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Effect of physical mutation on gladiolus traits and studies on gladiolus (*Gladiolus grandiflorus*) mutant

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ABSTRACT

The study was carried out over five years (2017–2022) at Banaras Hindu University, Varanasi, Uttar Pradesh to assess the effect of gamma irradiation on morphological, flowering, and post-harvest traits in nine gladiolus (*Gladiolus grandiflorus* L.) cultivars i.e. Gulal, Jyotsna, Mohini, Pusa Kiran, Pusa Srijana, Pusa Vidushi, Swarnima, Pusa Shubham and Urvashi with the objective to identify stable mutants using randomised block design (RBD) with gamma doses of 0, 20, 30, 40, and 50 Gy. Dose-response analysis revealed significant variation in survival rate, sprouting, floral traits, and vase life. Probit analysis estimated LD_{50} values across cultivars to average /~46 Gy, with optimal mutation induction between 30–40 Gy. Regression models indicated quadratic responses for days to sprouting, number of sprouts/hill, and number of open florets per spike, peaking at 30–40 Gy, while higher doses resulted in physiological damage. Floral longevity declined linearly with increasing dose. Significant cultivar-specific responses were in Gulal and Jyotsna, responded positively to moderate doses, while Pusa Srijana and Pusa Kiran showed greater sensitivity to higher doses. Mutation frequency ranged from 2% (20 Gy) to 9% (40 Gy), with the highest mutation effectiveness observed at 40 Gy (4.00%), followed by 30 Gy (0.17%). Notable phenotypic mutants included changes in tepal colour, floret shape, and bract morphology. A stable purplish-red floral mutant was isolated from cv. Gulal at 40 Gy and confirmed across VM_1–VM_5 generations. The results confirmed gamma irradiation as an effective tool for inducing beneficial mutations and support 30–40 Gy as the optimal dose range for gladiolus improvement.

Keywords: DUS, Floral mutant, Gamma irradiation, Gladiolus, Mutation breeding, Post-harvest quality

Floriculture, one of the most significant subfields of ornamental plant cultivation. The primary goal is to satisfy public aesthetic demand. *Gladiolus* spp., a perennial geophyte, was recognised over 2000 years ago in Asia minor (Cantor and Tolety 2011). They are highly valued in floriculture for their vibrant colours, long vase life and diverse range of cultivars (Singh 2014). The development of molecular genetics and DNA technologies has ushered in a molecular era in plant breeding, particularly mutation breeding. Mutations are changes that are inherited in the phenotypic of an organism. These variations are the result of chemical changes at gene level. Novel and heritable character variants in crop plants could result from these alterations (Yali and Mitiku 2022). According to Ahloowalia *et al.* (2004), X-rays are used to create 22% of the radiation induced mutant types, whereas gamma rays are used to create 64%. The majority of the 552 mutant cultivars of floricultural plants, as reported in the FAO/IAEA Database, were chrysanthemum (232), followed by alstroemeria (35), dahlia (36), bougainvillaea (12), rose (61), achimenes (8), begonia (25), carnation (18), streptocarpus (30), and azalea (15) (Maluszynski *et al.* 2000). Since gladiolus is cultivated flawlessly through vegetative means, mutation breeding presents significant opportunities since the modified portion can be easily reproduced through vegetative means, leading to the creation of novel forms. Hence, the present investigation was carried out to find desirable variations as well as a stable mutant caused by physical mutagens, affecting the vegetative, flowering and post-harvest parameters in gladiolus.

MATERIALS AND METHODS

The study was carried out over five consecutive growing seasons from 2017–2018 to 2021–2022 at Banaras Hindu University, Varanasi (25°16' N, 82°59' E; at an elevation of 80.71 m amsl), Uttar Pradesh. The study aimed to assess the effect of gamma irradiation on morphological, flowering, and post-harvest traits in gladiolus and to identify stable mutants.

Plant material and gamma irradiation: Uniform, healthy, and disease-free dormant corms of nine Indian gladiolus cultivars (Gulal, Jyotsna, Mohini, Pusa Kiran, Pusa Srijana, Pusa Vidushi, Swarnima, Pusa Shubham and Urvashi) were selected. A total of 100 corms/cultivar/

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treatment were irradiated in October 2017 (one time irradiation) at the Division of Floriculture, Botanic Garden and Eco-education, CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, using a Cobalt-60 gamma source. The gamma irradiation doses applied were 0 Gy (Control), 20 Gy, 30 Gy, 40 Gy, and 50 Gy. All irradiated corms were handled and transported under ambient conditions and planted in November 2017.

Experimental design and cultivation: The experiment was laid out in a randomised block design (RBD) with three replications. Each treatment plot contained 100 corms/cultivar, with a spacing of 30 cm × 20 cm (row-to row × plant-to-plant). Recommended agronomic practices for gladiolus cultivation were followed uniformly throughout the experiment.

Trait evaluation and mutant identification: Observations on vegetative, floral, and post-harvest traits were recorded in the M_1 (2017–2018) and M_2 (2018–2019) generations. Traits observed included days to sprouting, number of sprouts/hill, number of open florets/spike, last floret opening, floret withering, and floral opening at a time/spike (in field and vase). Mutants showing phenotypic deviations in tepal colour, floret shape, bract structure, and plant vigour were isolated and advanced.

Stable mutants were evaluated further during M_3 (2019–2020), M_4 (2020–2021), and M_5 (2021–2022) generations based on Distinctiveness, Uniformity, and Stability (DUS) guidelines (Anonymous 2015). A notable floral mutant of cv. Gulal was identified at 40 Gy with purplish-red tepals and triangular floret shape, which remained stable across generations.

