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# Selection in the $M_2$ Generation of Soybeans (*Glycine Max* (L.) Merill) irradiated with Cobalt – 60 Gamma Irradiation in the Guinea Savannah Agroecology of Ghana

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## Abstract

*Field studies were conducted at the research fields of the University for Development Studies, Ghana from July to November, 2014 and June to October 2015. The studies were aimed at investigating the effect of gamma irradiation on growth and grain yield of soybean. Seeds of soybean variety Jenguma were subjected to gamma irradiation at 150, 200, 250 and 300 Gy from the 60Co source at the Biotechnology and Nuclear Agricultural Research Institute of the Ghana Atomic Energy Commission in Accra, Ghana. The irradiated seeds and some unirradiated control (0 Gy) were planted during the 2014 cropping season in a randomized complete block design with three replications. At harvest, all $M_1$ seeds for respective gamma ray doses were harvested and composited, and advanced to the $M_2$ generation during the cropping season of 2015. In $M_1$, gamma irradiation significantly ($P < 0.05$) affected seedling emergence. There was significant reduction in survival of seedlings especially from the 150 Gy. Seedling heights of the irradiated species were significantly ($P < 0.05$) reduced. Number of days to 50% flowering was also significantly ($P < 0.05$) affected. In $M_2$, more desired traits were found from plants irradiated with the 200 Gy and 250 Gy doses, with only few in the 150 Gy and 300 Gy treated plants. There was a potential for total grain yield improvement. Numbers of pods per plant and seed weight were the key parameters found to influence grain yield. Maturity period was also found to be shorter in the selected plants. The shattering resistance of plants in the 200 Gy and 250 Gy was found to be a potential improvement over the parental variety 'Jenguma' which was originally bred for that purpose. Selected plants would be advanced into $M_3$ generation for further studies and results will be published*

***Keywords:*** *Gamma irradiation, dose, mutation, mutagen, Soybean, growth and grain yield*

## INTRODUCTION

Legumes are generally important sources of protein (higher protein than cereals), vitamins, carbohydrates and dietary fibre (Tharanathan and Mahadevamma, 2003; Costa *et al*., 2006; Udensi *et al*., 2011). Soybean is ranked the third most important grain legume in Ghana after groundnut and cowpea. The crop is gaining prominence in northern Ghana largely because of its multi-purpose usage (Rheenen *et al*., 2012). Yield levels and growth characteristics of most genotypes of soybean cultivated by farmers in northern Ghana are not encouraging. There is therefore the need to overcome this problem. Recent crop genetic improvement programmes may usually have the major objective of increasing yield, while maintaining quality.

In general, plant breeding requires genetic variation of useful traits for crop improvement but often, however, the desired variation is lacking (Novak and Brunner, 1992) and creation of variability has therefore been the essence of plant breeding. Hybridization though may be useful, the various problems often encountered in effecting crosses, the longer period in evolving a superior variety and the non-availability of parents with desirable genes has enforced a limitation on the use of hybridization (Shanthala, *et al.*, 2013). Transgenesis has also shown to offer a promising future for crop genetic improvement. However, issues of health, religion, social, and ethical interest concerning the release of transgenic plants to the environment still remain a course for discussion.

Mutagenesis could provide a solution to the poor yielding ability of the varieties used in northern Ghana and numerous challenges facing the production of the crop in general. Mutagenic agents, either physical or chemical can be used to induce mutations and generate genetic variations from which desired mutants may be selected. Mutation induction has become a proven way of creating variation within a crop variety (Novak and Brunner, 1992). Mutation is thus considered as a valuable tool in crop improvement and a source to increase genetic variability resources from which useful variants could be obtained either directly or after recombination (Shanthala, *et al.*, 2013). Gamma and X-rays are the most commonly used physical mutagens and different doses have successfully been used to induce mutation in several crop species including blackgram (Ignacimuthu and Babu, 1990), and cowpea (Thirugnanakumar, 1986). The objectives of the present studies were to create variation in soybean and select genotypes with improved growth and grain yield.

