

# EFFECT OF GAMMA RADIATION ON MORPHOLOGICAL AND MOLECULAR CHARACTER OF *Sphenostylis stenocarpa* (Hochst. ex A. Rich.) Harms.

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## ABSTRACT

Genetic variation in two varieties of *Sphenostylis stenocarpa* (Hochst.ex A.Rich.) Harms was created using different doses of gamma-rays with the aim of increasing variability among the landraces for better morpho-agronomic characteristics. The two varieties responded differently to the doses of the treatment. The mutagen stimulated the germination percentage in both varieties. The highest germination percentage was observed at 200Gy for Tss86 while for Tss10 it was observed at 25Gy. It showed variation on the germination and growth parameters evaluated. The highest plant height (74.17±4.42) for Tss10 was observed at 25Gy while for Tss86, 100Gy gave the highest mean of 79.6±8.02. Molecular evaluation revealed variation in the patterns of RAPD profile between irradiated plants and control. The RAPD primers showed 82.9% polymorphism. Also, as observed from the similarity coefficient values, the trend of the genetic variability was not proportional to the doses of the gamma ray. The similarity indices ranged from 0.09 to 0.60 with 3 distinct cluster groups identified at 0.64. The variant of Tss86 at 250Gy was found to be the most diverse. Thus, this integrated approach can be used for carrying out the mutation-assisted breeding and subsequent selection of desired mutants using molecular markers in AYB.

**Keywords:** Gamma ray, *Sphenostylis stenocarpa*, RAPD, genetic variability, mutation breeding

## Introduction

Globally, several species of plants, that are cultivated for food production and generation of revenue are underutilized and or neglected, although they perform an important role in the livelihood of poor people in rural settlements (Magbagbeola *et al.*, 2010). These species of crops which includes *Sphenostylis stenocarpa* (Hochst.ex A.Rich.) Harms are rich in nutrient. Consequently, their extinction will have adverse effect on food security hence the need to improve them through crop improvement programs to increase their nutritional values

and to promote food security, especially among the poorer communities (Dansu *et al.*, 2012). *Sphenostylis stenocarpa* (Hochst.ex A.Rich.) Harms also referred to as African yam bean (AYB), is grown mostly in Africa for their seeds and tubers especially in West Africa. The protein content found in the seed and tubers ranges from 15 to 29% and its amino acid values are more than those found in other legumes like; cowpea, bambara groundnut and pigeon pea (Adewale & Dumet, 2010). Although, it is well known in the eastern part of Nigeria, for its nutritious beans and revenue

generation, it is still highly underutilized in most Nigerian homes due to long pre-cooking soaking to manually remove the seed coat followed by long duration (4-6 hours) of cooking of the beans (Thomas *et al.*, 2005).

The use of mutation breeding to create variation in the genome of crop plants for desired traits has been well reported in literatures (Melchinger, 2015; Asare *et al.*, 2017). However, the use of mutation induction can be associated with creation of undesired traits alongside the desired (Roychowdhury & Tah, 2011; Oladosu *et al.*, 2016). Mutation breeding can be achieved using three types of mutagenesis: mutagenesis using physical mutagens (irradiation); mutagenesis using chemical mutagens and biological mutagens (transposable elements). Among the physical mutagens, gamma ( $\gamma$  -rays) have so far been used extensively for mutation breeding aimed at improving agronomic traits of different crop species (Akhtar, 2014; Laskar *et al.*, 2018).

The application of molecular markers for analysis of genetic diversity and variety identification is considered best because it is not affected by stage of development of the crop, crop's environmental or management practices. In recent times, the molecular markers commonly used are Polymerase chain reaction (PCR) based markers which includes Random Amplified Polymorphic DNA (RAPD) (Selvi *et al.*, 2007; Abebe *et al.*, 2020).

According to Selvi *et al.* (2007) genetic variability and diversity analysis in African Yam Bean (AYB) has been achieved using identifiable phenotypic traits. Also, Popoola *et al.* (2017) has reported the use of RAPD in the evaluation of intraspecific variability among some African Yam Bean accessions. However, no report has been made on the use of molecular markers to estimate genetic variability and diversity studies created as a

result of mutation induction using gamma rays. The success in the use of RAPD in determination of genetic diversity in crops has been reported hence, its use in this study for diversity studies. RAPD has been employed in diversity study of crops like rice, maize, wheat and barley (Salem *et al.* 2007). This study aimed to create genetic variation in African yam bean (*Sphenostylis stenocarpa*) using gamma rays for crop improvement and germplasm conservation and to evaluate the variation as a result of the irradiation using RAPD.

