Arc Sin $\sqrt{\%}$ transformation before ANOVA and data were analysed using R package software. Their means were calculated based on ‘Tukey HSD at a probability level of 5%.

RESULTS AND DISCUSSION

Survival of cuttings: A gradual decrease in survival of cuttings was observed for cv. ‘Pusa Virangana’ with increasing doses of gamma rays at different intervals *i.e.* after 30, 60 and 90 days of irradiation (Fig. 1). Total 100% cuttings were dried when irradiated at higher dose of gamma rays (>40Gy). Similar results were observed by Pal *et al.* (2017) and Bharti *et al.* (2021) in dahlia to show direct correlation of increased gamma ray doses and high mortality. Reduction in survival after exposure to gamma rays was explained due to inactivation and/or decreases in auxin content affecting cell division resulting in poor establishment and survival (Kapadiya *et al.* 2016). The decrease in plant survival percentage with higher gamma-ray doses may be due to genetic loss at the gene level and chromosomal aberrations (Tiwari and Kumar 2011).

Estimation of LD_{50} dose: In the present study, radio sensitivity was tested to find out the most effective dose of gamma rays to induce variation under *in vivo* conditions. The lethal dose 50 (LD_{50}) for gamma irradiation was determined on the basis of survival percentage of cuttings using Probit analysis values, which were 27.9, 16.2 and 11.2 Gy after 30, 60 and 90 days of planting, respectively (Fig. 2). It is supported by Wang *et al.* (2020) in chrysanthemum ‘Donglinruixue’ exhibiting significantly increased death rate along with increase in radiation doses and determined approximately 35 Gy as semi-lethal (LD_{50}) dose compared with unirradiated control. Similar to present studies, the effects of ^{60}Co - γ ray on cuttings of nine gardens rose seedlings irradiated at different doses (0, 25, 45, 60 and 80 Gy) was also exhibited by Wenquan *et al.* (2020) and they also found that semi-lethal radiation dose (LD_{50}) was distributed between 26 and 55 Gy among different rose seedlings.

Effect of gamma (γ) irradiation on vegetative characters: Gamma rays affected plant growth characters and it was



Model: $Y=mx + C$, LD_{50} Value: 30.31, MSE: 0.8126, R^2 : 0.7692

Fig. 2 Probit analysis based on mortality percentage of garden rose cuttings in cv. 'Pusa Virangana'.

observed that data pertaining to all the characters decreased with increased dose of gamma rays. Maximum survival percentage after 30 days (56.69%), 60 days (29.67%), 90 days (18.12%) was observed in 20 Gy gamma rays treatment (T_1). The findings of the study were supported by Kim *et al.* (2017) who irradiated rooted and unrooted cuttings of roses with gamma rays at 70 Gy and found that after 30 weeks, survival percentage for gamma-ray irradiated rooted plants was 16.4–50.8% and for unrooted cuttings, it was 0–5.1%. Among the growth characters, minimum days to bud sprouting (23.40 days) and maximum plant height (68.00 cm), internodal length (3.50 cm), number of primary branches (6.00 nos.) and plant spread (55.50 cm) was observed in 20 Gy among the all gamma rays treatments. While, number of primary branches in all the treatments along with control are at par with each other (Table 1). The results of the finding were supported by Bajpayee and Dwivedi (2019) in chrysanthemum by exhibiting more number of shoots and plant height at lower dose. The reduction in plant growth characteristics at higher doses of gamma rays may be due to consequence of somatic competition in the plants.

The irradiated plants showed stable growth and produced new leaves after 60 days of irradiation. In case of leaf characters, results showed random effects on leaflet length and width and compound leaf area. Maximum compound leaf area (51.00 cm), leaflet width (2.73 cm) was recorded in 20 Gy gamma irradiation, however, maximum leaflet length (4.43 cm) was observed in 30 Gy in comparison to the other treatments. However, compound leaf area and leaflet width are at par in all the treatments along with control.

