**Determination of lethal (LD) and growth reduction (GR) doses on acute and chronic gamma-irradiated Bambara groundnut [Vigna subterran^a^aia (L.) Verdc.] varieties**

Ismaila Muhammad^a,b^, Mohd Y. Rafi^a,c^, Muhamad Hazim Nazli^a,d^, Shairul Izan Ramlee^e^, Abdul Rahim Harun^e^ and Yusuff Oladosu^a^.

^a^Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, Serdang, Selangor, Malaysia; ^b^Department of Biological Sciences, Faculty of Science, Gombe State University, Gombe, Nigeria; ^c^Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia; ^d^Agrotechnology and Bioscience Division, Malaysian Nuclear Agency, Kajang, Selangor, Malaysia.

**ABSTRACT**

Bambara groundnut is a highly nutritious underutilized legume with enormous potential to sustain food security in resource-poor countries. However, its potential for improvement through conventional breeding (< 2% success rate) limitation due to the nature of the flowers. Thus, the most viable method of improving this crop is by creating genetic variability through induced mutagenesis. The present study was conducted to evaluate the radiosensitivity of two Bambara groundnut varieties irradiated with acute and chronic gamma irradiations to determine the lethal dose (LD) and growth reduction dose (GR). Healthy seeds of both varieties were exposed to acute gamma irradiation using Cesium-137 at 0, 25, 50, 75, 100, 125, 150, 175, 200, 250, and 300 Gy. For chronic irradiation, two-week-old seedlings of the two genotypes were exposed to accumulated doses of 0, 8.52, 17.04, 35.56, 34.09, 42.61, 59.65, 93.74, 144.87, 235.64, and 570.94 Gy, respectively, in Gamma Green House (GGH) for 60 days. The result from the variance analysis indicated highly significant differences (P < 0.01) for all evaluated traits except for internode length. A linear regression model was developed to determine the mean LD and GR of both genotypes. The established lethal doses (LD25, 50, 75) for acute gamma irradiation on Ex-Sokoto variety were 75, 160, and 250 Gy while 68, 148, and 227 Gy were recorded for Karo variety, respectively. For chronic irradiation, the established growth reduction doses for Ex-Sokoto were 47, 250, and 444 Gy, whereas 70, 264, and 452 Gy were observed in Karo. Variations were observed between the gamma-irradiated genotypes and the methods of irradiations. Generally, the growth, development, and survival rate of Bambara groundnut increase with a decrease in gamma-irradiation doses. The established LD and GR doses from this study can be utilized in large-scale mutagenesis breeding programs for generating a wide range of mutants in Bambara groundnut.

**1. Introduction**

Bambara groundnut [Vigna subterran^a^aia (L.) Verdc.] is one of the minor indigenous tropical African legumes that is mostly cultivated at the subsistence level. It is predominantly cultivated by low-income farmers under the fringe and poorly drained environments across sub-Saharan Africa. This crop is tolerant to drought stress and temperature extremities with resistance to a wide array of pests and diseases (Muhammad et al., 2020). Traditionally, Bambara groundnut is used as a complete diet, providing food security and a source of income for the farmers (Olayide et al., 2018). Being a legume crop, Rhizobia strains found in Bambara groundnut can fix atmospheric nitrogen for soil improvement. Economically, the seed (grain) composition is fairly similar to cowpea and chickpea, with the protein content ranging between 20% and 24%, carbohydrate 57% and 63%, and 5% and 7% oil. Lysine and methionine are the main essential amino acids found in this crop at the range of 80.2 ± 5.2 mg/g and 6.4 ± 0.1 mg/g, respectively, accounting for 10.3% of the entire amino acid contents (Halimi et al., 2019; Musa et al., 2020). Additionally, a wide range of essential minerals deposited in Bambara seeds helped improve micronutrient requirements in the human diet, particularly among rural dwellers.

Previous researchers have observed reasonable variations in yields and quality parameters among landraces of this crop with yield fluctuations between 650 and 850 kg ha^-1^ (Alake & Ayo-Vaughan, 2016). However, Pungulani et al. (2012) reported that the yield potential could be as high as 3000–4000 kg ha^-1^.

Presently, there are no Bambara groundnut varieties for specified agro-ecological system or farming conditions due to difficulties in hybridization. Control crosses through hybridization have recorded less than...
2% success due to the small size and nature of the flowers couple with low seed set success. Therefore, crops with restricted genetic variations such as Bambara groundnut require induced mutation or mutagenesis to create heritable and desirable variations for improvement of yield and yield-related traits (Aisha et al., 2017; Oladosu et al., 2014).