## Experimental

### *Planting materials*

Two hundred and fifty healthy certified seeds each of accessions TSs10 and TSs86 of African yam bean used for this research were collected from the Gene bank of the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The description and geographic origin of the varieties selected according to the International Institute of Tropical Agriculture are presented in (Table 1).

### *Gamma source and irradiation of seeds*

About two hundred and twenty-four (224) seeds of each accession of AYP were irradiated. Thirty-two seeds (32) were used for each dose rate and placed in a glass Petri dish before irradiation at the following dose rates: 25Gy/h, 50 Gy/h, 100 Gy/h, 150 Gy/h, 200 Gy/h and 250 Gy/h using Cobalt-60 gamma source (Gamma Chamber 900, BRIT India make), while 0Gy/h served as control at the National Institute of Radiation Protection and Research (NIRP) at the University of Ibadan.

### *Experimental location, design and planting method*

The laboratory experiment was conducted in the Genetics Laboratory, Department of botany while the field experiments were carried out at the Department of Botany Nursery, University of Ibadan, Nigeria (Latitude 7°26'N and Longitude 3°53'E). The Molecular characterization was conducted at the Bioscience Laboratory International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. A plot of land measuring 10m X 10m was map out for the experiment.

Germination tests of irradiated seeds was carried out in the laboratory on Whatman No.1 filter paper in eight (8) replicates (per dose per accession) of 5 cm Petri dishes previously moistened with water. Each replicate contains a total of four (4) seeds making it Thirty-two (32) seeds per dose per accession.

For the field experiment, six seedlings in three replicates per dose per accession were sown 1m apart on a single role plot of length 10m. Weeding of the experimental plots was done at an interval of three weeks, pest control practices were also observed two weeks after planting following the method of Adewale *et al.* (2012). Staking was done three weeks after germination using strong stake each measuring about 3m high. Each seedling was independently staked to prevent mixing the accessional yields.

### *Experimental design*

The laboratory experiment was carried out in a Completely Randomized Design (CRD) with eight replicates, while Randomized Completely Block Design (RCBD) with three replicates, was adopted for the field experiment.

### *Evaluation of agronomic and morphological traits*

After 11 days of sowing, the germination percentage was evaluated, the length of hypocotyl and radicle were measured using a metre rule and the number of lateral roots were also counted. A total of six (6) morphological characters were evaluated using IITA descriptors of AYB. These characters are: plant height, number of branches, number of leaves, terminal leaf length, terminal leaf width and petiole length.

### *Determination of germination percentage and rate*

Estimation of the germination percentage (GP) and rate of germination (MGT) were calculated following the method adopted by Khaje-Hosseini *et al.* (2003).

GP = Number of germinated seeds after 11 days/ Total number of seeds planted X 100

$$MGT = \frac{\sum(fx)}{\sum f}$$

Where; MGT= mean germination time; F= the number of newly germinated seeds on each day; X= the day of counting.

### *DNA extraction and amplification*

The method used for the DNA extraction was as described by Dellaporta *et al.* (1983). Young leaves (0.2 g) of each accession were lyophilized and grounded into fine powder. The protocol incorporates a plant extraction buffer (PEB). The PEB is composed of 637.5ml of double distilled water, 100ml of 1 M Tris-HCl (pH 8.0), 100 ml of 0.5M Methylenediaminetetraacetic acid (EDTA) (pH 8.0), 100 ml of 5 M NaCl<sub>2</sub> and 62.5 ml of 20% sodiumdodecylsulphate (SDS). Seven hundred (700) ul of hot (65°C) of PEB was poured into Eppendorf tube. The PEB is pre- warmed and just before use, one percent