## MATERIALS AND METHODS

### Experimental site

Two experiments were conducted to generate $M_1$ and $M_2$ populations of soybean at the University for Development Studies, Ghana during the 2014 and 2015 cropping seasons. The experimental location is found on altitude 183 m, latitude 09$^o$ 25' N and longitude 0$^o$ 58'W. The place has a unimodal rainfall of about 1000 mm which is evenly distributed from May to October with the peak in August and September. The average minimum temperature is 25˚C whilst the maximum average temperature is 35˚C (Lawson *et al*., 2013). The area lies within the interior Guinea savannah of Ghana and is characterized with natural vegetation dominated by grasses with few shrubs. Soils of the area are moderately drained and are free from concretions; they are shallow with hardpan under the top few centimetres and were derived from Voltaian sandstone. The soils, according to FAO (1988), are classified as Nyankpala series or Plinthic Acrisol. The area has grassland vegetation and it is interspersed with short trees such as *Parkia biglobosa*, and *Azadirachta indica* and weed species such as *Centrosema pubescens*, *Cyperus difformis* and *Striga hermontheca.*

### Plant culture

Field was ploughed and harrowed with a tractor and leveled manually in both the 2014 and 2015 cropping seasons. Field was demarcated into plots, each measuring 1 m x 1 m with 0.8 m and 1 m alleys between plots and replications respectively. Seeds of soybean variety Jenguma were used as the initial breeding material and were exposed to gamma ray doses of 150, 200, 250 or 300 Gy from $^{60}$Co source at the Biotechnology and Nuclear Agricultural Research Institute of the Ghana

Atomic Energy Commission in Accra, Ghana. The irradiated seeds and some unirradiated control (0 Gy) were planted during the 2014 cropping season in a randomized complete block design with three replications. All seeds in the $M_1$ generation were harvested and advanced to $M_2$ generation during the 2015 farming season. Screening and selection of desirable mutant lines were conducted in $M_2$. Desirable plants were selected for advancement into the $M_3$ generation, and results of screening in this generation will as well be published in the future. Weed control was done manually at 3, 6 and 9 weeks after planting during the $M_1$ and $M_2$ generations.

**Data collection and statistical analysis**

Data collected included plant height, number of branches, number of leaves, chlorophyll content, percentage pod shattering, number of days to 50% flowering, number of days to maturity, length of pods, hundred seed weight, number of seeds per pod, number of pods per plant and total grain yield. The data were subjected to analysis of variation using Genstat discovery, 12th edition and means separated using LSD at 5% probability level.

## RESULTS

**Earliness in flowering and maturity period**

Gamma irradiation significantly affected number of days to 50% flowering and maturity period in both $M_1$ and $M_2$ generations. Plants from the control, 150, 200 and 250 Gy plots took relatively lesser days to attain 50% flowering and varied significantly ($P < 0.05$) from plants in the 300 Gy plots in the $M_1$ generation. In the $M_2$ generation, plants from all gamma ray treated plots took similar number of days to reach 50% flowering and varied significantly from the control (Figure 1). Plants from 200 and 250 Gy treatments took the least days to reach maturity and varied significantly ($P < 0.05$) from all other treatments, whilst the control, 150 and 300 Gy took significantly ($P > 0.05$) similar number of days to reach maturity in the $M_1$ generation. However, in the $M_2$ generation, 200 Gy took the least number of days to reach maturity and varied significantly ($P < 0.05$) from all other treatments. Plants from 150 and 250 Gy plots took significantly ($P > 0.05$) similar number of days to reach maturity, as were those from the control and 300 Gy treatments (Figure 2).

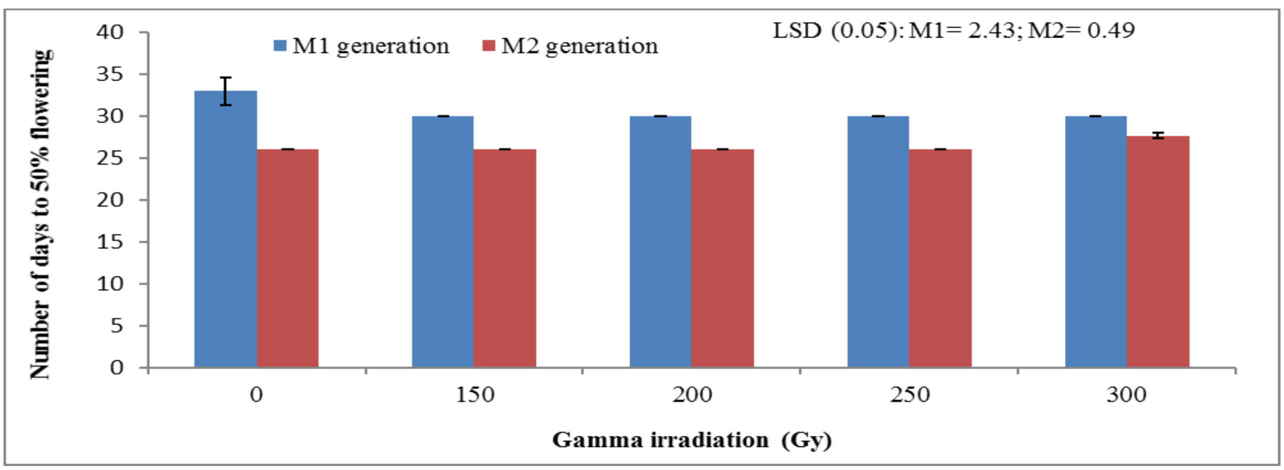


**Figure 1: Effect of gamma irradiation on number of days to (a) 50% flowering (b) maturity. Bars indicate mean ± standard error of the means.**