The utilization of nuclear technology such as gamma radiation for the benefit of humanity is gaining global popularity, especially in agro-technology for crop protection, soil fertility improvement, food preservation, and crop plant breeding. Such technology is also adopted to enhance physiological, agronomical, and plant resistance to biotic and abiotic stresses (Oladosu et al., 2016). To achieve optimal plant mutagenesis, an appropriate gamma-irradiation dose must be determined individually for each genotype (Hasan et al., 2020; Sani Haliru et al., 2020). Similarly, Sparrow et al. (1961) reported that in a chronic gamma-irradiation procedure for mutagenesis studies with no prior information on the required exposure period to achieve reasonable growth reduction (survival rate), a dose range of 10–2000 Gy might be essential.

Radiosensitivity test or optimum dose determination in plant mutagenesis are terms used to describe a relative amount of detectable effects of exposure to radiation on irradiated samples (Surakshitha et al., 2017). It is a prerequisite to determine the lethality or growth inhibition, somatic mutation and chromosome breakage of large-scale-induced mutations. Various chemical, physical, and biological factors can modify the radiation effects in plants. Mba (2013) described optimal mutagen dose frequency of mutation induction with a minimal unintended injury. Tshilenge-Lukanda et al. (2012) stated that the optimal doses of mutation induction might be established through determining seed germination percentages, seedlings survival percentages, lengths of hypocotyl, and epicotyl among others. Generally, mutagen doses that induce 50% lethality (LD_{50}) among the generation 1 mutant (M_1) population may be suitable due to its ability to produce a higher range of mutations (Ke et al., 2019).

The purpose of mutation induction is to create genotypic and phenotypic variations that allow the selection of plant with desirable characteristics. The maximum probability of generating viable and useful mutants for breeding and crop improvements using acute and chronic mutagenesis approaches is achieved at the dose where 50% (LD_{50}) of the irradiated samples die or when 50% of plants growth have decline (GR_{50}) (Álvarez-Holguín et al., 2019). However, the dose range below and above the LD_{50} (LD_{25%} and LD_{75%}) was reported by Aminah et al. (2015). Generally, LD_{50} and GR_{50} are established on the hypothesis that lower doses of gamma irradiation can produce the least impact on the plant genome which may result in morphological changes; however, higher gamma-irradiation doses may bring about several effects on the entire genome leading to negative mutations (Álvarez-Holguín et al., 2019). Therefore, this study aimed to investigate the biological responses (LD_{25}, 50, 75 and GR_{25}, 50, 75) induced by acute and chronic gamma exposure of two (Ex-Sokoto and Karo) Bambara groundnut seeds and seedlings to achieve an optimal dose of gamma irradiation for effective mutagenesis induction.

2. Materials and methods

2.1. Planting materials

Two Bambara groundnuts Landraces viz: Karo (KR) and Ex-Sokoto (SK) used in this study were sourced from the Genetic Resources Centre of the International Institute of Tropical Agriculture (IITA), Kano station, Nigeria.

2.2. Acute and chronic gamma-irradiation treatments

For acute gamma irradiation, a total of 100 seeds for each genotype were subjected to 25, 50, 75, 100, 125, 150, 175, 200, 250, and 300 Gray (Gy) at the dose rate of 5.20k Gy/Min using Mark I research irradiator emitted from the Cesium-137 source using Biobeam GM 8000. For chronic gamma-irradiation treatment, a total of 110 seedlings from each of the two genotypes were raised in 18 × 18 inches poly bags at a glasshouse in the Faculty of Agriculture, Universiti Putra Malaysia, Selangor for two weeks after germination and transferred to Gamma Green House (GGH) at the Nuclear Malaysia, Bangi, Selangor, Malaysia. Ten healthy seedlings from each genotype were exposed to ten different doses (rings) (Figure 1) for chronic gamma irradiation at 0 Gy (control), 0.67 Gy (ring 2), 0.30 Gy (ring 3), 0.17 Gy (ring 4), 0.11 Gy (ring 5), 0.07 Gy (ring 6), 0.05 Gy (ring 7), 0.04 Gy (ring 8), 0.03 Gy (ring 9), 0.02 Gy (ring 11), and 0.01 Gy (ring 15) kGy/day for 15 hours daily until physiological maturity. The current gamma-irradiation rate for GGH is 2.7 × 105 (5%) mR/h or 2.67 Gy/h at 1 m distance from the Cesium-137 (^{137}Cs) source (Yasmine et al., 2019).

2.3. Experimental design and radiation sensitivity test

The experiment was conducted under glasshouse condition, followed by acute gamma irradiation. Seeds from different gamma-irradiated treatments were sown alongside the non-irradiated ones (control) using the sand bed technique. The experiment was laid in a completely randomized design (CRD) with eleven different treatments of gamma rays at
a planting distance of 10 × 20 cm within and between rows. For chronic gamma irradiation, treated plants were also laid in a completely randomized design (CRD) with eleven gamma-irradiation treatments and two varieties with a distance of 1 m × 1 m.

