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Preliminary assessment of the effects of gamma rays on germination and seedling growth in Bambara groundnut (*Vigna subterranean* l. verdc.) landraces

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Abstract
Dry and healthy seeds of three Bambara Groundnut Landraces; Alkaleri cream (Alk-C), Alkaleri mottled (Alk-M) and Alkaleri black (Alk-B) were irradiated with varying doses of Gamma rays (0, 50, 100, 150, and 200 Gy) from ^{60}Co source and planted in polythene pots under nursery conditions, which were arranged in Complete Randomized Design (CRD) format and replicated three times. There was significant reduction in seed germination rate with the increase in gamma radiation in all landraces, with maximum germination rate (89%) recorded in the control treatment. Seedlings of the irradiated population showed deleterious effects of Gamma rays at the higher doses (150 and 200 Gy), with significant reduction in all parameters studied (plant height, number of branches, number of leaves, terminal leaf length and terminal leaf width). Considerable increase in plant height and number of branches at the low dose (50 Gy) was observed, compared to the control population as indicted in the positive shift in their mean values. The Alk-B landrace showed more tolerance to the gamma radiation treatment in seedling establishment by recording significantly higher mean values in most of the parameters compared to the other landraces. The positive shift in mean values of some of the parameters studied, due to Gamma radiation treatment at 50 Gy dose implied that this mutagenic agent has the potential to improve certain characters in Bambara Groundnut landraces.

Keywords: Bambara Groundnut, Gamma radiation, Crop improvement, Tolerance, Landrace.

Introduction

Grain legumes, commonly known as pulses, occupy a pivotal position in meeting the protein needs of people in most developing countries like Nigeria. Bambara groundnut (*Vigna subterranea* L. verdc.) as an underutilized grain legume is an important, self-pollinating pulse crop grown in most parts of Sub-Saharan Africa. It is a leguminous plant species which belongs to the family *Fabacaeae*. It originated from West Africa and is cultivated in sub-Saharan areas, particularly Nigeria [1]. It is recognized for its high nutritional composition, tolerance to poor soils, drought and it has good atmospheric nitrogen fixing ability. On average, the seeds of Bambara Groundnut contain 63% carbohydrate, 19% protein and 6.5% fat, thereby making it a complete food [2]. Approximately 165,000 tons of this species is produced in Africa each year, but yields are low because efforts to improve Bambara Groundnut have not been given the needed research attention for many years [3]. The crop is mostly grown by women, intercropped with other crops such as maize, millet, sorghum, cassava, yam, peanut and cowpea. According to [4], Bambara Groundnut was traditionally cultivated in extreme tropical environments by small-scale farmers with no access to irrigation and fertilizers, who had little or no guidance on improved production practices. The potential of Bambara groundnut as a crop-based approach for the sustainable reduction of protein–energy malnutrition prevalent in Nigeria is still being explored [5]. However, one of the limitations to sustainable production of this crop legume is low and unstable yield. This low yields have been associated with poor germination percentage and variable germination rates which lead to poor crop establishment in the dry regions where the crop is grown [1]. Furthermore, the crop largely exists as local landraces with few varieties developed through controlled breeding [6].

Attempts to develop Bambara Groundnut cultivars with increased pod yield, quality components and stability is still in its infancy [7]. Thus, one way to improve the crop is to assess the genetic variation present in the population, which may be important for selection. Induced mutagenesis is a highly recognized and recommended method used in crop improvement programs. With the availability of mutagenic agents (physical and chemical), plant breeders have the ability to induce genetic variability, which can be utilized successfully in the improvement of Bambara Groundnut. Physical mutagen such as Ionizing radiation has proven to be an effective method to increase the genetic variability of the crop according to [8]. As the most preferred physical mutagen by plant breeders, Gamma radiation was used in this present investigation to assess the germination and early growth response of three Bambara Groundnut landraces with a view to identify the most tolerant landrace, suitable for use in crop improvement trials.

