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Research Article
Mutagenesis for Improvement of Growth and Yield of Groundnut (*Arachis hypogea* L.) in the Guinea Savannah Agro-Ecology of Ghana

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Abstract
Background and Objective: In recent times, groundnut production has recorded low yields in the Northern part of Ghana and this could be attributed to the use of unimproved varieties, the incidence of diseases and pests and the prevalence of drought. The objective of this study was to compare agronomic traits of M_1, M_2 and M_3 mutant lines of groundnut produced over the years in the Guinea Savannah agro-ecological zone of Ghana. **Materials and Methods:** The M_1, M_2 and M_3 mutant lines of groundnut were screened for improved agronomic traits. The trials were laid out using Randomized Complete Block Design (RCBD) with 3 replications. The experimental plots measured 3×3 m. Data collected were subjected to combined analysis for variation in factorial experiments using Genstat (18 edition) statistical package. Means were separated using the Least Significant Difference (LSD) approach at a 5% probability level. **Results:** Results of the study indicated that mutagenesis had great potential to improve the yield characteristics of groundnut. More desired traits were found with the 150 and 200 Gy doses. The number of pods per plant and seed weight was the key parameters found to influence grain yield. The maturity period was found to be shorter in the selected mutant genotypes. **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, generation, groundnut, mutagenesis, Guinea Savannah, agroecology, mutant lines

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

In Ghana and especially in the Northern Region, groundnuts are the most cultivated crops for their oil and protein content[1]. Groundnut is a self-pollinated, annual leguminous crop that is fairly drought resistant and mainly cultivated in dry tropical areas[2]. The crop increases the nitrogen content of the soil through its nodulation and biomass production. Therefore, subsequent crops that are cultivated after the harvest of the groundnut crop, benefit a lot from the residual nitrogen especially, when groundnut residues are incorporated into the soil during ploughing. Despite the high local demands for groundnuts, yields in Ghana continue to be low, averaging 0.1 t ha^{-1} of dry shelled seeds. Groundnut is an important oil, food and feed legume crop grown in over 100 countries in Sub-Saharan Africa. It covered 24 million ha area worldwide with a total production of 38 million tons in 2010[3]. Groundnut is the 6th most important oilseed crop in the world. It contains 48-50% oil and 26-28% protein and is a rich source of dietary fibre, minerals and vitamins.

It has been demonstrated in many studies that genetic variability for several desired characters can be induced successfully through mutations and the practical value of mutation in plant improvement programmes has been well established[4]. Mutation breeding is the process of generating mutants with desirable traits when plants are exposed to chemicals or radiation. It is sometimes referred to as "variation breeding". Plants created using mutagenesis are sometimes called mutagenic plants or mutagenic seeds. Induced mutations can provide beneficial variations to breed high yielding groundnut. More than 2252 mutant varieties of different crops have been officially released in the world[5]. There has been fruitful gamma irradiation for the development of new mutant varieties[6,7]. Previously[8] reported beneficial use of grain legume mutation breeding for the development of improved cultivars. Mutation breeding serves as a source of creating variability and could confer specific improvement in a crop without significantly altering its phenotype. The successful utilization of gamma rays to generate genetic variability in plant breeding has been reported in groundnut[9]. From 1930-2014 more than 3200 mutagenic plant varietals have been released that have been derived either as direct mutants (70%) or from their progeny (30%). Crop plants account for 75% of released mutagenic species with the remaining 25% being ornamentals. There are different kinds of mutagenic breeding, a few of such includes: The use of chemical mutagens, radiation and also transposons are used to generate mutants.