Radiation Sensitivity test (biological effects of mutagenic treatments on seed and seedlings) was carried out following the procedures outlined by Olasupo et al. (2016) to determine the effects of acute and chronic gamma irradiation on the two Bambara groundnut varieties. The radiation sensitivity was assessed based on survival percentage (%) recorded after seeds exposure to gamma irradiation. Lethal dose percentages (LD_{25\%}, LD_{50\%}, and LD_{75\%}) were determined after six weeks of germination in the acute phase. For chronic gamma irradiation, the effects were determined based on the growth reduction percentage (GR_{25\%}, GR_{50\%}, and GR_{75\%}), specifically the plant’s height. The plant height was measured weekly starting at two weeks after the plants were moved to gamma greenhouse until week 7. Lethal dose and growth reduction doses were then determined using a simple linear regression model graph of absorbed dose against survival percentage using a straight line (best fit). The equation for the model is: y = mx+c; where y is the response variable (germination percentage), x is the independent variable (gamma irradiation dose), while m and c represent the slope and constant, respectively, a reading corresponding to 25%, 50%, and 75% reductions were obtained.

2.4. Data collection

Observations on plant morphological traits such as plant height, number of petioles, number of leaves, number of branches and internode length were recorded. Data were also collected on germination rates and seedling survival for each of the gamma-irradiation treatments as follows:

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\text{Germination percentage(\%)} = \frac{\text{Total number of germinated seeds}}{\text{Total number of seeds sown}} \times 100
\]

\[
\text{Survival percentage(\%)} = \frac{\text{Number of plants failed to survive after germination}}{\text{Total number of germinated seeds}} \times 100
\]

2.5. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using SAS program version 9.4 to determine the variation among the gamma-ray doses and between the two genotypes of Bambara groundnut using the general linear model procedure (PROC GLM). Mean comparisons were performed using LSD.

3. Results and discussion

3.1. Percentage seed germination (%)

The percentage germination was one of the most important variables to be considered in any plant mutagenesis experiment because plant mortality rate signifies the extent of damage caused as a result of exposure of seeds or seedlings to gamma radiation treatments. Results of the acute gamma irradiation indicated high significant differences (P < 0.01) in germination percentage among the 0, 25, 50, 75, 100, 125, 150, 175, 200, 250, and 300 Gy treatments (doses).

| Dose (Gy) | Number of seeds sown | Percentage germination (%) | Percentage survival (%) | Survival reduction over control (%) |
|-----------|----------------------|---------------------------|------------------------|----------------------------------|
| 0 Gy      | 100                  | 97.75                     | 99.04                  | 100.00                           |
| 25 Gy     | 100                  | 89.27                     | 83.00                  | 87.63                           |
| 50 Gy     | 100                  | 85.60                     | 86.67                  | 82.97                           |
| 75 Gy     | 100                  | 89.67                     | 78.33                  | 79.27                           |
| 100 Gy    | 100                  | 86.63                     | 76.67                  | 85.20                           |
| 125 Gy    | 100                  | 78.53                     | 70.10                  | 52.23                           |
| 150 Gy    | 100                  | 71.50                     | 67.63                  | 54.80                           |
| 175 Gy    | 100                  | 74.53                     | 57.67                  | 45.20                           |
| 200 Gy    | 100                  | 57.07                     | 43.33                  | 37.40                           |
| 250 Gy    | 100                  | 50.00                     | 43.33                  | 31.10                           |
| 300 Gy    | 100                  | 50.00                     | 43.33                  | 31.10                           |

Table 1. Germination and plant survival percentages in two Bambara groundnut varieties exposed to different acute gamma-irradiation doses.
Generally, the result indicates a gradual reduction (Table 1) in the percentage germination with increased gamma-irradiation doses except for doses of 25 Gy (89.27%) and 75 Gy (89.67%). No significant differences were observed in the Ex-Sokoto variety, however, in the Karo variety doses of 250 Gy and 300 Gy exhibit similar results (43.33). The results of the chronic gamma irradiation (Table 2) show that there were no significant differences in percentage germination because no treatment was imposed until after two weeks after seedlings emergence. Therefore, a similar trend of germination was also observed in the seedlings with slight variations in terms of seedling height and number of leaves.

