



RESEARCH ARTICLE

Enhancing genetic variability in *Solanum surattense* (Burm.f.) through EMS and gamma ray-induced mutagenesis

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Abstract

Solanum surattense Burm.f. is an important medicinal plant with limited genetic variability due to presence of heavy thorns. The objective of this study represents the first report on the induction of mutation in *Solanum surattense* Burm.f. using EMS and gamma ray irradiation to enhance its genetic variation. A total of 100 pre-soaked Kantakari seeds were treated with varying concentrations of EMS (0.2 %, 0.4 %, 0.6 %, 0.8 % and 1 %), gamma rays (50 Gy, 100 Gy, 150 Gy, 200 Gy and 250 Gy) and their combined treatments (50 Gy + 0.2 % EMS, 100 Gy + 0.4 % EMS, 50 Gy + 0.6 % EMS, 200 Gy + 0.8 % EMS and 250 Gy + 1 % EMS). The results revealed a progressive decline in germination rate, survival percentage, root length (cm) and shoot length (cm) with increasing doses of both individual and combined mutagens in the M₁ generation. The LD₅₀ values were determined as 0.8 % EMS (47.48 %), 200 Gy gamma rays (46.47 %) and a combination treatment of 0.4 % EMS + 100 Gy (53.54 %). Mutation breeding using gamma rays, EMS and their combinations significantly influenced growth and yield parameters, in addition to, reducing thorn density. Among the tested treatments, the combination of 0.4 % EMS + 100 Gy (6.23, 5.42) and 200 Gy gamma rays (8.69, 7.31) was the most effective in reducing the number of thorns on both the upper and lower surfaces of the leaves. These findings suggest that mutation breeding through gamma irradiation and combination treatments holds great potential for developing high-yielding, low-thorn mutants of Kantakari, making it a valuable approach for future crop improvement programs.

Keywords: EMS; gamma ray irradiation; germination percentage; induced mutation; lethal dose; *Solanum surattense* (Kantakari)

Introduction

Solanum surattense Burm.f. (syn. *Solanum xanthocarpum* Schard. and *Solanum virgianum* L.) (2n = 24) belongs to the family Solanaceae. It is an important medicinal plant in Indian traditional systems of medicine. It is commonly known as yellow berried nightshade (1). The species is majorly distributed in dry tracts of the world, especially in Southeast Asia (2). Major chemical constituents of *Solanum surattense* are β-carotene, diosgenin, solasodine and solamargine (3, 4). Due to its well-known ethnobotanical properties, this plant has attracted researchers from around the world. In the ayurvedic system of medicine, Kantakari is described as pungent, bitter, digestive and alternative astringent. Fruits are edible and used in traditional medicines to treat a variety of illnesses (5, 6).

Currently, germplasm collection involves harvesting plants from wild habitats. However, the presence of heavy thorns poses a major challenge in germplasm collection. Moreover,

these densely distributed thorns across all plant parts hinder commercial cultivation, despite the availability of high-yielding genotypes in this crop (7). Efforts to develop thornless varieties in Kantakari has been attempted through conventional crop improvement techniques such as selection and hybridization. As the natural variability in this crop is very low, mutation breeding has been resorted to generate variability using physical and chemical mutagens (8, 9). The dose of the mutagen that induces a higher rate of mutations in the treated samples with less biological damage is considered to be optimal. Hence, determining the lethal dose that causes 50 % seed mortality, or the tolerable dose at which 50 % of the seeds survive (LD₅₀), is a prerequisite in mutation breeding. With this objective, the present study aimed to evaluate different doses of physical and chemical mutagens, as well as their combination, i.e., gamma-ray irradiation, EMS and EMS + gamma-rays to determine the optimal dose of mutagens to induce the desired variability in Kantakari crop more effectively.

