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Research Article

Evaluation of Mutant Generations of Irradiated Soybean (*Glycine max* (L.) Merrill) in the Guinea Savannah Agroecology of Ghana

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Abstract

Background and Objective: Gamma rays at different doses have been used to induce mutation in several species. The objective of the study was to determine mutant lines with improved agronomic traits. **Materials and Methods:** Seeds of soybean variety 'Jenguma' were irradiated with gamma rays at 150, 200, 250 and 300 Gy using un-irradiated (0 Gy) as control. The M_2 seeds were screened and the promising lines were selected and were advanced to M_3 generation. Desired M_3 mutants were evaluated for improved agronomic traits in RCBD in the Guinea Savannah Agro-ecology. **Results:** The irradiation was found to have had great potential to improve the yield and other important agronomic traits for the crop. Desired characteristics were found with 200, 250 and 150 Gy treated seeds. Maturity periods for seeds selected were shorter. The shattering resistance of the selected M_2 seeds of 200 and 250 Gy were also found to be a potential improvement over the parental variety 'Jenguma' which was originally bred to control pod shattering. More desired traits were found in the 200 and 250 Gy mutant lines with only a few in the 150 and 300 Gy. **Conclusion:** Promising mutant genotypes should be tested in multi-locational trials to determine their suitability in the various agro-ecologies for release as varieties and production by farmers in these areas.

Key words: Gamma irradiation, genetic variation, mutant lines, interactions, pod shattering, agronomic traits, agro-ecologies, legume crop

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is an annual legume crop belonging to the botanical family *Fabaceae*. It is an economically important leguminous crop worldwide and also the most important legume in Ghana[1]. Among soybean growing regions in Ghana, the largest production occurs in the northern part of Ghana, which lies within the Guinea Savannah and Sahel agro-ecological zones[2]. Soybean is gaining prominence in Ghana mainly because of its multipurpose usage[3]. In Ghana, soy cake is an excellent source of protein feed for the livestock industry[4]. Soybean serves as a good source of unsaturated fatty acids, minerals (Ca and P) and vitamins A, B, C and D[5]. It is also used to fortify various traditional foods such as gari, sauce, stew, soup, banku and kenkey to improve their nutritional levels[4]. Soybean research and production in Ghana are besieged with many constraints. The use of unimproved low yielding varieties, poor seed viability, low research effort, pests and disease infestation, the narrow genetic base of cultivars and pod shattering among others continue to bedevil production of this crop. As a result, high soybean production is not obtained and there is a big gap between what is currently produced and what is needed. As a way of improving the production level, one of the major areas of research in the country would be the development of high yielding varieties that are also resistant to pod shattering.

Introducing new improved soybean genotypes may increase genetic diversity, which would facilitate the development of new varieties that can address some of the constraints in soybean production[6]. The production of soybean crops with new desirable traits has recently been achieved through genetic engineering. However, health, religious, social and ethical considerations concerning the release of transgenic crops to the environment remains a course for discussion in the country. Mutagenesis could be one of the key solutions to numerous constraints facing soybean production and agriculture in Ghana. Mutagenic agents, either physical or chemical, can be used to induce mutations and generate genetic variations from which desired mutants would be selected. In the present study, M_2 seeds were screened and the selected promising lines advanced to M_3 generation. The desired M_3 mutants were evaluated for improved agronomic traits.

The study therefore aimed at determining the effectiveness of selection in the M_2 and M_3 generations with the view to comparing the progress made in the selection of desirable agronomic traits in the 2 generations.

