American Journal of Plant Sciences, 2024, 15, 651-676
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Genetic Enhancement of Indigenous Cowpea with Gamma-Ray Induced Trait Variation

Mathurin Tiergnin Dabiré[1,2], Minimassom Philippe Nikiéma[2], Djibril Yonli[2], Safiatou Sanna[2], Wossoguim Josué Gouba[2], Siébou Palé[2], Hamidou Traoré[2], Joseph T. B. Batieno[2], Varra Prasad[3], Zacharia Stewart[4], Jan B. Middendorf[3], Abhishek Rathore[5], Anupama J. Hingane[6]

[1]Unité de Formation et de Recherches en Sciences et Technologies, Département des Sciences de la Vie et de la Terre, Université Norbert ZONGO, Koudougou, Burkina Faso
[2]Institut de l'Environnement et de Recherches Agricoles (INERA), Ouagadougou, Burkina Faso
[3]Department of Agronomy, Kansas State University, Manhattan, USA
[4]Center for Agriculture-Led Growth Bureau for Resilience and Food Security United States Agency for International Development, United States Agency for International Development, Washington DC, USA
[5]International Maize and Wheat Improvement Center (CIMMYT), ICRISAT Campus, Patancheru, India
[6]Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture Department of Nuclear Sciences and Applications, Plant Breeding and Genetics Laboratory, International Atomic Energy Agency (IAEA) Seibersdorf, Vienna, Austria
Email: d.yonli313@gmail.com

How to cite this paper: Dabiré, M.T., Nikiéma, M.P., Yonli, D., Sanna, S., Gouba, W.J., Palé, S., Traoré, H., Batieno, J.T.B., Prasad, V., Stewart, Z., Middendorf, J.B., Rathore, A. and Hingane, A.J. (2024) Genetic Enhancement of Indigenous Cowpea with Gamma-Ray Induced Trait Variation. *American Journal of Plant Sciences*, **15**, 651-676.
https://doi.org/10.4236/ajps.2024.158044

Received: May 14, 2024
Accepted: August 25, 2024
Published: August 28, 2024

Abstract

Production of cowpea (*Vigna unguiculata* (L.) Walp.), a staple legume crop in Sub-Saharan Africa, faces challenges due to biotic and abiotic constraints. Induced mutagenesis was deployed to create genetic variation in two cowpea varieties (KVX396-4-5-2D and Moussa local). The radio-sensitivity tests led to determe the lethal dose 50 (LD_{50}) corresponding to 230 Gy and 220 Gy for KVX396-4-5-2D and Moussa local varieties, respectively. Dried seeds (M0) of each variety were gamma-ray irradiated with $LD_{50} - 50$, LD_{50} and $LD_{50} + 50$. M1 seeds were advanced to generate M2, M3 and M4 mutants using the single-seed-descent method. M4 mutant lines were evaluated in rain-fed conditions using a randomized complete block design to assess phenotypic differences. Data on seven qualitative and eleven quantitative traits were collected. The results indicated that the mutation induced variability in three qualitative traits: in KVX 396-4-5-2D mutant lines, with flower and seed color frequencies at 2.61% and 0.56% respectively, and pod dehiscence at a frequency of 0.24%. While in Moussa local mutants, a pod color changed at a frequency of 17%. ANOVA results revealed significant differences between mutants of both varieties for all quantitative traits, including photosynthetic parameters. Positive correlations were observed between leaf diameter and 100-seed weight, and between branch number and 100-seed weight. Hierarchical clustering revealed three clusters among KVX 396-4-5-2D mutants and six clusters among Moussa local mutants. Early maturity and high foliage were induced traits in Cluster

3 of KVX 396-4-5-2D mutants while high hundred-seed weight was induced in Cluster 6 of Moussa local mutants.

