



RESEARCH PAPER

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Genetic variability induced by gamma radiation on second generation of mutants M₂ of Bambara groundnut [*Vigna subterranea* (L.) Verdcourt] in Burkina Faso

Brahime Tinguéri¹, Mahamadi Hamed Ouedraogo¹, Wendmanegda Hermann Tonde¹, Tégawendé Odette Bonkougou², Celestin Thiombiano³, Adjima Ouoba^{3,4}, Daouda Ouedraogo³, Mahamadou Sawadogo¹

¹Plant Genetic and Breeding Team, Department of Plant Biology and Physiology, University Joseph Ki-Zerbo, Burkina Faso

²Pan African University, Life and Earth Sciences Institute (PAULESI), Nigeria

³Department of Plant Production, Institute for the Environment and Agricultural Research (INERA), Burkina Faso

⁴Polytechnic University Center of Ziniaré, University Joseph Ki-Zerbo, Burkina Faso

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Abstract

Bambara groundnut is a highly nutritious food legume. However, the low genetic variability in Bambara groundnut constitutes a serious barrier for its improvement. The mutagenesis is one of the techniques able to create genetic variability of crops species for breeding. This study aims at evaluating the genetic variability induced by gamma irradiations within the second generation of mutants (M₂). Thus, 40 families of M₂ mutants resulting from the mutagenesis of three Bambara groundnut varieties (KVS115, KVS234 and KVS259) were characterized using a Fisher block design with three replicates. Twenty-two (22) characters related to phenology, morphology and yield were used for the characterization. The results showed highly significant differences between M₂ mutant families for all the evaluated traits. Significant variability was recorded for the traits number of pods per plant, weight of pods, seeds weight, yield and number of leaves for which high coefficients of variation were observed (CV > 44%). The number of pods per plant, grain yield, seed weight and pod weight per plant, terminal leaflet length and width, and number of leaves presented high phenotypic and genotypic coefficients of variation (>20%). High heritability (H² > 60%) associated with high genetic advance value (GA > 72%) were registered for number of pods per plant, pods weight per plant, seed weight per plant and grain yield. Three classes were revealed from the Hierarchical ascending classification of the studied mutants. The first class comprised of fairly late maturing mutants (> 88 days), the second of and high yielding mutants (102-258 g/m²), while the third class comprised mutants with important aerial biomass. This significant variability could be used in Bambara groundnut improvement programs for creating highly performing varieties adapted to various biotic and abiotic constraints.

*Corresponding Author: Brahime Tinguéri ✉ tingueribrah@gmail.com

Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdcourt) also called voandzou is a legume which belongs to the Fabaceae family (Bamshaiye *et al.*, 2011). It is one of the five most important sources of protein for many Africans (Chittaranjan, 2007). According to Dapaah and Sangwan (2004), Bambara groundnut seeds are considered as a balanced food because of their richness in iron (4.9-48 mg/100 g), protein (18.0-24.0%, with high contents of lysine and methionine), mineral salts (3.0-5.0%), fat (5.0-7.0%), fiber (5.0-12.0%), potassium (1144-1935 mg/100 g), sodium (2.9-12.0 mg/100 g), calcium (95.8-99 mg/100 g), carbohydrates (51-70%) and in energy (367-414 kcal/100 mg). In Burkina Faso, Bambara groundnut cultivation constitutes a source of economic income for women (Ouoba *et al.*, 2016). Agronomically, Bambara groundnut contributes to improving soil fertility through the fixation of atmospheric nitrogen in symbiosis with Rhizobia (Muhammad *et al.*, 2021).

Regarding food importance, Bambara groundnut appears essential in strategies for consolidating the resilience of populations in food and nutritional insecurity, in the current context of climate change (Nadembega, 2016). However, Bambara groundnut production and promotion are severely hampered by several constraints. Indeed, the low production potential of existing varieties limits its production and productivity despite the favorable pedoclimatic conditions. The current maximum yield potential is 1,600 kg.ha⁻¹, while according to Pungulani *et al.* (2012), the crop yield potential could reach 3,000 to 4,000 kg.ha⁻¹. In addition, anti-nutritional factors such as phytates, tannins and fiber (House, 1999) which are abundant in Bambara groundnut seeds and which cause bloating and flatulence constitute a barrier to large-scale consumption. In addition, its grains preparation requires a long cooking time which constitutes a concern for processors.

Thus, the varietal improvement will allow the wide range production of the crop and establish a lasting basis for its promotion. Indeed, through the existence

of a genetic variability, genes of interest can be incorporated in a genotype and favor its adoption by producers. However, several studies (Ouoba *et al.*, 2017) reported low variability within Bambara weight accessions in Burkina Faso. The classical method of plant improvement, namely the hybridization of lines has been revealed unsuccessful in most of cases (Massawe *et al.*, 2003) or very difficult to carry out on a crop like the Bambara groundnut due to the small size and nature of its flowers. The success rate of this method reported to date is less than 2% (Muhammad *et al.*, 2021). Therefore, the use of the mutagenesis by gamma irradiation appears one of the alternatives to the classic method of varietal development.

