



RESEARCH ARTICLE

Impact of gamma irradiation on mutation induction and flowering performance in tuberose genotypes

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Received: 20 January 2026; Accepted: 15 March 2026; Available online: Version 1.0: 12 May 2026

Cite this article: Ajay M, Anuj K, Jyoti K, Roshan G, Mayank P, Rishika C. Impact of gamma irradiation on mutation induction and flowering performance in tuberose genotypes. Plant Science Today. 2026; 13(sp2): 1-8. <https://doi.org/10.14719/pst.13676>

Abstract

The present study was carried out to see the effect of gamma irradiation on the flowering performance of different tuberose varieties. This experiment was laid out in a factorial randomized block design (FRBD) with 3 replications. Four cultivars, namely Calcutta single (V₁), Hyderabad single (V₂), Prajwal (V₃) and Shringar (V₄) were treated with various gamma irradiation doses, 0.75 kilorad (kR) (G₂), 1.50 kR (G₃), 2.25 kR (G₄) and 3.00 kR (G₅) along with a control (untreated). The study revealed that the varieties showed significant differences with respect to flowering and flower quality parameters. Among the tuberose varieties studied, V₂ showed the best performance with respect to spike emergence, length of spikes, days taken to opening of first floret, number of florets per spike, diameter of floret and length of floret. However, V₁ shows poor performance with respect to spike emergence, spike girth, days taken to opening of first florets, number of florets and length of floret. Among the gamma-ray doses, G₂ (0.75 kR) treatment produced the earliest spike emergence (101.57 days). While G₄ (2.25 kR) took maximum days for spike emergence (158.25 days) and first floret opening (183.80 days). Among the interaction earliest spike emergence (75.80 days) was observed in V₁ at 2.25 kR and V₄ produce maximum number of spikes (2.53) at control group. The overall mutation frequency was observed to be 2.67 %. A total of 16 mutants (5 in Calcutta single, 3 in V₂, 3 in V₃ and 5 in V₄) were observed at 0.75–2.25 kR irradiation doses. A higher dose of gamma irradiation (3.0 kR) showed the lethal effect and not a single plant survived in the experiment at 50 days after planting.

Keywords: *Agave amica*; gamma radiation; induced mutagenesis; mutation breeding; mutation frequency; *Polianthes tuberosa*

Introduction

Tuberose, scientifically referred to as *Agave amica* (Medik.) Thiede & Govaerts, previously known as *Polianthes tuberosa* L., belongs to the Asparagaceae family and native to Mexico (1). This plant is primarily cultivated for both cut and loose flowers, as well as for the extraction of natural flower oil, which is utilised in cosmetics and perfumes (2). Tuberose is also used in the making of buttonholes, bouquets, floral ornaments and artistic garlands and with the arrangement of the flower spikes in vases for decoration (3). Nowadays, it is extensively cultivated in tropical and subtropical regions across Africa, Asia and the Americas underscore may lead to high demand for its flowers and spikes (4). Tuberose produces white flowers, in which single flowers are highly fragrant and bloom in clusters and exhibit a waxy white appearance (5). Tuberose is primarily propagated through bulbs and bulblets. Traditional breeding methods are effective for developing new varieties, but they may also be time consuming (6). Employing advanced techniques such as mutation breeding, polyploidy, tissue culture and gene transfer can contribute significant benefits in the improvement of tuberose. Mutation plays a pivotal role in generating variation in numerous crops, both through natural processes and induced mutations through mutagens such as gamma rays and x-rays. Induced mutation methods are particularly advantageous for increasing genetic

diversity, especially concerning carotenoid pigments in tuberose leaf bases, notably in crown leafy varieties. Mutations may lead to changes in floret colouration (7). It is noteworthy that nearly 70 % of all mutant species worldwide have been produced through gamma irradiation (8). Gamma rays, being one of the most potent forms of electromagnetic radiation, have been employed as ionising radiation in plant breeding and genetic research for extended periods (9). Low doses of gamma irradiation are used in tuberose mutation breeding to induce genetic variability while minimising lethal effects. Despite limited studies on gamma irradiation in tuberose, the optimal mutation-inducing dose for Indian varieties remains undefined. Consequently, this study was initiated with the following objectives: (1) to evaluate the dose-dependent effects of gamma rays on flowering characteristics, (2) to identify the optimal and lethal doses for inducing desirable mutations and (3) to isolate and characterise the mutants.

Materials and Methods

Experimental site and irradiation treatment

The experiment was conducted at the experimental farm of the Department of Floriculture and Landscaping, KNK College of Horticulture, Mandsaur, Madhya Pradesh, India, during the kharif

season 2023–24. Mandsaur is situated in the Malwa plateau of Madhya Pradesh at a latitude of 23.45–24.13° N and 74.44–75.18° E longitudes and an altitude of 435.02 m above mean sea level. Tuberose bulbs were treated with different gamma irradiation doses, viz. 0.75 kilorad (kR) (G_2), 1.5 kR (G_3), 2.25 kR (G_4) and 3.00 kR (G_5) in the ^{60}Co gamma source chamber at ICAR-Indian Agricultural Research Institute (IARI), New Delhi. Untreated bulbs, 0 kR (G_1), were taken as a control.

The measurement of the amount of ionising radiation in air through unit kR, while gray (Gy) measures the absorbed dose by biological tissue (10). In mutation breeding, gamma ray doses are most reported in kR. Studies on various crops such as gladiolus, guar, pomegranate and safflower consistently use kR to describe effective mutagenic doses (11–14). Although Gy is the SI unit for absorbed dose, the mutation breeding literature predominantly uses kR due to historical and practical reasons related to radiation source calibration ^{60}Co (11). Therefore, kR remains the standard unit in mutation breeding research despite the scientific preference for Gy in radiation dosimetry.