MATERIALS AND METHODS

Experimental site: The researches were conducted at the experimental field of the University for Development Studies (UDS) in the Faculty of Agriculture, Nyankpala campus in the Northern Region of Ghana. Nyankpala is located about 16 km west of Tamale Metropolis and lies on the latitude 09°24′15.9″N and longitudes 01°00′12.1′W and an altitude of 183 M (msl) of the interior Guinea Savannah agro-ecological zone of Ghana. The studies were carried out during the cropping seasons of 2015, 2016 and 2017, respectively. The experimental site is characterized by tree and weed species such as Neem tree (*Azadirachta indica*), Shea tree (*Vitellaria paradoxa*), Dawadawa tree (*Parkia biglobosa*), Teak (*Tectona grandis*), Broom weed (*Sida acuta*), Speargrass (*Imperata cylindrica* L.), Pigweed (*Boerhavia diffusa*) *Andropogon gayanus*[7]. The soil in the area is Nyankpala series and is made up of mainly sand and loam. Rainfall in the experimental area starts in late April and reaches a peak in July-September, there is a sharp decline and little or no rain in November and December[8]. The area experiences total precipitation of about 1,100 mm per annum, with a range from about 800-1500 mm (Table 1). The experimental area has an average ambient temperature usually high all year round (about 28°C) but the *harmattan* months of December and January, are characterized by the minimum temperature that may fall to 13°C at night, while March and April, may experience 40°C in the early afternoon (Table 2). The experimental site also has an average Relative Humidity (RH) of 64 during May-December, (Table 3).

Land preparation, experimental design and crop husbandry: The field was ploughed and harrowed by a tractor and later manually levelled, ridged and demarcated according to the planting distance of 50×20 cm for soybean in the various cropping seasons. This was done with the aid of tape measure, lines and pegs. A plot size measuring 2×2 m, with 1.5 m space between blocks and 1 m space between plots and replication were considered.

The trials were laid out using Randomized Complete Block Design (RCBD) with three replications. Soybean variety (Jenguma) were irradiated to generate M_1 seeds in 2015 using 150, 200, 250 and 300 Gy doses of gamma irradiation from Cobalt-60 source and planted. Some un-irradiated seeds (0 Gy) were also planted to serve as the control. The harvested M_1 seeds were planted to obtain M_2 seeds in 2016. Selected M_2 seeds were planted and evaluated for improved agro-morphological traits in 2017.

Table 1: Total rainfall and its' distribution during the experimental period

	2015		2016		2017	
Rainfall (mL)	Total rainfall (mL)	Number of rain days	Total rainfall (mL)	Number of rain days	Total rainfall (mL)	Number of rain days
May	40.0	5	58.7	6	38.4	4
June	158.9	8	157	8	68.9	5
July	222.7	11	236.4	14	146.4	12
August	238.0	14	240.2	14	180.5	15
September	260.9	16	256.7	17	227.5	16
October	132.7	12	133.9	11	124.3	8
November	19.5	1	20	2	7.6	1
December	0	0	0	0	0	0
Total	1072.7	67	1102.9	72	793.6	61
Mean	134.1	8.4	137.9	9.0	99.2	7.6

Table 2: Temperature distribution during the experimental period

	2015			2016			2017		
Temperature (°C)	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean
May	25.3	34.3	29.8	26.8	35.5	31.2	25.9	35.5	30.7
June	24.3	31.1	27.8	25.6	33.2	29.4	25.3	34.3	29.8
July	23.6	29.7	27.8	24.9	30.9	27.9	24.2	30.7	27.5
August	22.9	28.9	26.7	23.3	30.4	26.9	24	29.8	30.8
September	22.6	30.2	25.9	23.1	30.5	26.8	24	27.5	27.4
October	23.2	32	26.4	23.5	32.7	28.1	24.5	32.5	28.5
November	22.9	35	29	24.3	35.6	30	23.2	35.7	29.5
December	19.6	35.5	27.5	20.7	35.8	28.4	18.6	34.2	26.4
Total	184.4	256.7	220.9	192.2	264.6	228.7	189.7	260.2	230.6
Mean	23.0	32.0	27.6	24.0	33.0	28.5	23.7	32.5	28.8

Table 3: Relative humidity distribution during the experimental period

	2015			2016			2017		
Relative humidity (RH)	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean
May	60	83	73	56	83	70	67	85	76
June	68	89	79	64	88	70	48	94	69
July	75	93	84	71	91	81	56	94	75
August	75	93	84	73	93	83	64	96	80
September	74	89	79	75	93	84	72	94	83
October	68	90	79	68	90	79	67	93	80
November	53	82	68	54	82	68	39	74	57
December	52	68	60	38	56	44	37	57	44
Total	525	687	606	499	676	579	450	687	564
Mean	65.6	85.8	75.7	62.3	84.5	72.3	56.2	85.8	70.5

Weeding was carried out manually on 2 weeks' interval basis to control the growth of weeds.

