# Effect of Gamma Irradiation on Morpho-Agronomic Characteristics of Soybeans (*Glycine max* L.)

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# ABSTRACT

Mutation breeding in crop plants is an effective approach in improvement of crop having narrow genetic base such as soybean. The main objective of the present study is to determine the effect of different doses of gamma irradiation on different morpho-agronomic characteristics. Agronomic traits that were analyzed included; grain yield, number of pods/plant, number of seeds/plant and weight of 100 seeds and numbers of days to 50% flowering. Morphometric characterization of the descriptive data included plant height, stem diameter, number of leaves/plant, leaflet length, leaflet width, number of ramifications/plant, and pod length and width at 3 lodge stage. The results of the present study revealed that the two gamma irradiation doses used (0.2 kGy and 0.4 kGy) decreased significantly most of agronomic and morphological traits evaluated in M1 populations. Different effects of 0.2 kGy and 0.4 kGy irradiation were observed in M2 populations with significant increase of grain yields and yield components in all the three soybean varieties. In general, a significant decrease or no changes of morphological traits were observed for the two irradiation doses in M2 populations. The levels of changes varied among varieties. Potential high yielding mutants were identified in progenies of irradiated seeds.

Keywords: Gamma Irradiation; Glycine max; DR-Congo; Genetic Variation; Crop Yield

## **1. Introduction**

The soybean (*Glycine max* L.) is native to East Asia and it is currently widely grown in many countries worldwide. The US, Brazil, Argentina, China and India are the world's largest soybean producers and represent more than 90% of global soybean production. The majority of African countries growing soybeans use tropical soybean varieties developed by the International Institute of tropical Agriculture (ITTA) [1-3].

Despite the richness of the soybean germplasm collections, the genetic base of soybean [*Glycine max* (L.) Merr.] cultivars developed in many breeding programs is very narrow. Several studies have shown that only a few accessions have contributed to the majority of the genes in current cultivars [4-10]. This may threaten the ability of breeders to sustain improvement for high yield, resistance to pests and abiotic stresses. Mutation breeding in crop plants is an effective approach in improvement of crops having narrow genetic base as such soybean. It is an important supplementary approach to crop improvement. Many mutant lines have been identified in soybean mutation breeding programs based on morphological characteristics [11-14].

Genetic variability can also be increased by inducing mutations with ionized radiations [3,15,16]. Genetic diversity of mutant lines can be monitored using morphological, agronomic and molecular characterizations. In addition, the effectiveness of gamma radiation in improving plant growth, seed quality, cooking time and physiological processes is highly related to the level of doses used [3,17,18]. For example, Hezazi and Hamideldin [17] showed that gamma irradiation at 0.4 kGy gave the highest effect in improving okra plant growth and seed yield compared to 0.3 kGy and 0.5 kGy. Lima et al. [18] demonstrated that gamma irradiation at doses varying from 1 kGy to 10 kGy were efficient for the destruction of the fungal genera found in cowpeas bean grains. The application of gamma irradiation in developing high grain vield soybean varieties is limited.

The main objective of the present study is to determine the effect of different doses of gamma irradiation on different morpho-agronomic characteristics and to identify mutant lines with a potential of high grain yield.



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## 2. Materials and Methods

#### 2.1. Gamma Radiation

The study was carried out in the DR-Congo. Soybean seeds were provided by the Mvuazi and Gandajika research stations of the national institute for agronomic and research studies (INERA). To determine the effects of gamma radiations on morphometric and agronomic traits, 168 seeds from Kitoko, Vuangi, and TGX814-49D varieties were irradiated with different doses of gamma radiations with a cesium 137 source using "Lisa 1 conservatome" equipment at the Regional Nuclear Energy Center of Kinshasa (CRENK) in the DR-Congo. The treatments include 0 kGy, 0.2 kGy, 0.4 kGy, 0.6 kGy, and 0.8 kGy of gamma rays. Irradiated seeds were grown and M1 and M2 populations were produced for field trials.