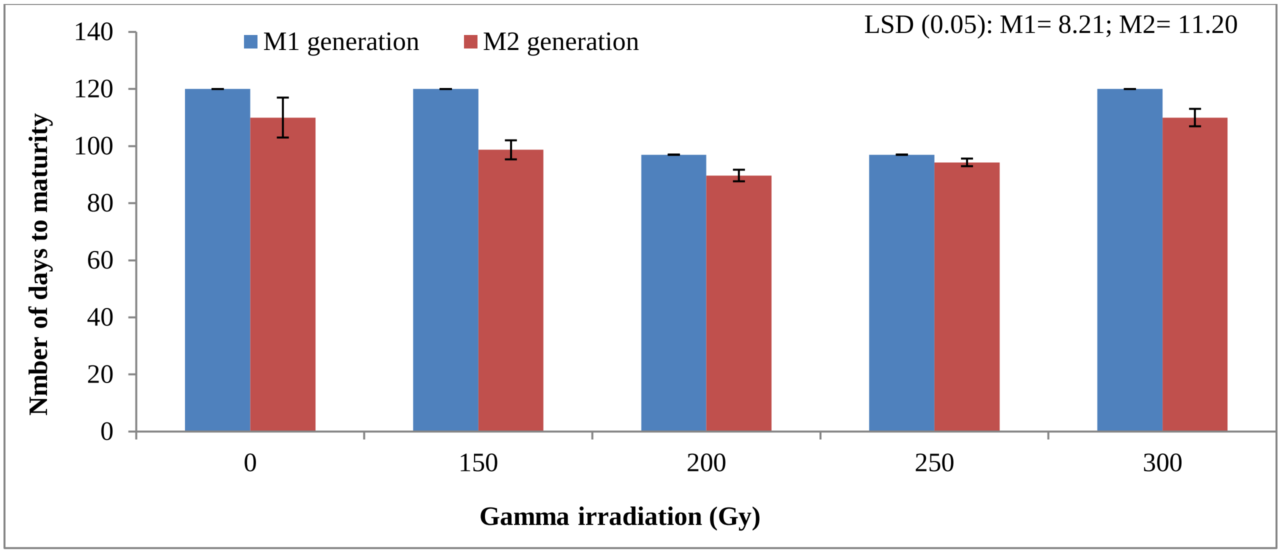


**Figure 2: Effect of gamma irradiation on days to (a) 50% flowering (b) maturity. Bars indicate mean ± standard error of the means.**

**Plant growth characteristics**

Plant height was significantly (P < 0.05) affected by gamma ray treatments in both mutant generations. The highest height in the $M_1$ generation was obtained by plants from the control, but was significantly (P > 0.05) similar to the height recorded by plants from 200, 250 and 300 Gy plots. In the $M_2$ generation, however, plants from 250 and 300 Gy plots recorded significantly (P > 0.05) similar heights, as were plants from the control, 150 and 200 Gy plots (Table 1). Gamma irradiation affected number of leaves only in the $M_1$ generation (Table 1). Chlorophyll content was also affected by gamma irradiation only in the $M_2$ generation. Plants from the control, 150, 200 and 250 Gy plots recorded significantly (P > 0.05) similar values for chlorophyll content, whilst 300 Gy recorded the highest (Table 1). Gamma irradiation significantly affected primary and secondary branching only in the segregating $M_2$ generation. Plants from 150 Gy treatment recorded the highest number of primary branches; it was however, significantly similar to the numbers recorded by plants from the control, 200 and 250 Gy plots (Table 1). Plants from 150 Gy plot recorded the highest number of secondary branches and was significantly similar to the number recorded by plants from 300 Gy. Plants from the control, 200 and 250 Gy plots recorded significantly (P > 0.05) similar number of secondary branches.

