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RESEARCH ARTICLE

Effect of physical and chemical mutagens on the growth performance of two soybean (TGX 1987-62F and TGX 1835-10E) varieties in the M_1 generation

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Abstract

Mutational breeding is a cost-effective and time-efficient technique that enhances genetic variability in crops by inducing mutations using physical agents like gamma rays or chemical agents like sodium azide, without involving genetic engineering. The aim of the study was to analyse the effect of gamma radiation and sodium azide (SA) on the growth performance of two soybean varieties (TGX 1987-62F and TGX 1835-10E). Growth parameters included germination percentage, plant height, number of leaves per plant, number of pods per plant, days to 50 % flowering, chlorophyll content, 100-seed weight and total grain yield. The experiment included 20 treatments and was laid out in a randomized complete block design (RCBD). Statistical analysis showed that among the two varieties, the highest rate of germination (60.0 % seedling/plot) was found in TGX 1835 (15Gy) while, 19.67 % seedling/plot was observed at 0.0.2 % SA. Average plant height (64.40 cm) was observed in TGX 1835 irradiated with 10Gy, as compared to the controls (TGX 1987: 55.46 cm and TGX 1835: 60.93 cm). Highest average leaf count in TGX 1987 was 125.67 leaves/plant, irradiated with 10Gy while, 183.33 leaves/plant, treated with 0.02 % SA. The highest total leaf area of 163.19 cm^2 was recorded in TGX 1835 in M1 (mutation 1 generation) plants and 163.56 cm^2, treated with 0.02 % SA. The gamma ray's treatment at 10Gy in TGX 1835 affirmed its potential in generating highest yield in M1 plants. Future breeding programs should focus on stabilizing these mutants (M1 and subsequent generations) to develop commercially viable high-yield varieties, for food security.

Keywords: gamma rays; *Glycine max*; mutation; physical mutagens; sodium azide

Introduction

The steadily growing global population is expected to surpass 9 billion by 2050 which presents a major challenge to food security. To meet this rising demand, crop production must be increased by 50-60 % over the next 25 years to feed the teeming millions (1). Heritable changes in a plant's genetic makeup that occur at the gene or chromosomal level are referred to as plant mutations. Mutation breeding serves as an effective strategy for enhancing both quality and yield-related traits in crops. Quality improvements include better disease resistance, superior fruit characteristics, early maturation and greater tolerance to stress conditions such as salinity, cold and heat. On the other hand, quantitative gains involve increases in seed yield, seed weight, total biomass and oil content. These genetic alterations act as powerful tools in modern crop improvement efforts. Thus, mutations are crucial for the genetic advancement of crops that are significant to the economy (2, 3). Mutation breeding, sometimes referred to as variant breeding, is a process that uses physical or chemical means to produce spontaneous genetic diversity in order to create new crop types. Mutation breeding is a simple, reliable, cost-effective, non-hazardous method for developing quality trait which are heritable. Nevertheless, this technique is not free from constraints, which include stringent screening of mutants exhibiting the desired characteristics, possibility of unpredictable random genetic recombination. Moreover, the effectiveness of this process relies on desirable genetic traits being present in the gene pool (4, 5). Mutagens are mainly classified into physical and chemical types. Physical mutagens include ionizing radiation like alpha, beta and gamma rays and non-ionizing ones like UV rays. Chemical mutagens involve agents such as alkylating compounds (e.g., EMS, MMS), deaminating agents (e.g., nitrous acid) and base analogues. Mutation breeding, using these mutagens, has been instrumental in enhancing crop traits, improving yields and developing better varieties, especially through crossbreeding programs (6, 7).

Soybean (*Glycine max* L. Merrill), commonly known as "golden bean" and "miracle crop" consists of 20 % high-quality oil, 40 % protein and 36-40 % starch and 8-10 % fibre (8). Moreover, it is a valuable source of vitamins A, B and D, as well as minerals such as calcium, iron, manganese, phosphorus, copper and thiamine (6-7 %) and phytochemicals (flavonoids, saponins, alkaloids, steroids and tannins), making it a versatile and health-promoting crop (9). Belonging to the Fabaceae family, soybeans are annual herbaceous plants reaching up to 1.5 m, with distinct leaf and flower structures, often covered in trichomes (10-13). Soybeans are widely used in the food industry for producing tofu, soy sauce, artificial milk, meat substitutes and cooking oil. The powdered form is used in animal feed, while industrial applications include functional foods, biofuels and products like yeasts, antibodies, soaps and disinfectants (14-16). Globally, over 90 % of soybean production comes from Brazil, the US, Argentina, China and India. Domesticated in eastern China between the 11th and 17th centuries BC, soybeans were introduced to the US in 1765 and Canada in 1893.

Mutation breeding enhances specific traits without altering the entire genome and morphological analysis is key to identifying favourable changes. Studies have shown that gamma rays, EMS, MMS and X-rays can induce beneficial mutations in crops like *Arachis hypogaea*, *Brassica napus*, mung bean, black gram, cowpea and pigeon pea improving traits such as germination, growth, leaf morphology and yield (17-21). However, while gene-editing tools like CRISPR/Cas9 offer precision and speed, they face limitations with complex traits and species lacking genomic data. EMS mutagenesis remains a powerful alternative, capable of generating diverse and desirable mutations across multiple loci. Sodium azide (NaN_3) is considered a potent chemical mutagen (induce point mutations) as it possesses high mutation efficiency, cause low chromosomal damage, remains effective at low concentrations and works well on seeds (barley: *Hordeum vulgare*, rice (*Oryza sativa*), wheat (*Triticum aestivum*), tomato (*Solanum lycopersicum*).