Plant material and planting

Healthy bulbs of 4 different viz Calcutta single (V_1), Hyderabad single (V_2), Prajwal (V_3) and Shringar (V_4) varieties of tuberose (*A. amica*), 2–3 cm with 18–25 g, were taken for this experiment. These varieties are single-flower types. The bulbs were planted in mid-July 2023 (kharif) at a depth of 6–8 cm by adopting a spacing of 30 cm between rows and 20 cm between plants in the field. Light irrigation was given immediately after planting (Fig. 1).

Statistical design and data collection

The experiment was carried out in a factorial randomized block design (FRBD) containing 20 treatments with 3 replications; a total of 30 bulbs/replications were planted in each treatment. Data was analysed. Analysis of variance (ANOVA) was performed following FRBD at $p \leq 0.05$. Various flowering parameters, viz. days taken to spike emergence, days taken to open first floret, number of spikes per plant, number of florets per spike, spike girth, spike length, floret diameter and floret length are subjected to be recorded.

Mutation frequency (%)

The mutation frequency data for the M_1 generation were computed using the following method (11).

Results

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

Significant varietal, gamma and interaction effects were observed across all flowering parameters evaluated ($p < 0.05$; CD at 5 % level). The 4 tuberose varieties exhibited distinct performance patterns reflecting their genetic background. Additionally, gamma irradiation successfully induced 16 morphologically distinct mutants, with substantial variation across the 4 varieties (Table 1; Plate 1–4).

Effect of variety on flowering parameters

The significant varietal differences were observed across all flowering parameters evaluated ($p < 0.05$) (Fig. 2). Among all 4 varieties, V_1 showed the earliest spike emergence (106.88 days) and first floret opening (126.63 days), while V_2 exhibited the longest duration for spike emergence (151.32 days), representing 41.6 % delay and first floret opening (172.45 days), exhibiting 36.2 % delay compared to V_1 . Despite these delays in floret opening, V_2 compensated by producing superior quality flowers. The critical difference value at 5 % significance level was 11.87 and 13.8 days respectively. Regarding floral quality attributes, V_2 recorded superior performance with maximum spike length (69.74 cm), florets per spike (36.90), floret diameter (4.45 cm), floret length (6.20 cm) and demonstrated its robust flowering potential. In contrast, V_3 exhibited the highest spike girth (0.77 cm), while V_4 produced the maximum spikes per plant, 1.67, indicating its prolific flowering habit. V_4 demonstrated the lowest spike length (56.16 cm), spike girth (0.60 cm) with a critical difference (CD) value of 0.05 cm, florets per spike (25.18) with a CD value of 3.42 florets and floret diameter (4.07 cm) with a CD value of 0.17 cm. V_1 observed a minimum floret length of 5.80 cm.

Effect of gamma irradiation dose on flowering parameters

Gamma irradiation doses significantly influenced all flowering parameters (Fig. 3). The control treatment G_1 (0.00 kR) produced maximum spike length (76.09 cm), florets per spike (35.68) and spikes per plant (1.52). However, G_2 (0.75 kR) treatment demonstrated the earliest spike emergence (101.57 days). This 13.6 % enhancement in earliness (as compared to control) was maintained for first floret opening (122.92 days), along with superior floret quality parameters including maximum spike girth (0.75 cm), floret diameter (4.43 cm) and floret length (6.35 cm). Progressive increase in gamma dose from G_3 (1.50 kR) to G_4 (2.25 kR) resulted in gradual deterioration of flowering performance. The highest dose G_4 (2.25 kR) exhibited significantly delayed spike emergence (158.25 days) and first floret opening (183.80 days), accompanied by substantial reductions in spike length (46.49 cm), spike girth (0.56 cm), florets per spike (21.90), spikes per plant (1.03), floret diameter (3.66 cm) and floret length

Table 1. Number and type of gamma irradiation-induced mutants recovered in 4 tuberose (*Agave amica*) varieties

Variety	Gamma dose (kR)	No. of mutants	Mutation type	Morphological features	Remarks
Calcutta single (V_1)	0.75 kR	1	Floral (petal number)	8 petals in 1 st floret	Plate 1: A
	1.50 kR	3	Floral (petal number)	3 petals (2 nd and 8 th floret),	B
				4 petals (7 th floret),	C
				5 petals (3 rd floret)	D
Hyderabad single (V_2)	2.25 kR	1	Floral (petal number)	5 petals (6 th floret)	E
	0.75 kR	2	Vegetative (leaf length/width)	Longer and wider leaves	Plate 2: F, G
	1.50 kR	1	Floral (petal number)	7 petals in 1 st floret	H
Prajwal (V_3)	0.75 kR	3	Floral (petal number)	5 petals (6 th floret), 7 petals (4 th floret), 14 petals (39 th floret)	Plate 3: I, J, K
Shringar (V_4)	0.75 kR	5	Floral (petal number/stigma structure)	5 petals (21 st floret), 8 petals (1 st /2 nd floret), 4-lobed stigma	Plate 4: L, M, N, O, P