From the results of this study, there was a continued decline in the percentage of germination among the two genotypes of Bambara groundnut as a result of an increase in the concentration of gamma rays. This result agrees with the findings of several researchers, such as in Wilman lovegrass by Álvarez-Holguín et al. (2019); in Grasspea by Ramezani and More (2013); in pigeon pea by Ariraman et al. (2018); and in Bambara groundnut by Bharatkumar et al. (2015) and Adebola and Esson (2017) where germination decreases as the gamma radiation dose increases. This decline was related to the higher gamma-irradiation doses which inhibited the cell vital functions that eventually lead to the death of the embryo or certain cells in the seeds. Similarly, the observed decline in the percentage of seeds germination from this study may be linked to the alteration in the enzymatic activities, which leads to the decrease in the seed germination or due to the inhibitory actions of the mutagens on the plumule and radicle. This result is supported with the work of Ke et al. (2019) in Cauliflower; in Garden Bean by Monica and Seetharaman (2016); in Panicum sumatrense by Ramkumar and Dhanavel (2019) and in wheat genotypes by Olaolorun et al. (2019). The above studies maintain that mutagen treatments especially at higher dose rates bring about an alteration in the enzyme actions and lead to inhibition and decline in the percentage of germination among the treated samples. Additionally, compounds such as protein, chlorophyll a, chlorophyll b, auxins, and ascorbic acids that are directly related to the plant metabolism may also be damaged or altered by the gamma irradiation and can potentially inhibit seedling germination.

It was also observed from this study that few seeds that were exposed to higher gamma irradiation were germinated but died a few later. This coincides with the study reported by Marcu et al. (2013) and Olasupo et al. (2016), that seeds exposed to higher mutagen doses may not germinate, and the germinated seedlings cannot survive more than a few days after germination. Similarly, a study conducted by Preuss and Britt (2003) on Arabidopsis indicated that gamma radiation can inhibit plant growth due to the presence of a signal transduction mechanism that monitors cellular destruction in plants. The process of cell division is halted by this mechanism whenever there is damage to the cell structure.

### 3.2. Seedling survival percentage (%)

The decrease in the percentage of plant survival among all the acutely gamma-irradiated treatments compared with control (0 Gy) at seven weeks after germination was observed. However, there were highly significant differences (P < 0.01) in the survival percentages among the gamma rays treatments as the plant’s survival chances decrease with an increase in the gamma-irradiation dose. The highest survival percentage (87.63%) was observed in the Ex-Sokoto variety treated with 25 Gy while the lowest survival percentage (21.10%) was observed from the highest gamma-irradiation dose (300 Gy) used. For the Karo variety, the highest survival percentage (78.38%) was also recorded at 25 Gy, whereas the lowest survival percentage (36.94%) was recorded at 300 Gy (Table 1). Higher survivability percentage was common among genotypes exposed to lower and moderate gamma-irradiation doses as compared with those exposed to higher gamma irradiation.

For chronic gamma irradiation, there was no mortality recorded because healthy growing seedlings (two weeks old) were used, and the plant’s height as

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**Table 2.** Germination and plant survival percentages in two Bambara groundnut varieties exposed to different chronic gamma-irradiation doses.

| Dose (Gy) | Number of seeds sown | Percentage germination (%) | Percentage survival (%) | Survival Reduction over control (%) |
|-----------|-----------------------|----------------------------|------------------------|-------------------------------------|
|           |                       | Ex-Sokoto | Karo | Ex-Sokoto | Karo | Ex-Sokoto | Karo |
| R0 (Control) | 20                    | 98.00     | 97.00 | 100.00    | 100.00 | -           | -    |
| R15 (8 Gy)  | 20                    | 91.12     | 91.74 | 84.29     | 80.85  | 15.71      | 19.15 |
| R11 (17 Gy)| 20                    | 85.78     | 88.45 | 79.57     | 77.87  | 20.43      | 22.13 |
| R9 (35 Gy)  | 20                    | 90.90     | 92.57 | 67.18     | 68.97  | 32.82      | 31.21 |
| R8 (44 Gy)  | 20                    | 96.02     | 80.35 | 59.48     | 54.09  | 40.52      | 45.91 |
| R7 (42 Gy)  | 20                    | 76.18     | 86.85 | 57.20     | 49.99  | 42.8       | 50.01 |
| R6 (59 Gy)  | 20                    | 89.10     | 93.43 | 52.56     | 49.79  | 47.44      | 50.21 |
| R5 (93 Gy)  | 20                    | 86.13     | 95.83 | 37.46     | 40.34  | 62.54      | 59.66 |
| R4 (144 Gy)| 20                    | 83.09     | 92.78 | 21.54     | 24.89  | 78.46      | 75.11 |
| R3 (255 Gy)| 20                    | 94.21     | 86.58 | 13.65     | 18.3   | 86.35      | 81.7  |
| R2 (570 Gy)| 20                    | 92.48     | 87.72 | 9.03      | 11.26  | 90.97      | 88.74 |
a parameter to measure the survival percentages was employed. Results from the control treatments (0 Gy) seedlings showed a 100% survival rate. Significant differences (P < 0.01) were observed among all the treatments (rings) and between the genotypes used. The highest survival percentage recorded in Ex-Sokoto and Karo varieties were (84.29%) and (80.85%), respectively. Both were observed in ring 15 (8.52 Gy) whereas the lowest survival percentage was (9.03%) and (11.26%), recorded in ring 2 (570.94 Gy), respectively. This indicated that the rings which were closer to the gamma cell dome received a higher amount of emitted gamma radiation than those that are further from the source. Therefore, the likelihood of fatality was high among the seedlings exposed to higher chronic gamma-irradiation doses than those exposed to lower gamma-irradiation dosage (Table 2).