Materials and Methods

The present study was conducted in the Department of Medicinal and Aromatic Crops, Tamil Nadu Agricultural University (TNAU), during 2023–2024. In a natural population, a high-yielding Kantakari accession (Ss-13) with high thorn density was identified. Berries were collected, seeds were extracted and used for conducting the experiment. Seeds were subjected to different doses of gamma rays (50–250 Gy) at the gamma chamber of Department of Genetics and Plant Breeding, TNAU, Coimbatore, where Cobalt- 60 served as source of gamma rays. The chemical ethyl methane sulphonate (EMS) was purchased from Sigma Chemical Company, Coimbatore, Tamil Nadu, India and solutions of varying concentrations (0.2 %, 0.4 %, 0.6 %, 0.8 % and 1 %) were prepared using freshly prepared phosphate buffer (pH 7.0) for seed treatment. Combination treatments of EMS and gamma rays were also administered as follows: 50 Gy + 0.2 % EMS, 100 Gy + 0.4 % EMS, 50 Gy + 0.6 % EMS, 200 Gy + 0.8 % EMS and 250 Gy + 1 % EMS. The experiment consisted of a total of 15 treatments, including a control, with three replications each.

Construction of the kill curve

The 'kill curve' analysis was carried out following the documented procedures (10) with slight modifications (11). For physical mutagenesis, dry seeds were irradiated with 50 Gy, 100 Gy, 150 Gy, 200 Gy and 250 Gy gamma rays. For chemical mutagenesis, the seeds were first pre-soaked in water for 12 hr to enhance the mutagen uptake. After pre-soaking, the seeds were treated with EMS at varying concentrations of 0.2 %, 0.4 %, 0.6 %, 0.8 % and 1 % for 8 hr at a constant temperature of 25 ± 2 °C. For combination treatments, the Kantakari seeds were initially pre-soaked in water for 12 hr before being exposed to gamma irradiation at doses of 50 Gy, 100 Gy, 150 Gy, 200 Gy and 250 Gy. Following irradiation, these pre-soaked and irradiated seeds were further subjected to EMS treatment at the same concentration levels (0.2 %, 0.4 %, 0.6 %, 0.8 % and 1 %) for 8 hr at 25 ± 2 °C. The combined treatments were designated as follows: 50 Gy + 0.2 % EMS, 100 Gy + 0.4 % EMS, 50 Gy + 0.6 % EMS, 200 Gy + 0.8 % EMS and 250 Gy + 1 % EMS. After EMS treatment, all seeds were thoroughly washed under running water for at least an hour to remove any residual mutagen before sowing. Treated seeds were sown separately in the trial using 100 seeds each in three replications along with parental controls (non-treated seeds) in pots. Germination count was taken on the 7th day after sowing. Morphological observations on survival percentage, root length (cm) and shoot length (cm) were recorded on the 14th days of germination in pots. The percentage of M_1 plants emerging in the pots were averaged over three replications and reduction in germination was calculated to determine the LD_{50} (46.47, 47.48 and 53.54) (12).

When the seedlings reached the 4 to 5 leaf stage, they were transplanted to a well-prepared experimental field following a randomized block design (RBD) with three replications for evaluating both morphological and yield traits. A spacing of 60 cm × 60 cm was maintained between rows and between plants. The M_1 generation was raised following agronomic practices for field preparation, sowing and crop management. Data were collected from five randomly selected plants per treatment in each replication for plant height (cm),

number of branches per plant, number of thorns on upper side of the leaf and lower side of the leaf, days to fifty per cent flowering, number of flower clusters per branch, number of berries per plant, fresh single berry weight (g), dry single berry weight (g), number of seeds per berry and fresh berry yield per plant (g) and dry berry yield per plant (g). Per-plant values were computed based on average data. Analysis of variance (ANOVA) was performed using the SPSS 21 statistical software package (IBM Corp., Armonk, NY, USA) at a significance level of $p \leq 0.05$. Pearson's correlation (r) analysis was conducted to assess the relationships between variables and simple regression analyses were performed to evaluate the effects of independent variables on the dependent variable (13).

Results and Discussion

LD_{50} dose

Evaluating the effects of mutagens observable in the M_1 generation is a common procedure in radiation breeding programmes. Research indicates that physical and chemical mutagens can induce physiological damage (injury), gene mutations (point mutations) and chromosomal mutations (chromosomal aberrations) in the biological material of the M_1 generation (14). Analysing morphological variations in M_1 is useful for predicting the efficiency of mutagens and identifying desirable mutants.