b-mercaptoethanol was added. The tube was then capped and gently inverted six to seven times to mix the buffer with the sample. To homogenize the samples, the solution was incubated at 65°C in water bath for 20 mins with occasional mixing. The samples were then removed from the water bath, the caps were opened and left for 2 minutes at room temperature to cool. Five hundred (500 µl) of 5M of potassium acetate was added to the content and covered, followed by gentle mix by inverting the tubes six to seven times. The samples were incubation on ice for 20 minutes after which the tubes were spin at 12,000 rpm for 10 minutes using a centrifuge (Kecheng HI-16k, China) at 4°C. The supernatant was transferred to a new clean 1.5ml Eppendorf tubes using a 1000 µl micropipette, care being taken to avoid the debris. Seven hundred (700 µl) chloroform: isoamyl alcohol was added to the supernatant and then spin at 10,000 rpm for 10 minutes using a centrifuge. The supernatant was collected and transferred into new marked tubes, followed by the addition of 700-µl ice-cold isopropanol and mixed gently by inverting the tubes 6-10 times. The tubes were then stored at -20°C for at least 1 hour to precipitate the DNA. In order not to dislodge the pellets from the bottom of the tubes; the supernatant was discharge with care and then drained on a clean paper towel inverted for 1 hour or more. Pellets of the DNA were double purified in 100 µl, cold 70% ethanol for 20 minutes and thoroughly air dried, after which 60 µl of 1×TE [10mM Tris-HCL (pH 8.0), 1mM EDTA (Ph-8.0)] was poured onto the pellets, followed by 2µl of 10ng/ml Rnase to extract the RNA. The solution was incubated for 40 minutes at 37°C with gentle mix at 10 minutes intervals.

PCR amplification was done using a thermal Cycler (Eppendorf Mastercycler pro S (US) with first denaturation step for 5 min at 94°C, followed by 35 cycles each of 1 minute at 94°C (denaturation), 1 min at 59.3°C (annealing), 2 min at 72°C (extension) followed by one final extension of 7 minutes at 72°C. The products of the amplification were electrophoresed in 1.8% agarose gels in 0.5× TBE (10× stock containing 1 M Tris, 0.8 M boric acid, 0.5 M EDTA) and stained with ethidium bromide (0.5 µg/ml). The gels placed were placed under a Gel Documentation System (Perkin Elmer Geliance 2000, state country of origin). The bands were scored for presence (score of 1) or (score of 0) for analysis.

#### *Molecular analysis*

Seven (7) RAPD primers (Table 6) showing polymorphism were employed in scoring and estimating the level of polymorphism. The gel bands were scored as present (1) or absent (0) and were assembled in a binary matrix. The data generated were analysed using Numerical Taxonomic System of Statistics (NTSYS version 2.21) and Power Marker Software (version 3.25).

Jaccard coefficient of similarity was used to estimate genetic distance and to construct dendrogram using Unweighted Pair Group Method of Arithmetic Means (UPGMA) to reveal phonetic representation of genetic relationship between mutants and control.

#### *Statistical analysis*

Data analysis was done using Statistical Analysis Software (SAS version 9.1). Means of ANOVA analysis showing significant

differences were separated by Duncan Multiple Range Test (DMRT) at 95% ( $p < 0.05$ ).

### Results and discussion

Table 1 shows the description and geographic origin of the varieties selected according to the International Institute of Tropical Agriculture are presented. The percentage germination at the different doses of treatment is all higher than the control except Tss 86 at 25Gy which has the minimum percentage germination and at 150Gy the two accessions had a germination percentage lower than the control (Fig. 1). Thus, Gamma irradiation showed a stimulatory effect on African Yam Bean Seeds.

A differential response of the mean germination time of the two varieties of AYB studied was reported in Table 2. For Tss10 in Table 2, 200 Gy had the lowest mean germination time which differed significantly from the other dose treatments while the same dose in Tss86 had the highest but only differed significantly from 250 Gy. Dhumal & Bolbhat (2010) reported a reduction in germination of gamma irradiated horsegram plant with increase in dose treatment which was observed in Tss10 accession. Varied mutagenic sensitivity to different doses of the mutagenic

treatment, in different genotypes has also been reported in production of viable mutants in *Nigella sativa* L. and in *Vignamungo* L. following gamma ray irradiation (Goyal & Khan, 2010). For Tss86, 150 Gy had the lowest MGT however, it did not differ significantly from other dose treatments applied (25, 50, 100, 200 and 250Gy). The irradiation dose had significant effect ( $p \leq 0.001$ ) on the hypocotyls length, the number of lateral roots and the root length (Table 3). The varietal effect on the hypocotyl's length was significant ( $p \leq 0.001$ ) while the interaction between variety and dose had significant effect ( $p \leq 0.05$ ) on hypocotyls length but not significant effect on the number of lateral roots and the root length, this can be compared to the work of Borzouei *et al.* (2010) on wheat and Badr *et al.* (2014) on cowpea.