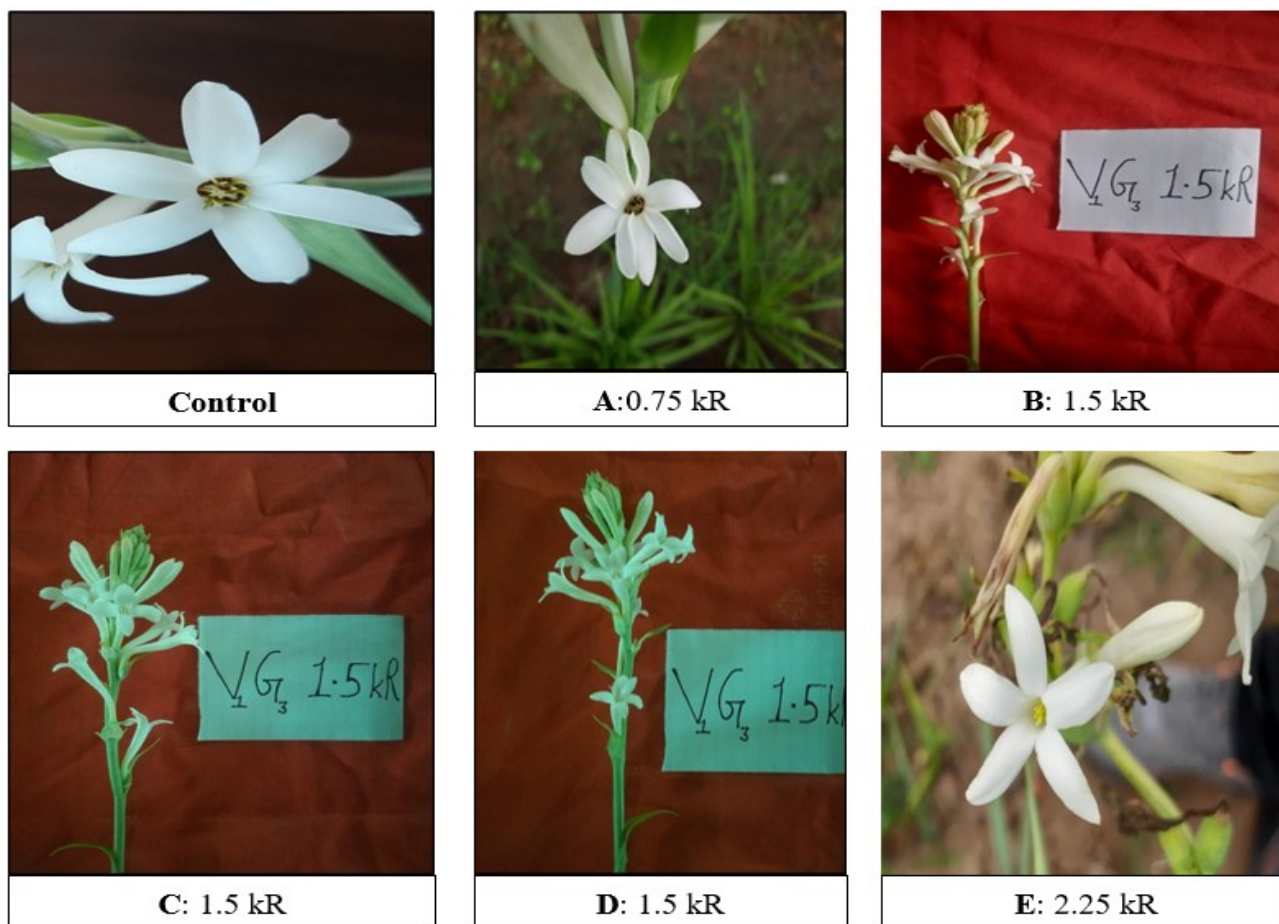


Plate 1. Identified mutants of tuberose variety Calcutta single through different gamma doses.

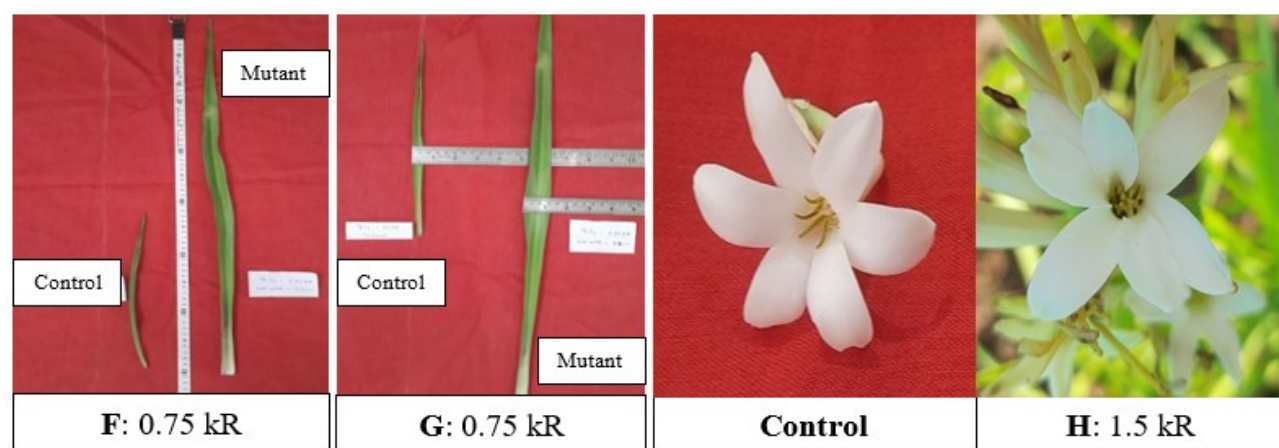


Plate 2. Identified mutants of tuberose variety Hyderabad single through different gamma doses.