Similar to the results on germination percentage, survival percentage from this study also indicates a significant decrease in survival rates with the increase in the gamma-irradiation doses for both acute and chronic gamma radiation treatments. Similar results were observed by other researchers such as in Cowpea by Horn et al. (2016); in Wilman lovegrass by Álvarez-Holgui et al. (2019); in Dianthus by Roychowdhury et al. (2012); in pigeon pea by Ariraman et al. (2018) and in Bambara groundnut by Adebola and Esson (2017). The findings of the studies indicated that higher survival rates are recorded at lower and intermediate mutagen treatments whereas lower survival rates are recorded at higher mutagenic treatments. Reduction in the plant survival percentage might be due to the inhibitory effects of gamma irradiation on the meristematic tissues of the seed which results in an injury to the chromosome structures. In a similar study, Talebi et al. (2012) indicated that the increasing rate of chromosomal damage with increasing gamma-irradiation dosage may perhaps be the reason for a decrease in plant survivability.

A stimulating effect of both acute and chronic gamma irradiations due to low and intermediate gamma-irradiation treatments were observed between the two Bambara groundnut varieties. Bambara groundnut varieties were used in this study most especially with respect to plant growth and physiological developments. Similar results were discovered in Vettiver by Roongtanakiat et al. (2012); in wheat genotypes by Ololuron et al. (2019); in garden bean by Monica and Seetharaman (2016) and in Cauliflower by Ke et al. (2019). Higher mutagens doses bring about the modification to the plant molecular properties that can lead to severe inhibition to the plant survival percentage among the mutagen treated plant materials. Equally, their studies revealed that compounds like protein, chlorophyll, and plant growth promoters which are directly linked to the plant metabolism can equally be injured or altered by the gamma-irradiation treatments and can potentially truncate the survival of the plants. Contrary to the findings of this study, Geng et al. (2019) reported that pre-treatments of osmanthus seedlings with different gamma-irradiation doses especially higher doses results in higher survival percentage and salinity stress tolerance. However, studies conducted on two Pepper cultivars (Capsicum annuum) by Kim et al. (2005) showed a contrasting outcome with no effects of acute gamma-irradiation doses of 2, 4, 8, and 16 Gy on seed germination and seedlings survival, whereas seedlings growth was stimulated at doses between 2 and 8 Gy.

### 3.3. The radiosensitivity test

In any successful induced crop mutagenesis procedure, it is imperative to determine the lethal dose (LD50) or growth reduction dose (GR50) values which will serve as a baseline for the subsequent doses that can be used to treat and study a larger population (samples). Subjecting planting materials such as seeds or seedlings to higher gamma radiation dosage can lead to deleterious consequences. The mean lethal dose (LD50) in acutely gamma-irradiated samples from this study was estimated from the resulting regression equation of the percentage survival rate (Figures 2 and 3), the same regression equation was used to determine a step below (LD25), a step above (LD75) and the mean lethal dose (LD50). For the Ex-Sokoto variety, the established lethal dose values were 73 Gy (LD25); 160 Gy (LD50), and 248 Gy (LD75), respectively (Figure 2). For the Karo variety, the identified lethal dose values are 68 Gy (LD25), 147 Gy (LD50), and 227 Gy (LD75), respectively (Figure 3). Based on these results, it can be seen that Ex-Sokoto has less sensitivity to gamma-irradiation treatment when compared to the Karo variety. This difference indicates that different genotypes from the same family can vary considerably in their sensitivity response to gamma irradiation (Hernández-Muñoz et al., 2017).

For chronic gamma irradiation, growth reduction was estimated for 25% (GR25), 50% (GR50), and 75% (GR75) (Figures 4 and 5). Based on the results of this analysis, using the resulting regression equation of the percentage survival rate, for Ex-Sokoto variety, the recorded dose responses are 47 Gy, (GR25), 250 Gy (GR50), and 444 Gy (GR75), respectively (Figure 4). For Karo variety, the values recorded are 70 Gy (GR25), 264 Gy (GR50), and 452 Gy (GR75) (Figure 5). This indicated that the Karo variety was less sensitive to the chronic gamma irradiation unlike in the acute phase where Ex-Sokoto appeared to be less sensitive to gamma irradiation. Therefore, these genotypes vary expressively to the type of gamma mutagenesis approach applied.

The results of the radiosensitivity test in this study among the gamma-irradiated treatments indicated a great decline in survival rate with the increase in
Figure 2. Lethal dose determination on Ex-Sokoto variety of Bambara groundnut irradiated with different doses of gamma-ray at 7 weeks after planting.

Figure 3. Lethal dose determination on Karo variety of Bambara groundnut irradiated with different doses of gamma-ray at 7 weeks after planting.