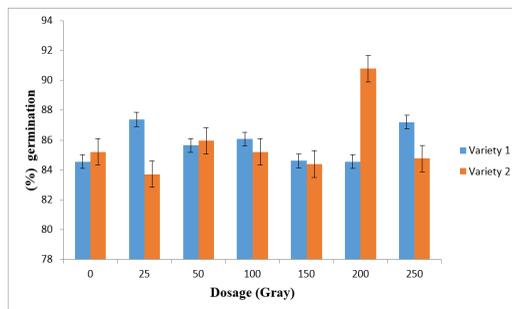


Fig. 1. Germination percentage (%) of the two varieties of AYB treated with gamma irradiation.

TABLE 1

*Morphological descriptions of the two Tropical Sphenostylis sternocarpa varieties (Tss10 and Tss86) based on seed coat colours*

S/N	Accessions name	Country of Origin	Seed coat colours	Colour codes in Methuen book of colour chart (Kornerup & Wanscher, 1978)
1	Tss10	Nigeria	Camel brown(V)	6D4
2	Tss86	Nigeria	Camel brown	6D4

TABLE 2

Mean Germination Time (MGT) of the two varieties of AYB treated with gamma irradiation

Variety	Dose (Gy)	Mean Germination Time (MGT)
Tss10	Control	3.14 ± 0.49a
	25	3.60 ± 0.07d
	50	3.15 ± 0.28a
	100	3.14 ± 0.30b
	150	3.14 ± 0.91a
	200	2.74 ± 0.15f
	250	3.56 ± 0.14b
Tss86	Control	3.85 ± 0.28b
	25	4.00 ± 0.49bc
	50	4.25 ± 0.14bc
	100	3.93 ± 0.21b
	150	3.79 ± 0.64b
	200	4.84 ± 0.49ab
	250	4.21 ± 0.21bc

Values with different letters as superscripts are significantly different at  $P \leq 0.05$ .

TABLE 3

Analysis of variance for germination parameters of the two varieties of AYB treated with gamma irradiation

	Df	HL (cm)	RL (cm)	NLR
Variety	1	15.02***	10.39**	5.5*
Dosage	6	11.76***	14.46***	8.48***
Variety*Dosage	6	3.17*	0.95 <sup>ns</sup>	0.94 <sup>ns</sup>
Error	42			
Total	55			

\* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ , \*\*\* =  $P \leq 0.001$ , ns = non-significant, Df- degree of freedom, HL-hypocotyl length, RL-root length, NLR- number of lateral roots.

Table 4 shows the response of AYB varieties to the mutagenic treatment. among the germination parameters with both varieties having the lowest mean at 200 Gy though not significantly different from other treatments this confirms the report of Laskar *et al.* (2018) who reported mutagenic sensitivity of different treatment doses in tomato plant. In Table 5, 25 Gy for Tss10 produced the highest mean across all the growth parameters except for leaf width. The plant height ranged from 49.00±2.36 to 74.17±4.42 cm in Tss10 and from 55.00±4.04 to 79.6±8.02 cm in Tss86. The highest plant height 79.6±8.02 was achieved at 100 Gy for Tss86 while for Tss10 the highest plant height 74.17±4.42 was recorded at 25Gy. This showed that though there were varying effect of the treatment on the two accessions of the AYB, the irradiation produced a higher plant height at a particular dose on each accession when compared to the control. At 200 Gy, all the growth characteristics measured in both varieties were low except in petiole length. The highest mean petiole length for Tss10 was observed at 25 Gy while for Tss86 irradiation at 100 Gy led to the development long petioles. This observation has also been made by Melki & Marouani (2010) on effect of irradiation on wheat. The effect of irradiation on growth may be attributed to chromosomal damage caused by the dose applied (Koing *et al.*, 2008).