Materials and Methods

Three (3) local Bambara Groundnuts landraces (Alk-C, Alk-M and Alk-B) were obtained from local farmers in Alkaleri LGA of Bauchi state (Plate 1). The seeds of these landraces were characterized by their seed testa colour. One hundred (100) dry and healthy seeds per landrace totalling 300 seeds, were selected and irradiated with five doses of Gamma rays; 0, 50, 100, 150 and 200 Gy. The Gamma Irradiation of Seed samples was carried out using Gamma Beam X 200 machine with Cobalt – 60 (^{60}Co) as the gamma source and dose rate of 0.01032 Gy/s, at the Institute for Radiation Protection and Research (NIRPR), University of Ibadan. The irradiated Bambara Groundnut landraces and the control (Un-irradiated seeds) were planted in polythene pots and arranged in a format of Complete Randomized Design (CRD) and replicated three times.

Data were collected for number of days to germination, number of days to 50% germination, germination rate, plant height, number of branches, number of leaves, terminal leaf length and terminal leaf width. These were subjected to Analysis of variance (ANOVA) using Minitab statistical software version 16.0. Means were separated using Least Significant Difference (LSD) at 5% level of significance to test for treatment effects.

Plate 1: Seed samples of the three Bambara Groundnut landraces used in this study; Alkaleri cream (Alk-C), Alkaleri mottled (Alk-M) and Alkaleri black (Alk-B)

Results

Different doses of gamma radiation had significantly influenced the germination and germination rate of the Bambara Groundnut landraces. Table 1 indicated that the control treatment (0 Gy) had emerged earlier than the irradiated population at 12.1 days after sowing and had also reached 50% germination earliest at 13.4 days. The higher radiation doses (150 and 200 Gy) took longer days to germinate and to reach 50% germination when compared with the control treatment. There was significant reduction in rate of seed germination as the radiation dose was increased, implying an inverse relationship between radiation dose and germination rate. The highest germination rate (89.6%) was found in the control population while the least germination rate (37.2%) was recorded in 200 Gy gamma dose as shown in Table 1.

Table 1: Effects of varying gamma doses on the germination and germination rate of M_1 generation of Bambara Groundnut

Gamma Rays Dose (Gy)	Number of Days to Germination	Number of Days to 50% Germination	Germination Rate (%)
0 Gy	12.0d	13.4b	89.6a
50 Gy	12.3cd	14.6b	85.3ab
100 Gy	14.3bc	15.6b	71.7bc
150 Gy	16.2b	20.1a	60.5c
200 Gy	18.4a	22.7a	37.2d
LSD	2.7	2.9	18.7

Means followed by the same letter(s) within the same column are not significantly different at 5% level of probability using LSD

Data on the effects of varying doses of gamma radiation on plant height, number of branches, number of leaves, terminal leaf length and terminal leaf width (Table 2) showed significant decrease in these parameters at higher doses (150 and 200 Gy). However, significant increase in mean values of these parameters were observed in the low radiation dose (50 Gy), it was also revealed that it is mostly at this low dose that the mean values shifted towards the positive side indicating significant improvement. However, at the higher Gamma radiation doses (150 and 200 Gy), drastic reduction in mean values of the evaluated parameters was observed (Table 2). Similarly, there was negative shift in the mean values at these higher doses. There was significantly high variation among the treatments in plant height and number of branches, especially at the higher radiation doses (150 and 200 Gy) as observed in the results.

Table 2: Effects of varying doses of gamma radiation on some growth characters in M_1 generation of Bambara groundnut seedlings at 27 days after sowing.

Parameter	Gamma Dose	Mean	Standard Error (SE)	Shift in Mean	Variance
Plant height (cm)	Control	16.24	± 0.48	-	6.14
	50 Gy	17.81	± 0.62	+1.5	6.87
	100 Gy	16.82	± 0.64	+1.2	9.86
	150 Gy	12.89	± 1.20	-1.3	14.32
	200 Gy	8.51	± 0.27	-2.4	10.98
Number of branches	Control	6.11	± 0.41	-	0.61
	50 Gy	6.67	± 0.88	+0.5	0.25
	100 Gy	6.33	± 0.33	-0.3	0.75
	150 Gy	5.44	± 1.02	-0.7	2.53
	200 Gy	4.67	± 1.19	-1.4	1.00
Number of leaves	Control	19.33	± 1.15	-	6.25
	50 Gy	20.22	± 2.64	+0.9	3.19
	100 Gy	18.89	± 1.00	-0.4	5.11
	150 Gy	16.33	± 3.06	-3.0	20.00