Estimation of the lethal dose (LD_{50}) values revealed an increase in lethality with an increase in the dose intensity of EMS, gamma ray and combination treatments (Fig. 1). The effect of different concentrations of EMS and gamma ray treatments, based on the pooled means of control and mutagen-treated seeds along with correlation and regression analyses revealed that the variance due to concentration was significant, indicating the existence of a wide range of variation among the treatments. Seed germination percentage was significantly and negatively correlated with the concentrations of gamma radiation, EMS and combination treatments. The LD_{50} for Kantakari was estimated to be 200 Gy for gamma radiation (46.47), 08. % for EMS (47.48) and 100 Gy + 0.4 % EMS for the combination treatment (53.54) (Table 1).

Seed germination and seedling survival percentage

The germination percent of treated seeds serves as a key indicator of mutagenic effectiveness, with values below 50 % considered lethal or undesirable. Assessing seed germination inhibition following exposure to different mutagens is a practical method for evaluating their impact on plant growth and development. A gradual decline in germination was observed with increasing concentrations of gamma radiation, EMS and their combination (86-31 %, 87-34 %, 54-18 %) (Table 1). This is likely due to the fact that short-wave photons (i.e., gamma-rays) are more energetic than visible light photons (> 400 nm) and therefore have a stronger effect on surface of plant cells. This leads to the ultimate breakdown of seed coat, thereby facilitating germination, as reported in an earlier study (15). The highest germination and survival rates were recorded in seeds treated with 0.2 % EMS (87 % and 82 % respectively), followed by 50 Gy gamma radiation alone (86 % and 75 %) and the combined treatment of 50 Gy + 0.2 % EMS (54 % and 48 %).

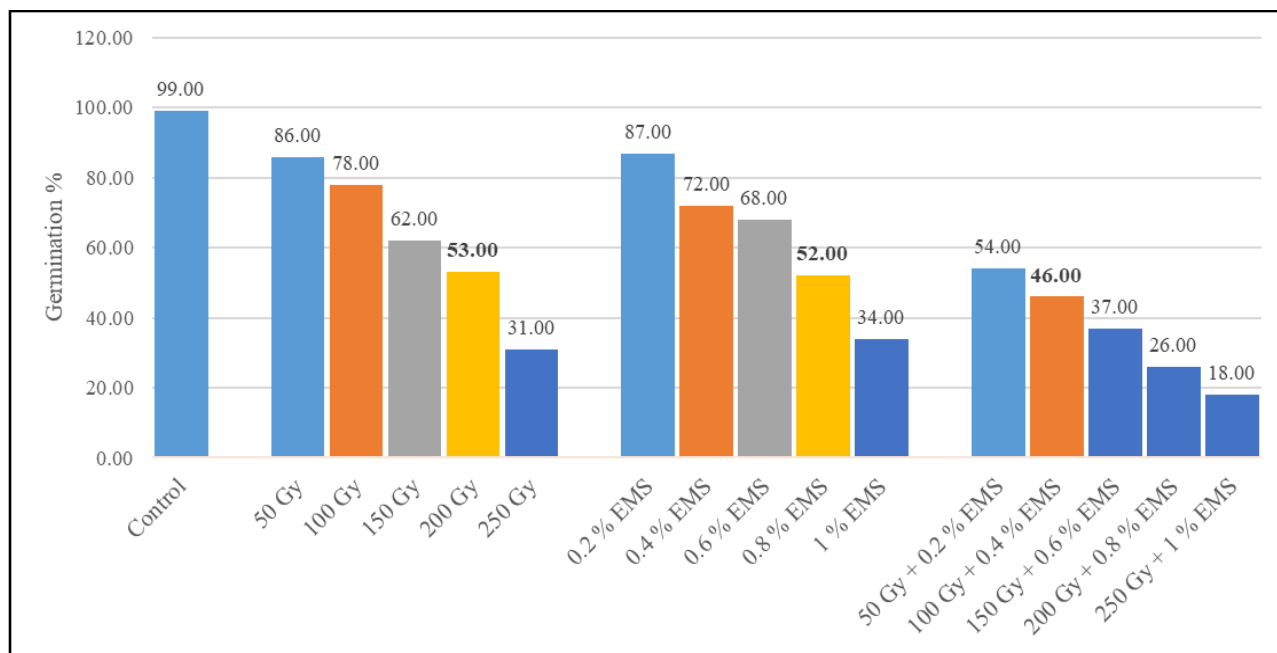


Fig. 1. Determination of the lethal dose (LD₅₀) of *Solanum surattense* Burm.f. to gamma rays and EMS.