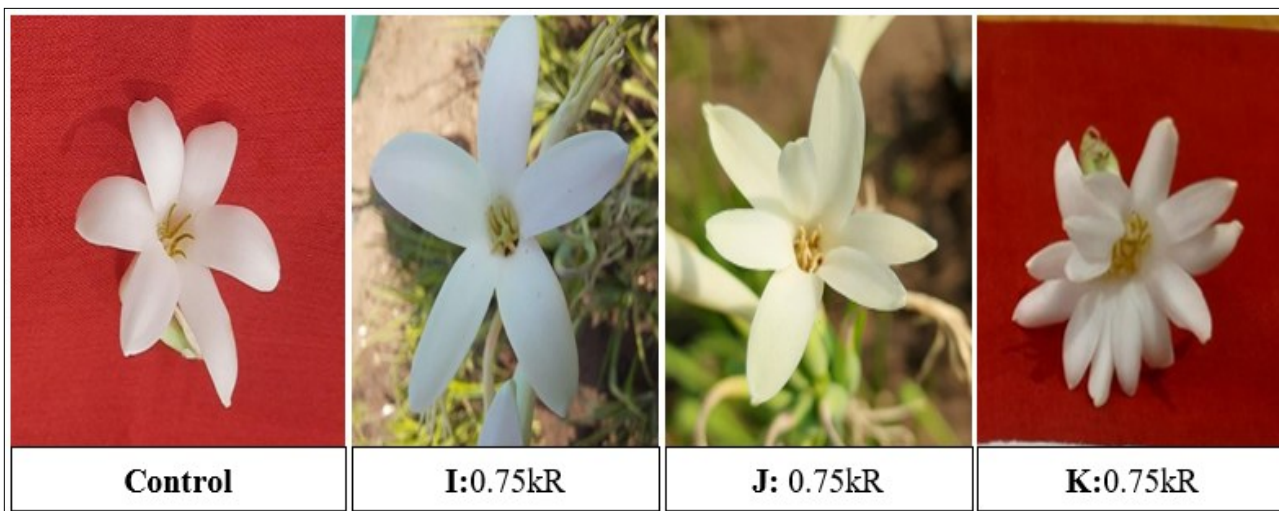


Plate 3. Identified mutants of tuberose variety Prajwal through gamma dose of 0.75 kR.

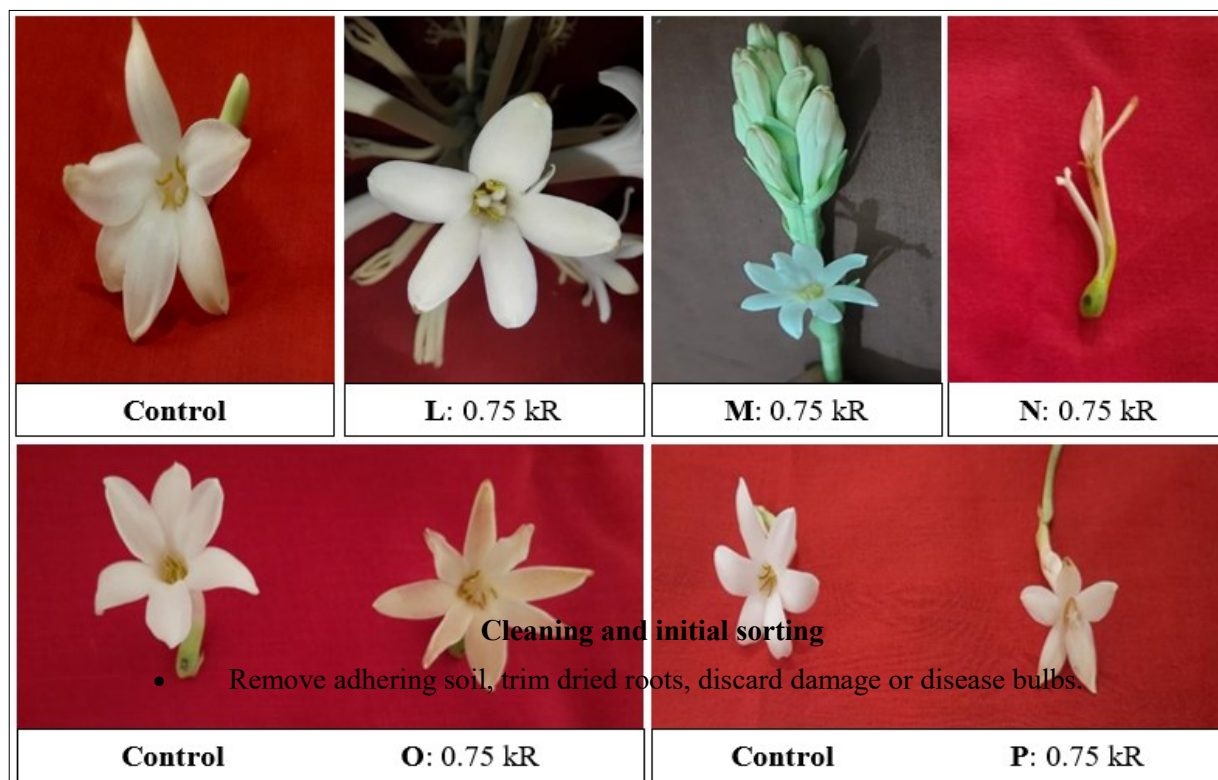


Plate 4. Identified mutants of tuberose variety Shringar through different gamma doses.