#### 2.2. Field Trials

Field experiments were conducted over 2 years (2008-2010) at two sites in Eastern Kasai (Gandajika) in the DR. Congo. Site 1 was located at INERA agricultural research station (23°57'E, 06°48'S and 754 m altitude) and site 2 in Mpiana (23°56'E, 06°36'S and 685 m altitude). The region falls within the Aw4 climate type according to Köppen classification characterized with 4 months of dry season (from mid-May to August) coupled with 8 months of rainy season, sometimes interrupted by a short dry season in January/February. Daily temperature averages 25°C and annual rainfall is close to 1500 mm. Typically, Gandajika soils consist of a collection of sandy on clay sediment more often based on a shallow lateritic old slab.

At each site, gross plot size was 11 m long and 2 m wide for M1 populations and 6.4 m  $\times$  2 m for M2 populations. The sub-plots were 2 m  $\times$  1.8 m for both trials. Two seeds were were sown at every 10 cm to a depth of about 2 cm. Manual weeding was carried out as to keep the field clean.

The experiment was a split plot design with five replicates. The varieties represented the main plot and the irradiation dose treatments were the sub-plots. The trials were conducted with no fertilizer or pesticide applications.

In total 13 characters were selected for germplasm characterization. The descriptive data included plant height, stem diameter, number of leaves/plant, leaflet length, leaflet width, number of ramifications/plant, pod length and width at 3 lodge stage. Plant height was measured as the length of the main stem from the soil surface to the terminal node at maturity. Agronomic data include grain yield/ha, number of pods/plant, number of seeds/plant, weight of 100 seeds, numbers of days to 50% flowering.

Data were subjected to analysis of variance (ANOVA) using GenStat Discovery Edition 3 and R software. Main effects were separated by least significant differences (LSD) at P = 0.05 level.

## 3. Results

Mutation breeding in crop plants is an effective tool in hands of plant breeders especially in crops having narrow genetic base such as soybean. In the present study, four main components of yields were analyzed in details. They include, number of pods per plant, number of seed per plant, grain yield per hectare, and weight of 100 seeds. **Tables 1** and **2** describe data for the M1 generation and **Tables 3** and **4** for M2 progenies. Grain yield varied from 370.7 kGy (for TGX 814-49D at 0.4 kGy) to 2156.4 Kg/ha (for KITOKO at 0 kGy) in M1 and from 1513.3 kGy) (for VUANGI at 0 KGy) to 2542.7 Kg/ha (for KITOKO at 0 kGy) in M2. In M1, gamma irradiation at any dose decreased significantly grain yield compared to control for the three varieties evaluated.

There was a high level of variability in the M2 generation for all characters evaluated. In general gamma irradiation increased significantly grain yield, number of pods/plant and number of seeds /plant. The highest changes for agronomic grain yield, number of pods and seeds per plants were observed in variety Vuangi. In fact the gamma irradiation at 0.2 kGy increased significantly grain yield by 17% for Vuangi and 11% for TGX-814-49D. An unexpected decrease of 32% was observed for Kitoko at the 0.2 kGy dose. The number of pods per plant was increased by 14% for Kitoko, 48% for Vuangi, and 7% for TGX814-49D. For the number of seeds per plant, there was a significant increase of 7% for Kitoko, 77% for Vuangi, and 7% for TGX 814-49D at 0.2 kGy dose. A similar trend was observed for the 0.4 kGy dose. For this treatment, there was a 13% grain yield increase for Vuangi and 15% for Kitoko. For the number of pods per plant, there was a 37% increase for Vuangi, 5% for Kitoko, and 13% for TGX814-49D. The number of seeds per plant was increased by 70% for Vuangi, 15% for Kitoko, and 6% for TGX814-49D. Several mutants showing significant increase of number of pods and seeds per plant were observed. The highest increase in pod production was observed in TGX814-49D variety where an individual plant from seeds subjected to 0.2 kGy irradiation produced 464 pods at M2 generation compared to the mean of 67 pods per plant produced in control treatment (0 kGy).