The exposure of plant materials to gamma radiation has been reported to give rise to morphological, physiological and biochemical mutants[10]. Gamma radiation doses at the range of 200-300 Gy are known to be suitable for the mutation induction in groundnut[10]. Plants with desirable traits were observed and identified from 200-250 Gy doses in groundnut and these showed fewer days to maturity apart from recording higher yield over the control[11]. Gamma radiation improved germination, plant height, seed per plant and seed yield per plant in wheat[12]. Some soybean seeds are irradiated with gamma doses at 0, 50, 100, 150 and 200 Gy[13]. Variations that were obtained from each dose at M_1 and M_2 generations influence the plant growth and development both qualitatively and quantitatively. The average highest genetic variation at M_2 generation was induced from the 200 Gy dose. The mutagenic treatments increased the mean values of plant height in the M_1 generation[14]. Exposure of cowpea seeds to gamma rays at a dose of 250 Gy resulted in a decrease in germination percentage[15]. The yield of several crops has been increased by creating a variation of the parental crops. Plant breeding requires a genetic variation of useful traits for crop improvement. Often, however, the desired variation is lacking[16]. Consequently, the extent to which groundnut cultivars may be improved through conventional breeding methods is limited. Genetic engineering, hybridization and induced mutation have been used in the generation of genotypic variation. Genetic engineering has several challenges concerning the release of transgenic plants into the environment in terms of religion, health, environment and ethical interest. Hybridization though may be useful, the various problems often encountered in effecting crosses such as a longer period in evolving a superior variety and the non-availability of parents with desirable genes have resulted in a limitation on the use of hybridization[17]. It has been demonstrated that genetic variability for several desired characters can be induced successfully through mutations and the practical value of mutagenesis in plant improvement programmes has been well established[4].

The objective of the study was to create genetic variation in groundnut through mutagenesis and hence, select desirable mutant genotypes for multilocational trials.

MATERIALS AND METHODS

Experimental area: The study was conducted at Nyankpala in the Northern Region of Ghana. This research project was conducted from 2015-2017. The experimental site is located in the Guinea Savanna agro-ecological zone of Ghana. The Guinea Savanna zone covers an area of 147,900 km^2, which is over one-third of the entire land area of Ghana[18]. The experimental site as well as the weather characteristics of the experimental area, such as rainfall, temperature and evaporation, have already been described in detail as contained[19].

Land preparation, experimental design and crop husbandry: In each of the experimental years, the field was ploughed and harrowed using a tractor and later manually levelled, ridged and demarcated into experimental plots measuring 3×3 m. The walking path between blocks/replications were 1.5 m whiles 1 m space was left in between plots. The trials were laid out using Randomized Complete Block Design (RCBD) with 3 replications. Groundnut variety (Chinese) were irradiated to generate M_1 seeds in 2015 using 150, 200, 250 and 300 Gy doses of gamma irradiation from Cobalt-60 sources and planted. Some un-irradiated seeds (0 Gy) were also planted to serve as a 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.

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

Data collection and analysis: Data on percentage emergence, plant height, number of days to 50% flowering, average pod length, number of days to maturity, number of seeds per pod, 100 seeds weight and total grain yield were recorded and subjected to combined analysis for variation in factorial experiments in RCBD in Genstat (18 edition) statistical package. Means were separated using the Least Significant Difference (LSD) approach at a 5% probability level.

RESULTS

Plant height: Variation in plant height for the 3 generations was highly significant (p<0.001). Mutants from the M_2 generation recorded the tallest followed by those from the M_3, with those from M_1 recording the least height (Fig. 1a). There was also a highly significant difference (p<0.001) among the mutant genotypes with 250 Gy recording the highest height, whilst those irradiated with 200 Gy were the shortest (Fig. 1b).

Number of leaves and chlorophyll content: Significant differences (p<0.05) were observed among the various generations in terms number of leaves. M_1 plants recorded the highest number of leaves among generations with

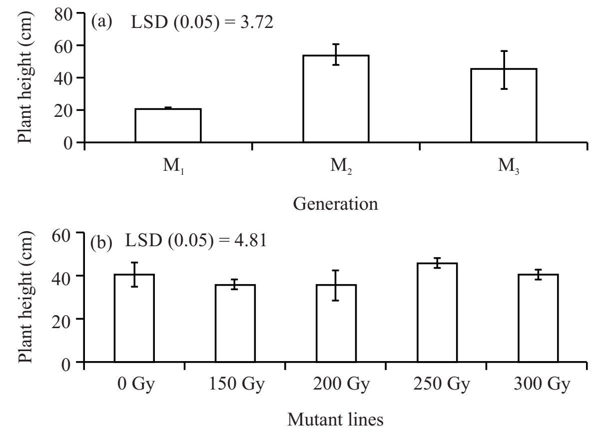

Fig. 1(a-b): Influence of (a) Irradiation dose and (b) Generations on plant height of groundnut (*Arachis hypogea* L.) mutants during field experimentation
Error bars represent Means±SEM