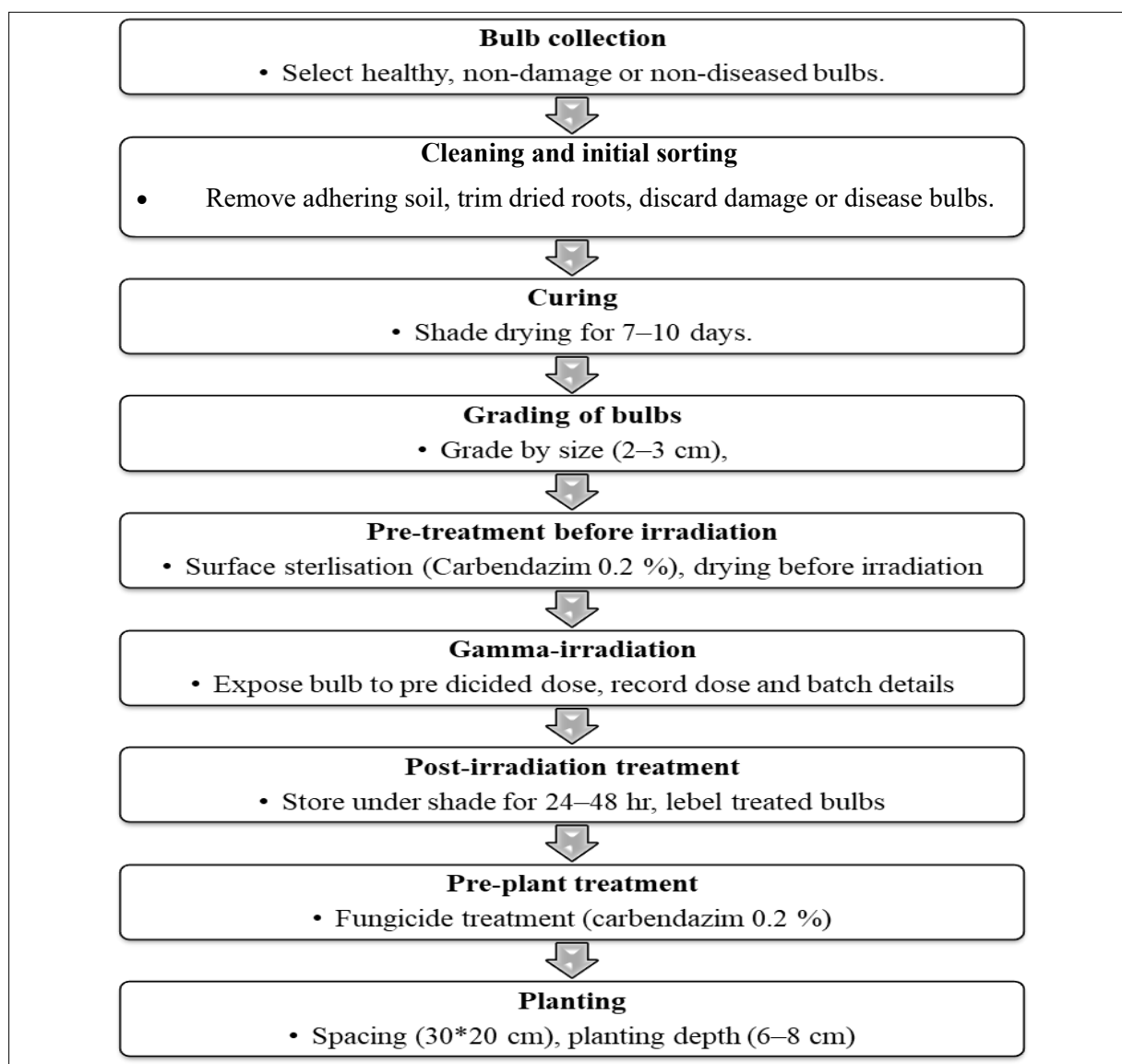


Fig. 1. Schematic flow diagram of the gamma-irradiation treatment of the tuberose.