Mutation frequency and effectiveness:

$$\text{Mutation frequency (\%)} = \frac{\text{Number of mutants observed}}{\text{Total number of plants treated}} \times 100$$

$$\text{Mutation effectiveness (\%)} = \frac{\text{Mutation frequency (\%)}}{\text{Dose (Gy)}}$$

LD_{50} estimation and dose-response analysis: To determine the lethal dose (LD_{50}) for 50% reduction in survival, probit analysis was performed using survival data across all doses. Additionally, dose-response analysis was conducted using regression models (linear and quadratic) for key traits such as days to sprouting, no. of sprouts per hill, open florets per spike, and vase life parameters. Trait responses were evaluated for significance using analysis of variance (ANOVA) at $p<0.05$ and $p<0.01$, followed by post hoc comparison.

Statistical analysis: Data were statistically analysed using SPSS v22.0 for data, regression and probit analysis. The cultivar × dose interaction was analysed to assess genotype-specific responses to gamma irradiation.

RESULTS AND DISCUSSION

Days to sprouting: A considerable variation was observed among varieties and doses during 2nd year only. In the 2nd year, 20 Gy followed by 30 Gy, accelerated sprouting significantly compared to control, whereas no such effect was noted in the first year (Table 1). Across the first year, cv. Jyotsna followed by Gulal and Swarnima sprouted earliest. This early sprouting aligned with the report of Yadav *et al.* (2025) where lower dose enhanced α-amylase activity, sugar mobilisation and oxygen uptake in corms (Karki *et al.* 2010). In the second year, Gulal led, trailed by Swarnima for earlier sprouting. Notably, Control × Jyotsna, 40 Gy × Swarnima, and Control × Gulal combinations sprouted earliest in 2nd year, while Control × Pusa Srijana exhibited delayed sprouting. These outcomes suggest that low gamma doses may enhance enzyme activity, whereas higher doses may disrupt auxin-mediated cell division and growth (Misra and Bajpai 1983).

Correlation: Days to sprouting exhibited a weak to moderate positive correlation with the number of sprouts/hill, suggesting that earlier sprouting genotypes generally produced fewer sprouts. Its correlation with reproductive traits such as open florets/spike and vase life indicators was weak or inconsistent across years, reflecting that sprouting behavior is more influenced by vegetative vigour than floral performance.

No. of sprouts/hill: Gamma irradiation influenced this trait significantly in the first year, but cultivars × dose interactions were non-significant across both years (Table 1). In 1st year, 40 Gy produced the maximum number of sprouts, statistically similar to 50 Gy. But cultivar, Pusa Kiran followed by Gulal and Mohini showed maximum sprouting in 1st year, whereas Pusa Shubham followed by Pusa Kiran dominated in 2nd year. These varietal differences likely reflect inherent genetic factors modulating mutagenic responsiveness (Sisodia and Singh 2015, Sah *et al.* 2017, Yadav *et al.* 2025).

Correlation: The trait showed positive associations with no. of florets/spike in both years, indicating that genotypes with more sprouts had stronger vegetative growth and potentially higher assimilate supply for spike development. However, the correlation was moderate rather than strong, suggesting that additional physiological factors also plays a role.

No. of florets open spike: The performance observed during 1st year peaked at 20 Gy, except versus 50 Gy (Table 2). Gulal recorded for the maximum number of florets open/spike in 1st year; while in 2nd year, Pusa Vidushi excelled, at par with cvs. Pusa Srijana, Jyotsna, Gulal and Urvashi. Interaction extremes were observed with 50 Gy × Gulal (maximum) and 30 Gy × Pusa Shubham (minimum) in 1st year; 40 Gy × Jyotsna excelled in 2nd year. These results reinforce that moderate irradiation may stimulate growth via repair or signaling pathways (Esnault *et al.* 2010, Sisodia and Singh 2014, Mounir *et al.* 2022).

Correlation: The trait was positively correlated with number of florets/spike and florets open at a time/spike, showing that longer spikes support maximum number of florets and synchronous blooming. The association with vase life traits was positive but weaker, implying that spike

Table 1 Effect of gamma irradiation on days to sprouting and no. of sprouts/hill in different gladiolus varieties