Qualitative characters of leaf i.e. shape of compound leaf and leaflets significantly differ in all the treatments. Maximum difference was recorded in 30 Gy gamma rays doses where compound leaf was observed more curved and leaflet shape was cordate type in comparison to the control (round type) (Fig. 3). Leaflet shape was found to be obtuse type at 20 and 40 Gy. Colour of all leaves was found normal green in all the treatments as compare to control with a dark-green colour. Morphological variations in the foliage such as changes in leaf shape and size were induced at higher doses of γ -rays. Based on the visual observations, leaf colour variations were also recorded which may be due to adverse

Table 1 Effect of different doses of gamma rays on plant growth in garden rose cv. Pusa Virangana

Treatment	Days to bud sprouts	Plant height (cm)	Number of primary branches	Plant spread (cm)	Internodal length (cm)	Leaflet length (cm)	Leaflet width (cm)	Compound leaf area (cm ²)
T_0 (control)	12.00 ^{ab}	71.50 ^c	5.00 ^b	49.50 ^{bc}	3.83 ^c	4.57 ^c	2.80 ^b	54.30 ^b
T_1 (20 Gy)	23.40 ^{bc}	68.00 ^c	6.00 ^b	55.50 ^c	3.50 ^c	4.33 ^{bc}	2.73 ^b	51.00 ^b
T_2 (30 Gy)	29.30 ^{bcd}	59.00 ^{bc}	4.67 ^b	47.20 ^{bc}	3.29 ^c	4.43 ^{bc}	2.57 ^b	43.90 ^b
T_3 (40 Gy)	45.00 ^{cde}	48.7 ^b	4.33 ^b	34.00 ^b	2.53 ^b	4.02 ^b	2.72 ^b	38.00 ^b
T_4 (50 Gy)	46.70 ^{de}	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
T_5 (60 Gy)	56.00 ^e	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
T_6 (70 Gy)	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
T_7 (80 Gy)	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
T_8 (90 Gy)	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
T_9 (100 Gy)	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a

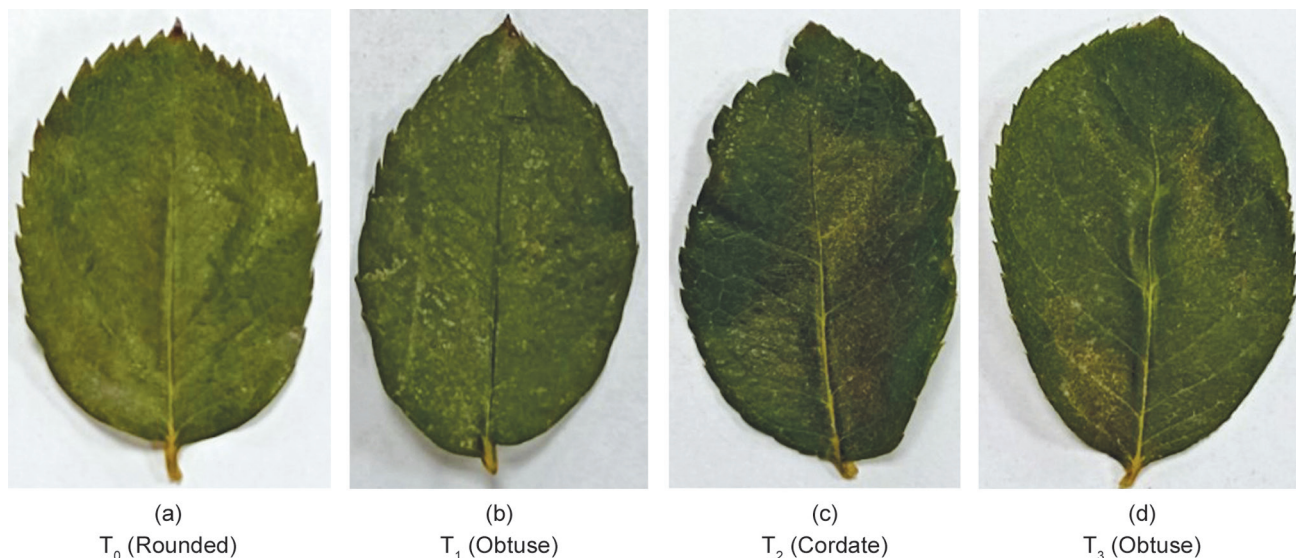


Fig. 3 Effect of different doses of gamma rays on leaf apex shape in rose cv. ‘Pusa Virangana’. (a) T₁, Control (b) T₂, 20 Gy (c) T₃, 30 Gy (d) T₄, 40 Gy.