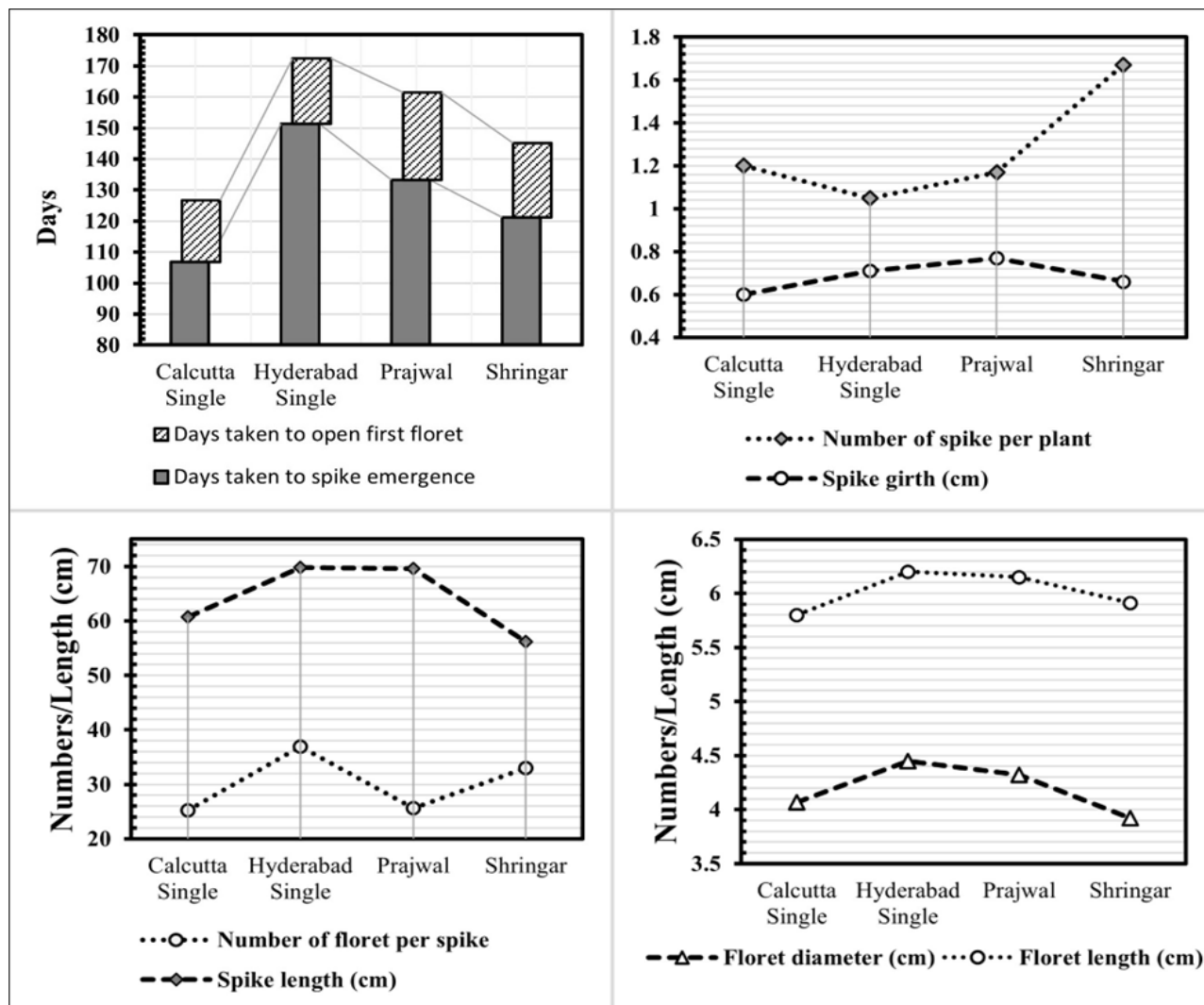


Fig. 2. Effect of the different varieties on flowering parameters of tuberose.

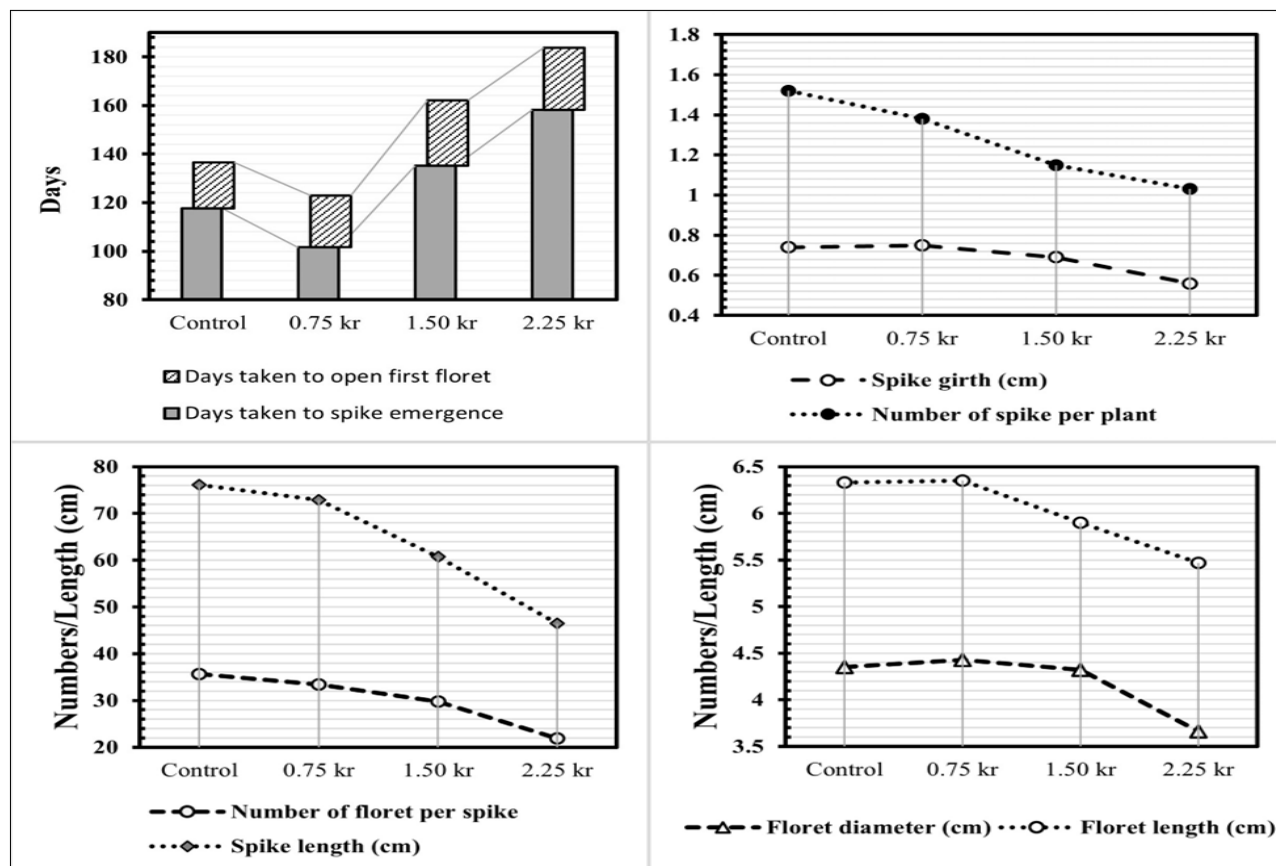


Fig. 3. Effect of gamma irradiation on the flowering of different varieties of tuberose.

(5.47 cm). Notably, the lethal dose G_5 (3.00 kR) led to complete mortality among plants by 50 days after planting across all the varieties. While sprouting patterns were similar to other doses, overall growth and development were considerably poor. As a result, the frequency of growth and development of the plants was drastically reduced, with not a single plant reaching the flowering phase.