Keywords

Cowpea, Mutagenesis, Gamma Rays, Radio-Sensitivity, Phenotyping

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is a legume largely grown in arid and semi-arid zones in Africa. Its nutritional traits include high proteins, vitamins, carbohydrates, fatty acids and antioxidant contents [1]. Consumption of cowpea contributes to food and nutritional security, thereby improving human and animal health [2]. Cowpea-based cropping systems also enhance soil nutrient content through their biological ability to fix atmospheric nitrogen [3]. Cowpea grain production is estimated at 8.9 million metric tons per year on a cultivated area of 14.4 million ha [4], with majority coming from Africa, particularly Nigeria, Niger, and Burkina Faso, which together account for 74.3% [5].

In Burkina Faso, cowpea is cultivated across all agroecological zones and ranks as the leading legume and the fourth staple food crop, after sorghum, maize, and pear millet [6]. The average grain yield is estimated at 4.18 $t \cdot ha^{-1}$, which is considerably low compared to the global average yield (6.16 $t \cdot ha^{-1}$) [4]. This lower yield can be attributed to the low productivity of local cowpea varieties [7] and various abiotic and biotic constraints [8]. It has been postulated that the low productivity of varieties could be linked to the low genetic variability within cultivated cowpea germplasm [9] [10], resulting in a narrow genetic base for cowpea breeding programs. Hence, there is an interest in enhancing genetic variability in cowpea varieties to exploit promising genotypes. One of the effective technologies for achieving this is induced mutagenesis, which can induce genetic variability in cowpea varieties [11] and enable the selection of genotypes with desired phenotypic traits, such as resistance to diseases and pests, drought tolerance, photosynthetic efficiency and high yield potential [12]. Mutagenesis therefore provides a strong genetic basis for breeding programs, having led to the development and release of 3402 varieties of crops of which only 16 cowpea mutant varieties have been developed and released (https://mvd.iaea.org). In Burkina Faso, induced mutagenesis has been successfully applied in cowpea, sorghum, and rice to identify *Striga*-resistant and drought-tolerant genotypes [13] [14] as well as drought-tolerant mutants in cowpea [15]. The aim of this study was to assess the phenotypic and photosynthetic variability induced by mutation breeding in cowpea for desirable agro-morphological traits.

2. Materials and Methods

2.1. Study Site

The development of mutant populations and their agro-morphological assessment were carried out at the Institute of Environment Agricultural Research (INERA)/CREAF experimental station at Kamboinsé, located at 12˚27 North latitude and 1˚32 West longitude, 296 m above sea level. It lies in the North Sudanian phytogeographical sector with an annual rainfall of 600 - 900 mm [16]. The rainfall recorded at the station from May to October 2021, which corresponds to the rainy period, was 775.6 mm.

2.2. Plant Material

The plant material consisted of 448 cowpea genotypes including 446 M4 mutants, and their two parents (KVX396-4-5-2D and Moussa local) which are considered as preferred farmers' cowpea varieties. The seeds of both parents were supplied by INERA's cowpea breeding program. KVX396-4-5-2D is an improved variety whereas Moussa local is a local variety. KVX396-4-5-2D has white seeds with a semi-erect habit. Grain yield varies from 1.5 to 2 $t \cdot ha^{-1}$, and the maturity duration is 70 days. The Moussa local ecotype has a creeping habit with white seeds and a maturity duration of 85 days.

Genetically, uniform cowpea seeds of the two parents were used for seed gamma-ray irradiation.

2.3. Methods

2.3.1. Generating Cowpea M4 Mutant Lines

1) Optimization of Gamma-ray irradiation doses on cowpea variety seeds

The viable seeds of the two cowpea varieties with moisture content of 12% - 15% were irradiated with five doses of gamma-rays (75, 150, 300, 450 and 600 Gy) along with control (0 Gy) using 180 seeds per dose. Irradiation was done using Cobalst-60 (^{60}Co) radioactive isotope source with an activity of 202 Gy/min, at the Plant Breeding and Genetics Laboratory of the International Atomic Energy Agency (IAEA), Seibersdorf in Austria.