**TABLE 4**  
*Mean effect of different dosages Gamma ray on germination parameters of the two varieties of AYB (African Yam Bean)*

Varieties	Dosage (Gy)	HL (cm)	RL (cm)	NLR
TSs10	0	10.6±0.29 <sup>a</sup>	7.38±0.83 <sup>ab</sup>	10.0±1.47 <sup>abc</sup>
	25	7.98±0.65 <sup>bcd</sup>	6.65±1.05 <sup>bc</sup>	7.00±0.00 <sup>bcd</sup>
	50	9.78±1.38 <sup>ab</sup>	8.8±0.53 <sup>a</sup>	14.5±2.33 <sup>a</sup>
	100	7.65±0.47 <sup>bcd</sup>	6.03±0.59 <sup>bc</sup>	13.5±1.44 <sup>a</sup>
	150	8.5±0.82 <sup>abcd</sup>	6.85±0.6 <sup>ab</sup>	10.75±1.49 <sup>ab</sup>
	200	6.08±0.78 <sup>d</sup>	3.13±0.42 <sup>de</sup>	5.5±0.65 <sup>cd</sup>
	250	6.55±1.43 <sup>cd</sup>	4.53±0.73 <sup>cde</sup>	8.25±2.17 <sup>bcd</sup>
TSs86	0	7.93±0.63 <sup>bcd</sup>	5.9±0.5 <sup>bc</sup>	7.5±0.87 <sup>bcd</sup>
	25	7.78±0.75 <sup>bcd</sup>	6.48±0.63 <sup>bc</sup>	8.5±1.19 <sup>bcd</sup>
	50	6.75±0.42 <sup>cd</sup>	6.95±0.88 <sup>ab</sup>	10.5±1.32 <sup>ab</sup>
	100	8.55±0.44 <sup>abcd</sup>	6.15±0.85 <sup>bc</sup>	13.5±2.33 <sup>a</sup>
	150	8.8±0.53 <sup>abc</sup>	4.65±0.56 <sup>cd</sup>	7.5±1.55 <sup>bcd</sup>
	200	3.78±0.99 <sup>c</sup>	2.53±0.33 <sup>e</sup>	4.25±0.85 <sup>d</sup>
	250	2.03±0.52 <sup>c</sup>	2.70±0.37 <sup>de</sup>	4.50±1.55 <sup>d</sup>

Values in the same column followed by different letters are significantly different ( $p \leq 0.05$ ). HL-hypocotyl length, RL-root length, NLR- number of lateral roots.

**TABLE 5**  
*Effect of different of doses of gamma rays on growth performance of the two varieties of AYB at 2 weeks after sowing*