effect of radiation on chlorophyll synthesis processes or may be due to reduced levels of substrates affecting biochemical pathways. These morphological variations were supported by the Singh and Bala (2019) in flower crops and reported that the variations such as light green or yellowish leaves, narrow or broad leaf, serrated margins, fused leaflets, and unequal development of lamina from gamma irradiated cuttings of rose and other ornamental crops.

Influence of gamma (γ)- irradiation on floral characters:

Floral characters were also affected by the different doses of γ-rays. Higher dose of gamma rays delayed the flower bud initiation and among the γ-rays treatments, minimum days to flower bud initiation (123.00) was recorded at 20 Gy dose. This might be an indirect effect of γ-rays through delayed sprouting and slower growth, or it is due to the deleterious effects of γ-irradiation on plant growth hormones such as auxins and gibberellins, or the induction of photo insensitivity due to irradiation. Similar results were reported in rose (Bala and Singh 2015) and in chrysanthemum (Bajpayee and Dwivedi 2019). Maximum flower stalk length (6.83 cm), number of flowers/plant (9.00), flower diameter (7.67 cm), number of petals/flower (30.00), petal length (4.62 cm) and single flower weight (5.09 g) were observed in 20 Gy gamma ray dose. However, minimum days to flower bud initiation (120 days), maximum flower bud length (2.71

cm) and petal width (3.79 cm) were observed in control. However, all the floral characters are at par with each other in all the treatments along with control (Table 2).

Treatment with higher doses delayed flowering and other flowering characters, depending on the dose. The probable cause of the reductions in flower diameter, the length of the flower bud, and the number of petals may be the reduced size of petals and/or abnormalities and variations in floral characters due to hindered development by irradiation. Similar to present findings, the positive effect of lower doses of gamma rays on growth related attributes as compared to higher doses have also been reported by Chawala *et al.* (2021) in chrysanthemum cv. Thai Chen Queen treated with different doses of gamma rays. The flowering characters decreased inversely with increasing doses of gamma rays. Reduction in flower weight may be due to reduced size of flower head and reduced number of petals in flower head. Findings of our study are also supported by Bala and Singh (2015) in rose, Singh *et al.* (2023) and Haspolat (2024) in chrysanthemum. Maximum flower variation observed in 20 Gy and 30 Gy treatments of gamma rays. Similar findings of inducing novel flower colour and other variations were also observed by Setia *et al.* (2020) in Chrysanthemum cultivars namely Thai Chen Queen and Purnima which were exposed to γ-irradiation at 10 or 15 Gy doses. This may be

Table 2 Effect of gamma rays on various flowering traits of garden rose cv. ‘Pusa Virangana’

Treatment	Days to flower bud initiation	Flower bud length (cm)	Flower stalk length (cm)	Number of flowers/plant	Flower diameter (cm)	No. of petals/flower	Petal length (cm)	Petal width (cm)	Single flower weight (g)
T ₀ (control)	120.00 ^a	2.71 ^a	6.12 ^a	8.00 ^a	6.95 ^a	26.20 ^a	4.17 ^a	3.79 ^a	4.52 ^a
T ₁ (20 Gy)	123.00 ^a	2.69 ^a	6.83 ^a	9.00 ^a	7.67 ^a	30.00 ^a	4.62 ^b	3.73 ^a	5.09 ^a
T ₂ (30 Gy)	140.00 ^b	2.48 ^a	6.47 ^a	7.00 ^a	7.52 ^a	28.80 ^a	3.99 ^a	3.49 ^a	4.89 ^a
T ₃ (40 Gy)	132.00 ^{ab}	3.62 ^a	5.60 ^a	4.00 ^a	7.43 ^a	26.30 ^a	3.77 ^a	3.42 ^a	3.45 ^a