Effect of interaction between variety and gamma irradiation on flowering parameters

The interaction between variety and gamma dose significantly affected all flowering parameters (Table 2). For earliness traits, the treatment combination V_4G_1 recorded the earliest spike emergence (75.80 days), while V_1G_2 showed the earliest first floret opening (96.60 days). Conversely, maximum delays were observed in V_4G_4 for spike emergence (170.80 days) and first floret opening (191.27 days). The superior interaction for floral quality was obtained with V_2G_2 , which produced maximum spike length (83.10 cm), spike girth (0.95 cm), florets per spike (45.00), floret diameter (4.89 cm) and floret length (6.64 cm). This treatment combination represents the optimal dose for V_2 , balancing mutagenic effects with excellent flowering performance. However, V_3G_1 recorded the longest spike length (83.25 cm), marginally exceeding V_2G_2 . Maximum spike production was achieved with V_4G_1 (2.53 spikes per plant), while several treatment combinations, including V_1G_2 , V_2G_3 , V_2G_4 , V_3G_1 , V_3G_4 and V_4G_4 , produced only one spike per plant. The most detrimental interaction effects were consistently observed with high-dose treatments, particularly V_1G_4 and V_4G_4 . Treatment V_1G_4 exhibited minimum spike length (38.19 cm), spike girth (0.54 cm) and floret length (4.63 cm), while V_4G_4 recorded minimum floret diameter (3.17 cm). The combination V_3G_4 produced the least number of florets per spike (15.13).

Identification of mutants and mutation frequency (%)

Gamma irradiation induced 16 distinct visible morphological mutants that were identified across all 600 treated bulbs of 4 varieties of tuberose. The mutation spectrum varied among varieties: V_1 produced 5 mutants, V_2 produced 3 mutants, V_3 produced 3 mutants and V_4 produced 5 mutants (Table 1).

The overall mutation frequency was observed to be 2.67 %. This frequency demonstrates that gamma irradiation is an effective tool for generating genetic variability in tuberose. The mutation induction response showed a clear dose-dependent relationship. The lowest gamma irradiation dose at 0.75 kR induced the highest number of mutations (11 mutants) with a mutation frequency of 9.16 %. This dose emerged as the optimal level for mutation breeding in tuberose, balancing effective mutation induction with acceptable plant survival and vigour. As gamma irradiation dose increased mutation frequency progressively declines. At 1.50 kR (4 mutants) and 2.25 kR (1 mutant) were identified with 3.33 % and 0.83 % of mutation frequency. The absence of irradiation dose in G_1 (control) confirms that mutation rates in untreated plants are negligible, while the absence of surviving plants at G_5 (3.00 kR) prevented evaluation of mutation frequency at lethal doses. This declining tendency of frequency suggests that higher radiation doses cause excessive cellular damage (Fig. 4).

Discussion

The varietal differences in radiation sensitivity emerged across all parameters (Fig. 2). The variety V_2 demonstrated superior performance in spike emergence, spike length, floret number and floret quality. In contrast, V_1 exhibited the poorest overall performance. These phenotypic differences reflect inherent genetic variation in radiation sensitivity, with varieties differing in DNA repair capacity and antioxidant enzyme expression.

The progressive delays in spike emergence and first floret opening (up to 55.8 % for spike emergence and 49.5 % for floret opening) has positive correlation with gamma dose are congruent with known effects of ionising radiation, which can disrupt cellular metabolism, delay cell division and prolong vegetative phases (11, 15). Reductions in spike length of up to 38.9 % have been observed, which correlates with decreased cell elongation due to disruptions in the auxin and gibberellin signaling pathways, as well as altered availability of photosynthetic assimilates. Additionally, there is a significant reduction in floret number (38.6 %), which

Table 2. Effect of interaction of varieties and gamma doses ($V \times G$) on flowering parameters of different tuberose varieties

Treatment	DTSE	DTOFF	NSPP	NFPS	SG	SL	FD	FL
V_1G_1	100.27	114.27	1.53	31.07	0.71	81.84	4.29	6.46
V_1G_2	84.27	96.60	1.00	26.13	0.60	75.11	4.33	6.45
V_1G_3	104.67	129.80	1.13	20.87	0.57	47.71	4.03	5.64
V_1G_4	138.33	165.87	1.13	22.67	0.54	38.19	3.62	4.63
V_1G_5	ND	ND	ND	ND	ND	ND	ND	ND
V_2G_1	142.40	159.67	1.00	37.27	0.66	79.49	4.44	6.54
V_2G_2	150.67	174.67	1.20	45.00	0.95	83.10	4.89	6.64
V_2G_3	144.13	167.00	1.00	37.00	0.66	61.91	4.60	5.85
V_2G_4	168.07	188.47	1.00	28.33	0.57	54.46	3.87	5.79
V_2G_5	ND	ND	ND	ND	ND	ND	ND	ND
V_3G_1	151.87	176.53	1.00	40.00	0.89	83.25	4.55	6.38
V_3G_2	76.80	100.87	1.47	24.53	0.71	69.79	4.28	6.31
V_3G_3	148.73	178.93	1.20	23.00	0.90	77.80	4.47	6.12
V_3G_4	155.80	189.60	1.00	15.13	0.58	47.39	3.97	5.77
V_3G_5	ND	ND	ND	ND	ND	ND	ND	ND
V_4G_1	75.80	97.40	2.53	34.40	0.72	59.76	4.13	5.94
V_4G_2	94.53	119.53	1.87	37.93	0.73	63.45	4.22	6.01
V_4G_3	143.40	172.67	1.27	38.07	0.63	55.50	4.17	6.01
V_4G_4	170.80	191.27	1.00	21.47	0.57	45.91	3.17	5.68
V_4G_5	ND	ND	ND	ND	ND	ND	ND	ND
S.Em+	8.22	9.56	0.11	2.37	0.04	3.74	0.12	0.16
CD at 5 %	23.74	27.61	0.31	6.84	0.10	10.79	0.34	0.48

DTSE: days taken to spike emergence; DTOFF: days taken to open first floret; FD: floret diameter; FL: floret length; ND: Not determined; NSPP: number of spikes per plant; NFPS: number of floret spikes; SG: spike girth; SL: spike length.