Mutation techniques have proven useful in obtaining new traits, creating genetic variability and complementing conventional breeding (Sangsiri *et al.*, 2005; Anbarasan *et al.*, 2013).

This genetic variability is necessary for crop improvement (Aliero, 2006; Bolbhat *et al.*, 2012) because the variability that exists in all organisms, including our cultivated plants, has been generated by the mutation and subsequent recombination. Mutation techniques have been used to obtain many varieties of cultivated plants around the world including rice, rapeseed and wheat (Meunier, 2005; FAO/IAEA, 2020), sorghum (Nikiema *et al.*, 2020) and cowpea (Gnankambary, 2019). The application of the mutagenesis to Bambara groundnut could therefore create new variations in the agromorphological characters of the crop, which can result to setting up more productive, nutritious and adapted varieties to meet the needs of producers and consumers. Thus, the present study aims to evaluate the genetic variability induced by gamma irradiation within the second generation of mutants (M2) of three varieties of Bambara groundnut in Burkina Faso.

Materials and methods

Study site

The study was carried out under rainfed conditions in 2023 at field in the research station of the *Institut de*

l'Environnement et de Agricultural Recherche Agricoles (INERA), Saria, Burkina Faso. The station is located in the Boulkiemde province, 23 km east of Koudougou, between 12°16' North latitude and 2°09' West longitude, at 300 m altitude. The station belongs to the northern-Sudanian type climate (Thiombiano and Kampman, 2010). The soils of the location are of tropical ferruginous type, deficient in phosphorus and poor in organic matter (Hien, 2004). During the 2023 rainy season, temperatures fluctuated between 23°C and 38°C (ANAM, 2023), and rainfall of 747.6 mm were recorded (INERA, 2023). During the study period (August - October), the average rainfall recorded was of 449.1 mm. The precipitation diagram of the study site during the year 2023 is summarized in Fig. 1. It indicates that the rainfall was early and poorly distributed over time. The rains reached their peak in August (312.2 mm).

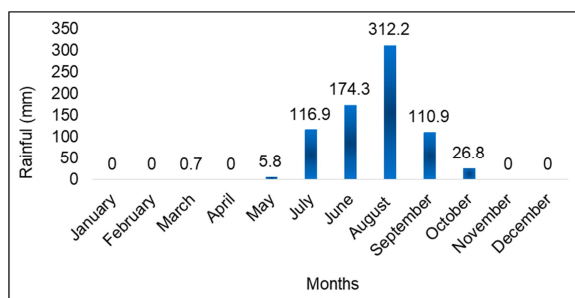


Fig. 1. Rainfall diagram for the INERA Saria experimental site in 2023 (INERA, 2023)

Plant material

The plant material consists of seeds second generation of M2 mutants and three non-irradiated parent varieties (KVS234, KVS259 and KVS115) used as controls. These seeds are divided into 40 mutant families, each family coming from the same first generation M1 mutant plant.

Studied mutants were derived from gamma irradiation of the three varieties at optimum doses of 200 gray (for KVS234 and KVS259 varieties) and 250 Gray (for KVS115 variety) using a labeled Cobalt source (^{60}Co) in the laboratory of the of the Insectarium of Bobo Dioulasso (IBD), Burkina Faso. These doses were chosen because germination assays indicated that 200 Gray and 250 Gray corresponded

to the optimal dose which induced 50% of seed germination of the three varieties.

Experimental design

The experimental design used for the development of the second generation of mutants M2 was a Fisher block with three replications. The spacing between two consecutive blocks was of 1 m. In each block were allocated all of the mutant families and the three non-irradiated control varieties. Each mutant family and each control variety was sown on a 3 m line per replication. The spacing between two consecutive lines was of 0.4 m and that between two consecutive pockets of 0.15 m. The experimental plot dimension was of 16.4 m x 12 m, either 180.4 m².

Cultivations practices

Soil preparation consisted of a ploughing using a tractor followed by a leveling. Sowing was carried out on August 4, 2023. Plant maintenance consisted of manual weeding to minimize competition with weeds and fertilization. The NPK (14-23-14) fertilizer was applied at a dose of 100 kg.ha⁻¹ at 21 days after sowing (DAS). Weeding was done twice, especially at 30 and 60 DAS. A hilling was carried out 45 days after flowering in order to create favorable conditions for fruiting.

Data collection

Twenty-two (22) qualitative and quantitative characters related to phenology, morphology and yield were collected.

Phenological traits

The collected phenological characters were:

Number of days to first flowering (DPF): it was obtained by counting the number of days from sowing to the date of the first flower appearance in each treatment.