The effect of gamma irradiation on plant height, stem diameter, number of leaves/plant, leaflet length, leaflet width, number of ramifications/plant and pod length and width at 3 lodge stage varied between treatments and varieties. Using the non-irradiated seeds as control or

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Accessions	Irradiation	Grain yield/ha	Number of pods/plant	Number of seeds/plant	Weight of 100 seeds	Numbers of days to 50% flowering	
	Doses (kGy)	Kg	Mean number	Mean number	Gram	Mean number	
	0	$2156\pm137$	71 ± 4.3	$98 \pm 5.7$	$9.8\pm0.5$	$43.4\pm0.8$	
	0.2	$1207\pm87$	$50 \pm 4$	61 ± 5.1	$9.8 \pm 0.6$	$44.5 \pm 1.7$	
KITOKO	0.4	$941 \pm 80$	$51 \pm 4.2$	$59 \pm 4.3$	$9.6 \pm 0.7$	$44.5 \pm 1.3$	
	mean	1435	57.2	72.3	9.7	44.1	
	0	$1510 \pm 163$	$43 \pm 3.2$	$64 \pm 4.8$	$10.1\pm0.6$	$41.0\pm0.4$	
VILANCI	0.2	$1085\pm131$	$49\pm4.7$	$63 \pm 4.0$	$10.4\pm0.8$	$41.2 \pm 1.1$	
VUANGI	0.4	$424\pm68$	$23 \pm 2.2$	$27 \pm 2.6$	$10.8\pm0.6$	$42.7\pm1.0$	
	mean	1006	38.3	51.1	10.5	41.6	
	0	$1728\pm169$	$60 \pm 6.2$	$81 \pm 6.4$	$9.5 \pm 0.4$	$43.0\pm1.1$	
TCV 014 40D	0.2	$907\pm99$	$57 \pm 4.1$	$68 \pm 5.4$	$9.3\pm0.6$	$42.2\pm0.5$	
TGX 814-49D	0.4	371 ± 51	$21 \pm 2$	21 ± 2.5	$9.9\pm0.6$	$43.3\pm0.5$	
	mean	1002	46.2	56.9	9.6	42.8	
LSD (p = 0.05)		110	3.9	4.6	0.6	0.8	

Table 1. Grain yield, number of pods and seeds per plant, weight of 100 seeds and days to 50% flowering in M-1 generation of three soybean accessions subjected to different doses of gamma irradiation.

Table 2. Plant height, stem diameter, number of leaves per plant, leaflet width, number of ramification per plant, pod length and width in M-1 generation of three soybean accessions irradiated with different doses of gamma radiations.

Accessions	Irradiation Doses (kGy) -	Plant height	Stem diameter	Number of leaves/plant	Leaflet length	Leaflet width	Number of ramifications/plant	Pod length 3 lodge stage	Pod width at 3 lodge stage
		cm	mm	Mean number	mm	mm	Mean number	mm	mm
КІТОКО	0	$41 \pm 33$	$6.2\pm0.6$	$20.3\pm2.2$	$10 \pm 1$	$4.7\pm0.4$	$3.9\pm0.6$	$3.9 \pm 0.2$	$8.4\pm0.2$
	0.2	$31 \pm 3.1$	$5.1\pm0.5$	$19.7\pm2.3$	$9.3\pm0.8$	$4.5\pm0.4$	$3.9\pm0.5$	$4.0\pm0.1$	$8.6\pm0.2$
	0.4	$30 \pm 2.8$	$5.8\pm0.6$	$19.8 \pm 1.7$	$9.6\pm0.7$	$4.9\pm0.5$	$3.9\pm0.4$	$4.1 \pm 0.4$	$8.4\pm0.4$
	mean	34.0	5.7	19.9	9.6	4.7	3.9	4.0	8.4
	0	$34 \pm 2.3$	$6.5\pm0.7$	$21.2 \pm 2$	$8.9\pm0.7$	$4.6\pm0.5$	$4.0\pm0.5$	$3.8\pm0.3$	$8.3\pm0.3$
	0.2	$30 \pm 2.2$	$5.0\pm0.4$	$19.1 \pm 1.9$	$9.3\pm0.6$	$4.2\pm0.4$	$3.4\pm0.6$	$3.9\pm0.2$	$8.9\pm0.3$
VUANGI	0.4	$25 \pm 1.8$	$5.3\pm0.6$	$16.6\pm1.9$	$8.3\pm0.6$	$3.7\pm0.4$	$2.5\pm0.3$	$3.9\pm0.2$	$8.6\pm0.4$
	mean	29.5	5.6	18.9	8.8	4.1	3.3	3.9	8.6
TGX 814-49D	0	$34 \pm 2.5$	$6.5\pm0.7$	$22.6\pm2.7$	$8.6\pm0.5$	$4.1\pm0.5$	$3.5\pm0.5$	$3.7 \pm 0.3$	$8.4\pm0.1$
	0.2	$30 \pm 3.1$	$5.3\pm0.5$	$19.3\pm2$	$8.8\pm0.8$	$4.1\pm0.4$	$3.7\pm0.5$	$3.8\pm0.2$	$8.6\pm0.5$
	0.4	$21 \pm 2.7$	$5.3\pm0.7$	$17.5 \pm 1.7$	$8.9\pm0.7$	$3.5\pm0.4$	$1.9\pm0.4$	$4.0\pm0.3$	$8.4\pm0.2$
	mean	29.21	5.7	19.8	8.8	3.9	3.0	3.9	8.5
LSD (p = 0.05)		2.5	0.5	1.6	0.7	0.4	0.4	0.3	0.3