Variety	Year	Days to sprouting						No. of sprouts/hill					
		Gamma irradiation doses (Treatment)						Gamma irradiation doses (Treatment)					
		Control	20 Gy	30 Gy	40 Gy	50 Gy	Mean	Control	20 Gy	30 Gy	40 Gy	50 Gy	Mean
Gulal	I	11.50	9.33	11.50	8.67	10.00	10.20	1.67	2.00	1.67	2.00	2.00	1.87
	II	11.77	12.50	12.23	11.93	12.32	12.15	2.22	2.83	2.89	2.67	1.83	2.49
Jyotsna	I	10.00	9.00	9.67	9.00	8.00	9.13	1.17	1.17	1.67	1.00	1.67	1.33
	II	10.00	12.14	14.17	15.31	16.67	13.66	2.42	2.80	2.64	2.47	2.25	2.52
Mohini	I	13.00	10.17	12.83	11.33	14.00	12.27	1.33	1.67	1.83	2.17	2.17	1.83
	II	16.73	12.48	16.24	16.25	16.67	15.68	2.44	2.14	2.22	1.92	1.91	2.13
Pusa Kiran	I	21.00	16.67	18.00	15.17	12.50	16.67	1.33	1.67	2.00	3.00	3.00	2.20
	II	13.33	18.33	13.94	17.17	13.58	15.27	1.33	2.67	3.22	2.83	3.64	2.74
Pusa Srijana	I	10.50	11.83	13.00	10.67	11.17	11.43	1.17	1.50	1.50	2.00	1.50	1.53
	II	27.00	14.67	15.44	16.94	13.83	17.58	2.00	2.50	2.72	2.83	2.39	2.49
Pusa Vidushi	I	17.33	13.17	9.67	10.83	10.67	12.33	1.00	1.50	1.33	1.67	1.83	1.47
	II	21.00	18.17	13.83	15.17	15.17	16.07	2.00	2.33	3.17	2.00	2.67	2.43
Swarnima	I	8.83	10.00	11.67	12.50	12.17	11.03	1.00	1.00	1.17	1.50	1.50	1.23
	II	12.50	12.50	13.43	11.73	12.83	12.60	2.67	2.50	2.22	2.50	1.67	2.31
Pusa Shubham	I	14.00	19.67	13.67	1300	16.50	15.37	1.17	1.17	1.17	1.17	1.00	1.13
	II	12.33	12.33	13.33	13.83	13.33	13.03	3.33	3.67	3.00	3.67	2.83	3.30
Urvashi	I	19.00	12.50	12.50	13.00	14.67	14.33	1.00	1.33	2.00	2.33	2.00	1.73
	II	13.67	14.50	13.23	15.49	16.67	14.71	2.67	3.17	2.00	2.33	2.83	2.60
Mean	I	13.91	12.48	12.50	11.57	12.19		1.20	1.44	1.59	1.87	1.85	
	II	15.37	13.85	13.99	14.87	14.56		2.34	2.73	2.68	2.58	2.45	
CD (p=0.05)		I year		II year					I year		II year		
Treatment (T)		NS		1.07					0.26		NS		
Variety (V)		2.24		1.44					0.35		0.50		
T×V		NS		3.21					NS		NS		

length indirectly contributes to extend display duration.

No. of florets open at a time per spike: The trait showed significant dose effects and interactions in both the years (Table 2). The maximum simultaneous floret opening occurred at 20 Gy and at par with 30 Gy during both years. Among cultivars, Mohini during 1st year and Jyotsna during 2nd year performed well, occurring maximum no. of florets open at a time/spike. Interactions of 20 Gy × Mohini and 50 Gy × Jyotsna indicated cultivar-specific peaks during 1st and 2nd year, respectively. Lower doses likely enhanced sugar metabolism and physiological readiness, improving floret opening at a time in gladiolus (Sisodia and Singh 2014) and tuberose (Kumar *et al.* 2003).

Correlation: The trait correlated strongly with florets open at a time in vase and last floret open. This indicates simultaneous opening of more flowers may prolong flowering, which is highly desirable for decorative use.

Days to withering of last floret (Vaselife): All cultivars and treatments significantly differed in both years, but interactions had no significant effect (Fig. 1A). Vase life was maximum preserved at 20 Gy, significantly outperforming 40 and 50 Gy during both years. Cv. Jyotsna consistently showed the late floret withering. Improved vase life may result from thicker stems, better turgidity, and maximum reserves, extending longevity (Anu *et al.* 2003, Sisodia and Singh 2014).

Correlation: In addition, it was positively associated with vase-life parameters (florets open at a time/spike and last floret open), as expected. Genotypes delaying first floret senescence tended to sustain longer flowering duration, an important quality trait. Its correlation with vegetative traits was negligible, confirming no correlation at early growth.

No. of florets open at a time per spike in vase: In vase conditions, Cultivar Mohini, at par with cv. Gulal during 1st year and, cv Jyotsna at par with Mohini, showed the maximum no. of florets open at a time/spike in postharvest condition (Fig. 1B). Among doses, 20 Gy performed maximum floret open at a time/spike during 1st year, while minimum was with 50 Gy. No significant dose effect was observed during 2nd year. Maximum number of florets open at a time/spike in vase was observed with 50 Gy × Jyotsna and at par with 30 Gy × Jyotsna and 20 Gy × Mohini during 2nd year alone. This likely reflects improved carbohydrate mobilization and water uptake (Singh *et al.* 2003).

Table 2 Effect of gamma irradiation on no. of open florets/spike and no. of florets open at a time/spike (field condition) in different gladiolus varieties