Number of days to maturity (DM): this variable was determined by counting the number of days from sowing to the date of harvest in each treatment.

Morphological characters

A total of ten agromorphological characters were collected ten (10) weeks after sowing on five

randomly selected plants per line and per mutant family. These variables were: petiole length (LP), terminal leaflet length (LoFoT), terminal leaflet width (LaFoT), fourth internode length (LEN), plant height (HP) and diameter of the plant (DP). The number of stems per plant (NTP) and the number of leaves (NF) per plant were obtained by counting the number of stems and leaves of the five (05) randomly selected plants. The number and arrangement of leaflets, the anthocyanin coloration of the petioles, the shape and coloration of the terminal leaflets, and the coloration of the seed coat were obtained by observation. Plant habit type was determined based on the $\frac{LP}{LEN}$ ratio. The plant's habit was considered erect when $\frac{LP}{LEN} \geq 9$, semi-erect if $7 < \frac{LP}{LEN} < 9$ and creeping if $\frac{LP}{LEN} \leq 7$ (IPGRI *et al.*, 2000).

Yield components

The yield components were collected at harvest or after drying the pods. These were:

Average number of pods per plant (NG), determined by counting the number of pods of five plants per replication, including plants that have not produced any pods.

Dry pods weight (PG), evaluated by weighing the pods of each individually harvested plant.

Weight of seeds per plant (PGr), obtained by weighing the seeds of each selected plant.

100 seeds weight (P100) determined by weighing one hundred dry seeds per plant.

Grain yield (RDT) per square meter was determined using the following formula:

$$RDT \left(\frac{g}{m^2} \right) = \frac{\text{Seeds weight per plant (PGr)}}{1m^2} \times NP ;$$

NP: number of plants harvested from an area of one square meter.

Statistical analyzes

The collected data were subjected to an analysis of variance (ANOVA) using XLSTAT software version 2023.2.0.1411. The Student-Newman-Keuls multiple

comparison test was performed with the aim of studying the variability between genotypes and identifying mutant families significantly performant compared to the parental lines.

Genetic parameters including phenotypic (σ^2p) and genotypic (σ^2g) variances, phenotypic (CVP) and genotypic (CVG) coefficients of variation, broad sense heritability (H^2), expected genetic advance (GA) and advance expected genetic percentage (GA%) were estimated using R software version 4.4.1. Phenotypic and genotypic coefficients of variation were classified according to their value into low (< 10%), medium (10-20%) and high (> 20%) (Sivasubramanian and Madhavamenon, 1973). Heritability in the broad sense was classified according to Robinson *et al.* (1949) into 3 classes, especially low (< 30%), medium (31-60%) and high (> 60%). The expected genetic advance in percentage was categorized as low (<10%), medium (10-20%), and high (>20%) as well (Johnson *et al.*, 1955). The R software was also used to carry out principal component analysis (PCA) and ascending hierarchical classification (CHA). The Pearson correlation test was performed likewise, to evaluate the different associations between the studied characters and grain yield.

Results

Genetic variability between Bambara groundnut M2 mutants

Variability of the qualitative characters

Populations of irradiated Bambara groundnut M2 plants showed visible morphological variations at different stages of their development. This variability involved variables such as color and shape of the terminal leaflet of the leaf, the color of the petioles, the type of plant habit and the seed coat color. The proportions of variations observed are illustrated in Table 1. Depending on the terminal leaflet color, 76.3 to 81.6% of the mutants populations presented terminal leaflets of dark green color (Fig. 2-B) similarly to the controls, while 18.4 to 23.7% of mutants showed light green terminal leaflets (Fig. 2-A).

Table 1. Variations of the qualitative characters in the mutant M2 population of Bambara groundnut

Population M2	Proportions of the variation of the characters (%)											
	Coloration FoT		Petiole coloring		Shape FoT		Type of stem			Grain color		
	LG	DG	Purple	Green	Ovale	Lanceolate	E	SE	C	Cream	DP	Black
KVS115	23.7	76.3	11.9	88.1	93.5	6.5	60	19	21	00	8.6	91.4
KVS234	19.5	80.5	00	100	91.8	8.2	60.9	24.4	14.3	100	00	00
KVS259	18.4	81.6	00	100	98.4	1.6	86.5	13.4	0.1	100	00	00

Note: FoT: terminal foliol, LG: light green, DG: dark green, E: Erected, SE: semi-erected, C: Creepy, DP: dark-purple.