	200 Gy	14.33	± 3.46	-5.0	8.00
Terminal leaf length (cm)	Control	7.64	± 0.26	-	0.36
	50 Gy	8.31	± 0.38	+0.9	0.71
	100 Gy	6.46	± 0.36	-1.2	0.92
	150 Gy	6.26	± 0.53	-1.5	1.18
	200 Gy	4.51	± 0.07	-3.1	0.94
Terminal leaf width (cm)	Control	1.72	± 0.14	-	0.04
	50 Gy	1.77	± 0.08	+0.4	0.04
	100 Gy	1.53	± 0.10	-0.4	0.09
	150 Gy	1.41	± 0.06	-0.3	0.18
	200 Gy	1.05	± 0.03	-0.7	0.12

The response of Bambara Groundnut landraces to different doses of gamma radiation varied significantly. The germination rate of the landraces was adversely affected by the varying gamma doses, and was found to be more deleterious for Alk-M landrace at the higher doses of 200 and 150 Gy with germination rates ranging from 23.2% - 37% as shown in Fig 1. However, Alk-C landrace demonstrated more tolerance to gamma irradiation than the other landraces having a range of 52.5% - 61.7% germination at 200 and 150 Gy respectively. The varying gamma doses significantly influenced seedling height in the three Bambara Groundnut landraces as shown in Fig 2. It was observed that, at 150 Gy and 200 Gy, Alk-B landrace grew taller than the other landraces, while at the 200 Gy significant reduction was observed in Alk-C and Alk-M landraces compared to the control population. Similarly, significant decrease in number of branches and number of leaves in all the landraces were observed at the higher radiation dose (200 Gy) as shown in Fig 3 and 4. Alk-B landrace was found to be more tolerant to the higher gamma radiation by having more number of branches and leaves compared to other landraces. Alk-C and Alk-M were very sensitive to gamma radiation because, significant decrease in terminal leaf length and terminal leaf width was observed in them at the 150 and 200 Gy, while in Alk-B, the irradiated populations were at par with the control population in these parameters as revealed in the results (Fig 5 and 6).

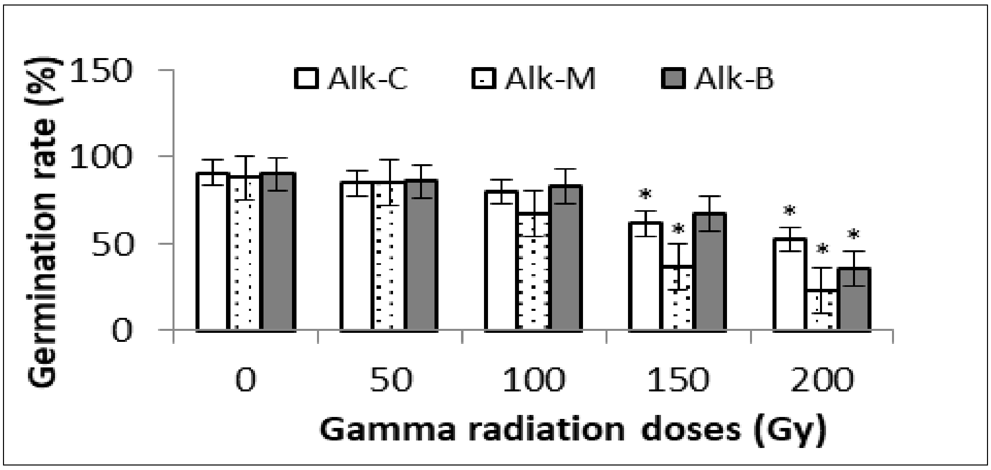

Fig. 1: Effects of different gamma radiation doses (Gy) on the germination percentage of three landraces of Bambara groundnut *Indicates significant differences compared to the control (Tukey (HSD), $P < 0.05$)