Data collection and analysis: Data generated from 2015-2017 from the various generations (plant height, chlorophyll content, number of branches, percentage pod shattering, number of days to 50% flowering, number of days to maturity, number of seeds per pod, number of pods per plant, pod length, 100 seed weight and total grain yield) were subjected to combined analysis for variation in factorial experiments (factor 1 being mutant genotypes and factor 2 being mutant generations) in RCBD. Genstat (18 edition) statistical package was used in the analysis. Means were separated using the Least Significant Difference test (LSD) at a 5% probability level.

RESULTS

Number of secondary branches: Irradiation dose and generation effect as well as their interactions significantly ($p<0.05$), influenced the number of branches. The 150 Gy mutant lines recorded the highest number of branches, while the 250 Gy mutant lines recorded the lowest number of branches (Fig. 2a). The M_3 generation in general recorded the highest number of branches whilst plants from the M_1 generation recorded the lowest number of branches (Fig. 2b). However, plants irradiated with 150 Gy recorded the highest number of branches at M_2 whilst those treated with 200 Gy dose recorded their highest number of branches at M_3 (Fig. 2c).

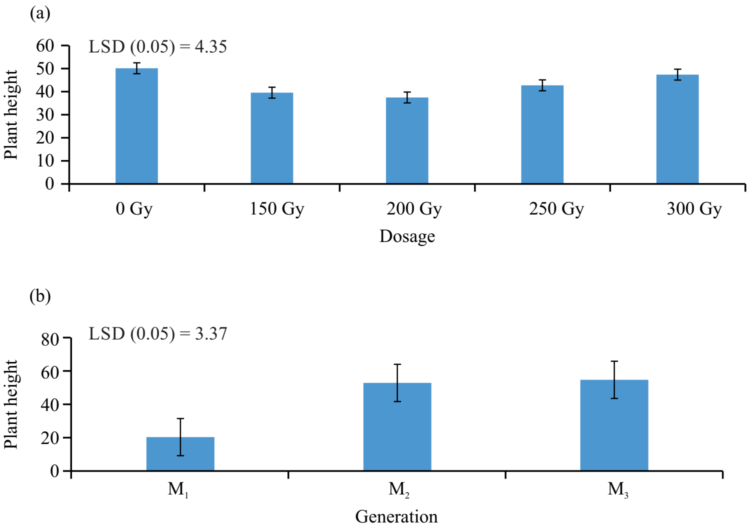

Fig. 1(a-b): Influence of (a) Irradiation dose and (b) Generations on plant height of soybean (*Glycine max* (L.) Merrill) mutants during field experimentation