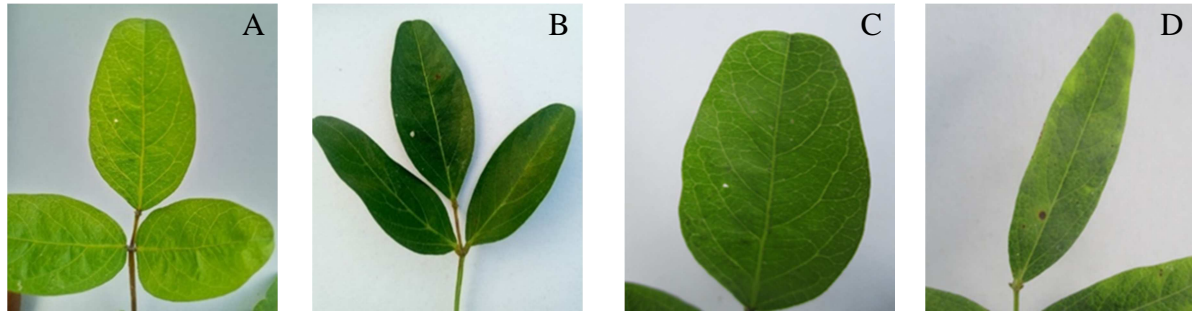


Fig. 2. Variation of the petioles color and the terminal folioles color and shape: leaf with green-light folioles and of violet petioles (A), leaf with green-dark foliole and green petiole (B), Oval foliole (C), lanceolate terminal foliole (D)



Fig. 3. Type of port: Erect port (A), Semi-erect port (B), Creepy port (C)



Fig. 4. Variability in folioles disposition

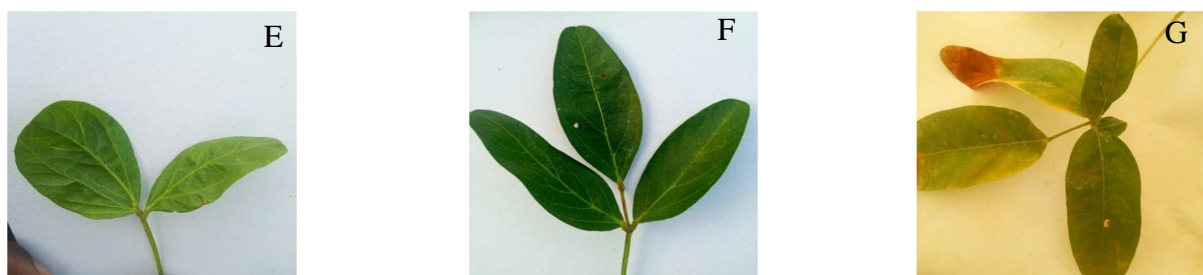


Fig. 5. Variation of the number of folioles of the leaves: Leaf with 2 folioles (E), Leaf with 3 folioles (F), Leaf with 4 folioles (G)

Table 2. Estimates of genetic parameters of various quantitative traits in M2 generation of Bambara groundnut

Parameters	Mean	MSG	MSE	σ^2g	σ^2p	GCV	PCV	H ²	GA	GA%	CV
NF	36.08	362.66 **	169.29	64.45	233.74	22.25	42.37	0.27	8.68	24.07	44.05
LP (mm)	109.96	1365.91***	63.48	434.14	497.62	18.95	20.28	0.87	40.09	36.46	21.94
LEN (mm)	11.28	37.00***	4.46	10.84	15.31	29.19	34.68	0.71	5.71	50.61	36.45
LoFoT(mm)	49.36	550.20***	10.76	179.81	190.57	27.16	27.96	0.94	26.83	54.35	24.59
LaFoT(mm)	21.39	98.29***	10.21	29.36	39.57	25.33	29.41	0.74	9.61	44.95	31.81
HP (Cm)	19.22	17.38***	1.18	5.40	6.58	12.09	13.35	0.82	4.3	22.56	13.28
NG	8.85	72.43***	7.90	21.50	29.41	52.42	61.30	0.73	8.17	92.36	66.16
PG (g)	5.54	22.81***	4.08	6.24	10.32	45.10	58.00	0.60	4.00	72.25	64.93
PGr (g)	4.46	15.58***	2.73	4.28	7.01	46.35	59.32	0.61	3.33	74.61	65.41
NTP	6.33	6.24***	1.70	1.51	3.21	19.41	28.31	0.47	1.73	27.42	28.45
DP (cm)	19.10	27.08***	0.97	8.70	9.68	15.45	16.29	0.89	5.76	30.17	16.13
P100 (g)	53.42	372.68***	84.50	96.06	180.56	18.34	25.15	0.53	14.73	27.56	29.42
RDT (g/m ²)	93.84	6906***	1078	1942.66	3020.64	46.97	58.56	0.64	72.81	77.58	65.08
DPF (JAS)	33.10	2.60***	0.06	0.84	0.91	2.78	2.88	0.93	1.83	5.52	3.12
DM (JAS)	85.01	116.75***	1.89	38.28	40.17	7.28	7.46	0.95	12.44	14.64	7.62