A similar study reported that a 100 Gy dose reduced germination rates by approximately 50 % across five eggplant genotypes (16). Our results are consistent with previous studies reporting reduced germination due to the inhibitory effects of chemical mutagens in tomato and fenugreek (17–20). The decrease in germination percentage among treated seeds may result from mutagen-induced disruptions in genetic and physiological processes essential for germination. These disruptions may involve altered enzyme activity, mitotic inhibition and hormonal imbalance (21). The decline in plant survival is attributed to cytogenetic damage, physiological disturbances and interference of mutagens with critical metabolic pathways (22). Additionally, reduction of root length and shoot length were observed in the 0.2 % EMS treatment (3.12 cm and 6.94 cm), followed by 50 Gy gamma radiation alone (3.09 cm and 6.12 cm) and the combined treatment of 50 Gy + 0.2 % EMS (3.10 cm and 6.06 cm) (Fig. 2). Similar reductions in root and shoot length following mutagenic

treatments have also been reported in previous studies on fenugreek (23) and cowpea (24).

Correlation and regression analysis

Simple correlation coefficients (*r*) and regression equations were worked out to study the extent and type of relationship between survival percentage and germination percentage, root length and shoot length. The analysis revealed that survival percentage of Kantakari was significantly and positively correlated with germination percentage, root length and shoot length (Fig. 3-5). Since the correlation coefficients were highly significant, a linear relationship was observed between survival percentage and the other parameters. The regression coefficients were worked out to estimate the quantum of change in survival percentage with the unit change in other parameters. Regression analysis indicated that the survival percentage of mutagen-treated Kantakari seeds was significantly influenced by germination percentage, root length and shoot length as observed in similar studies (25, 26).

Table 1. Effect of mutagens on the lethality (LD₅₀), germination %, survival %, root length (cm) and shoot length (cm) of *Solanum surattense* Burm.f. seeds

Mutagenic treatments	Total treated seeds	Germination (%)	Lethality	Survival (%)	Root length (cm)	Shoot length (cm)
Control (C)	100	99.00 (84.26)	--	95.00 (77.07)	3.54	8.24
Gamma rays						
50 Gy	100	86.00 (68.02)	13.14	75.00 (60.00)	3.09	6.12
100 Gy	100	78.00 (62.02)	21.22	60.00 (50.76)	2.18	5.98
150 Gy	100	62.00 (51.94)	37.38	58.00 (49.60)	1.98	5.12
200 Gy	100	53.00 (46.71)	46.47	44.00 (41.55)	1.52	4.06
250 Gy	100	31.00 (33.83)	68.69	28.00 (31.94)	1.11	3.38
Ethyl Methane Sulfonate (EMS)						
0.2 % EMS	100	87.00 (68.86)	12.13	82.00 (64.89)	3.12	6.94
0.4 % EMS	100	72.00 (58.05)	27.28	68.00 (55.55)	2.54	6.08
0.6 % EMS	100	68.00 (55.55)	31.32	60.00 (50.76)	2.02	5.42
0.8 % EMS	100	52.00 (46.14)	47.48	46.00 (42.70)	1.38	4.69
1 % EMS	100	34.00 (35.66)	65.66	27.00 (31.30)	1.25	3.10
Combination treatments (Gamma rays + EMS)						
50 Gy + 0.2 % EMS	100	54.00 (47.29)	45.46	48.00 (43.85)	3.10	6.06
100 Gy + 0.4 % EMS	100	46.00 (42.70)	53.54	32.00 (34.44)	2.88	5.25
150 Gy + 0.6 % EMS	100	37.00 (37.46)	62.63	25.00 (30.00)	2.14	4.88
200 Gy + 0.8 % EMS	100	26.00 (30.65)	73.74	20.00 (26.56)	1.12	4.01
250 Gy + 1 % EMS	100	18.00 (25.10)	81.82	12.00 (20.26)	1.08	3.94
SE (m)		0.024		0.027	0.003	0.003
CD @ 5 %		0.072		0.083	0.011	0.011

**SE (m)- Standard error of the mean and CD- Critical difference at the 5 % level of significance.



Fig. 2. Effect of physical and chemical mutagens on the root and shoot length (cm) of *Solanum surattense* Burm.f. seeds.