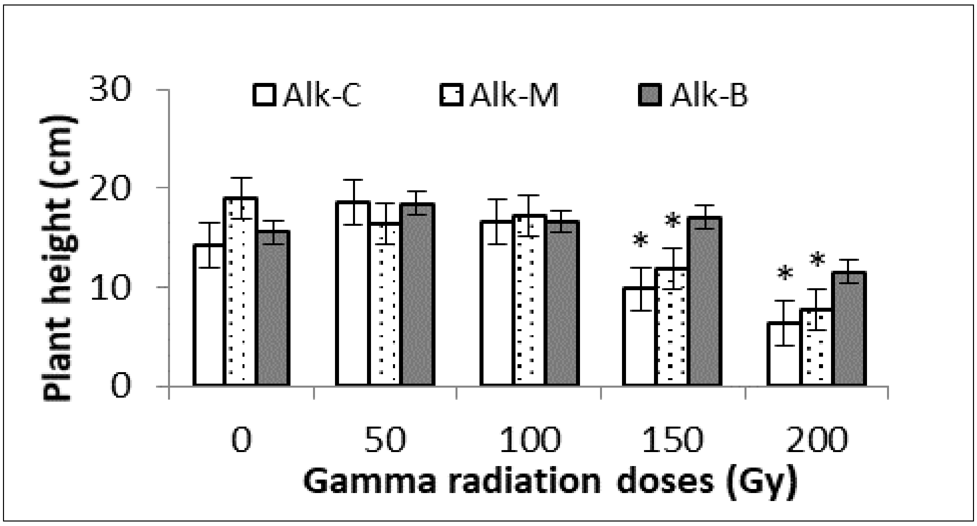

Fig 2: Effects of different gamma radiation doses (Gy) on the plant height of three landraces of Bambara groundnut *Indicates significant differences compared to the control (Tukey (HSD), $P < 0.05$)

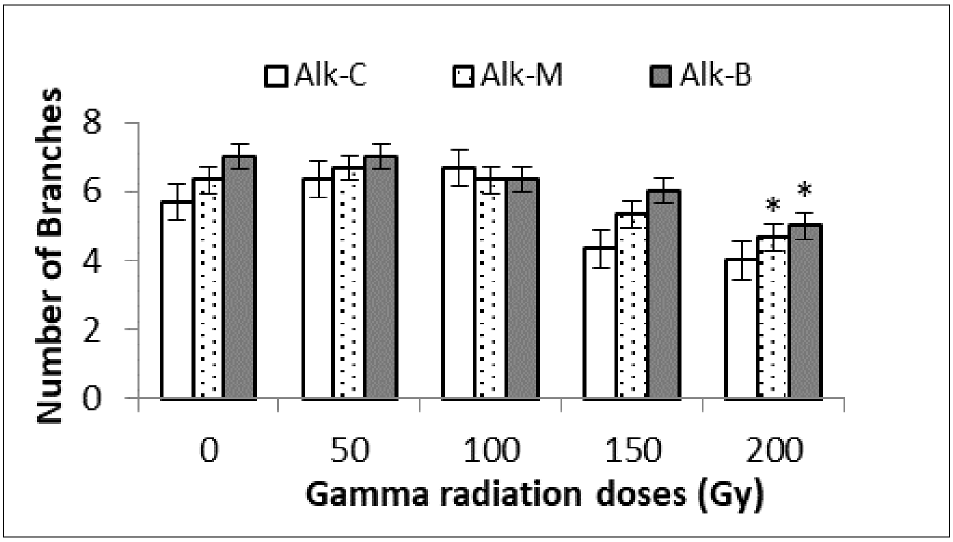

Fig 3: Effects of different gamma radiation doses (Gy) on the number of branches of three landraces of Bambara groundnut *Indicates significant differences compared to the control (Tukey (HSD), $P < 0.05$)