Variety	Year	No. of open florets/spike						No. of florets open at a time/spike					
		Gamma irradiation doses (Treatment)						Gamma irradiation doses (Treatment)					
		Control	20 Gy	30 Gy	40 Gy	50 Gy	Mean	Control	20 Gy	30 Gy	40 Gy	50 Gy	Mean
Gulal	I	11.67	12.33	10.00	9.00	13.33	11.27	4.33	4.50	5.17	5.33	4.00	4.67
	II	10.17	12.00	11.33	9.56	10.75	10.76	4.17	4.67	5.17	4.39	4.33	4.55
Jyotsna	I	10.67	10.67	9.33	10.67	8.00	9.87	4.67	5.33	6.00	5.33	4.67	5.20
	II	8.67	11.00	12.00	14.67	9.00	11.07	4.83	5.50	6.00	5.75	6.75	5.77
Mohini	I	10.67	12.33	10.67	10.33	8.33	10.47	7.00	7.67	7.33	7.00	6.67	7.13
	II	10.00	12.44	8.87	9.83	9.00	10.03	5.83	5.94	5.67	5.11	5.33	5.58
Pusa Kiran	I	11.67	11.83	12.17	9.67	9.17	10.90	6.17	6.33	5.83	5.67	4.67	5.73
	II	10.17	13.33	10.17	8.67	9.17	10.30	3.67	5.33	5.00	5.33	5.00	4.87
Pusa Srijana	I	11.33	12.67	9.67	8.33	10.67	10.53	3.67	3.50	3.50	2.67	3.33	3.33
	II	10.50	11.33	12.00	8.67	13.33	11.17	4.00	4.17	3.67	4.33	3.83	4.00
Pusa Vidushi	I	10.00	10.00	10.00	12.00	9.00	10.20	4.17	4.00	5.33	4.67	3.67	4.37
	II	13.00	10.33	12.67	10.83	11.00	11.57	4.33	4.83	4.33	5.33	4.00	4.57
Swarnima	I	10.00	9.00	10.00	9.00	8.67	9.33	3.33	3.17	3.33	3.00	3.00	3.17
	II	9.33	9.83	11.22	8.39	10.17	9.79	3.17	4.17	3.83	4.00	3.50	3.73
Pusa Shubham	I	10.00	9.00	7.67	8.00	8.17	8.57	4.00	4.00	3.17	3.33	3.00	3.50
	II	9.33	7.00	6.67	9.67	8.67	8.27	4.33	4.00	3.33	3.33	3.00	3.60
Urvashi	I	8.50	7.67	10.00	8.00	8.00	8.43	5.33	5.67	4.33	4.00	4.00	4.67
	II	11.00	11.17	11.50	10.00	9.00	10.53	4.33	4.67	5.00	4.67	4.00	4.53
Mean	I	10.50	10.61	9.94	9.44	9.26		4.74	4.91	4.89	4.56	4.11	
	II	10.24	10.94	10.71	10.03	10.01		4.30	4.81	4.67	4.69	4.42	
CD (p=0.05)		I year		II year					I year		II year		
Treatment (T)		0.88		NS					0.26		0.31		
Variety (V)		1.18		1.24					0.35		0.41		
T×V		2.64		2.78					0.78		0.91		

Correlation: Further, this character displayed strong positive correlation with no. of open florets/spike and last florets open in vase, in both years. This indicated that genotypes capable of opening more florets simultaneously also sustain prolonged flowering, which is highly desirable for ornamental purpose.

Days to opening last floret: Late opening of last floret in vase was observed with control which was statistically at par with 20 and 30 Gy of gamma doses and significant to 40 and 50 Gy (Fig. 1C). While during 2nd year, a maximum day taken to opening of last floret was registered with 20 Gy and at par with control and 30 Gy. Among cultivars, late opening of last floret was observed with cv. Jyotsna during both years. All the interactions were failed to exert any pronounced effect in both years of investigation. These findings suggest higher gamma doses accelerate floral senescence, likely by disrupting respiration and photosynthesis process (Pranom *et al.* 1986, Sisodia and Singh 2014).

Correlation: The strongest correlation was recorded between this trait and other floral traits, particularly days to withering of floret and floret open at a time/spike. This reinforces that last floret opening is a robust indicator of vase life. Its relationship with vegetative traits was generally weak, supporting its classification as performed using mean values across three replicates (R_1-R_3) for each trait and variety, separately for year Ist and IInd year (Table 3). Pearson correlation coefficients are presented above. Strong associations ($|r| \geq 0.70$) are specifically highlighted. Trait associations varied between years, indicating genotype × environment interactions and temporal instability of some relationships.

Genotype × Environment (G × E) character stability: The two years of evaluation revealed that while the direction of correlations remained generally consistent, the magnitude of association varied across years. In this research, the correlation among vegetative traits (days to sprouting and no. of sprouts/hill) with reproductive traits were stronger in 1st year but attenuated in 2nd year. Conversely, vase life characters (first floret withering, florets open at a time/spike and last floret open) maintained consistently strong interrelationship across both environments, reflecting their relative stability. These shifts highlight the influence of environmental factors such as temperature, humidity, and soil fertility on trait expression. Overall, The G × E