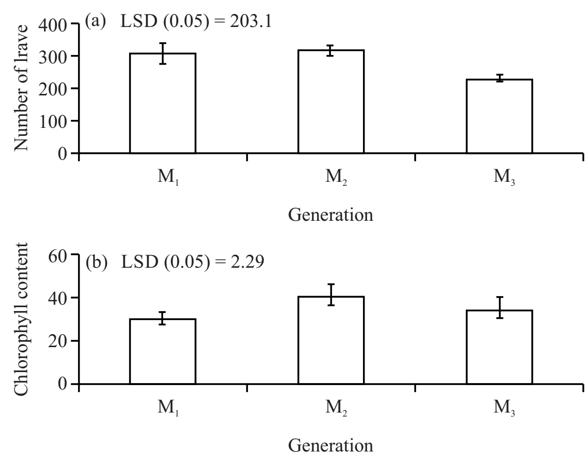

Fig. 2(a-b): Variation in (a) Number of leaves and (b) Chlorophyll content for generation of mutant groundnut (*Arachis hypogea* L.)
Error bars represent Means±SEM

M_2 recording the lowest (Fig. 2a). Differences among the mutant genotypes and the interactions between the mutants and generations were not significant (p>0.05). Chlorophyll content was significantly different (p<0.001) among the generations of selection of mutant groundnut. The M_2 plants had the highest whilst M_1 had the lowest (Fig. 2b). The single effect of mutant groundnut and the interactions did not show any significant differences (p>0.05).

Number of branches: Significant differences (p>0.05) in the number of primary branches were observed for the interactions between mutant groundnut and generations.

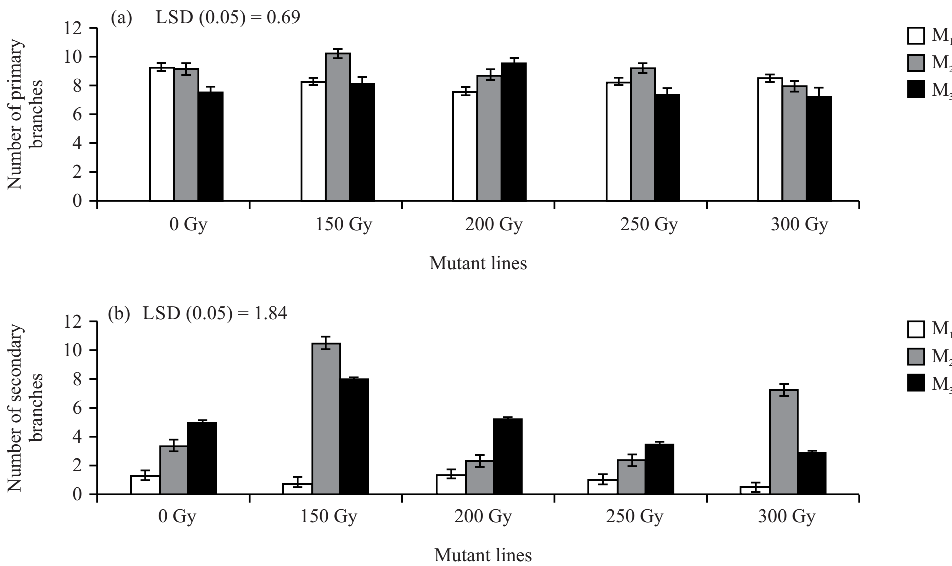

Fig. 3(a-b): Variation in (a) Number of primary branches and (b) Number of secondary branches of mutant groundnut (*Arachis hypogea* L.)
Error bars represent Means±SEM