Error bars represent Mean±SEM

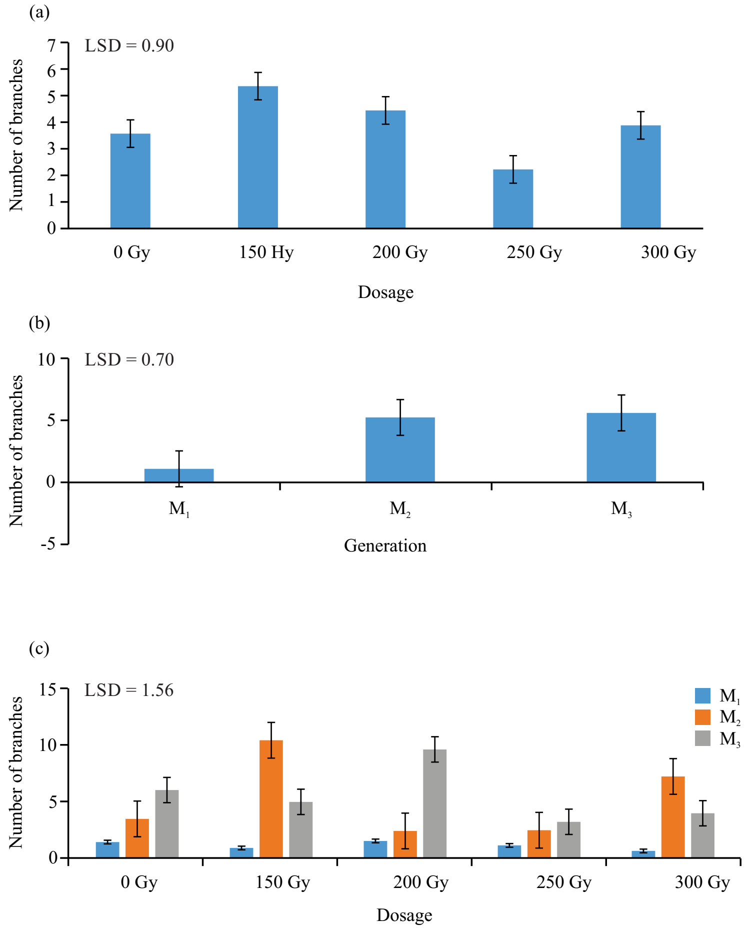

Fig. 2(a-c): Influence of (a) Irradiation, (b) Generations and (c) Genotype×generation on the number of branches of soybean (*Glycine max* (L.) Merrill) mutants

Error bars represent Mean±SEM

Days to 50% flowering: Treatments with gamma irradiation doses and generation effect as well as the interaction between the 2 factors significantly affected ($p<0.05$) number of days to 50% flowering. Plants from 150, 200 and 250 Gy recorded earliness in terms of flowering (Fig. 3a), whilst plants from the M_2 and M_3 showed the same number of days to 50% flowering (Fig. 3b). Plants from 150, 200 and 250 Gy had a similar number of days to 50% flowering at the M_2 and M_3 generations (Fig. 3c).

Number of days to maturity: The number of days to maturity followed a similar trend as the number of days to 50% flowering (Fig. 4a-c).

Pod shattering: Pod shattering was highly significant ($p<0.001$) among dosages and also single effect of generation and the interaction between the 2 factors had a significant ($p<0.05$) influence on pod shattering. The 0 Gy application dose recorded the highest number of pod-shattering, whilst the 250 Gy dose recorded the least number of pod-shattering (Fig. 5a). The M_1 generation recorded the highest number of pod-shattering per plant, whilst the M_2 generation recorded the least (Fig. 5b). The M_1 generation at 0 and 150 Gy recorded the highest pod shattering, followed by M_3 and M_2 at 0 Gy. Plants from the 200 Gy in all generations showed resistance to pod shattering (Fig. 5c).

Total grain and components of yield: Plants that were irradiated with 200 Gy of gamma-ray recorded relatively higher grain yield and components of yield especially at the M_2 generation (Table 4).