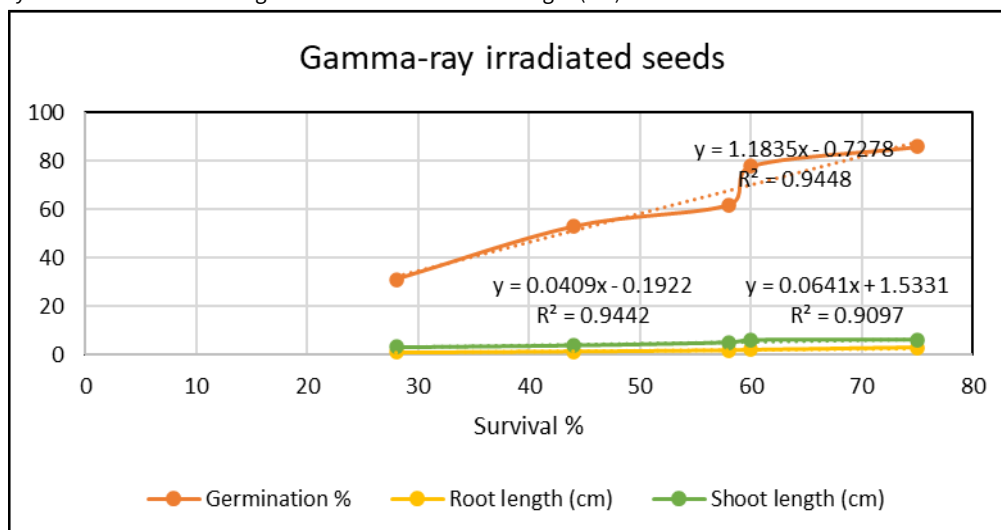


Fig. 3. Scatter plot and regression equation for the survival percentage of gamma ray-irradiated seeds of *Solanum surattense* Burm.f. with germination percentage, root length (cm) and shoot length (cm).

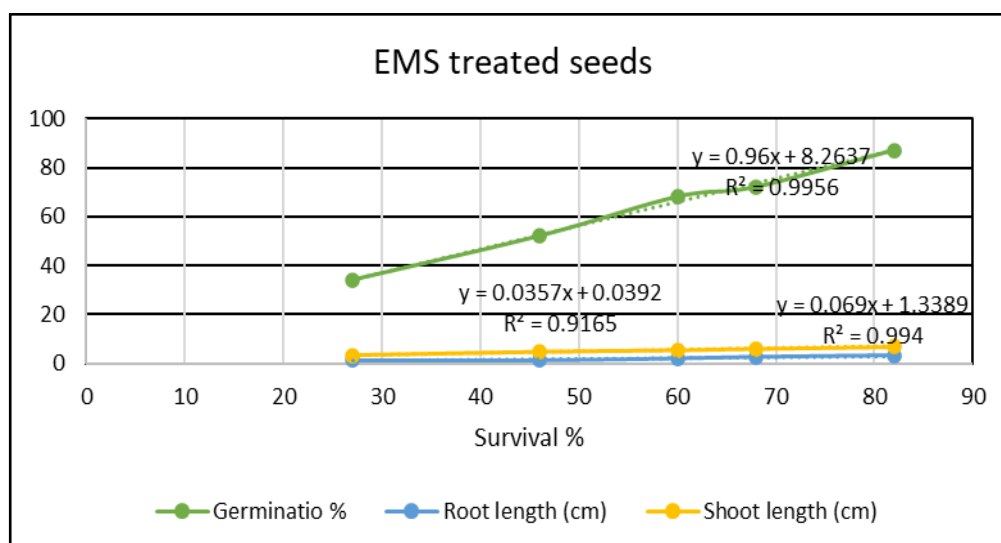


Fig. 4. Scatter plot and regression equation for the survival percentage of EMS treated seeds of *Solanum surattense* Burm.f. with germination percentage, root length (cm) and shoot length (cm).

