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Research Article
Selection of Mutants with Improved Growth and Total Grain Yield in the M_2 Generation of Pearl Millet (*Pennicetum glaceum* L.) in the Northern Region of Ghana

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Abstract

Gamma rays are the most commonly used mutagen in mutation breeding, owing to its ability to induce mutations at relatively higher frequencies. In the present study, gamma irradiation techniques were applied to pearl millet (Var. Naara) in order to determine the response in terms of growth and total grain yield of the crop to the mutagen in Northern region of Ghana, which is located in the Guinea Savannah Agroecology. Seeds were irradiated with gamma rays at 100, 200 or 300 Gy. Irradiated seeds and some unirradiated control seeds were planted in randomized complete block design with four replications at the experimental field of the University for Development studies, Nyankpala in the Northern region of Ghana. Data were collected on increases in height of plants, number of tillers, number of productive tillers, earliness to flowering, head length, head width, seed weight and total grain yield. Plants that received mutagen doses such as 100 or 200 Gy revealed stimulatory effects for all characters studied in the segregating M_2 generation relative to the control. Plants from the 300 Gy performed significantly similar in most of the characters measured as those subjected to 200 Gy. In general, superior strains were screened based on their growth rates and grain yield characteristics in the M_2 for advancement to M_3 generation. This study discusses the results of the M_1 and M_2 populations.

Key words: Gamma irradiation, dose, variety, mutation, mutagen, pearl millet

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

INTRODUCTION

Millets are cereal crops with relatively high global production due to its relative resilience and productivity under dry temperature and low nutrient conditions. Millets are of immense importance in tropical Africa, contributing significantly to the daily calorific intake in the sub region (McDonough *et al*., 2000). The most popular and widely grown species among the millets is the pearl millet (*Pennisetum glaceum* L.) (FAO., 1997).

Millets provide 13.40 kg year^{-1} per capita food use. They are nutritionally equivalent or superior to other cereals. They generally contain high protein (up to 9.5 g/100 g), ash, calcium (up to 344 mg/100 g), phosphorus and potassium (up to 250 mg/100 g and 314 mg/100 g, respectively), iron and zinc levels (Obilana and Manyasa, 2002).

Plant breeders exploit natural and induced genetic variation in crop improvement programmes. This type of variability helps in the selection of desirable traits in terms of growth, total grain yield and nutritional characteristics (Jain, 2010). However, the efforts by most plant breeders in developing crops with improved desirable characteristics are thwarted because the desired variability may be lacking in certain crop species. Different breeding techniques have successfully been utilized in breeding crops and many crop plants with improved desirable characteristics have been produced (Huang *et al*., 2002).

Frequency of spontaneous mutations is rather very low and difficult to exploit by the plant breeder (Maluszynski, 1990; Jain, 2010). Experimental mutagenesis aims at inducing mutations at higher frequencies in the treated populations (Micke, 1996). Induced mutation has widespread usage in improvement of crops as it can help to create and regenerate the required variability needed for crop improvement (Khan and Goyal, 2009). Gamma rays have been effective and are more commonly used in mutation breeding studies than other ionizing radiations because of their availability and relatively high power of penetration. The penetration power of gamma rays helps in its wider application for the improvement of various plant species (Moussa, 2010).

Millet remains a neglected and under-utilized crop with low yields especially under the local environmental conditions. For the last 35 years in all of Africa, the area planted to millets has increased by 50% but yields, averaging 620 kg ha^{-1} have not shown any significant change (Axtell, 1998). Yield levels and growth characteristics of most genotypes of pearl millet cultivated by farmers in Northern Ghana are not encouraging. Therefore, there is the need to overcome this problem though plant breeding. This study was conducted to subject millet to experimental mutagenesis and select mutants with improved growth and total grain yield.

MATERIALS AND METHODS

Study area: Two experiments (generation of M_1 and M_2 populations of pearl millet) were conducted from August to November, 2014 and December, 2014 to March, 2015, respectively at the University for Development Studies, Ghana. The study area is located on altitude 183 m, latitude 09°25′ N and longitude 0°58′ W. The area has only one rainy season in each year with a total annual rainfall of about 1000 mm. The months of August and September generally record the highest rainfall in the year. The average minimum temperature is 25°C whilst, the maximum average temperature is 35°C (Lawson *et al*., 2013). The area is found in the interior Guinea Savannah Agroecology of Ghana and is characterized with natural vegetation dominated by grasses with few shrubs. The experimental sites have moderately drained soils which are free from concretions and are shallow with hardpan under the top few centimeters and were derived from voltaian sandstone. The soils, according to FAO (1988) are classified as plinthic acrisol. The area has grassland vegetation and it is interspersed with short trees such as *Parkia biglobosa* and *Azadirachta indica* and weeds such as *Centrosema pubescens, Cyperus difformis* and *Striga hermontheca*.