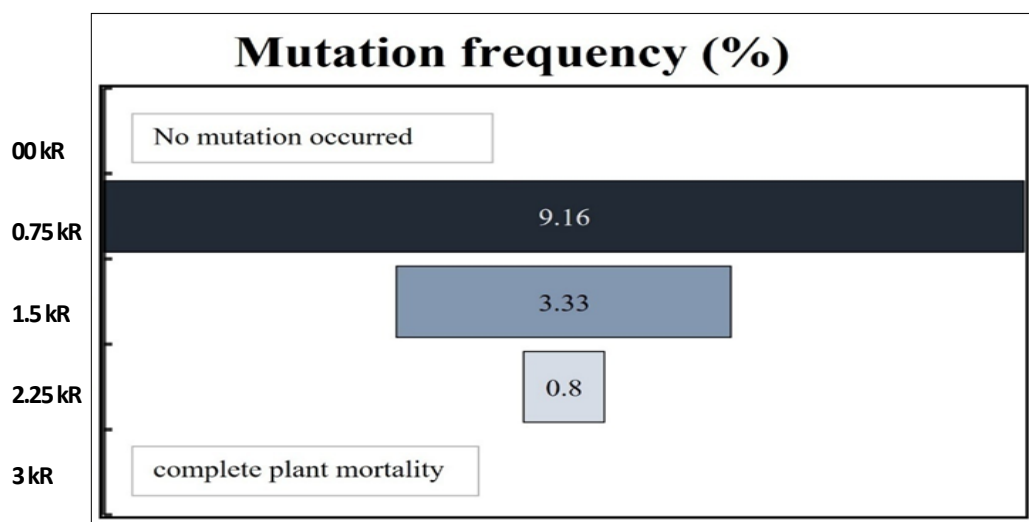


Fig. 4. Mutation frequency (%) induced by different gamma irradiation doses in 4 varieties of tuberose.

directly reflects a decrease in flower primordia initiation in developing meristems that are exposed to genotoxic stress. The floret size also decreased by 13.9 % at higher doses, indicating limitations in cell expansion that are associated with disrupted cell cycle progression and turgor regulation. These findings align with earlier researches on tuberose and chrysanthemum (16–20). It is noteworthy that spike girth and quality attributes reach their peak at lower treatment doses, suggesting a threshold; beyond this point, the benefits of mutagenic stimulation are outweighed by toxic effects.

Higher gamma irradiation doses (G_4 at 2.25 kR and G_5 at 3.0 kR) consistently suppressed flowering quality and quantity across all measured parameters. These treatments produced the minimum spike length (46.49 cm at G_4), delayed spike emergence, extended days to first floret opening (183.80 days at G_4) and markedly reduced floret number per spike (21.90 florets at G_4 compared to 35.68 in the control). The dose-dependent deterioration in flowering attributes reflects severe cytogenetic damage, including chromosomal aberrations, DNA strand breaks and disruption of cell division patterns at the meristematic level. Similar dose-response relationships have been extensively documented in gladiolus (20–22) and chrysanthemum (23, 24), where higher radiation exposures consistently resulted in stunted inflorescence development and reduced floral abundance. The mechanism of plant mortality involves damage to cellular components such as DNA, leading to reduced cell division and growth. Increased damage and impaired photosynthesis (25). Higher doses cause geoelectrolyte leakage and decreased chlorophyll content indicate membrane netic mutations that disrupt normal development, resulting in stunted growth or death (26).

The significant interaction effects between varieties and gamma doses (F -value significance at 5 % level) confirm that radiation response patterns are genotype-dependent, indicating differential activation of stress-responsive pathways and variable capacity to tolerate or compensate for genotoxic stress (Table 2). The interaction data ($V \times G$) demonstrated that V_2G_2 (Hyderabad single at 0.75 kR) achieved superior performance across multiple parameters, viz. spike length, spike girth, florets per spike and floret diameter. Conversely, both the treatments V_1G_4 and V_4G_4 demonstrated the poorest performance. V_1G_4 shows minimum spike length, floret diameter and V_4G_4 shows minimum floret diameter. The systematic patterns in these interactions indicate that genetic background

determines the interaction trajectory with radiation dose: variety with inherent radiation tolerance V_2 is suitable for the induction of genetic variability with respect of enhance desirable traits, while the V_1 radiation-sensitive variety shows steeper performance declines at elevated doses. Authors suggested that V_2 can be used for future mutation breeding program and stable mutants can also be used for hybridisation as a parental line.

Conclusion

This study establishes 0.75 kilorad (kR) as the optimal gamma irradiation dose for inducing maximum mutations (9.16 % mutation frequency) with acceptable plant survival in tuberose. The variety Hyderabad single (V_2) exhibited superior flowering performance. Sixteen morphologically distinct mutants (6 in Calcutta single (V_1), 3 in V_2 , 3 in V_3 and 5 in V_4) were recovered at doses of 0.75–2.25 kR, while 3.0 kR proved lethal across all varieties.

Acknowledgements

The authors are highly thankful to the Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh, for providing funds to conduct this research.

Authors' contributions

AM conducted the experiment, collected data, performed the analysis, and drafted the manuscript. AK designed the methodology, supervised the study, interpreted the results, and contributed to the discussion section. JK and RG participated in the review and editing of the manuscript. MP and RC contributed to the introduction and reference sections. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Grammarly and QuillBot for grammar correction in the manuscript. After using this tool, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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