reference, the effects of different doses were determined. In general 0.2 kGy and 0.4 kGy treatments resulted in reduction or no significant effect for the targeted descriptive traits. In the M1 generation, there was a 25% reduction of height for Kitoko, 13% for Vuangi, and TGX814-49D irradiated at 0.2 kGy dose. The levels of reduction were 18%, 23%, and 19% for stem diameter for Kitoko, Vuangi, and TGX814-49D, respectively. A similar level

Accessions	Irradiation	Grain yield	Number of pods/plant	Number of seeds/plant	Weight of 100 seeds	Numbers of days to 50% flowering	
	Doses (kGy)	Kg	Mean number	Mean number	Gram	Mean number	
	0	$2543\pm179$	$61.8 \pm 3.5$	$88.3\pm3.5$	$10.7\pm0.5$	$44\pm0.7$	
VITOVO	0.2	$1919\pm121$	$70.7\pm3.6$	$946\pm4.9$	$9.4 \pm 0.5$	$46.8\pm0.8$	
KITOKO	0.4	$2206 \pm 136$	$64.8\pm2.8$	$101.2 \pm 2.1$	$8.8\pm0.3$	$47.1 \pm 0.6$	
	mean	2222	65.8	94.7	9.6	45.9	
	0	$1513 \pm 135$	$48.6\pm2.7$	$53.8\pm4.1$	$9.6\pm0.6$	$41\pm0.7$	
VUANGI	0.2	$1777\pm181$	$72.1 \pm 3.8$	$95.5\pm2.9$	$9.3 \pm 0.1$	$42.7\pm0.8$	
VUANGI	0.4	$1704 \pm 138$	$66.6 \pm 4.1$	$91.9\pm3.2$	$9.9\pm0.6$	$43.3\pm0.9$	
	mean	1664.9	62.4	80.4	9.6	42.3	
	0	$1869 \pm 139.4$	$64.5\pm5.2$	$88.1\pm5.2$	$9.4 \pm 0.5$	$42.5 \pm 0$	
TGX 814-49D	0.2	$2075 \pm 183.1$	$638\pm3.9$	$94.3\pm4.5$	$9.6\pm0.5$	$46.7 \pm 1.5$	
1UA 814-49D	0.4	$1821 \pm 146.3$	$73.1 \pm 5.1$	$93.3\pm4.8$	$11.9\pm0.4$	$47\pm0.8$	
	mean	1921.8	67.2	91.9	10.3	45.4	
LSD (p = 0.05)		145	4.0	3.8	0.5	0.8	

Table 3. Grain yield, number of pods and seeds per plant, weight of 100 seeds and days to 50% flowering in M-2 generation
of three soybean accessions subjected to different doses of gamma irradiation.