** and *** Significant at 0.01 and 0.001 probability levels; NF: number of leaves, LP: length of the petiole, LEN: length of the 4th internode, LoFoT: length of the terminal leaflet, LaFoT: width of the terminal leaflet, HP: height of the plant, NG: number of pods per plant, PG: pods weight, PGr: seed weight per plant, NTP: number of stems per plant, DP: plant diameter, P100: weight of 100 seeds, RDT: yield, DPF: date of first flowering, DM: date maturity, MSG: Mean square of genotype, MSE: Mean square of error, σ^2g : Genotypic variance, σ^2p : Phenotypic variance, GCV: Coefficient of Genetic Variation, PCV: Coefficient of Phenotypic Variation, H²: heritability in the broad sense, GA : Genetic advance expected at 5% of the mean, CV: Coefficient of variation.

Plants with purple petioles (Fig. 2-A) were observed only in the KVS115 mutant populations with a proportion of 11.9%. The plants of the two other mutant populations (KVS234 and KVS259) presented at 100% leaves with green petioles (Fig. 2-B), like the control varieties. As for the type of plant habit, the majority of the mutant populations showed plants with an erect habit (Fig. 3-A), with respectively 60% to 86.5% of the plants depending on the populations. Plants with a semi-upright habit (Fig. 3-B) and a creeping habit (Fig. 3-C) represented 13 to 24.4% and 0.1 to 21%, respectively depending on the populations. All the control varieties presented an erect habit. Hundred per cent (100%) of the seeds collected from the KVS234 and KVS259 mutant plant populations were uniformly cream colored like the those from the control varieties. In the KVS115 mutant population, 8.6% of dark-purple seeds was observed compared to the control for which seeds 91.4% of seeds were black. In addition to these variations, variability in leaflets arrangement (Fig. 4) was observed in all mutant populations.

Variability of the quantitative traits of M2 mutants

Most of the mutant population plants presented leaves with three (3) leaflets, with proportion varying

between 94% to 96.6% depending on the populations (Fig. 5). Leaves with two (2) leaflets and four leaflets were poorly represented in the populations (3.3 to 5.6% and 0.1 to 0.4% respectively depending on the populations).

The analysis of the variance showed significant differences between the mutant families for all the studied traits (Table 2). The highest values of the coefficient of variation were registered from yield attributes, especially the number of pods per plant (66.16%), the grains weight per plant (65.41%), the grain yield (65.08%), the pods weight per plant (64.93%), the number of leaves per plant (44.05), the 4th internode length (36.45%), the terminal leaflet width (31.81%), the number of stems per plant (28.45%), the terminal leaflet length (24.59%), the petiole length (21.94%) and the plant diameter (16.13%). For the other parameters, coefficients of variation were less than 15%.

The results revealed that for all the studied traits, the phenotypic variance (σ^2p) was greater than the genotypic variance (σ^2g). The highest genotypic and phenotypic variance values were recorded with grain yield ($\sigma^2g= 1942.66$ and $\sigma^2p = 3020.64$) followed by

the petiole length ($\sigma^2g= 434.14$ and $\sigma^2p = 497.62$), terminal leaflet length ($\sigma^2g = 179.81$ and $\sigma^2p = 190.57$) and the 100 seeds weight ($\sigma^2g = 96.06$ and $\sigma^2p = 180.56$).

For all the traits, the phenotypic coefficients of variation (PCV) were higher than the genotypic coefficients of variation (GCV). The highest GCV values (>20%) were observed with the number of pods per plant (52.42%), the grain yield (46.97%), seed and pod weight per plant (46.35% and 45.10% respectively), the 4th internode length (29.19%), the terminal leaflets length and width (27.16% and 25.33% respectively) and the number of leaves per plant (22.25%). The lowest GCV (<10%) was registered from the number of days to first flowering (2.78%) and the number of days to maturity (7.8%). Similar trend was observed for the phenotypic coefficients of variation (PCV) of the characters.

The expected genetic advance compared to the recorded average significantly varied depending on the trait. The highest genetic advance (>30%) were recorded from the number of pods per plant (92.36%), followed by the grain yield (77.58%), seeds weight per plant (72.25%), the pods weight per plant (74.61%), the 4th internode length (50.61%), the terminal leaflet length and width (54.35% and 44.95%), the petiole length (36.46%) and the plant diameter (30, 17%). The lowest genetic advance was registered from the number of days to first flowering (5.52%).