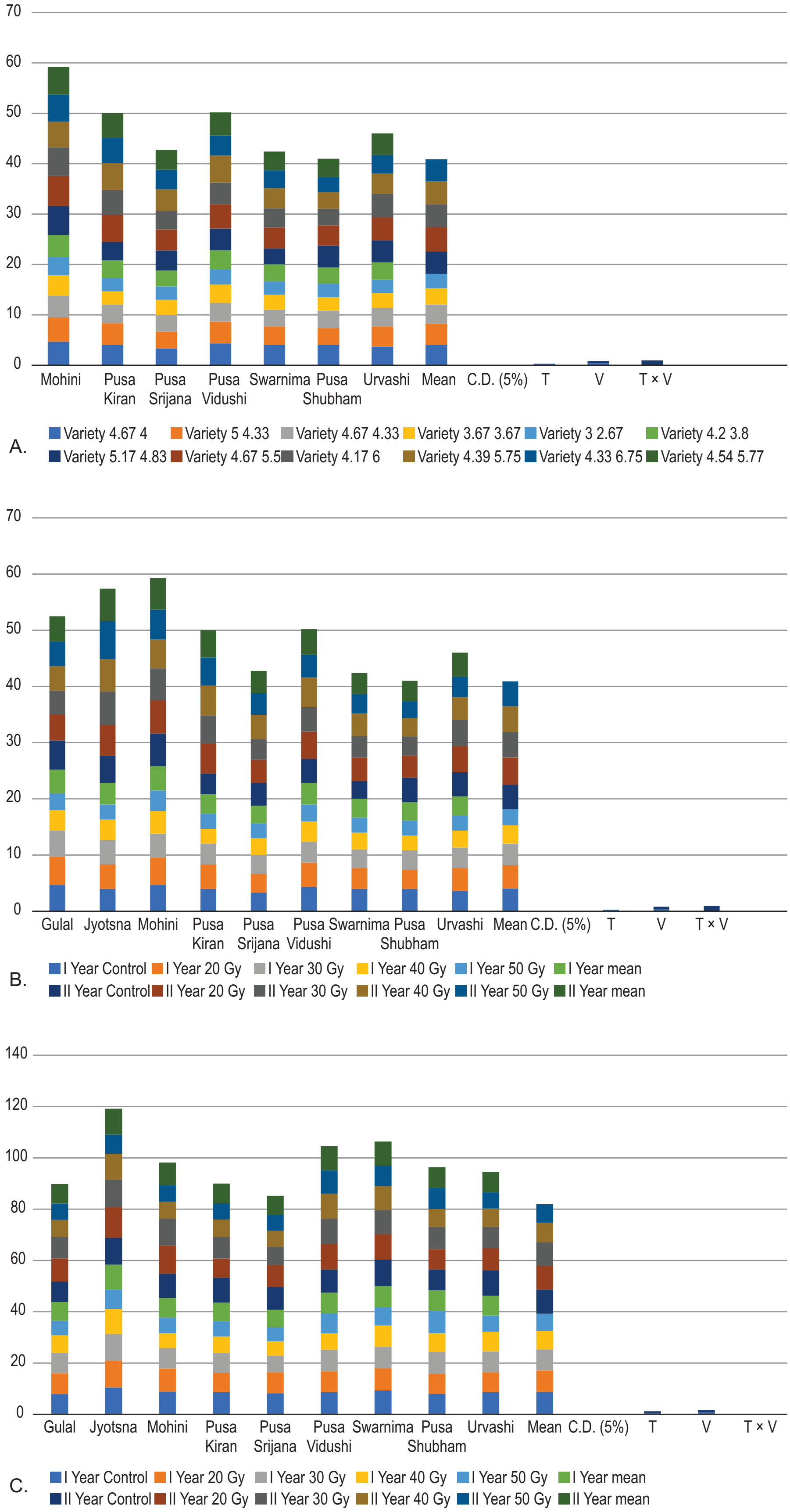

Fig. 1 Effect of gamma irradiation on A) days to withering of 1st floret; B) no. of florets open at a time/spike; C) days to opening of last floret in different gladiolus varieties during post-harvest observation.

analysis emphasizes that stable floral longevity traits should be prioritized in breeding, while vegetative vigour may require environment-specific selection.

Dose-response analysis and LD_{50} estimation: To better understand the effects of gamma irradiation on gladiolus cultivars, a dose-response analysis was conducted using regression models for key traits such as survival rate, number of sprouts/hill, days to sprouting, number of open florets/spike and days to withering of 1st floret in vase. The research determined the LD_{50} (Lethal dose at which 50% reduction in a given trait or survival occurs) and identify traits showing or non-liner (e.g. quadratic) responses to increasing irradiation doses.

LD_{50} determination: Using probit analysis on plant survival rates across doses (0, 20, 30, 40, 50 Gy), the estimated LD_{50} values varied slightly among cultivars. The average LD_{50} values for gladiolus across cultivars were found to be ~46 Gy, with cultivar-wise estimates were Gulal: LD_{50} ≈48.2 Gy; Pusa Srijana: LD_{50}≈44.6 Gy; Jyotsna: LD_{50}≈47.5 Gy; Mohini: LD_{50}≈45.3 Gy; and Pusa Subham: LD_{50}≈46.0 Gy [Although only discrete doses (20, 30, 40, and 50 Gy) were applied, LD_{50} values were estimated using probit regression, allowing interpolation of the dose corresponding to 50% survival]. This suggests that gamma doses beyond 50 Gy may have detrimental effects on most cultivars and optimal mutagenic response lies between 30–40 Gy.

Liner and quadratic dose-response

Sprouting traits (Days to sprouting, No. of sprouts/hill): Quadratic relationship (p<0.01) was observed with days to sprouting, indicating that moderate doses (30–40 Gy) promoted early sprouting, while higher doses delayed it. No. of sprouts/hill showed a significant quadratic trend (p<0.05), with a peak at 40 Gy and a sharp decline at 50 Gy.