Seed irradiation and plant culture: Seeds of pearl millet variety Naara were procured from Savannah seeds in Tamale, Ghana. Twenty grammes of well filled and healthy seeds were packed in brown paper envelops for treatment with gamma irradiation at the ^{60}Co gamma source of the Radiation Technology Center of Ghana Atomic Energy Commission (GAEC) in Accra, Ghana in August, 2014. The gamma irradiation doses used were 100, 200 or 300 Gy. The irradiated planting materials together with some unirradiated control were planted. During planting, treatments were replicated four times using randomized complete block design at a spacing of 25×75 cm. Plot size per treatment was 2.5×2.5 m. The M_1 seeds were all harvested and planted to obtain the M_2 generation. All M_2 plants were monitored individually in the field and screened for superior characteristics. During selection, parameters such as plant height, number of productive tillers, earliness to flowering, head length, head width and seed weight were considered. Weed control was done by hoeing at 4 and 7 weeks after planting. Scaring of birds was done from seed filling stage till harvest.

RESULTS AND DISCUSSION

Vegetative growth and development: The influence of gamma irradiation on vegetative parameters such as plant height, number of tillers, number of productive tillers and earliness to flowering is as shown in Table 1. Gamma irradiation had significant ($p<0.05$) influence on plant height. In the first generation (M_1), plants subjected to 100 Gy plots recorded the highest height. It was, however, similar to the value recorded by plants in the control plot, which in turn was similar to plants in 200 Gy plots. In the second generation (M_2), however, growth of plants in the 100 Gy plots were significantly the same as plants in 200 Gy plots, which was in turn similar to plants in 300 Gy plots (Table 1). The hypothetic origin of these stimulations of gamma ray doses could be attributable to cell division as well as an activation of growth hormone, such as auxin (Gunckel and Sparrow, 1961). It has been established that the lower doses of gamma rays are known to have stimulatory effect on plant growth through modification in the pattern of hormonal functioning in plant cell (Wi *et al*., 2007).

Gamma irradiation had no significant ($p>0.05$) effect on tillering in M_1, however, it significantly ($p<0.05$) influenced the parameter in M_2. Productive tillers were significantly ($p<0.05$) affected by gamma irradiation treatment in both M_1 and M_2 generations. Plants in 100 Gy plots recorded the highest tiller number, followed by those subjected to 200 Gy, the performances of, which were significantly the same as mutants produced from 300 Gy (Table 1). In general, tillering was greatly influenced when plants were subjected to 100 and 200 Gy of gamma irradiation in M_1. Subsequently in M_2, performance of plants irradiated with 100 Gy were at par with plants from the control plots, while plants irradiated with 200 Gy performed significantly the same as those that received 300 Gy of gamma irradiation (Table 1). In general, it is known that auxiliary bud development and floral differentiation occur at an early stage in plant development. It is also possible that mutants produced from irradiation of 100 and 200 Gy of gamma rays were affected through biochemical processes giving rise to increased auxin levels and these modified the rate and pattern of apical differentiation at these stages. This could have had a direct effect on subsequent vegetative growth of plants from the present study through the production and proliferation of tillers as has been reported by El-Ashry *et al*. (1992) and Koli and Sharma (2002).

Gamma irradiation also significantly ($p<0.05$) impacted on days to flowering. Pattern of flowering as influenced by gamma rays was similar in both M_1 and M_2 generations. Plants in 100 Gy plots took the least days to attain 50% flowering and differed significantly from plants in 200 Gy, 300 Gy and control plots (Table 1). The efficiency of lower levels of the gamma irradiation in stimulating early flowering could be due to the fact that biological damage such as seedling injury, lethality and sterility increased with increase in doses at faster rate than the mutations as reported by Konzak *et al*. (1965).