The selection of acceptable genotypes, evaluation of the selected genotypes and eventually multiplication and release of new cultivars are all steps involved in the process of plant breeding. The information base created by mutational breeding will help future users of mutation technology for crop development (22-24). Soybean production, due to its lower water requirements, can be an effective candidate for crop rotation regimes. The data generated from mutational breeding forms a crucial foundation for future use of mutation technology in crop development. This study investigates the impact of physical and chemical mutagens on two soybean varieties through field trials, aiming to explore their potential in generating genetic variability and identifying improved soybean varieties with higher yield potential.

Materials and Methods

Study area

The research was conducted at the Bayero University Fadama Teaching and Research Farm, located in Kano, Nigeria (11.58°N latitude and 8.33°E longitude), during the 2023 rainy season. This area falls within the Sudan Savanna Agro-ecological zone, characterized by erratic and limited rainfall, typically lasting for about five months (May to September), followed by a long dry period from October to April. The region has a semi-arid climate, with annual rainfall ranging from 550 to 850 mm. During the dry season, relative humidity varies between 21 % and 47 %, while it ranges from 51 % to 79 % during the rainy season. Temperatures fluctuate between 14 °C and 30 °C in the dry season and between 27 °C and 41 °C in the rainy season. A land area of 568 m^2 was subdivided into 60 plots (arranged in three columns of 20 plots each), each measuring 4.5 x 4 m^2, with a 0.75 m spacing between plots and a 0.5 m gap between replications to serve as irrigation channels. Before planting, the soil was ploughed, harrowed and ridged to eliminate debris, weeds and clods, which provides adequate aeration and moisture for crop growth. Seeds were sown in the third week of May 2023, promptly following rains, with a spacing of 75 cm along each ridge. The experimental design utilized a randomized complete block design (RCBD) with three replications. Each plot comprised of four ridges, separated by 0.37 m, with the two innermost ridges labelled as net plot. The row alleys were used for both destructive and non-destructive sampling, whereas the border rows were designated for discard sampling. Three seeds were manually sown per hole and at two weeks after-sowing (WAS), they were reduced to two plants per stand. Harvesting was manually done with a hoe and sickle and physiologically mature pods were gathered. The pods were collected and sundried to reduce moisture levels, followed by manual threshing to segregate the seeds from the chaff (25).

Seed germination test

Two soybean seed varieties (TGX 1987-62F and TGX 1835-10E), were procured from the International Institute of Tropical Agriculture (IITA), 11.58° N latitude and 8.33°E longitude, Kano, Nigeria. These varieties were of Sudan savannah agroecological zones, with early and extra-early growth characteristics. The germination percentage test was carried out using two earthen pots filled with fertile organic soil. Hundred seeds were broadcasted in each of the pots and lightly covered with soil. The pots were watered regularly and germination percentage was monitored after five days and up to 95 % germination was recorded in both the varieties, two weeks after sowing. Before planting, the soil was plowed, harrowed and ridged to remove debris, weeds and clods, for proper aeration and moisture for crop growth. Seeds were sown at a spacing of 75 cm on each ridge immediately after the rain during the third week of May 2023. Three seeds were manually sown per hole and later thinned into two plants per stand at 2 weeks after sowing (WAS). The experimental design followed a randomized complete block design (RCBD) with three replications measuring 8 x 60 m^2 (25).

Seed treatments

Exposure to gamma radiation

Soybean seed (*Glycine max* L.) was irradiated to varying gamma ray's doses (5Gy, 10Gy, 15Gy and 20Gy) at the Oncology Department of the Ahmadu Bello University Teaching Hospital in Zaria, Nigeria. The gamma radiation doses were obtained from a source of Cobalt-60 (^{60}CO).

Sodium azide (SA) treatment

The seeds were soaked in distilled water for 6 hrs and later treated with SA for 6 hrs. They were immersed at different concentrations (0.00 % to 0.04 % SA), respectively at room temperature (25-30 °C). The 0.0 % concentration was the control. Fifty mL of phosphate buffer solution was added to each treatment to maintain the osmotic content of the cell at a pH of 3. Another set of seeds were soaked in distilled water and phosphate buffer solution, as a control. After the treatment, seeds were extensively rinsed with running tap water approximately 8-10 times. Subsequently, both treated and untreated control seeds were sown in the field using a randomized complete block design with three replications to induce mutation generation (Table 1).

Data Collection

Plant growth characteristics

The evaluation of plant growth characteristics including seed germination, plant height, leaf count, leaf area, days to 50 % flowering in control plants and M1 plants in two soybean cultivars was done at different growth stages. The research utilized 85 seeds in each planting area for determining germination rates. The seedling count determined the seed germination percentage of experimental and control plot that was recorded through statistical analysis (Fig. 1). The average germination percentages appeared in bar charts through MS Excel version 13 while displaying chemical treatments along the x-axis and germination percentages on the y-axis. The plant height was measured at three specific timepoints starting from 4 to 12 weeks after sowing (WAS). Each plot contained five randomly plants measured from base to apex using tape measure. The mean height of M1 plants was compared with the control 25). Five randomly chosen plants from each plot underwent measurement of leaf count and leaf area during each growth stage. Chlorophyll concentrations in control and M1 plant leaves were measured sequentially by a SPAD meter from Minolta, Europe (25).

Statistical Analysis

Five randomly chosen plants from each experimental plot (Fig. 1) were used for data collection. The data were analyzed using SPSS software (version 22) through two-way ANOVA-test while the findings were separated by Duncan's multiple range test (DMRT) at a 5 % level of probability.