**Pod shattering and components of yield**

Gamma irradiation significantly (P < 0.05) affected pod shattering in both mutant generations. Notably, plants from 200 and 250 Gy plots recorded the lowest percentages of pods shattered and varied significantly from all other treatments in both $M_1$ and $M_2$ generations (Table 2). For the yield components, gamma ray significantly (P < 0.05) affected number of pods per plant, pod length and 100 seed weight only in the $M_2$ generation, but had no influence on number of seeds per pod in both mutant generations. Plants from the control and 150 Gy plots recorded significantly similar pod numbers as were those from 200, 250 and 300 Gy plots. Similar values for pod length were recorded by plants from the control, 150, 200 and 250

Gy plots, whilst plants from 300 Gy plots recorded the least length (Table 2). Plants from 150 Gy plot recorded the highest 100 seed weight, it was, however significantly similar to seed weights recorded by plants from the control, 200 and 250 plots (Table 2). Improvements in agronomic traits were observed at the $M_2$ generation. In particular, the improvements made in selection for earliness, pod shattering and total grain yield were remarkable. The 150 and 200 Gy treated populations recorded improvement in earliness and pod shattering whilst the 200 and 300 Gy treated plants recorded improvement in total grain yield (Table 2).

**Table 1: Effect of gamma irradiation on vegetative growth and chlorophyll content of soya bean**

| Gamma doses | Plant height (cm) | | Number of leaves | | Chlorophyll content | | Primary branches | | Secondary branches | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $M_1$ | $M_2$ | $M_1$ | $M_2$ | $M_1$ | $M_2$ | $M_1$ | $M_2$ | $M_1$ | $M_2$ |
| Control | 24.88a | 51.80ac | 68.00a | 83.80 | 34.07 | 41.55a | 9.00 | 9.00ab | 1.00 | 3.00a |
| 150 Gy | 16.31b | 51.00ac | 58.00ab | 74.00 | 34.00 | 41.65a | 8.00 | 10.00a | 1.00 | 8.00b |
| 200 Gy | 20.40ab | 45.10a | 65.00ab | 66.60 | 33.83 | 40.85a | 8.00 | 9.00ab | 1.00 | 2.00a |
| 250 Gy | 22.37a | 65.40b | 60.00ab | 86.60 | 33.57 | 40.35a | 8.00 | 9.00ab | 1.00 | 2.00a |
| 300 Gy | 20.78ab | 56.10bc | 56.00b | 79.70 | 32.50 | 44.92b | 9.00 | 8.00b | 1.00 | 7.00b |
| LSD (0.05) | 4.52 | 9.73 | 12.00 | NS | NS | 2.58 | NS | 2.00 | NS | 2.00 |

**NS = Not significantly different; Means followed by the same letter (s) in each column are not significantly different**

**Table 2: Effect of gamma irradiation on earliness and yield components of soya bean**

| Gamma doses | % pod shattered | | Pods per plant | | Pod length | | Seeds per pod | | 100 seed weight | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $M_1$ | $M_2$ | $M_1$ | $M_2$ | $M_1$ | $M_2$ | $M_1$ | $M_2$ | $M_1$ | $M_2$ |
| Control | 58.30a | 56.40a | 16.00 | 69.00a | 2.36 | 2.44a | 2.00 | 2.00 | 32.43 | 33.87ab |
| 150 Gy | 58.70a | 35.60b | 15.00 | 62.00a | 2.45 | 2.35ab | 2.00 | 2.00 | 35.66 | 35.81b |
| 200 Gy | 0.90b | 0.50c | 19.00 | 50.00b | 2.51 | 2.24ab | 2.00 | 2.00 | 35.81 | 34.36ab |
| 250 Gy | 2.10b | 1.80c | 19.00 | 49.00b | 2.34 | 2.28ab | 2.00 | 2.00 | 38.90 | 32.73ab |

**NS = Not significantly different; Means followed by the same letter (s) in each column are not significantly different.**

### Total grain yield

Gamma ray treatments had no significant ($P > 0.05$) effect on total grain yield in the $M_1$ generation. However, in the $M_2$ generation, total grain yield was significantly ($P < 0.05$) affected by gamma ray treatment. The highest grain yield of 3393 kg/ha was recorded by plants from 200 Gy treated plants and this value varied significantly ($P < 0.05$) from all other treatments (Figure 3). The 150 Gy treated plants recorded the least total grain yield.