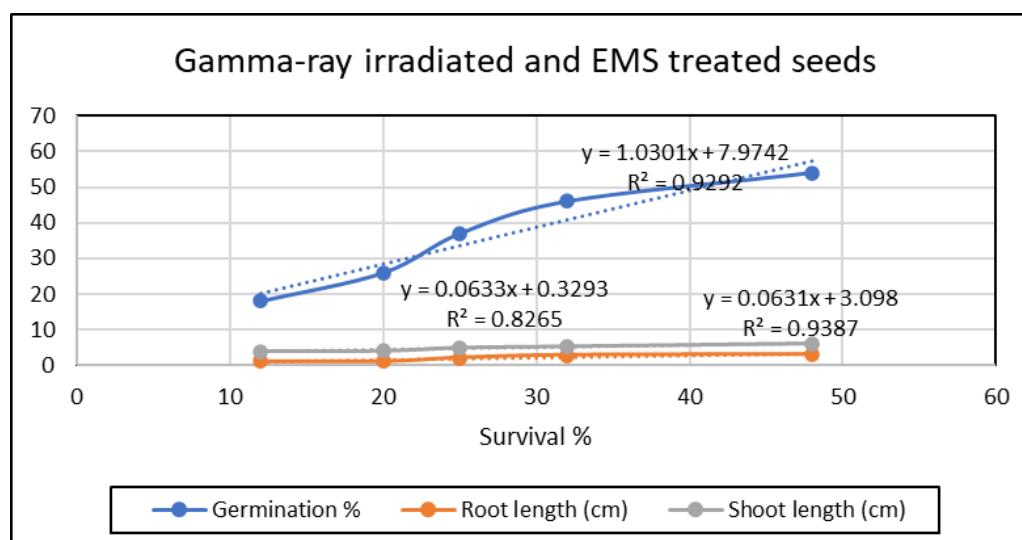


Fig. 5. Scatter plot and regression equation for the survival percentage of combinations of both EMS and gamma ray-irradiated seeds of *Solanum surattense* Burm.f. with germination percentage, root length (cm) and shoot length (cm).

Mutagen effect on growth attributes

Mutagenic treatment of Kantakari accession Ss-13 revealed significant differences in all the growth attributes studied (Table 2). An increase in plant height was observed under gamma rays and combination treatments compared to EMS treatment and the control. Maximum plant height was recorded under 100 Gy + 0.4 % EMS (29.25 cm) and 200 Gy (27.87 cm) respectively (Fig. 6). The number of branches increased by 10.22 and 8.94 under combination treatment (100 Gy + 0.4 % EMS) and gamma rays (200 Gy) respectively compared to EMS and the control. These results are consistent with a previous study in okra, where mutagenic treatment at 500 Gy resulted in maximum plant height (27). A similar trend was also reported in another study (28). The number of thorns on both the upper and lower leaf surfaces gradually reduced with increasing concentrations of mutagens. Both the combination treatment (100 Gy + 0.4 % EMS) and gamma rays

(200 Gy) recorded lesser number of thorns (6.23 and 8.69; 5.42 and 7.3 respectively) compared to EMS and the control (Fig. 7). Additionally, the combination treatment (100 Gy + 0.4 % EMS) and gamma rays (200 Gy) induced early flowering by approximately 28 and 30 days respectively, compared to the control. The number of flower clusters contributing for fruit formation and yield were also maximum in both combination treatments (24.57, 20.79) and gamma rays (21.61, 18.48) respectively over the control. A wide variation in flower colour, ranging from dark purple to purplish white, was observed, indicating the influence of mutagens in altering floral traits (Fig. 8), which may further impact fruiting and yield potential. Similar observations were reported in previous studies on eggplant (29), sesamum (30) and legumes (31), where mutagenic treatments induced significant alterations in flower morphology and pigmentation, highlighting their potential for generating novel variations beneficial for crop improvement.