Results

Effect of physical and chemical mutagens on seed germination

Fig. 1 shows the data recorded from various treatments applied to two soybean varieties (TGX 1987-62F and TGX 1835-10E). The results indicated significant differences among the treatments and compared to the untreated control plots ($P < 0.05$) across most data collection periods. The highest seed germination rate was observed in TGX 1835-10E with 15 Gy (60.0 % seedlings per plot) and in TGX 1987-62F with 0.03 % SA (19.69 %). In contrast, the lowest germination rate (13.34 % seedlings per plot) was recorded for TGX 1835-10E treated with 0.02 % SA among all treatments. For the untreated control, seed germination rates were 22.34 % for TGX 1987-62F and 57.00 % for TGX 1835-10E.

Table 2 presents the impact of physical and chemical

Table 1. Details of mutagenic treatment given to soybeans seeds

Mutagen used	Treatments	Duration of treatments (hr) TGX 1987-62F	Duration of treatments (H) TGX 1835-10E
Untreated control	C1	-	-
Gamma rays (Gy)	5Gy	5.98Gy/ min	5.98Gy/ min
	10Gy	11.97 Gy/min	11.97Gy/min
	15Gy	17.95Gy/ min	17.95Gy/ min
	20Gy	23.94Gy/ min	23.94Gy/ min
		Duration of pre-Soaking (H) TGX 1987-62F	Duration of treatments (H) TGX 1835-10E
Sodium Azide (%)	C2	6	6
	0.01 %SA	6	6
	0.02 %SA	6	6
	0.03 %SA	6	6
	0.04 %SA	6	6

C1=untreated control, 5Gy, 10Gy, 15Gy, 20Gy, 0.01 %SA, 0.02 %SA, 0.03 %SA, 0.04 %SA, C2=untreated control.

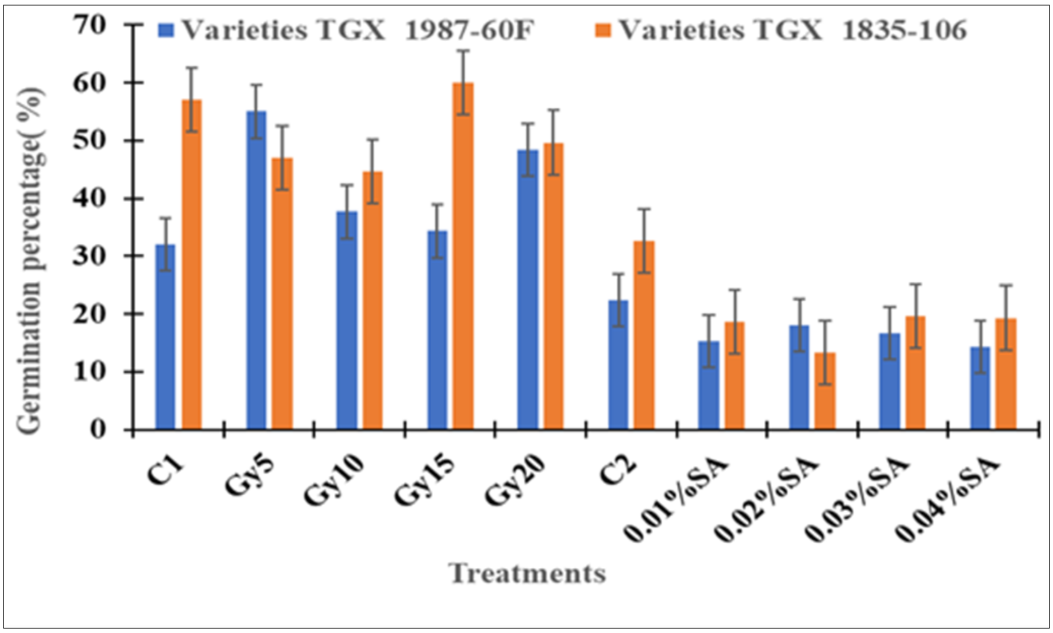

Fig. 1. Effect of physical and chemical mutagens on germination percentage. C1 =Untreated control, Gy= Gamma radiation, SA=Sodium azide.

mutagens on the plant height of control and M1 soybean plants. The treatments varied significantly from one another and from the untreated controls (P = 0.05) across most observation periods. At 10 WAS, the tallest plants in the TGX 1835-10E variety were recorded in T2 (5 Gy), reaching 64.40 cm, followed by T9 (59.17 cm) with 0.02 % SA. The shortest height (58.38 cm) was observed in T5 (20 Gy). Meanwhile, in the TGX 1987-62F variety, T10 (0.04 % SA) resulted in a plant height of 42.83 cm. These findings showed significant differences from the untreated controls, which measured 60.93 cm for TGX 1835-10E and 70.02 cm for TGX 1987-62F.

Effect of physical and chemical mutagens on number of leaves per plant

The results revealed that at 6 WAS, T3 (10 Gy) recorded the highest number of leaves per plant (41.67), while T8 (0.02 % SA) had the maximum count (66.67 leaves per plant). At 8 and 10 WAS, T3 (10 Gy) continued to show the highest leaf count, reaching 99.00 and 125.67 leaves per plant, respectively. Meanwhile, T7 and T8 recorded 183.67 and 183.33 leaves per plant, respectively. These values were significantly different from all other treatments, including the untreated control, which had 127.78 leaves per plant at 8 WAS and 142.33 leaves per plant at 10 WAS (Table 3).