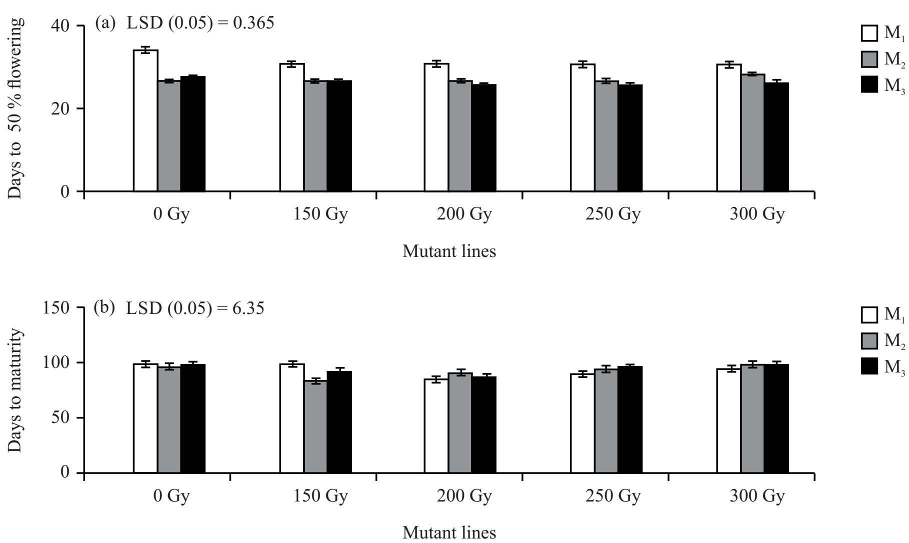

Fig. 4(a-b): Variation in (a) days to 50% flowering and (b) days to maturity of mutant groundnut (*Arachis hypogea* L)
Error bars represent Means±SEM

The number of primary branches from M_2 plants from the 150 Gy irradiation dose recorded the highest value whilst those of M_3 from 250 Gy and 300 Gy recorded the least (Fig. 3a). Similar observations were made for the number of secondary branches (Fig. 3b).

Days to 50% flowering and maturity: Variation in the number of days to 50% flowering was highly significant (p<0.001) for the interaction between mutant groundnut and generation. The 200 and 300 Gy mutant plants from the M_3 generation recorded the least value of days to 50% flowering whilst unirradiated plants in the M_1 were the latest to flower (Fig. 4a). The maturity period mimicked closely the pattern displayed by flowering except that in the former plant from the 150 Gy treated plants in the M_2 generation were the earliest to mature (Fig. 4b).

Total grain yield and yield components: The M_3 mutant genotype at 150 Gy dosage application recorded the highest grain yield of 2.31 t ha^{-1}. At 0 Gray application, M_3 recorded

Table 1: Variation in grain yield and yield components of mutant groundnut (*Arachis hypogea* L.)

	M_1			M_2			M_3		
Dosage	Seed weight (g)	Numbers of pod/plant	Total grain yield (t ha^{-1})	Seed weight (g)	Numbers of pod/plant	Total grain yield (t ha^{-1})	Seed weight (g)	Numbers of pod/plant	Total grain yield (t ha^{-1})
0 Gy	16.54	2.29	1.02	33.87	14.73	1.49	32.43	35.87	1.19
150 Gy	27.27	2.30	0.89	35.81	16.20	1.87	35.66	59.33	2.31
200 Gy	26.51	2.21	0.74	34.36	19.10	2.02	35.81	39.47	2.14
250 Gy	26.18	2.36	1.25	32.73	19.60	1.63	38.90	38.87	1.60
300 Gy	25.06	2.30	1.06	31.54	15.60	1.60	37.33	37.27	1.31
LSD (0.05)	7.834	9.87	0.380	7.834	9.87	0.380	7.834	9.87	0.380

LSD: Least significant digit

the lowest yield of 1.19 t ha^{-1} (Table 1). There were significant ($p<0.05$) variations of grain yield among the various dosage applications for M_3. Grain yield for M_3 (2.31 t ha^{-1}) at 150 Gy was significantly higher than that of M_2 at the same dosage application. The highest number of pod per plant was recorded by M_3 (59 pods) at Gray application of 150 Gy and this was significantly ($p<0.05$) higher than the number of pods produced by both M_2 and M_1 at similar Gray applications (Table 1).