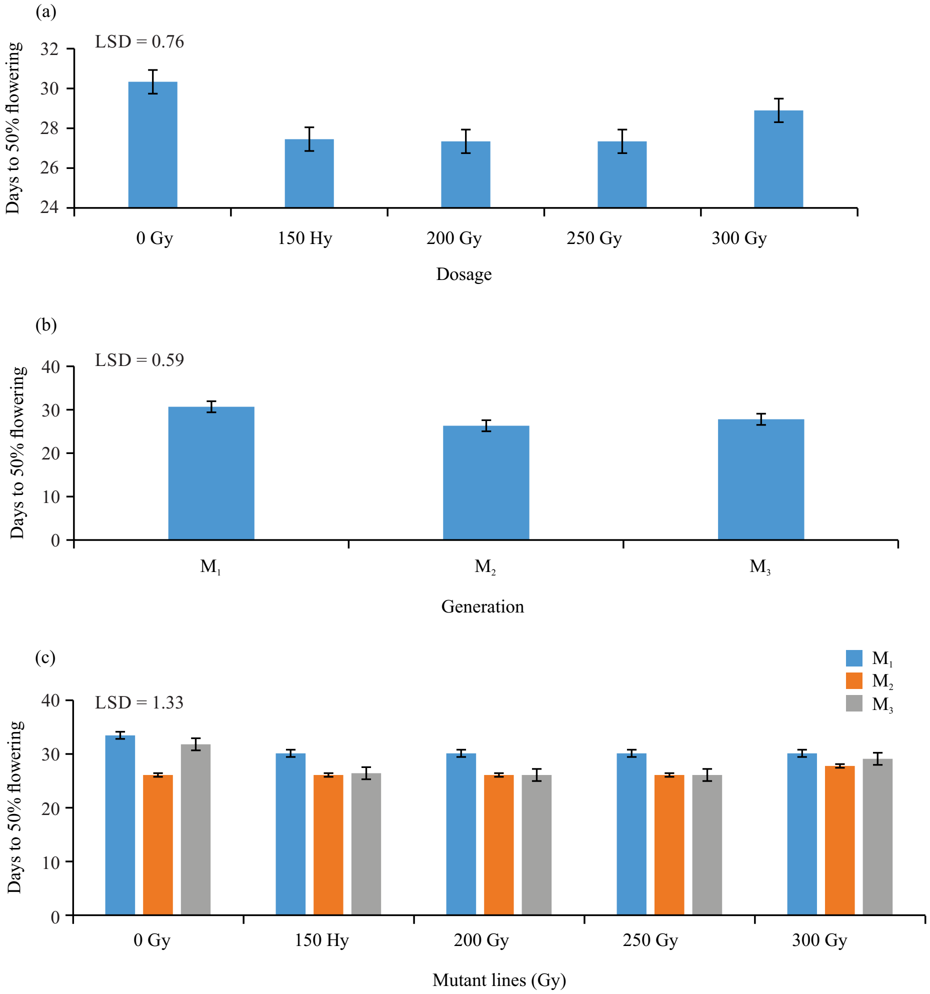

Fig. 3(a-c): Influence of (a) Irradiation, (b) Generations and (c) genotype×generation on the number of days to 50% flowering of soybean (*Glycine max* (L.) Merrill) mutants
Error bars represent Mean±SEM

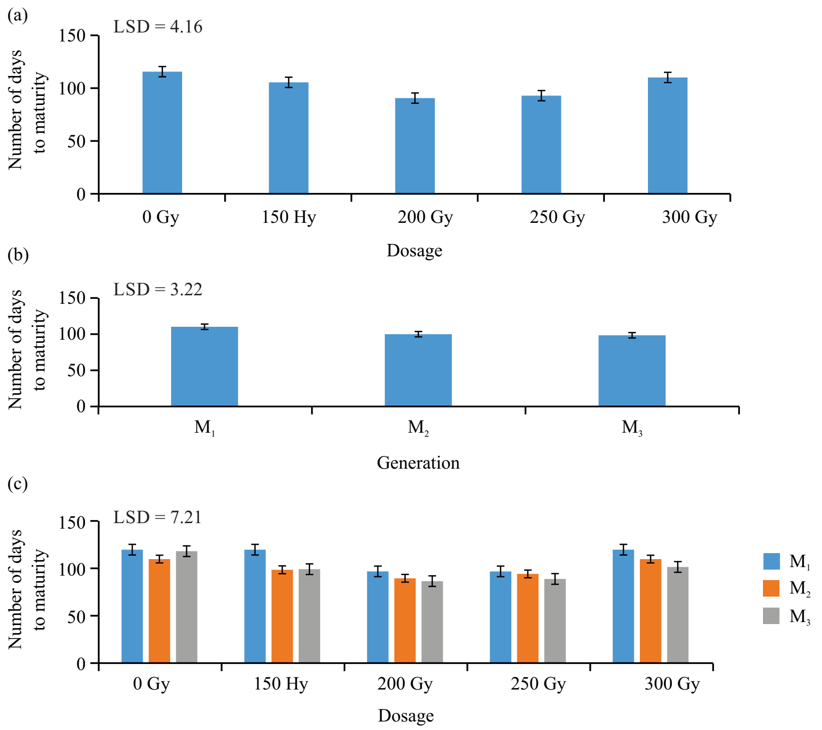

Fig. 4(a-c): Influence of (a) Irradiation, (b) Generations and (c) Genotype×generation on the number of days to maturity of soybean (*Glycine max* (L.) Merrill) mutants
Error bars represent Mean±SEM