Effect of physical and chemical mutagens on leaf variation

Chemical treatments, including gamma radiation (5Gy, 10Gy, 15Gy, 20Gy) and SA at 0.01 %, 0.02 %, 0.03 % and 0.04 %, led to variations in leaf form, such as unifoliate, bifoliate, tetrafoliate and pentafoliate leaves. In TGX 1987, broader trifoliate leaves were observed with 5Gy, 10Gy and 15Gy, while narrower, pointed leaves with thinner stems appeared at 20Gy. In TGX1835, broad leaves were seen with 0.01 % SA, while narrower leaves with pointed tips and thinner stems were noted with 0.02 % SA and 0.03 % SA. The untreated controls showed TGX 1987 with broad trifoliate leaves and TGX 1835 with narrow trifoliate leaves and chlorosis (Fig. 2). Additionally, pentafoliate leaves were narrower and had a smaller area compared to tetrafoliate leaves, which had a broader base. These results demonstrate how chemical mutagens can induce morphological changes, useful for breeding programs aimed at developing new crop varieties.

Effect of physical and chemical mutagens on leaf area (cm^2)

Table 4 presents the effects of physical and chemical mutagens on leaf area in control and M1 plants. The results indicated that at 6 WAS, the treated plot T3 (10Gy) had the largest leaf area in TGX 1835, with a measurement of 163.19 cm^2, while T8 (0.02 % SA) had a leaf area of 163.56 cm^2 in TGX 1835. However, these treated plots showed significant differences ($P \leq 5$ %) compared to the untreated plots, which had leaf areas of 168.46 cm^2 and 151.61 cm^2, respectively (Table 4).

Effect of physical and chemical mutagens on chlorophyll content (nmol/cm^2)

Table 5 shows the effects of physical and chemical mutagens on plant chlorophyll content at 6 and 10 WAS. At 10 WAS, chlorophyll content was found to be highest (36.93 nmol/cm^2) in T_3 M1 plants (10Gy) and lowest (32.34 nmol/cm^2) in T_2 (5Gy) M1 plants of TGX 1987 variety. While, in TGX 1835, the highest chlorophyll content (46.129 nmol/cm^2) was found in T_3 plot and the lowest (40.42 nmol/cm^2) in T_4plot.However, at 10WAS, the chlorophyll content was highest (33.89 nmol/cm^2) in T_9 (0.03 % SA) plot of TGX 1987 variety and lowest (24.56nmol/cm^2) in T_{10} (0.04SA). While, in TGX 1835, the highest chlorophyll content

Table 2. Effect of physical and chemical mutagens on plant height (cm)

Interval of data collection						
Six weeks after sowing			Eight weeks after sowing		Ten weeks after sowing	
Treatments	TGX 1987	TGX 1835	TGX 1987	TGX 1835	TGX 1987	TGX 1835
C1	28.83^{cde}	33.17^{bcd}	45.12^{b-e}	47.70^{a-e}	55.46^{bc}	60.93^{abc}
Gy5	36.47^{b}	31.05^{b-e}	54.12^{a-d}	61.02^{ab}	59.83^{ab}	64.40^{ab}
Gy10	38.82^{bc}	36.36^{b}	55.05^{abc}	57.21^{abc}	62.23^{ab}	63.50^{ab}
Gy15	31.90^{b-e}	30.32^{b-c}	47.96^{a-e}	53.50^{a-d}	53.18^{bcd}	58.15^{abc}
Gy20	36.12^{b}	36.20^{b}	53.59^{a-d}	58.32^{abc}	61.82^{ab}	58.38^{abc}
C2	44.76^{a}	33.01^{bcd}	58.67^{a}	48.76^{a-e}	70.02^{a}	60.93^{ab}
0.01 %SA	28.65^{cde}	37.43^{b}	41.87^{c-f}	50.64^{a-d}	53.67^{bcd}	58.45^{abc}
0.02 %SA	28.67^{cde}	29.83^{b-e}	27.41^{a-e}	29.12^{f}	54.02^{bcd}	49.90^{bcd}
0.03 %SA	27.30^{de}	33.56^{bcd}	41.23^{d-f}	55.31^{ab}	46.33^{cd}	59.17^{ab}
0.04 %SA	25.77^{e}	31.12^{b-e}	35.75^{f}	46.54^{a-e}	42.83^{d}	53.18^{bcd}
SE ± 2.47				4.77		4.43

The same common letters within the same column indicate no significant difference from one another (P < 0.05). SE± = Standard Error, WAS = Weeks After Sowing, C1 = Untreated Control, 5Gy, 10Gy, 15Gy, 20Gy, 0.01 % SA, 0.02 % SA, 0.03 % SA, 0.04 % SA, C2 = Untreated Control

Table 3. Effect of physical and chemical mutagens on number of leaves per plant

Treatment	Data taking periods					
	6 WAS		8 WAS		10 WAS	
	TGX 1987	TGX 1835	TGX 1987	TGX 1835	TGX 1987	TGX 1835
C1	37.33^{cd}	31.00^{d}	79.78^{d-f}	68.33^{e-f}	127.78^{b-a}	96.67^{cd}
Gy5	38.33^{cd}	32.67^{d}	79.69^{d-f}	79.$^{67d-f}$	115.11^{b-d}	105.33^{b-d}
Gy10	41.67^{b-d}	31.00^{d}	99.00^{c-e}	91.00^{d}	125.67^{b-d}	124.33^{b-d}
Gy15	32.33^{d}	38.33^{cd}	70.00^{c-f}	76.78^{df}	122.11^{b-d}	108.78^{b-d}
Gy20	40.33^{d}	37.67^{cd}	95.33^{c-f}	63.67^{f}	121.33^{b-d}	95.26^{c-f}
C2	68.00^{a}	32.67^{d}	118.67^{bc}	87.33^{c-f}	142.33^{b}	122.11^{b-d}
0.01 %SA	43.67^{b-d}	47.44^{bc}	89.67^{cf}	48.67^{ab}	113.92^{b-d}	183.67^{a}
0.02 %SA	66.67^{a}	41.67^{bcd}	162.62^{a}	76.00^{df}	183.33^{a}	113.67^{b-d}
0.03 %SA	39.00^{cd}	60.67^{b}	80.00^{df}	104.33^{cd}	112.00^{b-d}	131.00^{b}
0.04 %SA	36.67^{d}	59.00^{b}	70.oo^{ef}	96.00^{cf}	99.32^{cd}	130.00^{bc}
SE±	7.62		11.43		13.44	