Figure 4. Effect of chronic gamma irradiation on growth reduction determination on Ex-Sokoto variety of Bambara groundnut irradiated with different doses of gamma rays at 7 weeks after transfer to gamma greenhouse.
Gamma radiation dosage both in acute and chronic gamma mutagenesis. This decline in survival percentage due to mutagenic treatments among the biological samples can be linked to the extent of cell differentiation and embryo development at the time of mutagenic treatments. This finding corresponds with the result by Kusmiyati et al. (2018) in soybean and in Hibiscus sabdariffa by Díaz-López et al. (2016). They reported that the sensitivity of biological samples to gamma mutagenesis treatments depends on the level of injury brought about by the mutagen with respect to growth and development processes such as cell elongation, division, biosynthesis, and numerous levels of hormone pathways.

The findings of this study based on radiosensitivity test and lethal dose determination indicated that acute gamma radiation dose at the range of 25–300 Gy can produce viable mutations in Bambara groundnut varieties. Equally, the results on growth reduction test in chronic gamma mutagenesis indicated that gamma-irradiation dosage at the range of 47–500 Gy has the potential to produce viable mutants among the two Bambara groundnut varieties (Ex-Sokoto and Karo) used in this study. This result conforms with the findings of Olasupo et al. (2016); Horn et al. (2016) in cowpea genotypes and Oladosu et al. (2017) in rice genotypes treated with different levels of gamma-irradiation doses, where after radiosensitivity test and lethal dose determination, they established that a dose range of 5–600 Gy is recommended as a potential dose capable of inducing viable mutations in cowpea. Equally, they further stated that, a similar dose range can be applied in the preliminary mutagenesis studies in other leguminous crops species. In a similar study conducted on soybean by Kusmiyati et al. (2018) using acute gamma irradiation, a lethal dose (LD50) value of 314.78 Gy was established based on the radiosensitivity test. Therefore, it can be concluded that gamma radiation doses between 5 and 400 Gy might be used in plant mutation breeding to screen and determine the appropriate dose for legumes improvement. The results of this study showed more damage to the two Bambara groundnut varieties when exposed to acute gamma irradiation as compared to the chronic gamma irradiation as revealed by the LD50 and GR50 values. For instance, a record of 160 and 264 Gy were determined as the LD50 and GR50 values for the Ex-Sokoto variety, while for Karo variety an established record of 147 and 250 Gy were identified as the LD50 and GR50 values, respectively. These results are supported by the findings of other researchers (Hong et al., 2018; Kamarudin et al., 2018; Roongtanakit et al., 2012). They concluded that seeds or plant parts that are exposed to acute gamma irradiation caused more damage to the plants than those exposed to chronic gamma mutagenesis. Taking into consideration that LD50 and GR50 values are the standards for radiosensitivity test, variation existed not only between Bambara groundnut varieties used for this study but also between the types of gamma radiation applied. Explicitly, the results have shown that different varieties varied considerably in their sensitivity to gamma irradiation as stated by Lee et al. (2019).

3.4. Gamma-irradiation effects on plant growth and development

Bambara groundnut seeds and seedlings were exposed to different gamma-irradiation doses to determine the effects of both acute and chronic gamma radiations on its growth and development. Fifteen days after germination of the acute gamma-irradiated seeds, the growing seedlings showed pragmatic retardation in growth among the treatments. However, for the chronic gamma-irradiated plants, a slight decline in plant growth and development was observed at the initial stage of chronic gamma radiation imposition.
Consequently, the impact of both acute and chronic gamma irradiations became more pronounced with the advancement in time of their exposure. Measurements of plant height, the number of petioles, the number of leaves, and the number of branches were recorded at time intervals during the plant growth process while internode length was determined after forty days.