Floral traits (No. of open florets/ spike, florets open at a time, vase

Table 3 Correlation analysis of traits-to-traits

Traits	Days to sprouting	No. of sprouts/hill	No. of open florets /spike	No. of florets open at a time/spike	Days to withering of 1st floret in vase	No. of florets open at a time/spike in vase	Last floret open in vase
Year I				Correlation matrix of traits			
Days to sprouting	1.00	-0.20	-0.04	0.16	-0.43	-0.14	-0.17
No. of sprouts/hill	-0.20	1.00	0.16	0.33	-0.24	-0.01	-0.38
No. of open florets /spike	-0.04	0.16	1.00	0.37	0.29	0.49	0.13
No. of florets open at a time/ spike	0.16	0.33	0.37	1.00	0.18	0.55	0.11
Days to withering of 1st floret in vase	-0.43	-0.24	0.29	0.18	1.00	0.44	0.72
No. of florets open at a time/ spike in vase	-0.14	-0.01	0.49	0.55	0.44	1.00	0.40
Last floret open in vase	-0.17	-0.38	0.13	0.11	0.72	0.40	1.00
Year II				Correlation matrix of traits			
Days to sprouting	1.00	-0.22	0.15	0.18	-0.24	0.13	-0.18
No. of sprouts/hill	-0.22	1.00	-0.24	-0.14	-0.06	-0.15	-0.12
No. of open florets /spike	0.15	-0.24	1.00	0.22	0.22	0.24	0.22
No. of florets open at a time/ spike	0.18	-0.14	0.22	1.00	0.45	0.96	0.16
Days to withering of 1st floret in vase	-0.24	-0.06	0.22	0.45	1.00	0.46	0.57
No. of florets open at a time/ spike in vase	0.13	-0.15	0.24	0.96	0.46	1.00	0.21
Last floret open in vase	-0.18	-0.12	0.22	0.16	0.57	0.21	1.00

life): No. of open florets/spike and no. of florets open at a time/spike followed a quadratic trend, peaking at 20–30 Gy. Regression models indicated a decline in performance at 50 Gy, confirming the threshold of physiological tolerance. Days to withering of first floret in vase exhibited a liner decrease (p<0.01) with increase gamma dose, suggesting that higher doses negatively impact floral longevity. Days to opening of last floret showed a liner increase up to 30 Gy, followed a plateau or slight decline, suggesting moderate irradiation may extend bloom period, while higher doses inhibit further floral development.

Interaction of cultivar × dose: Interaction plots and two-way ANOVA regression coefficients revealed that cultivars differed in their sensitivity. Gulal and Jyotsna responded positively to moderate doses (30–40 Gy), while Pusa Srijana and Pusa Kiran showed more pronounced negative effects at higher doses. This genotype-specific response underscores the importance of dose optimization in mutation breeding. The findings align with previous reports indicating that gamma irradiation can show stimulatory effects at lower/ moderate doses and inhibitory effects at higher doses (Esnault *et al.* 2010, Sisodia and Singh 2014). The observed quadratic trends are typical in radiation mutagenesis studies, where there's an optimal dose window for beneficial mutations without compromising plant viability (Ahloowalia and Maluszynski 2001, Kumari *et al.* 2013).

Isolation of a stable mutant in cultivar Gulal at 40 Gy gamma dose: From cultivar Gulal, a stable mutant was obtained at gamma radiation of 40 Gy (Supplementary Fig. 1). This mutant was first discovered in 2017–2018 (VM_1), then isolated in 2018–2019 (VM_2) and it continued to exist in third generation (VM_3) (Supplementary Fig. 1B). On the other hand, its stability was noted between VM_3 and VM_5 (2019–2020 to 2021–2022). There were notable differences in a number of general traits, such as leaf, flower, spike and corm characteristics, in addition to tepal colour (Table 4 and Supplementary Fig. 1A-D).

Colour variation in tepals: The mutant tepals were purplish red in colour (Supplementary Fig. 1D), while the parent tepals were strong red (Supplementary Fig. 1A). In parent colour of 1st, 2ndand 3rd tepals was same i.e. primary colour was strong red B (Red group 53) and secondary colour was strong red A (Red group 51). However, colour of 1st, 2nd and 3rd tepals in mutant was different than parent; it was vivid purplish red C (Red purple group 61) as primary colour and strong red C (Red group 53) as secondary colour. However, there was much similarity in colour of 4th, 5th and 6th tepals in parent. Colour of these parent tepals was strong red C (Red group 53). Shades of light purplish grey D (Greyed purple group N187) and pinkish white B (White group N155) arises from base to center of the 1st, 2nd, 3rd tepals and as primary colour in 4th and 5th tepals in

Table 4 Comparison between characteristics of stable mutant along with parent cv. Gulal at 40 Gy gamma irradiation dose

Sl. no.	Characters	Parentage Gulal	Mutant
		2019–2022	2019–2022
1.	Plant height	55.50 cm	56.60 cm
2.	Corm: Number per plant	2.16	2.25
3.	Corm: Diameter	4.37 cm	4.74 cm
4.	Corm: Fresh weight	25.02 g	26.44 g
5.	Number of cormels/plant	52.00	28.20
6.	Leaf blade length	44.30 cm	45.60 cm
7.	Leaf blade width	3.00 cm	2.50 cm
8.	Leaf blade main colour	Moderate yellowish green C (Green group 139)	Moderate yellowish green C (Green group 139)
9.	Leaf: Curvature of distal half	Absent	Absent
10.	Inflorescence Lateral branches	Absent	Absent
11.	Width of scape	2.10 cm	2.00 cm
12.	Number of leaves	6.00	7.00
13.	Spike length	68.40 cm	78.60 cm
14.	Rachis length	51.00 cm	65.30 cm
15.	Internodal length	4.00 cm	3.86 cm
16.	Number of florets/spike	13.00	15.00
17.	Number of florets open at a time	6.00	7.00
18.	Number of open flowers	12.00	13.00
19.	Arrangement of flower	Zigzag	Zigzag
20.	Inner floret undulation	Weak	Absent
21.	Outer floret undulation	Weak	Absent
22.	Median inner tepal: Attitude	Erect	Erect
23.	Median inner tepal: Attitude of apex	Straight	Straight
24.	Inner tepal: Width of marginal zone	Absent	Absent
25.	Inner tepal: Border of marginal zone	Absent	Absent
26.	Inner tepal: Colour of marginal zon	Absent	Absent
27.	Tepal texture	Papery	Papery
28.	Length of floret	10.07 cm	10.48 cm
29.	Width of floret	10.50 cm	9.50 cm
30.	Floret colour	Strong red	Purplish red
31.	Floret shape	Round	Triangular
32.	Floret attitude	Semi upright	Semi upright
33.	Macule colour	Absent	Absent
34.	Macule shape	Absent	Absent
35.	Macule position	Absent	Absent
36.	Macule length	Absent	Absent
37.	Macule width	Absent	Absent
38.	Stripe colour	Absent	Absent
39.	Stripe position	Absent	Absent
40.	Stripe length	Absent	Absent
41.	Outer tepal shape	Ovate, ovate, ovate	Ovate, obovate, ovate
42.	Inner tepal shape	Ovate, ovate, ovate	Elliptical, Elliptical, Elliptical
43.	Length of 1st tepal	6.10 cm	6.10 cm
44.	Length of 2nd tepal	5.90 cm	5.50 cm
45.	Length of 3rd tepal	6.00 cm	5.70 cm