Table 2. Mean performance of mutagens on different characters in the M_1 generation

Mutagenic treatments	PH	NB	NTUSL	NTLSL	DFF	NFCPB	NBPP	FSBW	DSBW	NSPB	FBYPP	DBYPP
Control	23.79 (0.024)	5.29 (0.082)	19.74 (0.020)	17.22 (0.170)	67.37 (0.527)	12.49 (0.204)	35.18 (0.538)	1.95 (0.017)	0.92 (0.014)	585.15 (9.556)	108.54 (1.109)	48.94 (1.109)
Gamma rays												
150 Gy	24.25 (0.288)	7.15 (0.068)	11.23 (0.111)	10.18 (0.007)	48.78 (0.012)	18.48 (0.125)	72.58 (0.225)	3.45 (0.034)	1.98 (0.004)	352.25 (2.876)	251.33 (4.191)	85.82 (4.191)
200 Gy	27.87 (0.075)	8.94 (0.129)	8.69 (0.095)	7.31 (0.093)	39.55 (0.187)	21.61 (0.037)	83.65 (0.340)	4.18 (0.024)	2.35 (0.011)	298.86 (2.136)	349.65 (1.069)	208.55 (1.069)
Ethyl Methane Sulfonate (EMS)												
0.6 %	23.66 (0.170)	5.83 (0.050)	19.26 (0.184)	17.08 (0.075)	57.25 (0.681)	15.30 (0.061)	41.98 (0.671)	2.40 (0.030)	1.05 (0.009)	516.54 (1.928)	120.15 (1.962)	57.87 (1.962)
0.8 %	23.83 (0.381)	6.45 (0.071)	18.40 (0.068)	16.57 (0.152)	51.79 (0.510)	16.14 (0.170)	54.32 (0.554)	2.91 (0.003)	1.24 (0.020)	426.78 (3.341)	162.81 (1.218)	69.25 (2.18)
Combination treatments (Gamma rays + EMS)												
50 Gy + 0.2 %	27.66 (0.370)	8.45 (0.129)	10.73 (0.109)	9.07 (0.093)	42.53 (0.043)	20.79 (0.347)	80.12 (1.282)	3.97 (0.048)	2.08 (0.030)	310.99 (2.962)	318.07 (2.274)	118.93 (2.274)
100 Gy + 0.4 %	29.25 (0.030)	10.22 (0.027)	6.23 (0.065)	5.42 (0.064)	37.26 (0.191)	24.57 (0.100)	92.43 (0.724)	4.74 (0.023)	2.66 (0.018)	280.25 (4.386)	428.11 (4.369)	220.15 (4.369)
CD @ 5 %	0.687	0.275	0.332	0.320	1.143	0.525	2.082	0.091	0.050	13.187	7.843	7.843
SE (m)	0.229	0.092	0.111	0.107	0.382	0.175	0.695	0.030	0.017	4.404	2.619	2.619
SE (d)	0.324	0.130	0.157	0.151	0.540	0.248	0.983	0.043	0.023	6.228	3.704	3.704
CV	1.781	2.455	1.647	1.803	1.551	1.899	2.113	1.808	1.887	2.225	2.109	2.109

** PH- Plant height (cm), NB- Number of branches per plant, NTUSL- Number of thorns on upper side of the leaf, NTLSL- Number of thorns on lower side of the leaf, DFF- Days to fifty percent flowering, NFCPB- Number of flower clusters per branch, NBPP- Number of berries per plant, FSBW- Fresh single berry weight (g), DSBW- Dry single berry weight (g), NSPB- Number of seeds per berry, FBYPP- Fresh berry yield per plant (g), DBYPP- Dry berry yield per plant (g).



Fig. 6. Effect of mutagens on plant height of *Solanum surattense* Burm.f.: a) Control; b) 200 Gy; c) 0.8 % EMS; d) Combination treatment (100 Gy + 0.4 % EMS).