Table 3 Characterization of novel mutants on the basis of morphological and DUS traits

Character	States			Notes		
	PV	PVM1	PVM2	PV	PVM1	PVM2
Flower Type	Double	Double	Double	3	3	3
Flower: Numbers of petals	Medium	Many	Many	3	5	5
Flower: Colour group	Red Purple	Red Purple	Red Purple	12	12	12
Flower: Diameter	Large	Medium	Large	5	3	5
Flower: Colour of the center	Red	Red	Pink	5	5	4
Flower: Density of petals	Medium	Medium	Dense	5	5	7
Flower: Shape View from above	Round	Irregularly Rounded	Round	1	2	1
Flower: Side view of upper part (fully open flower)	Flat	Flattened convex	Flat	1	2	1
Flower: Side view of lower part (fully open flower)	Flattened convex	concave	Concave	3	1	1
Flower: Fragrance (recording during early morning)	Weak	Weak	Weak	3	3	3
Sepal: Extension	Many	Many	Few	7	7	3
Petal: Reflexing of petals one by one	Absent	Present	Absent	1	9	1
Petal: Shape	Ovate	Obovate	Ovate	2	2	2
Petal: Incisions	Absent	Absent	Absent	1	1	1
Petal: Undulation of margin	weak	Strong	Medium	3	7	5
Petal: Length	Long	Medium	Medium	7	5	5
Petal: Width	Medium	Medium	Medium	5	5	5
Petal: Number of colour on inner side (basal spot excluded)	One (Single)	Two (Double)	One (Single)	1	2	1
Petal: Colour of the majority portion of the petal	Red purple Group N 66A (Vivid Red Pink)	Red Purple Group N 58 C	Red Purple group N66 A	-	-	-
Varieties with one colour on inner side of petal: Intensity of colour excluding basal spot	Uniform	-	Uniform	2	-	2
Varieties with two or more colours on inner side of petals: Secondary colour of petal (basal spot excluded)	-	Red Purple Group N 57 A	-	-	-	-
Varieties with two or more colours on inner side of petals: Petal distribution of Secondary colour on inner side (basal spot excluded)	-	As segment or Strip	-	-	5	-
Petal: Spot at base of inner side	Present	Present	Present	9	9	9
Petal: Size of spot at base of inner size	Small	Small	Small	3	3	3
Petal main colour on the outer size (only if clearly different of inner side)	Red Purple Group N 57 (Vivid Purplish Red)	Red Purple Group N 58 C	Red Purple group N 66 A	-	--	-
Petal: Spots at the base of outer side	Absent	Absent	Absent	1	1	1
Outer stamen predominant colour of the filament	Brown Red	Brown Red	Light Yellow	8	8	3
Flower: Length of pedicel	Medium (5.2 cm)	Medium (5.9 cm)	Medium (4.8cm)	5	5	5

an indirect effect of γ -irradiation through delayed sprouting and slower growth or deleterious effect of gamma rays on plant growth hormones namely auxins and gibberellins or photoinsensitivity induction due to irradiation has already been reported by various researchers.

Characterization of novel mutants based on morphological and DUS traits: Two mutants were isolated from *in vivo* mutagenesis of rose cv. 'Pusa Virangana' on the basis of morphological and DUS traits (Table 3). 'Pusa Virangana Mutant-1'(PVM-1) was isolated from cv. 'Pusa Virangana' at 30 Gy of gamma rays treatment (Supplementary Fig. 1). It is bicolour (primary colour Red Purple Group 58 C and secondary colour Red Purple Group N57A according to the RHS colour chart), medium size, many numbers of petals (42.00) (Supplementary Fig. 2).

The overall findings suggested that employing gamma rays irradiation may induce variability in roses for further use in breeding program on various commercial traits. To summarize the present study, among the treatments, 20 and 30 Gy doses of gamma rays are most suitable for induction of variation in flowering and vegetative traits as compare to other treatments.

ACKNOWLEDGEMENT

Research was supported by the Indian Council of Agricultural Research, Department of Agricultural Research and Education, Government of India.

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