Variety	Dosage	PH (cm)	NL	PL (cm)	LL (cm)	LW (cm)	NB
Tss10	0	69.33±4.76 <sup>abc</sup>	35.33±4.70 <sup>ab</sup>	5.20±0.47 <sup>ab</sup>	7.73±0.46 <sup>abc</sup>	3.00±0.36 <sup>ab</sup>	4.00±0.58 <sup>a</sup>
	25	74.17±4.42 <sup>ab</sup>	39.00±7.00 <sup>a</sup>	5.33±0.44 <sup>a</sup>	8.13±0.63 <sup>a</sup>	2.80±0.25 <sup>b</sup>	5.33±0.88 <sup>a</sup>
	50	59.00±4.91 <sup>bc</sup>	32.67±4.06 <sup>bc</sup>	4.83±0.60 <sup>ab</sup>	7.50±0.58 <sup>abc</sup>	2.80±0.35 <sup>b</sup>	3.33±0.88 <sup>ab</sup>
	100	55.67±3.84 <sup>bc</sup>	22.33±1.86 <sup>abc</sup>	4.13±0.35 <sup>abc</sup>	6.63±0.55 <sup>abc</sup>	3.27±0.35 <sup>ab</sup>	3.00±0.58 <sup>ab</sup>
	150	56.33±3.71 <sup>bc</sup>	31±3.61 <sup>abc</sup>	5.50±0.58 <sup>a</sup>	7.5±0.58 <sup>abc</sup>	3.03±0.29 <sup>ab</sup>	3.00±0.58 <sup>ab</sup>
	200	49.00±2.36 <sup>c</sup>	20.33±1.76 <sup>bc</sup>	4.23±0.15 <sup>abc</sup>	6.33±0.44 <sup>abc</sup>	2.47±0.09 <sup>b</sup>	3.00±0.58 <sup>ab</sup>
	250	61.67±8.19 <sup>abc</sup>	27±3.46 <sup>abc</sup>	4.40±0.49 <sup>abc</sup>	6.63±0.19 <sup>abc</sup>	2.60±0.21 <sup>b</sup>	3.67±1.2 <sup>ab</sup>
Tss86	0	69.00±6.35 <sup>abc</sup>	28.67±4.7 <sup>abc</sup>	5.33±0.6 <sup>a</sup>	8.00±0.58 <sup>a</sup>	2.70±0.40 <sup>b</sup>	3.33±0.33 <sup>ab</sup>
	25	58.83±3.35 <sup>bc</sup>	24.33±5.46 <sup>abc</sup>	4.5±0.36 <sup>abc</sup>	7.97±0.79 <sup>ab</sup>	3.53±0.54 <sup>ab</sup>	4.00±0.58 <sup>a</sup>
	50	64.67±7.31 <sup>abc</sup>	25.67±6.98 <sup>abc</sup>	4.5±0.87 <sup>abc</sup>	7.33±0.73 <sup>abc</sup>	4.70±1.77 <sup>a</sup>	3.00±0.58 <sup>ab</sup>
	100	79.6±8.02 <sup>a</sup>	30.67±2.33 <sup>abc</sup>	5.47±0.64 <sup>a</sup>	7.67±0.44 <sup>abc</sup>	3.30±0.32 <sup>ab</sup>	3.67±0.33 <sup>ab</sup>
	150	63.17±4.85 <sup>abc</sup>	38.33±3.18 <sup>a</sup>	5.33±0.67 <sup>a</sup>	7.57±0.74 <sup>abc</sup>	2.97±0.15 <sup>ab</sup>	3.00±0.58 <sup>ab</sup>
	200	55.00±4.04 <sup>bc</sup>	18.33±1.20 <sup>c</sup>	3.5±0.29 <sup>bc</sup>	5.67±0.73 <sup>c</sup>	2.33±0.07 <sup>b</sup>	1.33±0.33 <sup>b</sup>
	250	55.00±11.79 <sup>bc</sup>	33.67±6.44 <sup>ab</sup>	2.97±0.15 <sup>c</sup>	5.9±0.83 <sup>bc</sup>	2.47±0.18 <sup>b</sup>	3.00±1.15 <sup>ab</sup>

Values in the same column with different letters as superscripts are significantly different. PH-plant height, NL-number of leaves, PL-petiole length, LL-leave length, LW-leave width, NB-number of branches.

Table 6 shows the primers used and their sequences, as well as the total number of bands produced by each primer. The DNA polymorphism obtained in the two varieties of AYB studied with the seven (7) primers revealed variation as a result of the irradiation. The polymorphic information content (PIC) as shown in (Table 7), ranged from 0.6953 (OPB-01 and OPT-06) to 0.9124 (OPT-20). The highest PIC (0.9124) was shown by primer OPT-20 followed by OPT-07 (0.9008). Thus, these primers provided useful information

which can be employed for further studies. Figs. 2 to 8 present the banding profile of irradiated plant materials and control with different RAPD primers. There was variation in banding pattern between the irradiated and non-irradiated beans which could be attributed to the mutagenic effect of the treatment which is similar to the work of Eze *et al.* (2015) who reported genetic variability on sodium azide induced maize plant. These effects of gamma rays may be due to structural re-arrangements in DNA strand caused as result damages (Selvi *et al.*, 2007; Gill *et al.*, 2015). The present findings are in agreement with reports of Erdem & Oldcay (2004) and Hussain *et al.* (2008).