Contd.

Table 4 (*Continued*)

Sl. no.	Characters	Parentage Gulal	Mutant
		2019–2022	2019–2022
46.	Length of 4th tepal	5.20 cm	5.10 cm
47.	Length of 5th tepal	5.20 cm	4.90 cm
48.	Length of 6th tepal	8.00 cm	7.30 cm
49.	Width of 1st tepal	4.50 cm	3.40 cm
50.	Width of 2nd tepal	3.60 cm	2.50 cm
51.	Width of 3rd tepal	4.70 cm	3.10 cm
52.	Width of 4th tepal	3.10 cm	2.10 cm
53.	Width of 5th tepal	2.40 cm	2.00 cm
54.	Width of 6th tepal	5.10 cm	4.00 cm
55.	Tepal reflexing	Weak	Very weak
56.	Bract length	7.40 cm	8.00 cm
57.	Bract width	3.00 cm	2.00 cm
58.	Bract: Shape of apex	Obtuse	Acute
59.	Bract anthocyanin colouration	Absent	Absent
60.	Androecium length	4.67 cm	5.13 cm
61.	Anther length	1.13 cm	1.67 cm
62.	Filament length	3.20 cm	3.70 cm
63.	Style length	6.40 cm	7.00 cm
64.	Lobe length	0.90 cm	0.90 cm
65.	Tepal 1 colour	Primary- Strong red B (Red group 53) Secondary- Strong red A (Red group 51)	Primary- Vivid purplish red C (Red purple group 61) Secondary- Strong red C (Red group 53) and strong purplish pink B (Red purple group 68) near macule region, less shades of light purplish grey D (Greyed purple group N187) and pinkish white B (White group N155) over tepal
66.	Tepal 2 colour	Primary- Strong red B (Red group 53) Secondary- Strong red A (Red group 51)	Primary- Vivid purplish red C (Red purple group 61) Secondary- Strong red C (Red group 53) with shades of deep purplish pink C (Purple group N78) near macule region and more shades of light purplish grey D (Greyed purple group N187) and pinkish white B (White group N155)
67.	Tepal 3 colour	Primary- Strong red B (Red group 53) Secondary- Strong red A (Red group 51)	Primary- Vivid purplish red C (Red purple group 61) Secondary- Strong red C (Red group 53) and shades of deep purplish pink C (Purple group N78) near macule region, shades of light purplish grey D (Greyed purple group N187) and pinkish white B (White group N155) near both sides of the margin
68.	Tepal 4 colour	Primary- Strong red C (Red group 53) Secondary- Deep purplish pink C (Red purple group N66)	Primary- Maximum portion covered with light purplish grey D (Greyed purple group N187) and pinkish white B (White group N155) i.e. up to half of tepal length (1.70 cm), Secondary- Shades of deep purplish pink C (Red purple group N66) and a small area of strong red C (Red group 53) near center of the margin

Contd.

Table 4 (*Concluded*)

Sl. no.	Characters	Parentage Gulal	Mutant
		2019–2022	2019–2022
69.	Tepal 5 colour	Primary- Strong red C (Red group 53) Secondary- Deep purplish pink C (Red purple group N66)	Primary- Maximum portion covered with light purplish grey D (Greyed purple group N187) and pinkish white B (White group N155) i.e., upto half of tepal length (1.80 cm), Secondary- Shades of deep purplish pink C (Red purple group N66) and a small area of strong red C (Red group 53) near center of the margin
70.	Tepal 6 colour	Primary- Strong red C (Red group 53) Secondary- Deep purplish pink C (Red purple group N66)	Primary- Vivid purplish red C (Red purple group 68) Secondary- Strong red C (Red group 53) and strong purplish pink B (Red purplish group 68) near to macule region
71.	Anther colour	Deep reddish purple A (Purple group 77)	Strong purple B (Violet group N87)
72.	Filament main colour	Apex- Vivid purplish red C (Red purple group 61) Base- Tinge of purplish red	Apex- White Base- Tinge of purplish red
73.	Filament: small spots at base	Present	Present
74.	Style colour	Apex- Reddish purple tinge Base- Light green	Apex- White Base- Pale greenish yellow
75.	Lobe colour	Strong purplish red B (Red purple group 64)	Strong reddish purple A (Red purple group 72)
76.	Time of beginning of flowering (days)	92 days	95 days
77.	Duration of flowering (days)	10 days	11 days