High significant differences (P < 0.01) were observed among all growth parameters except for internode length where no significant difference was observed in both acute and chronic gamma irradiations and between the varieties. Plant height (Figure 6(a)) varied considerably among the gamma-irradiated varieties when compared to the non-irradiated control. Among the Ex-Sokoto variety, the maximum plant height recorded was 18.16 ± 4.10 cm (150 Gy), followed by 17.96 ± 4.27 cm (75 Gy) while the least height observed was 9.99 ± 0.42 cm (300 Gy). However, for the Karo variety, the maximum plant height recorded was 23.71 ± 2.18 cm (150 Gy), followed by 20.33 ± 4.45 cm at (175 Gy). While the least plant height was 13.67 ± 3.88 at (300 Gy) and the control recorded 22.02 ± 1.97. Generally, the number of petioles per plant in both genotypes (Figure 6(b)) significantly declined with increasing gamma-irradiation doses when compared to control. In the Ex-Sokoto variety, the mean number of petioles recorded was 46 ± 9.00 (25 Gy), followed by 44 ± 4.58 (75 Gy), while the least plant height recorded was 10 ± 1.53 (250 and 300 Gy), respectively, and the control recorded 45 ± 9.17. In Karo variety, the highest mean number of petioles per plant recorded was 50 ± 1.53 (25 Gy), followed by 44 ± 7.64 (50 and 75 Gy), while the least number of petioles recorded was 21 ± 10.44 (300 Gy) and the control recorded 42 ± 7.12. Results on the mean number of leaves per plant (Figure 6(c)) indicated a significant reduction among all gamma-irradiated treatments. The highest mean value recorded for Ex-Sokoto was 138 ± 27.00 (25 Gy), followed by 132 ± 13.75 (75 Gy), and the minimum value recorded was 31 ± 4.58 (250 and 300 Gy) respectively, as compared to the control which recorded 135. In Karo variety, the highest mean number of leaves recorded was 149 ± 4.58 (25 Gy), followed by 133.16 (75 Gy) and the lowest mean observed was 63 ± 30.75 (300 Gy) while control recorded 134 ± 14.80. From the results on the mean number of branches per plant (Figure 6(d)), in the Ex-Sokoto variety, the highest mean observed for the number of branches per plant was 14 ± 3.00 (25 Gy), whereas the least value recorded was 3 ± 10 (250 and 300 Gy) while the control recorded 14 ± 2.52. For Karo variety, the highest mean number of branches recorded was 15 ± 0.58 (25 Gy), followed by 13 ± 2.31 (50 and 75 Gy) while the minimum recorded was 6 ± 2.89 (300 Gy) whereas the control had 13 ± 1.15. The results on the mean internode length (Figure 6(e)) indicated that there was no significant difference (P < 0.05) among all the treatments and between the genotypes used in this study.

Similarly, results of the chronic gamma-irradiated plants indicate highly significant differences among the parameters examined except for internode length. For the Ex-Sokoto variety, the highest mean plant height (Figure 7(a)) recorded was 24.01 ± 2.44 (ring 8), followed by 23.04 ± 1.76 (ring 9) while the least mean observed was 11.20 ± 1.45 (ring 2) and the control recorded 22.88 ± 2.69. In Karo variety, the highest mean plant height recorded among the gamma-irradiated plants was 21.50 ± 1.35 (ring 15), followed by 20.58 ± 1.14 (ring 7) whereas the least mean observed was 10.66 ± 1.26 (ring 2) as against the control which recorded 22.49 ± 1.13. There was a significant variation in the mean number of petioles per plant (Figure 7(b)). In the Ex-Sokoto variety, the highest mean number of petioles recorded was 46 ± 4.36 (ring 8), followed by 45 ± 4.73 (ring 11) and the lowest mean value recorded was 15 ± 2.52 (ring 2) against the control that recorded 43 ± 2.00. Results on the number of leaves per plant showed significant differences amongst treatments (Figure 7(c)). The highest mean number of leaves per plant recorded in Ex-Sokoto was 125 ± 15.39 (ring 8), followed by 115 ± 7.55 (ring 15) while the least value recorded for the mean number of leaves was 37 ± 4.58 (ring 2) while the control recorded 120 ± 7.94. For Karo variety, the highest mean number of leaves recorded was 138 ± 13.08 (ring 8) followed by 136 ± 14.18 (ring 11) while the least mean value observed was 44 ± 7.55 (ring 2) as compared with the control which recorded 129 ± 6.00. For the mean number of branches per plant (Figure 7(d)) with significant variation among the different gamma-irradiation treatments, the highest mean number recorded for Ex-Sokoto variety was 13 ± 1.53 (ring 8) followed by 12 ± 2.08 (ring 11) and the lowest mean recorded was 4 ± 0.58 (ring 2) as compared with 12 ± 1.00 in the control. Consequently, in Karo variety the highest mean value observed was 14 ± 2 (ring 11), followed by 13 ± 2 (ring 9) whereas the least mean value recorded was 5 ± 12 (ring 2), while the control recorded 13 ± 1. Conversely, results on the internode length (Figure 7(e)) as in the acute did not show any significant difference among all the treatments and the control parents.

Generally, it was observed from this study that high doses of gamma irradiation inhibit plant’s physiological processes which in turn affect their growth and developmental processes. In this study, plant physiological activities among all the parameters studied were significantly affected by the increase in high gamma-irradiation doses, and similarly with the
extended period of chronic gamma-irradiation exposure. These results concur with those reported by other researchers such as in soybean varieties by Yasmin et al. (2019); in groundnut by Tshilenge-Lukanda et al. (2013); Malek et al. (2012) in mustard and blackgram by Yasmin et al. (2020). They maintained that exposure of plants (seeds, seedlings, or propagules) to higher doses of acute or chronic gamma radiation doses can seriously bring about a decline in plant growth and physiological developments. Similarly, they also maintain that gamma irradiation at higher dosage appeared to be inhibitory in their action, while exposure to lower and moderate doses was stimulatory in many instances.