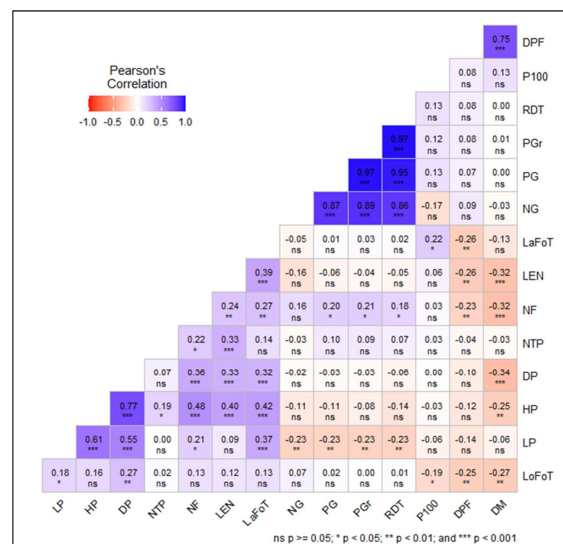
The broad sense Heritability was high (>60%) for most of the studied traits (number of days to first flowering, number of days to maturity, petiole length, the 4th internode length, the terminal foliole length, the terminal foliole width, the plant height, the number of pods per plant, grain weight per plant, the plant diameter and the yield), moderate (30-60%) for the number of stems per plant, the hundred seeds weight and the pod weight per plant. Lower value (<30%) was registered from the number of leaves per plant.

Correlations between various quantitative traits in M2 mutants generation

The results of the correlations analysis are presented in Table 3. Positive and significant correlation were

observed between grain yield and number of pods per plant ($r = 0.89$), pod weight per plant ($r = 0.92$), seed weight per plant ($r = 0.99$) and number of days to first flowering ($r = 0.16$). However, the the grain yield was negatively and significantly correlated with the petiole length ($r = -0.19$) and plant height ($r = -0.04$). Similarly, plant height was negatively and significantly correlated with yield components such as number of pods per plant ($r = -0.20$), pod weight per plant ($r = -0.19$) and seeds weight ($r = -0.17$) and positively correlated with the number of leaves per plant ($r = 0.42$), the petiole length ($r = 0.60$), the terminal leaflet length and width ($r = 0.43$), the number of stems per plant ($r = 0.16$) and the plant diameter ($r = 0.74$).

Table 3. Pearson correlation coefficients between various quantitative traits in M2 generation of Bambara groundnut



NF: number of leaves, LP: length of the petiole, LEN: length of the 4th internode, LoFoT: length of the terminal leaflet, LaFoT: width of the terminal leaflet, HP: height of the plant, NG: number of pods per plant, PG: pod weight, PGr: seed weight per plant, NTP: number of stems per plant, DP: plant diameter, P100: weight of 100 seeds, RDT: yield, DPF: date of first flowering, DM: date of maturity

Principal Component Analysis (PCA) and Hierarchical Ascending Classification (CAH)

Association of agromorphological traits

Principal component analysis (PCA) was carried out on quantitative traits. The eigenvalue, the

variance, and the latent vectors of the traits extracted from the principal component analysis (PCA) presented in Table 4 revealed that the first four factorial axes are significant with eigenvalues > 1 . These four cumulated axes allow the representation of approximately 70% overall variability, including 30.8% for axis 1; 21.8% for axis 2; 09% for axis 3 and 08,5% for axis 4.

Considering axis 1 which represents 30.83% of the total variation, the start of flowering, the maturity date and the yield components (P100, NG, PG, PGr, RDT) are opposed to the other characters. It can be said that axis 1 reflects the relationship between phenology and yield related and morphological traits. Axis 2 (21.8% of the total variation) shows that phonological parameters are opposed to all other agromorphological traits.

Table 4. Eigenvalues and percentage of variation expressed for the first four axes from the quantitative traits in principal component analysis

Characters	Dim1	Dim2	Dim3	Dim4
NF	0.008648011	0.3193399850	9.840003e-03	0.1536130780
LP	0.195899161	0.3189416627	4.673652e-05	0.1378862174
LoFoT	0.006205166	0.1762163369	1.215731e-01	0.0449398320
LaFoT	0.054844885	0.2807211316	1.311312e-01	0.0090897213
HP	0.228662276	0.5627329225	9.959442e-05	0.0016002627
NG	0.774678045	0.0818147444	6.164958e-02	0.0021949464
PG	0.805713649	0.1040985921	6.056568e-03	0.0009122294
PGr	0.858175635	0.1135637665	2.903951e-03	0.0012684232
NTP	0.003222964	0.0773726950	1.386533e-02	0.4939856133
DP	0.099251581	0.5570000130	3.968928e-05	0.0912118293
P100	0.005728102	0.0003991905	8.197734e-01	0.0214818229
RDT	0.862179986	0.1054310931	2.541567e-03	0.0019529559
DPF	0.105132016	0.1343533021	5.763021e-03	0.1438741665
Variances	4.008	2.832	1.175	1.104
% of variances	30.833	21.785	9.041	8.492
Cumulative % of variances	30.833	52.618	61.659	70.151