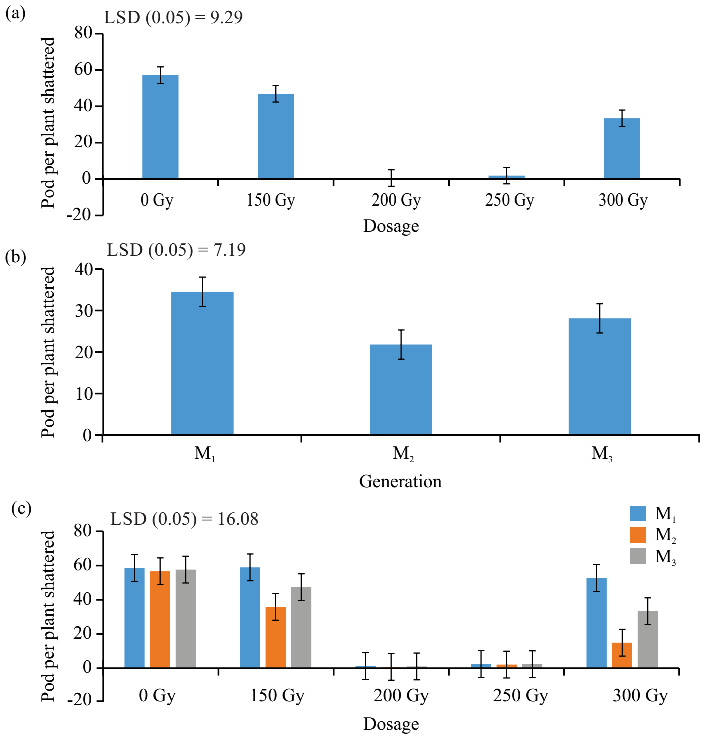

Fig. 5(a-c): Influence of (a) Irradiation, (b) Generations and (c) Genotype x generation on pod-shattering of soybean (*Glycine max* (L.) Merrill) mutants
Error bars represent Mean±SEM

Table 4: Grain yield and yield components of mutant lines of soybean (*Glycine max* (L.) Merrill) planted in Nyankpala for improved growth and yield

Dosage	Grain yield (t ha^{-1})	100-seed weight (g)	Number of pods per plant	Pod length (cm)
0	1.76	28.27	68	2.5
150	1.27	30.74	60	2.8
200	2.06	29.89	55	2.9
250	1.76	30.26	68	2.4
300	1.81	29.17	60	2.2
LSD (0.05)	3.56	2.53	9.29	0.11
Generation				
M_1	0.99	36.03	15	2.5
M_2	2.48	33.66	50	2.4
M_3	1.73	19.31	106	2.9
LSD (0.05)	0.75	1.96	7.19	0.23

DISCUSSION

There was significant variation among the irradiation doses, mutant generations and their interaction for grain yield and other agronomic traits measured. Gamma irradiation and selection significantly affected plant height. This agrees with the findings of the previous studies[9,10], who reported a reduction in height of basmati rice following gamma irradiation. The un-irradiated plants initially grew very slowly but later became the tallest, flowering lately and reaching maturity lately whilst plants irradiated with 150 and 200 Gy stopped growing tall and produced more branches. The biological effect of gamma-rays is based on the interaction with atoms or molecules in the cell[11]. Induced mutation using gamma irradiation might have resulted in retardation of growth. The observation made here is similar to those researchers[12,13], who also observed the effect of high dose treatment on plant derivatives of M_1 mungbean (*Vigna radiata* (L.) Wilczek), where plant height decreased due to the treatment of gamma irradiation. Gamma irradiation treatment increased the average plant height compared with the unirradiated controls[14]. Many studies have shown that treatment with a higher dose of gamma rays was inhibitory, whereas lower exposures were sometimes stimulatory[15,16]. This could be due to mutagenic induced chromosomal damage during cell division[17]. Gamma irradiation disturbs the synthesis of protein, hormone balance, leaf gas exchange, water exchange and enzyme activity[18]. However, previously[19] reported an increased plant height in soybean due to gamma rays. A similar observation was made in the M_3 plants that were initially treated with a 300 Gy dose of gamma-ray because these plants outgrew the 200 and 250 Gy treated plants. In-plant production, reduction in height may be advantageous as short plants generally resist lodging and hence avoid yield losses that may be associated with it. A reduction in plant height has also been reported, following the application of 250 and 200 Gy in soybeans[20,21]. The height difference was therefore more distinct in the M_3 and M_2 populations.