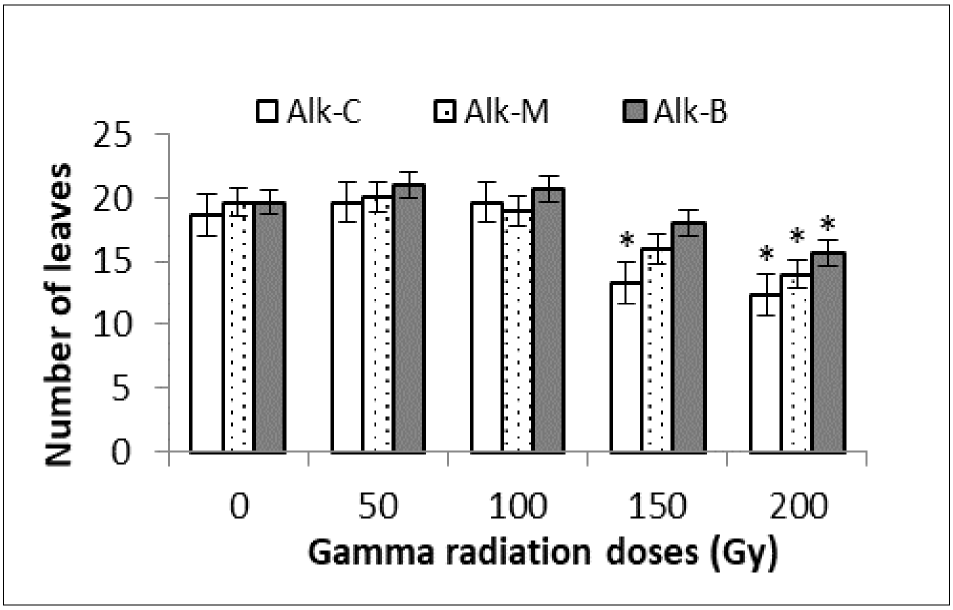

Fig 4: Effects of different gamma radiation doses (Gy) on the number of leaves of three landraces of Bambara groundnut *Indicates significant differences compared to the control (Tukey (HSD), $P < 0.05$)

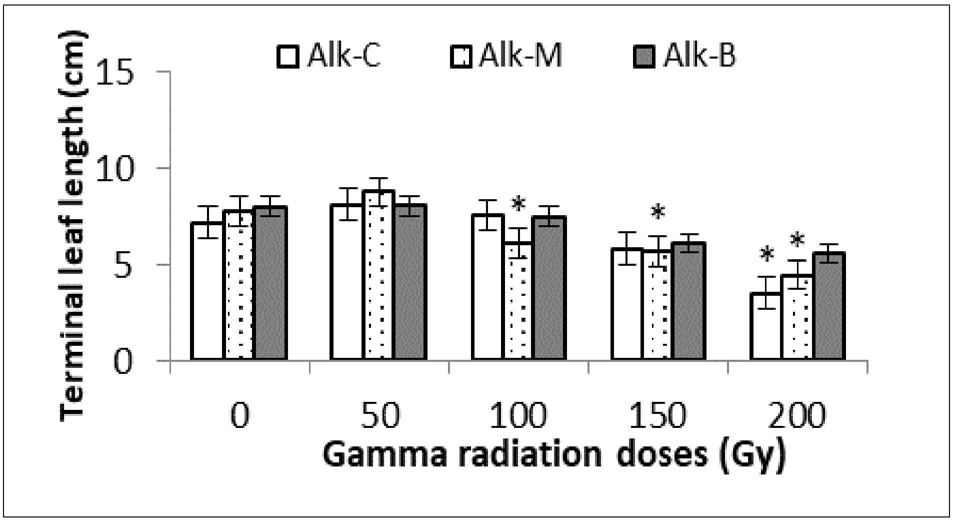

Fig 5: Effects of different gamma radiation doses (Gy) on the terminal leaf length of three landraces of Bambara groundnut *Indicates significant differences compared to the control (Tukey (HSD), $P < 0.05$)