Subsequently, based on the findings from this study, it was also evident that Bambara groundnut

Figure 6. Performance among two varieties of Bambara groundnut irradiated with different doses of acute gamma irradiation at 7 weeks after germination (a) plant, (b) number of petioles, (c) number of leaves, (d) number of branches, (e) internode length, (f) biomass fresh weight, and (g) biomass dry weight.
varieties were subjected to lower gamma-irradiation rates of acute 25–100 Gy. Chronic gamma irradiation at the range of 8–50 Gy gamma irradiations showed good performance in terms of growth and development among all the parameters studied, with lower fatality effects. These results coincide with the previous work by other researchers (Ariraman et al., 2018; Yasmine et al., 2019). Their studies established that the positive influence of lower doses of acute (100 Gy) and chronic (1–

Figure 7. Performance among two varieties of Bambara groundnut irradiated with different doses of chronic gamma irradiation at 6 weeks after germination, (a) plant, (b) number of petioles, (c) number of leaves, (d) number of branches, (e) internode length, (f) biomass fresh weight, and (g) biomass dry weight.
50 Gy) gamma mutagens on plant growth and physiological processes may be as a result of stimulation to the cell elongation, division, or modification of the metabolic activities which in turn affects the composition of nucleic acids or plant hormones. Also, Olasupo et al. (2016) stated that in some instances, exposure of seeds or planting materials to low levels of gamma irradiations appeared to be beneficial to plant growth and development due to hormesis.

Accordingly, several variations were observed with fluctuations between the responses of the two Bambara groundnut varieties to different levels of gamma-irradiation treatments with some parameters severely affected while others were not. These results agree with the work of other researchers (Oladosu et al., 2014; Ariraman et al., 2018; Thisaweck et al., 2020). They stated that some plant physiological processes such as plant height, number of leaves, number of branches, and biomass weight were severely altered due to the inhibitory effects of high mutagen doses on panting materials. They further illustrated that mutagens such as gamma rays (acute and chronic) may cause either negative or positive genetic effects on plant growth and development depending on the nature and quantity of the dosage applied. This suggests that researchers can have a wide range of traits to consider for selection.

The findings from their study maintained that treating plants with higher gamma-irradiation doses has shown the detrimental effect on plant physiological processes base on the interactions with cellular structure, molecules or atoms mainly water by increasing free radicals which in turn injure or alter the essential mechanisms of plant cellular metabolism such as thylakoid membrane dilation and were said to have affected several plant physiological, morphological, biochemical, and anatomical processes based on the gamma-irradiation dosage used and consequently. Their effects would manifest in photosynthesis alteration, phenolic compounds accumulation, and antioxidative system modulation (Borzouei et al., 2010; Malek et al., 2014). In the contrary, research conducted by Khah et al. (2020) in determining the mutagenic effectiveness and efficiency of gamma irradiation of two cultivars of bread wheat (Triticum aestivum L.) revealed that treating both cultivars with higher gamma-irradiation doses appeared to be more effective in inducing mutagenic effectiveness when compared with the lower doses. They further explained that the decrease in the efficiency of the gamma rays at low dose rate could be attributed to the lack of sufficient stimulation effects of any type of micro mutation at their DNA like transversion or translocation.

4. Conclusion

The radiation sensitivity test for Ex-Sokoto and Karo Bambara groundnut varieties has been optimized and established successfully for both acute and chronic gamma mutagenesis in this study. Following the exposure of Bambara groundnut seeds and seedlings to various acute and chronic gamma-irradiation doses, their growth pattern correlated inversely with the gamma-irradiated doses and appeared to be inhibitory at higher doses. Generally, an increase in gamma-irradiation doses (acute and chronic) resulted in a subsequent decrease and inhibition in plant growth and survival rate, while low doses of gamma rays stimulated certain growth and development traits in the two Bambara groundnut varieties. It was established that the lethal (LD_{25, 50, 75}) and growth reduction dose (GR_{25, 50, 75}) values for the two Bambara groundnut varieties in this study ranged between 63–250 Gy and 47–452 Gy, respectively. The outcome of the present study confirmed that induced mutagenesis (acute and chronic) can be used effectively to induce genetic variability for the improvement of certain characters or traits in crop species. This will further offer a realistic foundation for research on Bambara groundnut mutagenesis using gamma irradiation. However, it should be noted that induced mutagenesis is not specific but random events, and therefore established gamma-irradiation conditions might probably not give the same result of mutation events for different genotypes of the same family.

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ORCID

Mohd Y. Rafii http://orcid.org/0000-0003-4763-6367
Muhammad Hazim Nazli http://orcid.org/0000-0001-8171-8660
Yusuff Oladosu http://orcid.org/0000-0002-2092-971X

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