Table 4. Plant height, stem diameter, number of leaves per plant, leaflet width, number of ramification per plant, pod length and width in M-2 generation of three soybean accessions irradiated with different doses of gamma radiations.

Accessions	Irradiation Doses (kGy)	Plant height	Stem diameter	Number of leaves/plant	Leaflet length	Leaflet width	Number of ramifications/plant	Pod length 3 lodge stage	Pod width at 3 lodge stage
		cm	mm	Mean number	mm	mm	Mean number	mm	mm
кітоко	0	$44.8 \pm 3$	$8.7\pm0.6$	$26.4 \pm 2$	$10.6\pm0.6$	$4.8\pm0.5$	$4.3\pm0.5$	$4.1 \pm 0.2$	$8.6 \pm 0.3$
	0.2	39.1 ± 2	$8.3\pm0.8$	$25.8\pm2.3$	$9.6\pm0.8$	$4.2\pm0.3$	$4.7\pm0.5$	$4\pm0.2$	$8.3\pm0.2$
	0.4	$38.7\pm2.6$	$8.6\pm0.6$	$23.7 \pm 1.3$	$9.8\pm0.5$	$4.3\pm0.4$	$4.4\pm0.4$	$4.2 \pm 0.2$	$8.7\pm0.3$
	mean	40.8	8.5	25.3	9.9	4.5	4.5	4.1	8.6
	0	$36.8\pm2.7$	$7.4\pm0.8$	$24.2\pm1.3$	$9.6 \pm 1$	$4.4\pm0.4$	$4\pm0.4$	$4\pm0.1$	$8.4\pm0.4$
	0.2	$37.9\pm2.4$	$7.7\pm0.4$	$21.8\pm1.5$	$9.9\pm0.7$	$3.9\pm0.5$	$4.2\pm0.4$	$4\pm0.2$	$8.7\pm0.3$
VUANGI	0.4	$38\pm2.6$	$6.5\pm0.5$	$20.9\pm2.4$	$8.5\pm0.6$	$3.6\pm0.3$	$4.4\pm0.5$	$3.9\pm0.2$	$8.1\pm0.1$
	mean	37.6	7.2	22.3	9.3	4	4.2	4	8.4
	0	$35.9\pm1.8$	$7.3\pm0.3$	$32.8\pm2.3$	$9.9\pm0.8$	$4.8\pm0.6$	$4.3\pm0.4$	$3.9\pm0.2$	$8.7\pm0.2$
TGX 814-49D	0.2	$37.2 \pm 2.5$	$8.6\pm0.8$	$23.1 \pm 1.8$	$107 \pm 1.1$	$5.2\pm0.5$	$4\pm0.5$	$4 \pm 0.3$	$8.7\pm0.2$
IGX 814-49D	0.4	$32.8\pm2.1$	$8.4\pm0.7$	$20.5\pm2.3$	$8.9\pm0.6$	$3.9 \pm 0.5$	$4.2\pm0.3$	$3.9\pm0.4$	$8\pm0.4$
	mean	35.3	8.1	25.5	9.8	4.6	4.2	3.9	8.7
LSD (p = 0.05)		2.2	0.7	1.8	0.6	0.4	0.4	0.3	0.3

of reduction was observed for the 0.4 kGy treatment with 25%, 13% and 38% decrease of plant height for Kitoko, Vuangi, and TGX814-49D, respectively. Reduction of stem diameter was 6.5%, for Kitoko, and 18.5% for

Vuangi and TGX814-49D. Significant decrease of the number of leaves per plant, leaflet width, and number of ramifications per plant was observed only for the 0.4 kGy treatment. For this treatment, 12% and 13% de-

crease of the number of leaves per plant were observed for Vuangi and TGX814-49D, respectively. Leaflet width change was observed at 0.4 kGy treatment for Vuangi (-20%) and TGX814-49D (-15%). The highest change for the M1 generation was noted in the number of ramifications per plant with a 38% and 46% reductions in Vuangi and TGX814-49D, respectively.