The same common letters within the same column indicate no significant difference from one another (P < 0.05). SE± = Standard Error, WAS = Weeks After Sowing, C1 = Untreated Control, 5Gy, 10Gy, 15Gy, 20Gy, 0.01 % SA, 0.02 % SA, 0.03 % SA, 0.04 % SA, C2 = Untreated Control

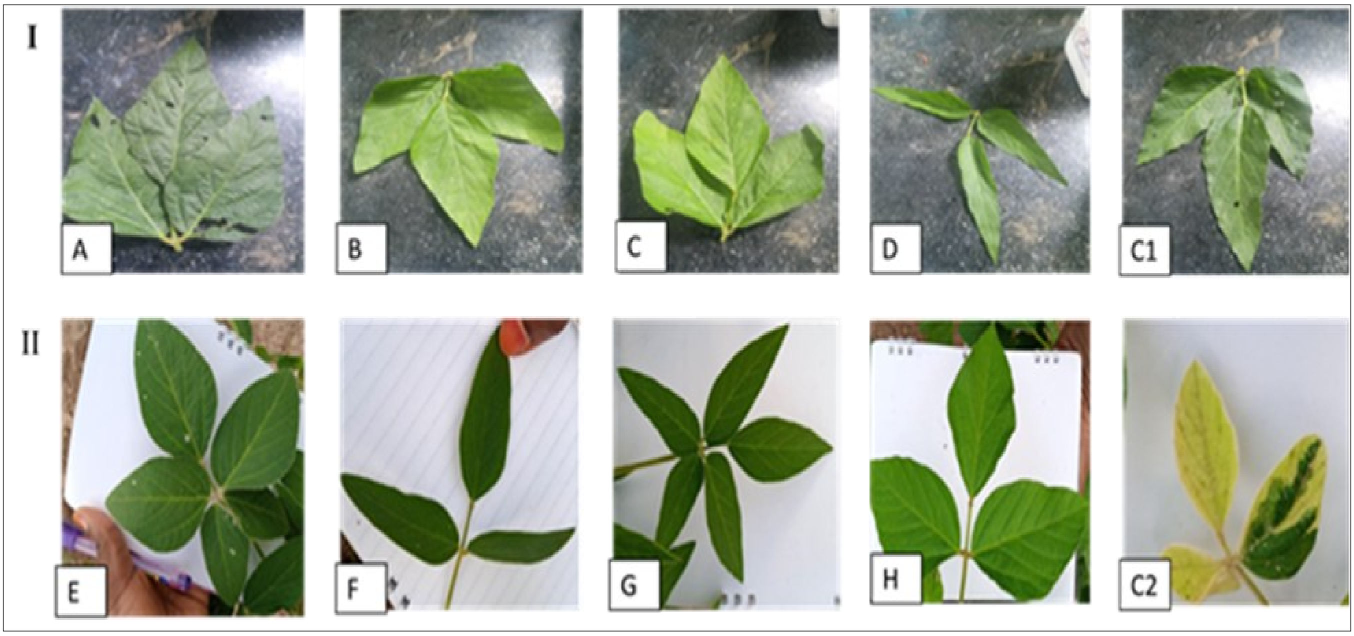

Fig. 2. Assessment of different concentrations of sodium azide on the leaf morphology of two soybean (*Glycine max* L.) varieties. (I) Variety I: TGX 1987-Mutagen I: Sodium azide (A) 0.01 %, (B) 0.02 %, (C) 0.03 %, (D) 0.04 % and (C1) TGX 1987-untreated control. (II) Effect of different doses of gamma radiation on the morphology of two soybean (*Glycine max* L.) varieties. Variety II: TGX 1835-Mutagen II: Gamma radiation (E) 5Gy, (F) 10Gy (G) 15Gy, (H) 20Gy and (C2) TGX 1835-untreated control.

Table 4. Effect of physical and chemical mutagens on leaf area (cm^2)

Treatment	Interval of data collection			
	Six weeks after sowing		Eight weeks after sowing	
	TGX 1987	TGX 1835	TGX 1987	TGX 1835
C1	84.68^{a-c}	87.92^{a-c}	168.46a	160.24^{a}
Gy5	63.83^{c}	81.52^{a-c}	135.68a	131.81^{a}
Gy10	76.66^{a-c}	92.84^{a-c}	159.24^{a}	163.19^{a}
Gy15	83.77^{a-c}	85.59^{a-c}	147.74^{a}	134.50^{a}
Gy20	82.71^{a-c}	92.76^{a-c}	127.84^{a}	151.55^{a}
C2	67.14^{bc}	88.45^{a-c}	151.61^{a}	121.94^{a}
0.01 %SA	99.40^{a}	91.47^{a-c}	122.51^{a}	152.53^{a}
0.02 %SA	94.33^{a-c}	76.60^{a-c}	131.63^{a}	163.56^{a}
0.03 %SA	93.33^{abc}	78.17^{a-c}	151.53^{a}	150.16^{a}
0.04 %SA	92.53^{a-c}	96.60^{ab}	125.30^{a}	148.33^{a}
SE±	8.54		17.13	