To determine the optimum irradiation dose that can cause optimum mutation frequency with least possible and unintended damage, twenty mutagenized seeds (M1) of each Gy dose along with 20 seeds of control (0 Gy) were planted on a growth medium consisting of peat, sand, and vermiculate at a ratio of 2:1:1. Additionally, 33 g phosphate and 27 g urea were also added. This was carried out, in a greenhouse using seedling trays at a temperature of 28˚C and a 12 h photoperiod. The experimental design consisted of a completely randomized block with three replications for each Gy dose. Seedlings were watered twice per week to ensure adequate soil moisture. Seed germination rate and plant mortality and height were recorded weakly. The plant height measured at 21 days after sowing of seeds (DAS) served as an indicator to estimate damage caused by mutagenic treatments. Data were subjected to analysis of variance (ANOVA) using linear models in SAS version 9.1. The means were separated using the Student-Newman-Keuls multiple-range test at a significant level of 5%. The lethal dose (LD_{50}) for each cowpea variety was estimated using a simple linear regression model by fitting the equation $y = mx + c$, where y is the response variable (plant height), x is the independent variable (irradiation dose), and *m* and *c* represent the slope and constant, respectively.

2) Development of cowpea M4 mutant lines

Cowpea seeds generated by single seed descent (SSD) method for each variety were irradiated at $LD_{50} - 50$, LD_{50} and $LD_{50} + 50$. Approximately 300 irradiated seeds (M1) from each Gy dose (treatment) were sown at Kamboinsé Research Station (plot, 12˚27'42.2"N and 1˚32'9.58"W), Burkina Faso during the cropping season of July-October 2019 along with respective parents following a randomized complete block design (RCBD). Each block comprised of one replication from each of the 8 treatments totaling 8 plots (9.6 × 7.2 m). Planting spacing was maintained at 0.40 m (seed to seed) within a row and 0.80 m between the rows within each plot (*i.e.* 100 seeds per plot). The M1 plants were individually harvested and 10 healthy M2 seeds from each harvested plant were sown in the 2020 rain-off season, using the one single-seed-descend method (on a plant progeny row basis), to obtain M3 seeds. Based on agronomic traits such as yield components, flowering time; and new morphological/color traits, 550 M3 mutant lines were selected and screened for *Striga gesnerioides* resistance during the 2020 cropping season in farmers' field with natural heavy *Striga*-infestation. From this field screening, 446 M4 mutants either with few Striga emergence (results not reported in this article) either other agronomic traits were recorded and then agro-morphological phenotyping. The distribution of M4 mutants according to the irradiation dose and cowpea variety is shown in Table 1.

Table 1. Number of cowpea M4 mutant lines selected per irradiation dose and variety.

Cowpea variety	Gamma ray irradiation doses (Gy)	Number of selected mutant lines
KVX396-4-5-2D	180	294
	230	81
	280	7
	Total	**382**
Moussa local	170	12
	220	52
	Total	**64**

2.3.2. Phenotyping of the Cowpea M4 Mutant Lines

The evaluation of the mutant lines was performed during the 2021 cropping season for phenotypic differences with parental varieties. The experiment was laid in an alpha lattice design. The trial consisted of 7 blocks of 65 lines. Each parent (M0 seeds) was replicated 3 times in each block. Each genotype was planted in five hills within a 4 m long row. Rows and holes were spaced 1 m apart for optimum expression of the traits. Two seeds were planted per hill and plants were thinned to one single-plant along with incorporation of NPK fertilizer (100 kg/ha) two weeks after the planting. Apron Star fungicide (20% Thiamethoxam + 20% Metalaxyl + 2% Difenoconazole) was also applied at a dose of 5 g/l. The rainfall recorded on the experimental site was 775.6 mm with 550 mm, while the average daily temperature ranged from 22˚C to 30˚C, during the cowpea growth period.

2.3.3. Data Collection

The agro-morphological data of seven qualitative and 11 quantitative variables (Table 2) were collected from each plant of each row.