The results from the 0.2 kGy treatment showed no significant differences in plant height and other morphological traits in the M2 generation for the three varieties with the exception of a 13% reduction of plant height for Kitoko and 30% decrease for the number of leaves per plant in TGX814-49D. The 0.4 kGy irradiation induced a significant reduction of plant height in Kitoko (14%), a decrease of the number of leaves per plant (37%) in TGX 814-49D, and a reduction of leaflet width (19%). Surprisingly an increase of 18% and 15% of the stem diameter was observed for stem diameter for the 0.2 kGy and 0.4 kGy treatments, respectively for TGX814-49D. No significant difference was observed for days to 50% flowering among different treatments for both M1 and M2 generations.

# 4. Discussion

By comparison of the different treatments (different radiation doses) and the control (not irradiated), it was observed that there were significant alterations in the plant development and production in the M1 generation for all the gamma rays doses. Significant reduction of all the agro-morphometric characteristics was observed at that stage. Seeds treated at irradiation dose of 0.6 kGy and 0.8 kGy did not survive to produce progenies for evaluation. Higher exposures of gamma rays may cause injury to seeds. These data are consistent with other irradiation results reported by Yakoob and Ahamad [19] in mung beans, and Kon et al. [20] in long beans. Many studies showed that treatment with higher dose of gamma rays were inhibitory, whereas lower exposures were sometimes stimulatory. Gamma rays produce radicals that can damage and affect differentially plant morphology, anatomy, biochemistry, and physiology depending on the irradiation level.

On the other hand, the results of the M2 generation reported in the present study showed that it is possible to increase grain yields components to a maximum dose of 0.4 kGy. Improvement of agronomic characteristics by using gamma radiation has been reported in several studies. Khan *et al.* [21] reported a significant increase of chickpea grain yield using gamma irradiation at 0.6 kGy. Gustafson *et al.* [22] developed a high yielding and early maturing barley by mutation breeding methods. Rahimi and Bahrani [23] reported the highest grain yield increase in canola irradiated with 0.2 kGy of gamma rays. Similarly, they reported also a significant improvement of 1000 kernel weight and harvest index for 0.1 kGy gamma ray treatments.

In the present study, an increase of the number of pods per plant was observed in all the varieties for gamma irradiation at 0.2 and 0.4 kGy doses. Khan *et al.* [21] reported a decrease of pod number at 0.4 kGy treatment and an increase at 50 kGy without a change in the number of seed per pod. Similar results have been reported by Shakoor *et al.* [24,25] in mungbean, Khan *et al.* [21,26] in sorghum and chickpea, and Bhatnagar [14] in chickpea.

The results of the present study on plant height revealed non-significant changes between different irradiation treatments compared to the control in M2 generation. This data are consistent with report by Khan *et al.* [21], Rao [27] and Shakoor *et al.* [24,25] in mungbean, and Ramani and Jadon [16] in groundnut. The reports of Rao [27] in pigeon pea and Khan *et al.* [26] in sorghum did not agree with these results. They observed that plant height increased with the application of gamma irradiation. This may be due to the different genetic material and environmental conditions.

The dose of gamma rays radiation is important for inducing genetic variation that can lead to positive mutant. Previous studies in soybean has shown that gamma ray irradiation at a dose of 0.2 kGy gamma rays effectively lead to genetic diversity in plants Mudibu *et al.* [3] demonstrated using ISSR markers that the level of polymorphic loci observed within soybean varieties was significantly increased by more than 10 % when 0.2 kGy gamma-rays treatment was compared with the control. Hanafiah *et al.* [6] reported also that the highest genetic variation at M2 generation of soybean was on 0.2 kGy doses.

## 5. Conclusion

The results of the present study revealed that the two irradiation doses used decreased significantly most of agronomic and morphological traits evaluated in the M1 populations. Different effects of 0.2 kGy and 0.4 kGy irradiation were observed in the M2 populations with significant increase of grain yields and yield components in all the three soybean varieties. In general, a significant decrease or no changes of morphological traits were observed for the two irradiation doses in the M2 populations. The levels of changes varied among varieties. Potential high yielding mutants were identified in M2 populations.

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