The same common letters within the same column indicate no significant difference from one another ($P < 0.05$). SE± = Standard Error, WAS = Weeks After Sowing, C1 = Untreated Control, 5Gy, 10Gy, 15Gy, 20Gy, 0.01 % SA, 0.02 % SA, 0.03 % SA, 0.04 % SA, C2 = Untreated Control

Table 5. Effect of physical and chemical mutagens on chlorophyll content (nmol/cm^2)

Treatment	Interval of data collection			
	Six weeks after sowing		Eight weeks after sowing	
	TGX 1987	TGX 1835	TGX 1987	TGX 1835
C1	33.74^{a}	26,00^{ab}	31.62^{d-f}	45.23^{a}
Gy5	23.20^{ab}	27.30^{a}	32.34^{d-f}	43.49^{abe}
Gy10	28.44^{a}	26.10^{ab}	36.93^{a-e}	46.129^{a}
Gy15	29.60^{a}	25.83^{ab}	33.74^{c-f}	40.42^{a-d}
Gy20	28.83^{a}	22.43^{ab}	36.83^{a-e}	46.13^{a}
C2	34.00^{a}	24.67^{ab}	34.87^{c-f}	36.85^{a-c}
0.01 %SA	27.06^{ab}	22.53^{ab}	33.35^{d-f}	27.18^{e-h}
0.02 %SA	28.07^{a}	23.76^{ab}	26.40^{f-h}	46.24^{a}
0.03 %SA	31.40^{a}	25.00^{ab}	33.89^{h}	35.99^{b-f}
0.04 %SA	34.33a	28.86^{ab}	24.56^{g}	30.67^{d-g}
SE±	5.61		3.21	

The same common letters within the same column indicate no significant difference from one another ($P < 0.05$). SE± = Standard Error, WAS = Weeks After Sowing, C1 = Untreated Control, 5Gy, 10Gy, 15Gy, 20Gy, 0.01 % SA, 0.02 % SA, 0.03 % SA, 0.04 % SA, C2 = Untreated Control

(46.24 nmol/cm^2) was found in T_8(0.02 % SA) plot and the lowest (27.18 nmol/cm^2) in T_7plot. These results differed significantly from all the treatments, including the untreated control plants (45.23 nmol/cm^2 and 34.87 nmol/cm^2 respectively).

Effect of physical and chemical mutagens on days to 50 % flowering

Fig. 3 shows the days to 50 % flowering was observed to be significantly different among the treatment at $P < 0.05$. The highest days to 50 % flowering (45.69 days) among both varieties was noted for untreated control, (TGX 1987 and TGX 1835). However, the treatments (0.01 % and 0.02 % SA) had similar results (45.35 days and 45.33 days) in TGX 1987. Meanwhile the lowest days to 50 % flowering (36.32 and 38.66 days) was observed in TGX 1835 irradiated with (20Gy) which was significantly lower among both varieties (Fig. 3).

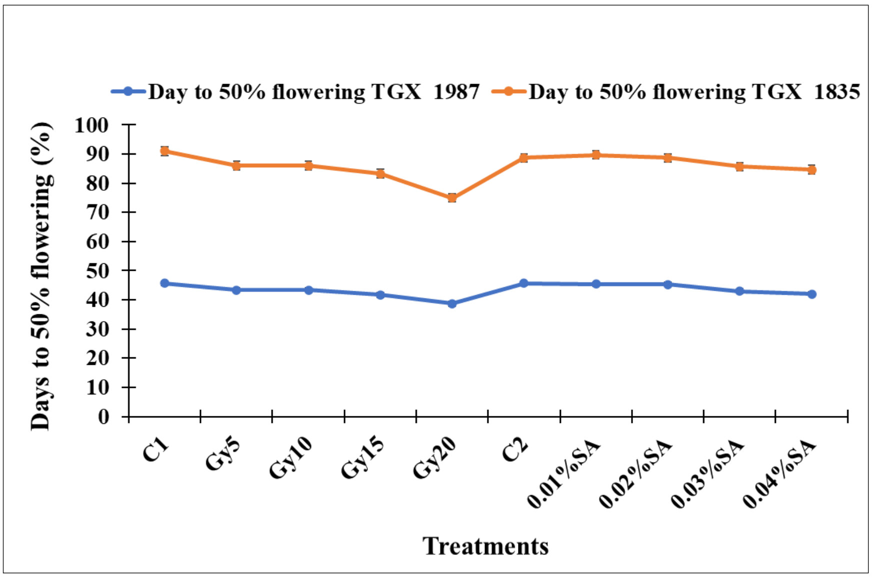

Fig. 3. Assessment of physical and chemical mutagens on days to 50 % flowering.

C1=untreated control, 5Gy, 10Gy, 15Gy, 20Gy, 0.01 %SA, 0.02 %SA, 0.03 %SA, 0.04 %SA, C2=untreated control

Effect of physical and chemical mutagens on number of pods per plant

The highest number of pods among both varieties and treatments, (255 pods/plant) was found in the M1 plants of TGX 1835 cultivar treated with (10Gy), while the least (52 pods/plant) was observed in TGX 1987 treated with (5Gy). Results indicate that T8 recorded 143 pods/plant with 0.02 % SA in TGX1835 and lowest (53 pods/plant) treated with (0.04 % SA) as compared with untreated plot (356 pods/plant in TGX 1987 and 166 pods/plant in TGX 1835 pods/plant) (Table 6).