Yield and yield components: Head length and head weight in M_1 did not significantly ($p>0.05$) vary following gamma irradiation, but in M_2 the two parameters varied significantly ($p<0.05$) as they were influenced by the irradiation. However, head width and seed weight significantly ($p<0.05$) differed as they were influenced by the gamma irradiation treatment in both M_1 and M_2. Plants subjected to 100 Gy gave the best performance in terms of head length especially in the M^2, it was followed closely by plants in 200 Gy plots, which performed similar to plants in 300 Gy plots (Table 2). The best head width in the M_1 generation was obtained from plants irradiated with 100 Gy but was significantly the same to the value obtained from the unirradiated control (Table 2). In the second generation (M_2) however, head width recorded by

Table 1: Growth, tillering and flowering response of pearl millet to gamma irradiation

	Plant height (cm)		Tillers		Productive tillers		Earliness to flowering (days)	
Gamma rays generations	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
Control	71.90^{ab}	97.70^{a}	6.03	3.66^{ad}	1.05^{b}	1.50^{b}	45.75^{a}	50.00^{a}
100 Gy	90.20^{a}	124.10^{b}	8.35	7.15^{b}	1.45^{a}	2.75^{a}	41.75^{b}	45.00^{b}
200 Gy	56.90^{bc}	115.40^{bc}	7.04	5.40^{c}	1.40^{a}	2.75^{a}	47.50^{a}	50.00^{a}
300 Gy	47.20^{c}	106.50^{c}	6.45	4.90^{cd}	1.20^{b}	1.50^{b}	48.75^{a}	50.25^{a}
LSD (0.05)	22.40	9.86	5.00^{NS}	1.39	0.18	1.08	3.52	3.90

Treatment means having the same letter (vertically) in each column are not significantly different, NS: Not significant

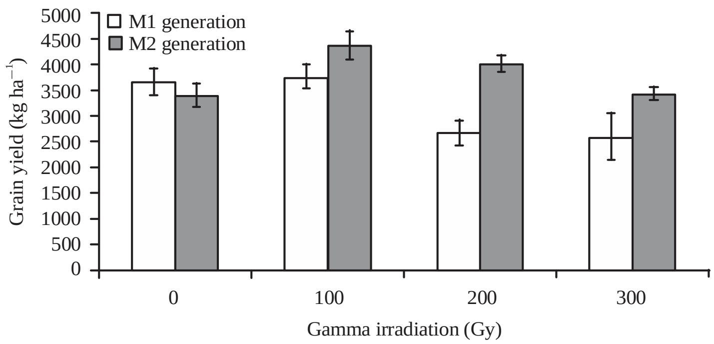

Fig. 1: Response in total grain yield of Pearl millet following gamma irradiation. Bars represent SEM

Table 2: Components of yield responses to gamma irradiation in pearl millet

	Head length (cm)		Head width (cm)		Head weight (g)		100 seed weight	
Gamma rays generations	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
Control	16.15	13.10^{c}	2.72^{ac}	2.36^{a}	12.29	25.1^{a}	1.41^{a}	0.85^{b}
100 Gy	16.64	16.74^{a}	2.78^{a}	2.67^{b}	16.16	22.40^{ab}	1.29^{ab}	1.07^{a}
200 Gy	16.23	15.32^{b}	2.57^{bc}	2.52^{c}	12.62	20.50^{ab}	1.29^{ab}	1.01^{a}
300 Gy	15.74	14.30^{bc}	2.45^{b}	2.48^{c}	15.09	16.3^{b}	1.24^{b}	0.95^{ab}
LSD (0.05)	1.02^{NS}	1.21	0.17	0.13	6.00^{NS}	6.46	0.16	0.13

Treatment means having the same letter (vertically) in each column are not significantly different, NS: Not significant

plants in 100 Gy plots varied significantly from all other treatments, while plants in 200 and 300 Gy plots recorded similar widths.

In the M_1 generation, plants from the unirradiated control plots recorded similar seed weight as those irradiated with 100 and 200 Gy. Seed weight recorded for plants in 100 Gy plots in M_2 were similar to those in 200 and 300 Gy plots (Table 2). The data obtained in this study on yield parameters confirm the earlier observation by Veeresh *et al.* (1995) and Kon *et al.* (2007) in winged and long bean, respectively, that lower irradiation doses resulted in increases in yield components.