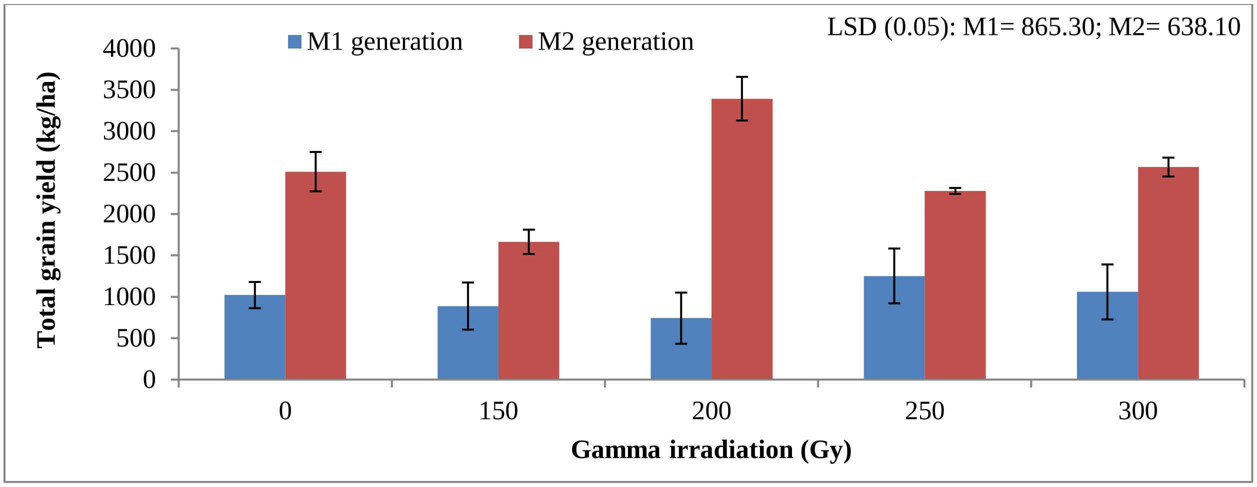


**Figure 3: Effect of gamma irradiation on total grain yield. Bars indicate mean ± standard error of the means.**

## DISCUSSION

Result obtained from the present study especially in the segregating $M_2$ generation implies that gamma irradiation would be appropriate in producing optimal effects in respect to the traits measured. This agrees with the findings of Mensah *et al*. (2005) and Warghat *et al.* (2011) who reported that appropriate application of dosage or concentration of mutagens improves agronomic traits of cowpea (*Vigna uguiculata*) and Musk okra (*Abelmoschus moschatus*). The variability in growth and yield parameters, as evident from the result of the study could be attributed to mitotic activities which are sustained from seedling stage till maturity, resulting in the proliferation of axilllary bud in response to gamma ray exposure. The marked differences observed in the studied characters even at the level of different dosages could have arisen from mutagenic effects of the gamma irradiations on the DNA and genetic structure of the plants. The observation made in this study is in tandem with the findings of Reddy and Smith (1994) who reported that mutagenesis induced notable vegetative characters in treated *Sorghum bicolar*.

The data obtained on number of days to flowering and maturity are in agreement with that of Mahala *et al.* (1990) who reported that mutagenesis could widen variability to either positive or negative direction which resulted in a sufficient variability in the treated population and this could be utilized for selecting early or late flowering plants. The gamma rays stimulated early flowering and this is probably due to the fact that biological damage increases with the increase in dose at faster rate than the mutations. The stimulatory effect of gamma irradiation on number of branches and yield components has been reported in literature (Youssef *et al*., 1998).

The potential of gamma rays to have stimulated some of the yield components especially in the segregating $M_2$ generation might have resulted in the variation created in these parameters. This observation agrees with the results of yield component reported by van Oosterom *et al*. (2002) in Pearl millet. Selection in the $M_2$ generation resulted in improvement in some agronomic traits. The observed improvements in earliness, pod shattering and total grain yield of the mutant lines, and the absence of any improvement in pod length or seed weight, among other reasons might have been due to the fact that some traits may require more than one segregating generation to achieve improvement while others may achieve gain in selection even within one generation of selection. The increased genetic advancement made in some of the traits measured in the treated populations is in conformity with the results obtained from sesame by Chavan and Chopde (1982) and in groundnut as reported by Mathur *et al.* (2000).

## CONCLUSION

More desired traits were found in the 200 Gy and 250 Gy treated plants with only few in the 150 Gy and 300 Gy. There was a potential for grain yield improvement. Maturity period for mutant lines was found to be shorter as compared with the unirradiated control. The shattering resistance of mutant lines particularly those of 200 Gy and 250 Gy was found to be a potential improvement over the parental variety 'Jenguma' which was originally bred for that purpose. Selected plants in this $M_2$ generation will be advanced into $M_3$ generation for further studies and results will also be published.

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