Effect of physical and chemical mutagens on one hundred seed weight (g)

The hundred-seed weight varied significantly between the mutagenic treatments and the control ($P < 0.05$). The highest seed weight (9.33 g) was observed in TGX 1835 treated with 15Gy, which was not significantly different from the 9.00g recorded in TGX 1835 treated with 5Gy. The lowest seed weight (6.33 g) was seen in TGX 1987 treated with 15Gy. The highest hundred-seed weight for the treatments (7.34 g) was found in TGX 1987 and TGX 1835 treated with 0.02 % SA, while the lowest (4.00 g) was recorded in TGX 1987 treated with 0.04 % SA for both varieties and treatments (Table 6).

Effect of physical and chemical mutagens on total grain yield (kg/ha)

The highest total grain yield (747.00 g) was observed in TGX 1835 treated with (10 Gy) and significantly higher in both varieties and the treatments. While 524.30g was observed in TGX 1835 treated with 0.02 % SA. The lowest (34.30 g) in both varieties and treatments was observed in TGX 1987 treated with (0.04 % SA) (Table 6).

Effect of physical and chemical mutagens on seed morphology

The soybean seeds exhibited changes in size and shape across the treatments. Treatments A (0.01 % SA), B (0.02 % SA), F (10Gy gamma radiation) and G (15Gy gamma radiation) show oval or rounded seeds. The colour tone of the seeds varies among treatments: G (15Gy gamma radiation) and H (20Gy gamma radiation) appear slightly darker compared to others, like C1 (TGX 1987-untreated control), C2 (TGX 1835-untreated control) and D (0.04 % SA). Treatments E (5Gy gamma radiation) and F (10Gy gamma radiation), have seeds that appear consistent in size and higher quality, while others, B (0.02 % SA) and H (20Gy radiation), have more variability (Fig. 4).

Discussion

The current findings suggest a strong potential for induced mutagenesis to play an important role in enhancing the genetic

Table 6. Effect of physical and chemical mutagens on number of pods per plant, one hundred seed weight and total grain yield

Treatment	Number of pods/plants		One hundred seed weight (g)		Total grain yield (kg/ha)	
	TGX 1987	TGX 1835	TGX 1987	TGX 1835	TGX 1987	TGX 1835
C1	356.00^{a}	345.00^{ab}	7.00^{b-d}	8.33^{a-d}	303.30^{d-e}	616.70^{a-d}
Gy5	52.70^{c}	89.00^{e}	7.66^{a-d}	9.00^{ab}	423.70^{a-g}	663.30^{a-c}
Gy10	126.30^{a-c}	255.00^{a-c}	6.33^{d}	9.33^{a}	487.30^{a-f}	747.00^{a}
Gy15	98.70^{c}	152.00^{a-c}	6.66^{cd}	8.33^{a-d}	349.70^{c-g}	551.30^{a-e}
Gy20	141.00^{a-c}	159.00^{a-c}	6.67^{ed}	8.33^{a-d}	419.30^{a-g}	716.30^{ab}
C2	85.00^{c}	166.30^{a-c}	6.33^{d}	8.67^{a-c}	213.70^{e-g}	694.30^{b}
0.01 %SA	88.70^{c}	122.30^{bc}	6.67^{cd}	6.68^{cd}	286.30^{d-g}	330.00^{c-g}
0.02 %SA	93.3^{c}	143.70^{a-c}	7.34^{a-d}	7.34^{a-d}	159.70^{f-g}	524.30^{a-e}
0.03 %SA	86.30^{c}	106.70^{c}	6.34^{d}	7.00^{b-d}	217.00^{e-g}	398.00^{b-f}
0.04 %SA	53.00^{c}	95.70^{c}	4.00^{e}	6.67^{cd}	34.30^{c-g}	119.70^{g}
SE±	86.05		0.78		118.09	

The same common letters within the same column indicate no significant difference from one another ($P < 0.05$). SE± = Standard Error, WAS = Weeks After Sowing, C1 = Untreated Control, 5Gy, 10Gy, 15Gy, 20Gy, 0.01 % SA, 0.02 % SA, 0.03 % SA, 0.04 % SA, C2 = Untreated Control

Fig. 4. (I) Assessment of different concentrations of sodium azide on the seed morphology of two soybean (*Glycine max* L.) varieties. Variety I: TGX 1987-Mutagen I: Sodium azide (A) 0.01 %, (B) 0.02 %, (C) 0.03 %, (D) 0.04 % and (C1) TGX 1987-untreated control. (II) Effect of different doses of gamma radiation on the morphology of two soybean (*Glycine max* L.) varieties. Variety II: TGX 1835-Mutagen II: Gamma radiation (E) 5Gy, (F) 10Gy, (G) 15Gy, (H) 20Gy and (C2) TGX 1835-untreated control.

composition of crop plants to help sustain the growing global population. Our study revealed that seed germination percentage increased (60.0 % and 19.69 seedlings/plot) at lower doses of gamma rays (15 Gy) and SA (0.03 % SA) but declined (48 % and 14.3 seedlings/plot) at higher doses of gamma rays (20 Gy) and SA (0.04 % SA) compared to the control plots (57 % and 22.34 seedlings/ plot). Every living cell needs energy (ATP molecules) to sustain biological reactions. Low ATP levels in the cells lead to decrease in the rate of biological processes inside the cell. Higher doses of SA (0.6 % SA) can inhibit ATP biosynthesis, leading to reduced ATP availability, which in turn slows down germination (26). Our findings align with previous studies that shows a decrease in germination percentage at higher SA doses (25 %) and an increase at lower doses (5 % SA) in cowpea seeds (27) . A similar trend was noted in sesame seeds, where higher SA doses (0.076 %) led to reduced germination, while lower doses (0.0473 % SA) resulted in increased germination. This decline in seed germination at higher SA concentrations may be attributed to both physiological disruptions and chromosomal damage (28).