DISCUSSION

The mutation is one of the genetic tools that can be used to create variation among plant species. Many studies have demonstrated that genetic variability can be induced successfully through mutation[4]. Mutation breeding is responsible for increasing the genetic variability for the desired traits in various crop plants and this has been proved beyond doubt by several scientists[20,21]. The analysis of the present study showed that much genetic advancement was made from M_1 through to M_3 generations in most of the parameters. In most cases, the valuable traits in mutation were heritable since those in M_1 reappeared in advance in M_2 and then in M_3 as reported previously[22]. The variation observed among the parameters of the various generations could be attributed to environmental and genetic influences. The genetic influence might be as a result of the effect of the gamma irradiation given to the parent lines. The analysis, therefore, showed that the plant height of groundnut mutants was significantly affected by both generation and gamma irradiation. The M_1 generation recorded the least height which increased significantly as the M_1 generation advanced to M_2 and then M_3. As observed[23] that plant height was increased in M_1 generation with gamma rays compared to the pure lines. In the case of dosages, 250 Gy mutant lines showed the highest plant height. This is in line with the findings of researchers[24], who reported an increase in plant height in soybean due to gamma rays. It has also been observed that gamma irradiation treatment increases plant height as compared with unirradiated seeds[25]. However, the height of 250 Gy was shorter than that of 300 Gy. Reduced height in plants help to prevent lodging that may lead to yield loss.

High gamma irradiation dose treatment of Mung bean (*Vigna radiata* (L.) Wilczek] decreased plant height[20,26]. Ramesh and Reddi[27] also observed a decrease in plant height in *Oryza sativa* as a result of gamma irradiation. The differences in plant height led to a significant difference in the number of leaves produced among the various generations of selections. In mutation breeding studies, exposure of plant materials to gamma radiation has been reported to give rise to morphological, physiological and biochemical mutants[10]. Though no significant difference was shown among mutant lines for the leaf number, plants from, 150 Gy showed the highest number of leaves compared to other higher doses which recorded almost the same number of leaves. Jan *et al*.[28] reported that irradiation with lower doses of gamma rays significantly improved vegetative traits while higher doses had inhibitory effects on vegetative parameters. Chlorophyll content varied significantly among the generations. The M_2 mutant lines followed by M_3 recorded the highest value in chlorophyll content. Increased dosages/concentrations of mutagens resulted in increases both in frequency and spectrum of chlorophyll mutation in M_2 compared to M_1 mutants. Pungulani *et al*.[29] indicated that parameters associated with photosynthesis are good characteristics for selection and this demonstrates the importance of chlorophyll content as a selection criterion for yield in the present study.

There was no significant difference in the number of primary branches among the dosages except for the number of secondary branches, with 150 Gy mutant lines recording the highest followed by 300 Gy. While the highest interactions were recorded between M_2 and 150 Gy, 0 Gy showed the least number of secondary branches. Treatment with gamma rays stimulated most of the quantitative characters of *Vigna unguiculate* including the number of branches[30]. The number of days to 50% flowering varied among all the treatments (generations and dosages) including their interactions. The M_1 and 0 Gy showed the highest number of days to flowering than plants from M_2 and 200 Gy which recorded the least number of days to flowering. Zaka *et al.*[31] had earlier reported a reduction in the number of days to flowering in pea (*Pisum sativum*) following irradiation and the results of the present study confirm this finding.

The maturity date varied significantly among the dosages. The 200 Gy followed by 150 Gy of M_2 and M_3 generation interestingly mature earlier whilst the 0 Gy took almost the same number of days as 300 Gy to mature. The maturity period of groundnut mutant lines varied due to the difference in the maximum percentage of mature fruits at harvest[32]. There was thus a positive correlation (coefficient not shown) between days to 50% flowering and the number of days to maturity in dosages.