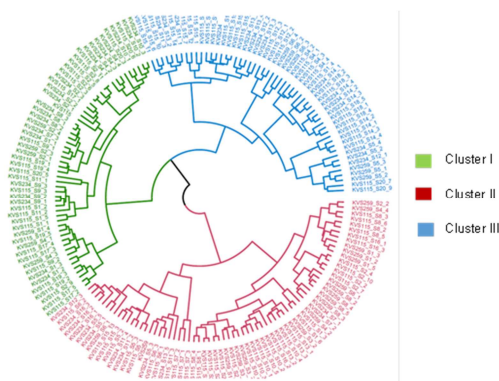


Fig. 6. Dendrogram from the hierarchical ascending classification of the individuals

Organization of variability

The Hierarchical Ascending Classification performed based on quantitative characters grouped the mutants into three clusters (Fig. 6). Fig. 7 shows the position of the genotypes on the $\frac{1}{2}$ plane of the Discriminant Factor Analysis. Cluster 1, comprising 54 mutants, includes late flowering and maturing individuals (high DPF and DM) with

low average values of DP, HP, LaFoT, LP, NF, LEN, PGr, RDT, LoFoT and PG. Cluster 2 contains 70 early flowering and maturing mutants (low DPF and DM) characterized by high average values of the variables PGr, RDT, PG, NG, NF and LoFoT. Cluster 3 consist of 54 mutants characterized by high values for the variables LP, HP, DP, LaFoT, LEN, NF and LoFoT and low values for the variables PG, RDT, PGr and NG.

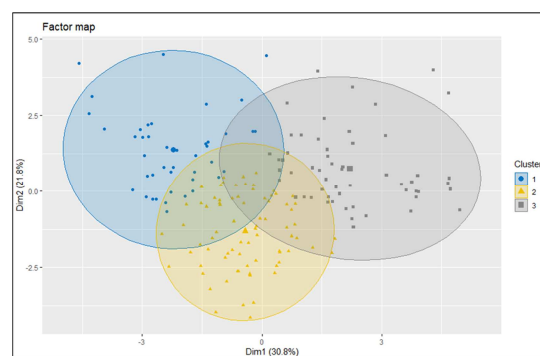


Fig. 7. Representation of groups on the $\frac{1}{2}$ axis of the AFD

Mutants of Interest

Results of means comparison test for the nine traits studied are listed in Table 5. There were families with mean values significantly different from the best control for all the traits. According to means comparison test, 27 M2 families superior to the control were determined for nine phenological and agronomical traits. Considering the number of leaves and plant height (characters linked to aerial biomass), one and three families respectively, presented mean values higher than that of the control. Concerning phenological characteristics, 13 families presented earlier flowering and 24 families presented earlier maturity. For the pods weight, only one mutant family showed means scored above the best control. For pods weight, seeds weight and seeds yield, two mutant families respectively showed a superior performance in comparison to the control. For 100 seeds weight, four mutant families presented mean values higher than that of the best control.

Table 5. Means comparison with the best control

Characters	Number of families inferior	Number of families superior
Number of leaves	3	1
Plant height	29	3
Number of pods	34	1
Pods weight	4	2
Seed weight per plant	12	2
Weight of 100 seeds	3	4
Seeds yield	12	2
Date of first flowering	13	12
Date maturity	24	0

Discussion

Gamma mutagenesis is one of the methods used to increase genetic variation, a prerequisite for plant breeding and improvement. In this study, gamma mutagenesis applied to three Bambara groundnut varieties induced variability in both qualitative and quantitative traits within the second generation of mutants. The most significant variations of the qualitative parameters were observed for plant habit, number and arrangement of leaflets and leaf color. According to Gaul (1964), the appearance of variants of observable qualitative characters is used as markers for the evaluation of the action of mutagens on genes.

The results of analysis of variance of the mutant populations showed significant differences between the

mutant families for all the studied traits. The high coefficients of variation reflect the variability that exists within the mutant's families. The maximum variability was observed for yield-related traits such as number of pods per plant, pods weight and seeds weight per plant, yield and number of leaves per plant with higher coefficients of variation ($CV > 20\%$). These results would reflect the existence of a strong heterogeneity within the families of the studied mutants for yield. According to FAO/IAEA (2020), the phenotypic manifestation of quantitative traits altered by mutagenic treatment can be detected by measuring dispersion coefficients or coefficients of variation. Phenotypic variability could result not only from the expression of strong genotypic heterogeneity, but also from the influence of environmental factors (Harouna *et al.*, 2018). The results are in agreement with those of Ibrahim *et al.* (2023) and Goli *et al.* (1997). Similar results were reported by Amri *et al.* (2018) in the M2 chickpea population and by Gnankambary *et al.* (2019) in M2 cowpea populations. These variations led to the identification of three statistically different groups in the studied M2 populations.