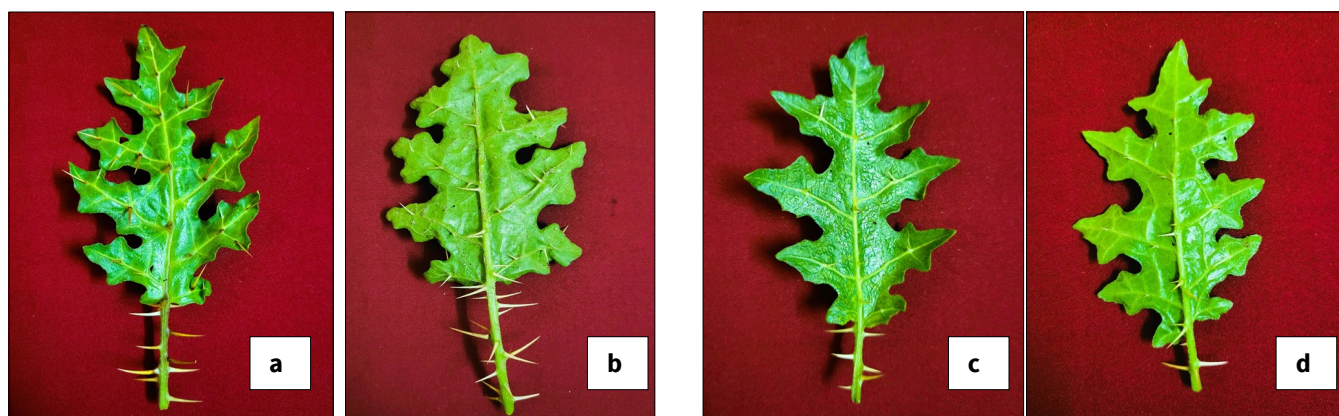


Fig. 7. Effect of mutagens on thorns in *Solanum surattense* Burm.f. leaves: a) number of thorns on the upper surface (control); b) number of thorns on the lower surface (control); c) number of thorns on the upper surface (mutated); d) number of thorns on lower surface (mutated).



Fig. 8. Effect of mutagens on flower color in *Solanum surattense* Burm.f.: a) Control; b) 0.8 % EMS; c) 200 Gy gamma ray; d) Combination treatment (100 Gy + 0.4 % EMS).

The results of the present study reveal that gamma rays, either alone or in combination with EMS, were comparatively more effective than EMS treatment alone in inducing favourable mutations. This finding is supported by an earlier study which reported that gamma ray applications were more effective (32). Similarly, previous research concluded that all growth parameters showed a positive response to both physical and chemical mutagens (33-35).

Mutagen effect on yield attributes

The M_1 generation exhibited significant differences in yield parameters (Table 2). The number of berries per plant was

higher under combination treatments (92.43, 80.12) and gamma ray treatments (83.65, 72.58) compared to the control, whereas only 6-10 % improvement was observed in EMS treatments. Fresh and dry single berry weight increased by 4.74, 4.18 g and 2.66, 2.35 g respectively, under combination treatments and gamma rays compared to the control. Similar trends were observed in total fresh and dry berry yield, with increases of 428.11, 349.65 g and 220.11, 208.55 g respectively, over the control. The number of seeds per berry decreased with increasing concentrations of mutagens. Both the combination treatment (100 Gy + 0.4 % EMS) and gamma rays (200 Gy) recorded less number of seeds (280.25 and 298.86 respectively)

compared to EMS and the control. An increase in yield parameters such as the number of fruits per plant, number of branches per plant and fruit weight per plant, was observed as the concentration of gamma rays increased above 200 Gy. This result aligns with the findings of an earlier study in okra, where specific doses of gamma irradiation (100 Gy and 500 Gy) increased the number of branches and fruits per plant (27). Similarly, researchers reported positive responses in okra and tomato for all yield parameters when exposed to both physical and chemical mutagens (36, 37).

Conclusion

Mutation breeding is a promising approach for generating genetic variation and enhancing crop improvement in Kantakari (*Solanum surattense* Burm.f.). This study demonstrated a consistent decline in germination percentage with increasing doses of both individual mutagens and their combined treatments. The LD₅₀ dose was determined as 0.8 % EMS, 200 Gy gamma rays, or a combination treatment of 100 Gy gamma rays and 0.4 % EMS. Mutagenic treatments using gamma rays, EMS, or their combinations significantly influenced growth and yield traits, particularly reducing thorn density and exhibiting flower colour variation. Among the treatments tested, the combination of 100 Gy gamma rays and of 0.4 % EMS was found to be the most effective in reducing thorn numbers on both the upper and lower surfaces of the leaves. Therefore, mutation breeding through gamma irradiation and combination treatments holds great potential for developing high-yielding, low-thorn mutants of Kantakari for future breeding programs.

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Authors' contributions

NL conceived the idea and acquired resources for the experiments. SP conducted the experiment and prepared the first draft of the manuscript. NL and GS generated source materials, monitored the experiment edited and finalized the manuscript. ST, MBN and CK reviewed the manuscript. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical issues: None

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