Induced mutation resulting from gamma irradiation in this study produced significant ($p<0.05$) variation in total grain yield. In M_1, significantly the same total grain yield were recorded by mutants produced from 100 Gy and the control plots, as were those from 200 and 300 Gy M_1 generation (Fig. 1). In the second generation (M_2), however, plants in 100 Gy recorded the highest grain yield and was statistically similar to plants in 200 Gy plots. Plants in 300 Gy and control plots recorded similar yields (Fig. 1). Earlier studies by Van Oosterom *et al.* (2002) indicated that tillering greatly and directly influenced total grain yield. The data obtained in this study is in agreement with this finding. In another but related studies, seed weight and head weight correlated well with total grain yield (Limon-Ortega *et al.*, 1998). The ability of gamma irradiation doses of 100 and 200 Gy to enhance profuse tillering and increases in seed weight relative to the unirradiated control and 300 Gy in the segregating M_2 generation consequently resulted in yield enhancement in the present study.

Genetic advancement: The efficiency and success of plant breeding is in the existence of genetic variability especially in segregating populations. The observed genetic advancement in the M_2 generation was highly variable. Some characters exhibited low percentage improvements, whiles others recorded higher percentages. Improvement in plant height, number of tillers and total grain yield were recorded by all gamma ray doses (Table 3). About 100, 200 and 300 Gy gamma ray doses improved plant height by 37.85, 102.81 and 125.64%, number of productive tillers by 89.66, 96.43 and 25.00% and total grain yield by 16.10, 50.19 and 32.21%. Improvement in head length (0.60%) and width (1.22%) were recorded by 100 and 300 Gy gamma ray doses, respectively (Table 3). The improvements made in selection in the present study are in conformity with results obtained for sesame as reported by Chavan and Chopde (1982) and in groundnut as reported by Mathur *et al.* (2000). In breeding, certain characters may require a couple of segregating generations to achieve stability and may do so in future segregating generations This explains the negative improvements recorded in some of the parameters (Table 3).

Table 3: Genetic advancement in M_2 population

Gamma irradiation	Characters	M_1 generation	M_2 generation	M_2-M_1	*Genetic advance (%)
100 Gy	Plant height	90.20	124.10	33.90	37.85
	Tiller number	8.35	7.15	-1.20	-14.00
	Productive tillers	1.45	2.75	1.30	89.66
	Earliness to flower	41.75	45.00	3.25	8.89
	Head length	16.64	16.74	0.11	0.60
	Head width	2.78	2.67	-0.11	-3.96
	Head weight	16.16	22.40	6.24	38.61
	100 seed weight	1.29	1.07	6.24	-17.05
	Grain yield	3758.00	4363.00	605.00	16.10
200 Gy	Plant height	56.90	115.40	58.50	102.81
	Tiller number	7.04	5.40	-1.64	-23.30
	Productive tiller	1.40	2.75	1.35	96.43
	Earliness to flower	47.50	50.00	2.50	5.26
	Head length	16.23	15.32	-0.91	-5.61
	Head width	2.57	2.52	-0.05	-1.95
	Head weight	12.62	20.50	7.88	65.56
	100 seed weight	1.29	1.01	-0.28	-21.71
	Grain yield	2668.00	4007.00	1339.00	50.19
300 Gy	Plant height	47.20	106.50	59.30	125.64
	Tiller number	6.45	4.90	-1.55	-24.03
	Productive tiller	1.20	1.50	0.30	25.00
	Earliness to flower	48.75	50.25	1.50	3.08
	Head length	15.74	14.30	-1.44	-9.15
	Head width	2.45	2.48	0.03	1.22
	Head weight	15.09	16.30	1.21	8.02
	100 seed weight	1.24	0.95	-0.29	-23.39
	Grain yield	2589.00	3423.00	834.00	32.21

*Genetic advance (%) = [(M2-M1/M1] × 100

CONCLUSION

Gamma irradiation has induced sufficient genetic variability in pearl millet in the present study. Mutants especially those from plants irradiated with 100 or 200 Gy of the mutagen recorded improved growth and yield characteristics following selection at M_2. These mutant lines have a potential in playing a major role in breaking the yield constraints in pearl millet in the study area and possibly improve the economic status of farmers. Genotypes with superior characteristics will be planted for screening and selection in subsequent generations followed by evaluations and possible release as varieties. Results obtained from future studies on this work will also be published.

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