*Flower colour (RHS colour chart, The Royal Horticultural Society, London). *Plant characters were recorded according to DUS for comparison.

mutant. Pinkish white B (White group N155) colour was observed in 4th and 5th tepals in mutant as primary colour that spread approximately half length of the tepals. Whereas, secondary colour of 4th and 5th tepals in parent had white shades of deep purplish pink C (Red purple group N66) was observed. In mutant the colour was involved both primary as well as secondary colour of these same tepals in parent. In 6th tepal, primary colour in mutant was vivid purplish red C (Red purplish group 68) and secondary colour was strong red C (Red group 53) (Supplementary Fig. 1E). The findings are consistent with those of Dhaduk *et al.* (1992), who identified five flower colour mutants from gamma irradiated corms across four gladiolus varieties. They attributed the changes in flower colour to alterations in anthocyanin content, suggesting that the reduction in these pigments resulted from disruptions in their biosynthesis. Additionally, the multiple propagation cycles are necessary to eliminate chimeras and achieve stable, uniform mutants (homo-histons) in vegetatively propagated plants following mutagenic treatment (Ahloowalia and Maluszynski 2001).

Colour variation in reproductive organs: In parent anther colour was noticed deep reddish purple A (Purple group 77). In mutant it was strong purple B (Violet group N87). The main colour at apex in filament was vivid purplish red C (Red purple group 61) and at base there was tinge of purplish red was seen in parent. While in mutant the apex colour of filament was white and a tinge of purplish red was seen near its base. Style colour in parent at apex had a reddish purple tinge and a light green colour was seen at its base. In mutant it was white colour at apex and a pale greenish yellow at base of style. Lobe colour in parent and mutant was also varied. In parent it was strong purplish red B (Red purple group 64) and in mutant it was strong reddish purple a (Red purple group 72) as per RHS colour chat (Supplementary Fig. 1F). Previous studies have reported that gamma rays recognized as one of the most potent forms of electromagnetic radiation. Various doses of gamma irradiation on mutation frequency of floret as well as floral parts colour and chimeras types in chrysanthemum has been notable (Boersen *et al.* 2006). Researches like Dai and Magnusson (2012) in buddleia, Kumari *et al.* (2013) in chrysanthemum, Sisodia and Singh (2014) in gladiolus were found the similar results.

Shape variation in floret and bract: Floret shape in parent was round (Supplementary Fig. 1A), whereas triangular shape in florets was observed in mutant (Supplementary Fig. 1D). Undulation in inner and outer tepals was absent in mutant, whereas it was weak in both outer and inner tepals of parent. Shape of outer and inner tepals was also varied greatly in parent i.e., ovate, ovate, ovate in both outer and inner tepals. However, in mutant it was ovate, obovate, ovate in outer and elliptical, elliptical and elliptical in inner tepal (Supplementary Fig. 1G,H). Bract shape of apex was obtuse in parent while in mutant it

Table 5 Mutation frequency and effectiveness across different doses of gamma radiation

Gamma dose (Gy)	No. of treated plants	No. of mutants observed	Mutation frequency (%)	Mutation effectiveness (%)
Control (0)	100	0	0.00	0.00
20 Gy	100	2	2.00	0.10
30 Gy	100	5	5.00	0.17
40 Gy	100	9	9.00	4.00
50 Gy	100	4	4.00	0.08

Mutants included both morphological and floral variants; confirmed across VM_1–VM_3.

was acute. Similar findings were also observed in a mutant isolated from cv. Tiger Flame at 4.5kR gamma dose (Singh and Sisodia 2015).

Mutation frequency or effectiveness: The frequency and effectiveness of induced mutations were estimated based on observable phenotypic changes in vegetative and floral traits, including sprouting behaviour, number of florets, tepal colour and floral shape (Table 5). The maximum mutation frequency was observed at 40 Gy, where a stable and heritable colour mutant (Purplish-red tepal) was identified in cv. Gulal.

Mutation effectiveness was maximum at 30 and 40 Gy, indicating these doses as the most efficient including useful mutations without excessive physiological damage. These results suggest that 40 Gy not only include the maximum number of observable mutants but also proved to be the most effective dose for gladiolus mutagenesis in the present study. However, 30 Gy also showed relatively high effectiveness with lower potential damage, making it suitable for large-scale mutation breeding programmes.

The observed mutation frequency and effectiveness trends clearly an optimal mutagenic window between 30–40 Gy, balancing mutation induction with plant viability. This aligns with earlier reports by Dhaduk *et al.* (1992) and Sisodia and Singh (2015), emphasizing moderate gamma irradiation doses as ideal for mutation breeding in gladiolus and similar vegetatively propagated ornamentals. These quantitative estimates provide a practical reference for optimizing gamma irradiation protocol in future gladiolus improvement programs.

The study demonstrated that gamma irradiation, particularly at lower to moderate doses (20–40 Gy), can significantly influence sprouting, floral traits and post-harvest quality in gladiolus. A stable mutant with distinct morphological and floral traits was successfully isolated from cv. Gulal at 40 Gy. Henceforth, 30–40 Gy was observed as the effective gamma dose for evolving mutants. This mutant holds promise for future cultivar development and commercial floriculture.

However, genotypes showing stable associations of vase life parameters across years can be considered more resilient to environmental variation and are better suited for consistent ornamental performance. Conversely, vegetative characters demonstrated more environmental sensitivity, implying that their contribution to floral quality may depend on year-specific growing condition.

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