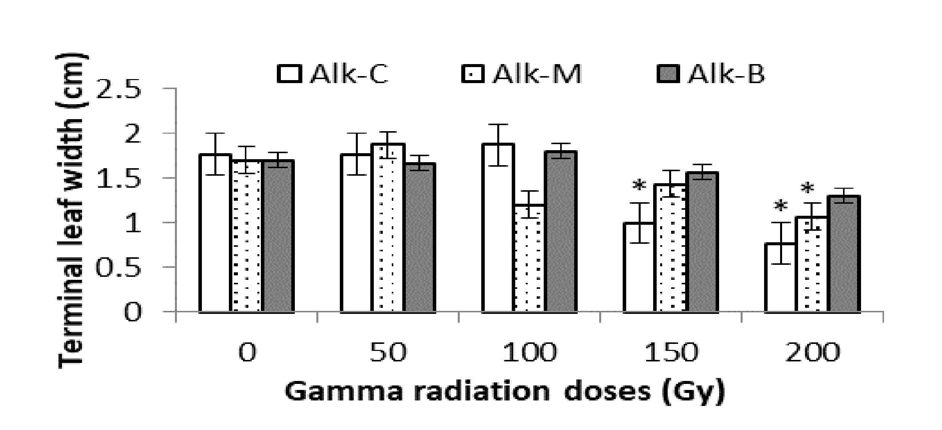

Fig 6: Effects of different gamma radiation doses (Gy) on the terminal leaf width of three landraces of Bambara groundnut *Indicates significant differences compared to the control (Tukey (HSD), $P < 0.05$)

Discussion

The different doses of gamma radiation as it affects the early growth parameters of Bambara Groundnut in this study varied considerably. The increase in the doses of gamma radiation significantly resulted in reduced seed germination and corresponding decrease in germination rate. This is in agreement with the findings of [9], who reported that reduced seed germination and germination rate due to gamma treatments results in delay or inhibition of metabolic activities necessary for seed germination. Furthermore, low germination rate of First Mutant generation (M_1) seed was observed by [10] in Cowpea and has attributed it to genetic and physiological damage to embryo cells or tissues, which can lead to abnormal cell division, cell death, tissue or organ failure and reduction in germination rate. The reduction in seed germination rate observed in this study was more severe at the 200 Gy for all the landraces evaluated. This could be as a result of inhibition of the mitotic proliferation in radicle and plumule as reported by [11]. The increasing doses of gamma radiation progressively inhibited the seedling height, number of branches and number of leaves for all the Bambara Groundnut landraces. However, at low (50 Gy) to moderate (100 Gy) gamma radiation dose, these parameters were not significantly affected, especially in Alk-B landrace. The reduction in seedling height at higher radiation doses according to [12, 13] can probably be due to the reduction of the bioactive content of Gibberellic Acid (GA) or the inhibition of GA signalling, modifying the expression of the coding sequence of the GA 2-oxidase gene that controls the plant height. Furthermore, the inhibition of cell division and cell elongation in the irradiated population may have resulted in plant growth retardation leading to decrease in seedling height, number of branches and number of leaves, this was corroborated by [14].

The reduced terminal leaf length and terminal leaf width as a result of high gamma radiation doses can be attributed to retarded plant growth due to the radiation effects. [15] reported in their study that, a decrease in mitotic activity as a result of irradiation led to a growth reduction in plants and that the destruction of many enzymes by radiation also contributes to the slowdown of cell division and plant growth and development. [16] also stated that, growth inhibition induced by high-dose irradiation has been attributed to the cell cycle arrest at G2/M phase during somatic cell division and other cellular or tissue damages that may affects vegetative growth and development in plants.

Conclusion

Preliminary dose response treatments of plant cultivars are necessary criteria for crop improvement studies using induced mutagenesis. Dose effects response can be accurately predicted by evaluating early quantitative characters of the treated plants. This study revealed that, Gamma Irradiation of Bambara Groundnut landraces significantly influenced the germination and early growth of seedlings. The positive shift in mean values of some of the parameters due to Gamma radiation treatment observed at lower doses is an indication that, this mutagenic agent can be very useful in the improvement of some certain characters in Bambara Groundnut. The Alk-B landrace have demonstrated high tolerance to Gamma radiation treatment in all the parameters evaluated in this study, which makes it suitable for use in yield improvement trials.

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