Table 2. Qualitative and quantitative traits recorded on cowpea plants.

N˚	Qualitative traits	Code	Collection method
1	Plant growth habit	Plant habit	Noted at flowering: Erect, semi-erect, spreading
2	Flower color	FlCol	White, white with purple outline, purple
3	Shape of median leaflet	ShaLeaf	Globular, sub-globular, lanceolate, sub-lanceolate, oval
4	Branch pigmentation	BranPig	Noted at maturity: Green, reddish, brown
5	Pod color	PodCol	Noted at maturity: Yellow, brown, reddish, dark red
6	Seed coat color	SeedCol	White, whitish, maroon, brown
7	Seed hilum color	HilCol	Brown, dark black, maroon, reddish
N˚	**Quantitative traits**	**Code**	**Collection method**
8	Days to 50% flowering	DaFl	Number of days from sowing to first flowering
9	Plant height	PlHeight	Measured from soil to plant apex
10	Branch number	NumBr	Counted primary branches at flowering
11	Leaflet diameter	LeafDia	Measured with a ruler on three median leaflets
12	Leaflet length	LeafLen	Measured with a ruler on three median leaflets
13	Petiole length	PetLen	Measured with a ruler on three petioles
14	Days to grain maturity	DaMa	Number of days from sowing to 95% grain maturity
15	Peduncle length	PdLen	Measured with a tape measure at maturity on three peduncles randomly taken
16	Pod number	PodNum	Pods/plant with seeds were counted at the harvest
17	Pod length	PodLen	Measured on five randomly selected pods
18	100-seed weight	100-SW	Weight of 100 seeds

Chlorophyll fluorescence and relative chlorophyll content (SPAD) were measured as physiological parameters using the MultispeQ v1.0 handheld device, which works in connection with the photosynq platform (Photosynq, Michigan, USA) [17]. The measured fluorescence parameters included the quantum yield of photosystem II (Phi2), quantum yield of Non-Photochemical Quenching (PhiNPQ) and, the yield of non-regulatory energy dissipation (PhiNO). Measurements were recorded from 8 a.m. to 11 a.m. during the period when the plants reached the reproductive development stage between flowering and pod formation. These measurements were recorded on the three central plants of each genotype, at the healthy median leaflet level of the third leaf located on the main stem from the apex.

Phi2 assesses the plant's ability to convert the light received by photosystem II into carbohydrates, considered as a good indicator of a plant's photosynthetic performance.

PhiNPQ represents the excess energy dissipated in the form of heat by the plant to prevent damage to photosynthetic structures. PhiNO indicates the unregulated energy lost by the plant.