Significantly greater plant height was recorded at lower doses (10Gy) of gamma radiation (64.40 cm) and (0.02 %SA) of SA (59.17 cm) in 1835-10E soybean variety while plant height decreased at higher doses (20Gy) of gamma rays (58.15 cm) in TGX 1835 variety and higher (0.04 % SA) doses of SA (42.82 cm). The effects of physical and chemical mutagens on plant growth hormones can lead to significant physiological and biochemical disturbances, including direct DNA damage, auxin degradation and alterations in ascorbic acid content (29, 30) These disruptions manifest phenotypically as reduced plant growth. Higher concentrations of mutagens often exacerbate these effects, resulting in more severe cellular injury. The observed reduction in plant height due to chemical mutagens can be attributed to these factors. Our findings align with those of previous studies that reported a decline in *Urd bean* height at higher EMS (12 %) concentrations due to extensive cellular damage (31). Similarly, former researchers observed an increase in wheat plant height (21.2 cm) at 0.1 % EMS but a decrease (16.3 cm) at 0.7 % EMS (32) .

A low dose of gamma radiation (10Gy) and SA (0.02 % SA) led to an increase in leaf number (125.67 and 183.33 leaves per plant, respectively), whereas higher doses (20Gy and 0.04 % SA) resulted in a decline (95.26 and 121.35 leaves per plant) in M1 plants. These findings align with previous studies, an increase in soybean leaf number at lower EMS doses (0.4 %) and a decrease at higher doses (6 %) (33). Similarly, former researchers observed a dose-independent effect of ionizing radiation on the leaf number of soybean (34). The stimulatory response at lower doses may be attributed to the influence of growth hormones.

In most treatments, the number of days to 50 % flowering was consistently reduced, indicating a shift toward earlier flowering, which is advantageous for developing varieties that can avoid pests, drought and other late-season stresses. Our study showed that as the mutagen dose increased, the time to 50 % flowering also increased, while lower doses resulted in earlier flowering. The shortest time to 50 % flowering was observed in TGX 1987 (36.32 days) with a 20Gy dose (T5) and in TGX 1835 (42.66 days) with a 0.04 % SA dose (T10). These results are consistent with one of the studies, who found that higher EMS doses (0.7 %) delayed flowering in cowpea (35). Similarly, the significant reduction in flowering time in *Dianthus caryophyllus* var. was due to mutagen effects that interfered with seed metabolism and DNA synthesis was reported earlier (36).

The number of pods per plant is a crucial yield-related trait that is consistently linked to the overall yield. In our findings, the highest pod count (255.00 pods/plant) was observed in TGX 1835 with a 10Gy treatment (T3), followed by a gradual decrease at higher gamma radiation doses (159.00 pods/plant). For SA, the highest pod number (143.70 pods/plant) was recorded with a 0.02 % SA treatment, while a decrease (95.70 pods/plant) was seen at 0.04 % SA. These results differ from those of former studies, that fenugreek plants (*Trigonella foenum-graecum*) yielded higher at a 0.5 % EMS dose and lower at a 0.1 % EMS dose (37). Additionally, previous researchers found that higher doses of SA (2.5 %) reduced yield, while lower doses (2 %) increased yield in barley plants (34).

The highest 100-seed weight (9.33g) in both varieties was observed at a lower dose of gamma radiation (10Gy) in TGX 1835. In contrast, the highest 100-seed weight (7.34g) in TGX 1987 was seen with a 0.02 % SA treatment. This could be attributed to the evolutionary conversion of plant habit genes, influenced by mutagens, which carry significant polygenic signals. Our results align with those of previous studies, that noted a decrease in 100-seed weight in chickpea plants at higher doses of gamma radiation and SA (38).

Total grain yield is a key factor in mutation breeding, as the ultimate goal is to enhance yield alongside other beneficial traits. The highest yield (747 kg/ha) for both varieties was recorded in TGX 1835 at a lower gamma radiation dose (10Gy). Similarly, TGX 1835 showed the highest yield (524.30 kg/ha) at a lower SA dose (0.02 % SA), compared to the untreated controls (694.30 kg/ha and 303.30 kg/ha). The increase in mean values for quantitative traits could be attributed to polygenic mutations with cumulative effects, as described previously (39).

Conclusion

The use of physical and chemical mutagens such as gamma rays and SA in crops serves as a straightforward and cost-effective approach for improving agronomic traits. Their mutagenic effects become evident soon after seed sowing and can be readily observed. Consequently, the mutagens have been widely used in various crops to improve yield and quality traits, as well as to enhance resistance to both biotic and abiotic stresses (39). This research has demonstrated the positive impact of gamma radiation treatment at 10 Gy, particularly in TGX 1835, which exhibited the highest yield (747.00 kg/ha) in M1 plants.

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Authors' contributions

The conceptualization of the study was undertaken by AH and BK. AH was responsible for materials preparation, data collection and analysis. The initial draft of the manuscript was authored by AH and subsequently reviewed and revised by BK, SS, PA and RD. All authors have thoroughly reviewed and approved the final version of the manuscript.

Compliance with ethical standards

This study was conducted in accordance with institutional, national and international guidelines for plant research and no endangered species were used.

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Ethical issues: None

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