**TABLE 6**

*List of primers and their sequences*

Primer	Sequence (5'-3')	% GC content	*TNB
OPT-01	GGGCCACTCA	70	8
OPH-05	AGTCGTCCCC	70	7
OPT-06	CAAGGGCAGA	60	5
OPT-07	GGCAGGCTGT	70	11
OPH-09	TGTAGCTGGG	60	9
OPT-20	GACCAATGCC	60	11
OPB-01	GTTTCGCTCC	60	6

\*TNB- Total number of bands

**TABLE 7**

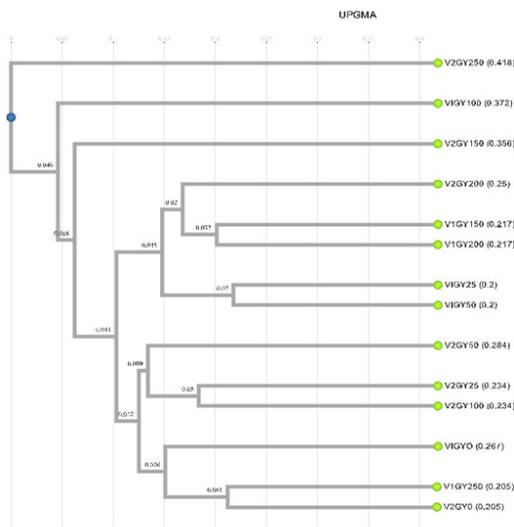
*List of primers and DNA fingerprint profile of gamma rays induced mutants in two varieties of AYB*

Primer	Major Allele Frequency	Sample Size	Allele Number	Number of Amplified Fragment	Gene Diversity	Polymorphic Information Content
				8.0000		
OPT-01	0.2143	14.0000	12.0000	6.0000	0.8980**	0.8899**
OPB-01	0.5000	14.0000	8.0000	7.0000	0.7143*	0.6953*
OPH-05	0.1429	14.0000	12.0000	5.0000	0.9082**	0.9008**
OPT-06	0.5000	14.0000	8.0000	11.0000	0.7143*	0.6953*
OPT-07	0.1429	14.0000	12.0000	9.0000	0.9082**	0.9008**
OPH-09	0.3571	14.0000	10.0000	11.0000	0.8265**	0.8129**
OPT-20	0.1429	14.0000	13.0000	8.1429	0.9184**	0.9124**
Mean	0.2857	14.0000	10.7143		0.8411	0.8297



The Jaccard's coefficient of similarity (Table 8) reflected the genetic relationship between the samples. The highest level of similarity was found between two irradiated plantlets; 25Gy of Tss10 and 50Gy of Tss10, with a similarity coefficient of 0.600, while a minimum similarity coefficient (0.095) was observed between 250Gy of Tss86 and 150Gy of Tss86 (the highest genetic distance).

The dendrogram based on Pearson's coefficients of Gamma ray-induced plants showed that three variants ( $V_2$  of 250Gy,  $V_1$  of 100Gy and  $V_2$  of 150Gy) were more distinct from the control, whereas others were quite similar to each other and with the control (Fig. 9). It can be deduced from these results that RAPD successfully detected variation between the non-irradiated and irradiated plants, which were morphologically indiscernible. This similar to the findings of Dhaksanamoorthy *et al.*, (2011) who reported polymorphism detection using RAPD on irradiated *Jatropha curcas*.



**Fig. 9.** UPGMA dendrogram based on Pearson's coefficient showing the genetic similarities and distance among Variety x Treatment of the two varieties of AYB studied.

## Conclusion

Gamma ray was able to induce genetic variability in the accessions of African yam bean and could be used as basis for mutation induction. selection in plant breeding considering the range of 25Gy dose through to 200Gy dose can be considered. There were differential responses of the two varieties to the mutagenic treatment. The gamma-rays exposure influenced the patterns of RAPD profile compared to the control, thus, proving the exploitation of RAPD analysis as a molecular marker for the identification of polymorphism in breeding of AYB useful. Progressive molecular characterization of irradiated plantlets may result in the establishment of an efficient mutation induction protocol, for example, identifying proper doses of mutagen by assaying DNA damage. The irradiated varieties with differences in their morphological characters, also, presented DNA polymorphism in PCR profile amplified by RAPD marker. It can be recommended that further analysis like proximate analysis be done on the seeds produced by irradiated plants to determine if there is a decrease or increase in protein content compared to the control.

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