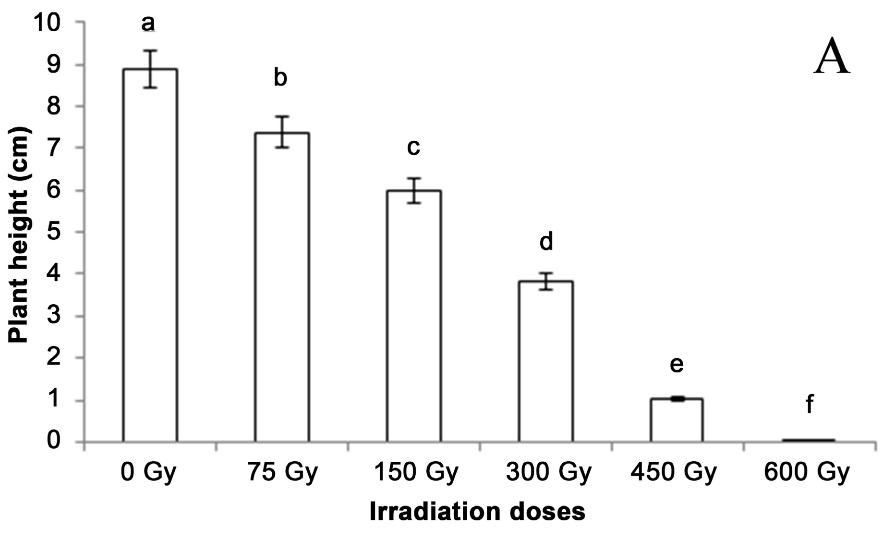
2.3.4. Statistical Analysis

Analysis of variance was performed to compare the mutant lines to the parents (controls). Significant difference between the parent and mutant means was determined using the Student-Newman-Keuls test at the 5% threshold. The relationship between quantitative data was established using Pearson's correlation coefficients, followed by principal component analysis to identify quantitative traits influencing phenotypic variation. Next, hierarchical ascending classification was used to assess the structure of the mutant population. These analyses were performed using the R software [18]. Qualitative data were used to calculate the frequency of occurrence.

3. Results

3.1. Determination of Lethal Dose 50 (LD_{50})

A significant ($P < 0.0001$) interaction was observed between the cowpea varieties and gamma radiation doses. The gamma-ray treatments significantly reduced survival rate above 200 Gy. Plants height decreased drastically with increasing irradiation doses in both varieties (**Figure 1**). According to the linear equation, the LD_{50} values of Moussa local variety and KVX396-4-5-2D variety were 223.87 Gy and 230.64 Gy respectively (**Figure 2**). For subsequent experiments, LD_{50} was considered to be 230 Gy for the KVX396-4-5-2D variety and 220 Gy for the Moussa Local variety.



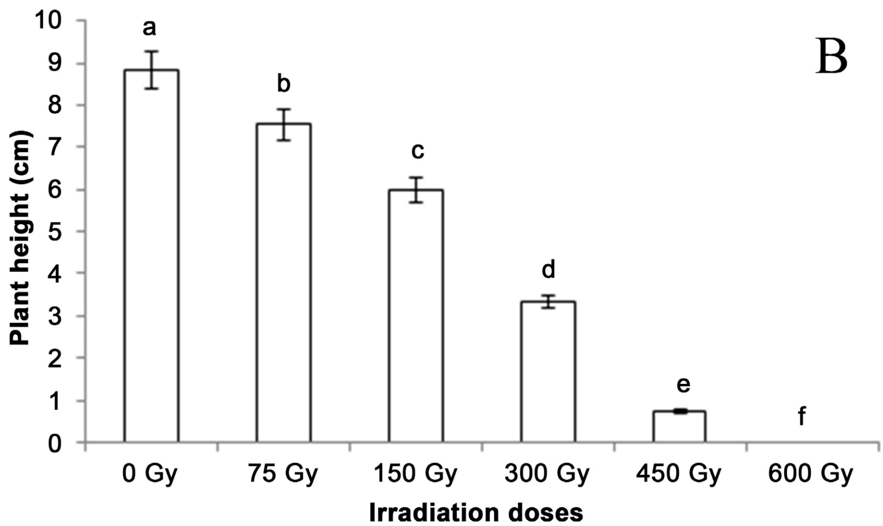


Figure 1. Effect of gamma ray irradiation on the seedling height of cowpea varieties KVX396-4-5-2D (A) and Moussa local (B) seeds irradiated with 0, 75, 150, 300, 450 and 600 Gy doses at 21th days old.