The number of days to (50%) flowering varied significantly among the generations of mutants of soybean. The difference was more distinct in 200 and 250 Gy mutants, which took about half the number of days taken by the 0 Gy. Zaka *et al.*[22] had earlier reported a reduction in the number of days to flowering in pea (*Pisum sativum*) following irradiation. Early maturity was observed in the soybean lines especially mutants from 150, 200 and 250 Gy. The 0 Gy took almost the same number of days as 300 Gy to mature. Ranjitha *et al.*[23] also selected mutants from gamma-irradiated soybean that proved to be early maturing and dwarf stature. Pod shattering is the opening of pods along both the dorsal and ventral sutures of the soybean pod. It is essential for the propagation of wild plant species bearing seeds in pods but is a major cause of yield loss in legume and crucifer crops. According to Valkama *et al.*[24], the gene for the shattering-resistant genotype, pdh1, is defective in soybean, having a premature stop codon. In this study, tolerance to pod shattering varied significantly among genotypes and was high in plants treated with 200 and 250 Gy. Ionizing radiation affects cellular components, thus, potentially inducing physiological changes in plants. This may be the mechanism that prevents the pod from shattering. The number of pods per plant differed significantly among the treatments. The result in part contradicts the findings of Pasupuleti *et al.*[25], who reported that the number of pods per plant decreased in all the genotypes (of a black gram) as the dose of irradiation increased. But Rahman *et al.*[26] observed that the treatment with gamma rays stimulated most of the quantitative characters of *Vigna unguiculata* such as number of branches, number of clusters, number of pods and yield. Khan and Goyal[27], also reported more number of pods per plant in mung beans treated with gamma rays. Hundred seed weight did not differ significantly among the treatments. A study of M_1 of cowpea, groundnut and soybean using gamma-ray at the same dose reported no significant differences[28]. Since hundred seeds weight did not significantly differ among the treatments, the number of pods that had a significant variation seemed to have caused the significant differences in the yields of soybean. The number of pods is a good selection criterion for increasing yield in grain legumes. This finding corroborates with the observation made by Rahimi and Bahrani[29], who reported the highest grain yield increase in canola irradiated with 0.2 kGy of gamma rays. Similarly, they also reported a significant improvement of 1000 kernel weight and harvest index for 0.1 kGy gamma-ray treatments. The sizes of grains in the mutants were comparably high as compared to the parent and were selected for high yield. The M_2 generation recorded the highest yield compared to the other generations. The results of the M_3 generation showed that it is possible to effectively increase grain yields components at a dose of 200 and 250 Gy. Similar observations were also made in other plants like black gram, cowpea and sesame[30]. Improvement of agronomic characteristics by using gamma radiation has also been reported in several studies. A study revealed a significant increase in chickpea grain yield using gamma irradiation at 0.6 kGy[31]. An increase has also been observed, in the number of pods per plant in all the varieties for gamma irradiation at 0.2 and 0.4 kGy doses[32]. However, Khan *et al.*[31], reported a decrease in pod number at 0.4 kGy treatment and an increase at 50 kGy without a change in the number of seeds per pod. The selected seeds especially of 200 and 250 Gy mutant lines should be advanced for multi-locational trials to determine the suitability of the selected mutants in various agro-ecologies for release as varieties for production by farmers.

CONCLUSION

The study showed that plants treated with 200 and 250 Gy were found to be highly resistant to shattering than the un-irradiated (0 Gy/control), believed to be an improved variety against field shattering. Field performance (agronomic traits) of soybean plants treated with 200, 250, 150 and 300 Gy were on the average higher than the parental line (control/0 Gy). These mutant lines also had shorter stems as compared to the parent line, which has an advantage in terms of lodging during heavy rains and strong winds. Yield and yield components results for soybean were higher among the M_2 populations followed by M_3 while M_1 had the least. Individual plants with desired characteristics than the parental variety 'Jenguma' were selected for multi-locational trials in the next generation. The number of pods per plant, seed size and seed weight were particularly considered during selection.

SIGNIFICANCE STATEMENT

This study discovered that mutagenesis could help improve yield, growth parameters and pod-shattering resistance of soya beans. Through this study, the researcher had discovered that plants treated with 200 and 250 Gy were found to produce more grain yield, improved growth parameters and were highly resistant to pod-shattering than the un-irradiated (0 Gy/control). Therefore, farmers who use these mutant lines are likely to get a better grain yield. This will result in increased soybean productivity in the Guinea Savannah Agroecology of Ghana.

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