Taking into account the correlation matrix can be a large measurement scale for a selection program (Adebisi *et al.*, 2004). Strong correlations were observed between plant height and number of leaves, petiole length and width, number of stems per plant and plant diameter as well as between grain yield, pod and seed weights and 100 seeds weight. Taller mutants were those that presented greater average values of vegetative developmental parameters (number of leaves per plant, number of stems per plant and plant diameter). The high grain yield productive mutants were those characterized by high number of pods per plant and higher pod and seed weights. Similar findings, especially the existence of strong correlations between seed weight per plant and pod weight per plant were reported by Harouna *et al.* (2018) as well. According to Ouedrago *et al.* (2008), traits such as number of pods per plant, number of seeds per plant and 100 seeds weight are positively correlated with grain yield of Bambara groundnut. The negative significant correlation observed between grain yield and plant height and plant diameter could be explained by the fact that significant vegetative development leads to low grain yields.

Phenotypic variance coefficients were higher than genotypic variances coefficients for all traits. This indicates that traits expression was mostly influenced by the environment, which means that phenotypic parameters play an important role in breeding programs (Chimdi *et al.*, 2021). These findings corroborate the results of several authors in Bambara groundnut, namely Tanimu *et al.* (1990), Tanimu and Aliyu (1997), Chimdi *et al.* (2021) and Ibrahim *et al.* (2023). Similar results were also been reported on lentil (Younis *et al.*, 2008; Rasheed *et al.*, 2008; Latif *et al.*, 2010; Tyagi and Khan, 2010), soybean (Malek *et al.*, 2014) and maize (Maphumulo *et al.* 2015; Sesay *et al.*, 2016; Jilo *et al.*, 2018). The high GVC values for pod number, seed weight and grain yield show that these traits are less affected by environmental fluctuations; which guarantees a selection progress for these traits (Chimdi *et al.*, 2021). However, the small relative differences between the phenotypic and genotypic coefficients of variation suggest that the environmental influence on the traits expression is weak. This is favourable for crop improvement (Umar *et al.*, 2014; Usman *et al.*, 2014; Mahmudul *et al.*, 2021).

Furthermore, traits with lower genotypic and phenotypic coefficients of variation (GCV and PCV), especially the number of days to first flowering, the number of days to maturity, showed lower variability between Bambara groundnut studied mutant populations. Therefore, these traits cannot be used to effectively discriminate the mutant families. Fewer successes should be expected for breeding programs based on these traits.

High heritability was observed for most of the studied traits. These results are in agreement with those of Nwakuche *et al.* (2019) who reported that high heritability exists between Bambara groundnut populations. However, these results contrast with those of Chimdi *et al.* (2021) who found low heritability in almost all agronomic traits of Bambara groundnut. These differences could be due to experimental conditions (environmental variations). Indeed, Collaku (1994) reported in a study on wheat that low heritability is due to drought stress. Similarly, the heritability of yield traits in fava bean was found to be higher in a well-

watered treatment than under drought stress conditions (Toker, 2004). The high heritability of traits reflects the weak influence of environmental factors on their expression. In this case the phenotype truly expresses the genotype of the accessions (Visscher *et al.*, 2008). However, Sawadogo *et al.* (2014) believe that heritability alone cannot predict whether selection will provide substantial improvement or not. However, the joint estimation of the heritability and the expected genetic advance can provide more reliable information (Govindaraj *et al.*, 2011). The 100 seeds weight ($h^2=96.85\%$ and $GA=52.97\%$) exhibited high broad-sense heritability and expected genetic advance. For Ridzuan *et al.* (2018), low to moderate heritability and genetic advance values can hinder the improvement of the parameter due to the high environmental effects compared to the genetic effects. Thus, only efficient selection can be achieved by choosing the traits with higher GCV, PCV, h^2 and GA, which means that the effect of additive genes is sufficiently robust than the environmental effect (Usman *et al.*, 2014). The concomitant high values of these parameters confirm that it is possible to select genotypes with desirable characteristics from the progeny (Oulmi *et al.*, 2016). The genetic advance (GA) expressed as a percent of the mean was high ($>30\%$) for number of pods per plant, seed and pod weights per plant, grain yield, 4th internode length and the terminal leaflet length and width. This suggests that genetic control of traits was primarily additive.

Conclusion

Mutagenesis induced significant genetic variability within the second generation (M₂) of Bambara groundnuts mutants. Structuring the variability has enabled us to identify 3 groups, each with interesting characteristics. The class 1 comprised of fairly late maturing mutants of fairly late maturing mutants (> 88 days). The class 2 is made up of individuals with high grain yield potential, while class 3 is consisting of individuals with a high above-ground biomass. The induced variability could serve as a basis for setting breeding programs with for purpose of creating new biotic and abiotic stresses tolerant varieties and that have a high yield potential.

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