In general, the irradiation rate at the M_3 generation produced negative results for most of the parameters but not those of yield components as shown by the analysis when compared with the M_1 and M_2. Tukamuhabwa *et al.*[33] reported that seeds treated with an irradiation dose of 600 Gy did not survive to produce offspring for assessment. Many studies have shown that treatment with a higher dose of gamma rays was inhibitory, whereas lower exposures were sometimes stimulatory[33]. Jan *et al.*[28], also reported that irradiation with lower doses of gamma rays significantly improved vegetative traits while higher doses had inhibitory effects on vegetative parameters.

The number of seeds per pod did not differ significantly among the treatments and their interaction. The number of pods per plant on the contrary differed among the treatments. The 150 Gy produced the highest number of pods in M_3 as compared with 200, 250 and 300 Gy in M_3 and other generations. The result in part contradicts the findings of Janila *et al.*[34], who reported that the number of pods per plant decreased in all the genotypes of a black gram as the dose of irradiation was increased. However, the 150 Gy mutants recorded more pods per plant than 0 Gy in all the generations. Mudibu *et al.*[30], observed that the treatment with gamma rays stimulated the number of pods of *Vigna unguiculate*. Singh *et al.*[35], also reported increases in the number of pods produced per plant in *Vigna radiata*. Seed size has been identified as a selection criterion for high yields in Bambara nut (*Vigna subterranean* (L.) Verdc)[29]. In the present study, the hundred seed weight differed significantly among the generations. Hundred seed weight together with the number of pods per plant rather than the number of seeds per pod and pod length which showed no significant variation seemed to have caused the significant differences in the yields. The number of pods, therefore, as suggested by Nwofia *et al.*[36], is a good selection criterion for increasing yield in grain legumes (groundnuts). Pod length is a criterion for selection, but in the present study that did not vary significantly. The treatments significantly affected the grain yield for M_1, M_2 and M_3 groundnuts. The 150 Gy mutants followed by 200 Gy in M_3 obtained the highest grain yield as compared to the other higher doses. Singh *et al.*[35], reported increases in total seed yield at lower doses of chemical mutagens in *Vigna radiata*. The 0 Gy performed the least among the irradiation doses in all the generations. This is in line with the report of Jamil and Khan[12] that in wheat, gamma radiations improved germination, plant height and seed yield per plant. Anning[11] reported that plants with desirable traits were observed and identified from 200-250 Gy doses in groundnut and these showed fewer days to maturity apart from recording higher yield over the control.

The implications of this study to the farmers, in and around the study locality, are numerous. The farmers will begin to appreciate the fact that if the genetic structure of the groundnut genotype is altered, grain yield is likely to increase or decrease depending on the level of genetic alteration. Knowledge and skills of mutation breeding should be disseminated to farmers so they can begin to practise mutation breeding in farmers' fields. It is recommended that mutant lines that were found to contain the desirable traits for yield and growth parameters, such as 150 Gy and 200 Gy, should be evaluated in multi-locations on farmers' fields. The on-farm evaluation of the desirable mutant lines will enable farmers to observe the practicability of mutagenesis and hence practice the new concept on their farms.

CONCLUSION

Desirable traits, such as shorter days to flowering, shorter days to maturity, the high number of pods per plant and high grain yield were observed in mutant lines 150 Gy and 200 Gy of groundnut. The yield and yield components recorded were highest among M_3 mutant generation, followed by M_2, whilst M_1 produced the least. The selected seeds of 150 Gy and 200 Gy mutant lines and the M_3 generation should be evaluated in multiplications for release to farmers for production.

SIGNIFICANCE STATEMENT

This study discovered that mutation breeding could help improve the yield and growth parameters of groundnut. Through this study, the researcher had discovered that the use of 150 Gy and 200 Gy dose application levels will result in increased groundnut productivity in the Guinea savannah agroecology of Ghana.

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