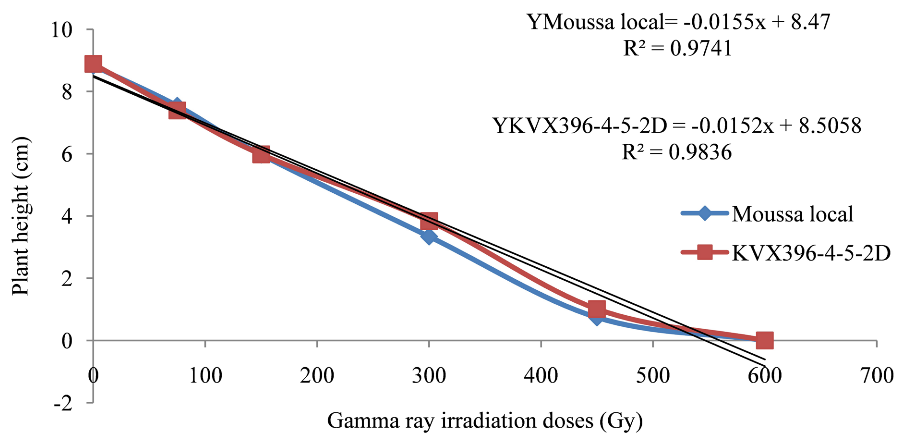


Figure 2. Seedling height and fitted straight line to estimate the LD_{50} in cowpea varieties KVX396-4-5-2D and Moussa local.

3.2. Frequency Distribution of Qualitative Traits in Cowpea M4 Mutant Lines

Gamma irradiation induced variability in some qualitative traits of cowpea (Table 3). White and purple flowers appeared with frequencies of 2.14% and 0.47%, respectively, in the KVX 396-4-5-2D variety, where white with a purple outline color was dominant (97.39%) (Figure 3). No difference in flower color of was observed with the Moussa local mutant lines. The mutation also induced variation in growth habit in both varieties. The majority of KVX 396-4-5-2D mutant lines (96.12%) showed a semi-erect habit similar to their parent, compared to 3.40% of mutants which have an erect growth habit and 0.47% with a creeping growth habit. Semi-erect, erect, and creeping growth habit appeared in the Moussa local mutant lines with frequencies of 47.33%, 1.53% and 51.14% respectively. Lanceolate and globular median leaflet shapes were observed in mutant lines of both varieties, with some KVX 396-4-5-2D mutants exhibiting an oval shape. Most of KVX 396-4-5-2D mutants (99%) and Moussa local mutants (52%) retained the pod color of their parents. However, color variations were observed in some mutants. The pods of some KVX 396-4-5-2D mutants showed a brown color at a frequency of 0.48%, and a reddish color at 17.18% in Moussa local. Dehiscent pods appeared only in the KVX 396-4-5-2D variety at a frequency of 0.24% (Figure 3). Some differences in seed color (whitish, brown, and maroon) were noted in some KVX 396-4-5-2D mutants.

Table 3. Frequency of variations in qualitative traits of cowpea KVX396-4-5-2D and Moussa local M4 mutant lines.

Qualitative traits	Phenotypic variation	Kvx396-4-5-2D		Moussa local	
		Number	Frequency (%)	Number	Frequency (%)
Flower color	White with purple outline	1230	97.39	-	-
	White	27	2.14	262	100
	Purple	6	0.47	-	-
Plant growth habit	Semi-erect	1214	96.12	124	47.33
	Erect	43	3.40	4	1.53
	Spreading	6	0.47	134	5114
Shape of median leaflet	Sub-lanceolate	1035	81.95	259	98.86
	Lanceolate	22	1.74	1	0.38
	Sub-globular	189	14.96	2	0.76
	Globular	9	0.71	-	-
	Oval	8	0.63	-	-
Branch pigmentation	Green	1234	97.70	-	-
	Reddish	23	1.82	262	100
	Brown	6	0.48	-	-
Pod color	Yellow	1257	99.52	-	-
	Brown	6	0.48	136	51.91
	Reddish	-	-	45	17.18
	Dark red	-	-	81	30.92
Pod dehiscence	Indehiscent	1260	99.76	262	100
	Dehiscent	3	0.24	-	-
Seed coat color	White	1256	99.44	262	100
	Whitish	1	0.08	-	-
	Maroon	3	0.24	-	-
	Brown	3	0.24	-	-
Seed hilum color	Brown	1243	98.42	253	96.56
	Dark black	15	1.19	-	-
	Maroon	1	0.08	-	-
	reddish	4	0.31	9	3.44

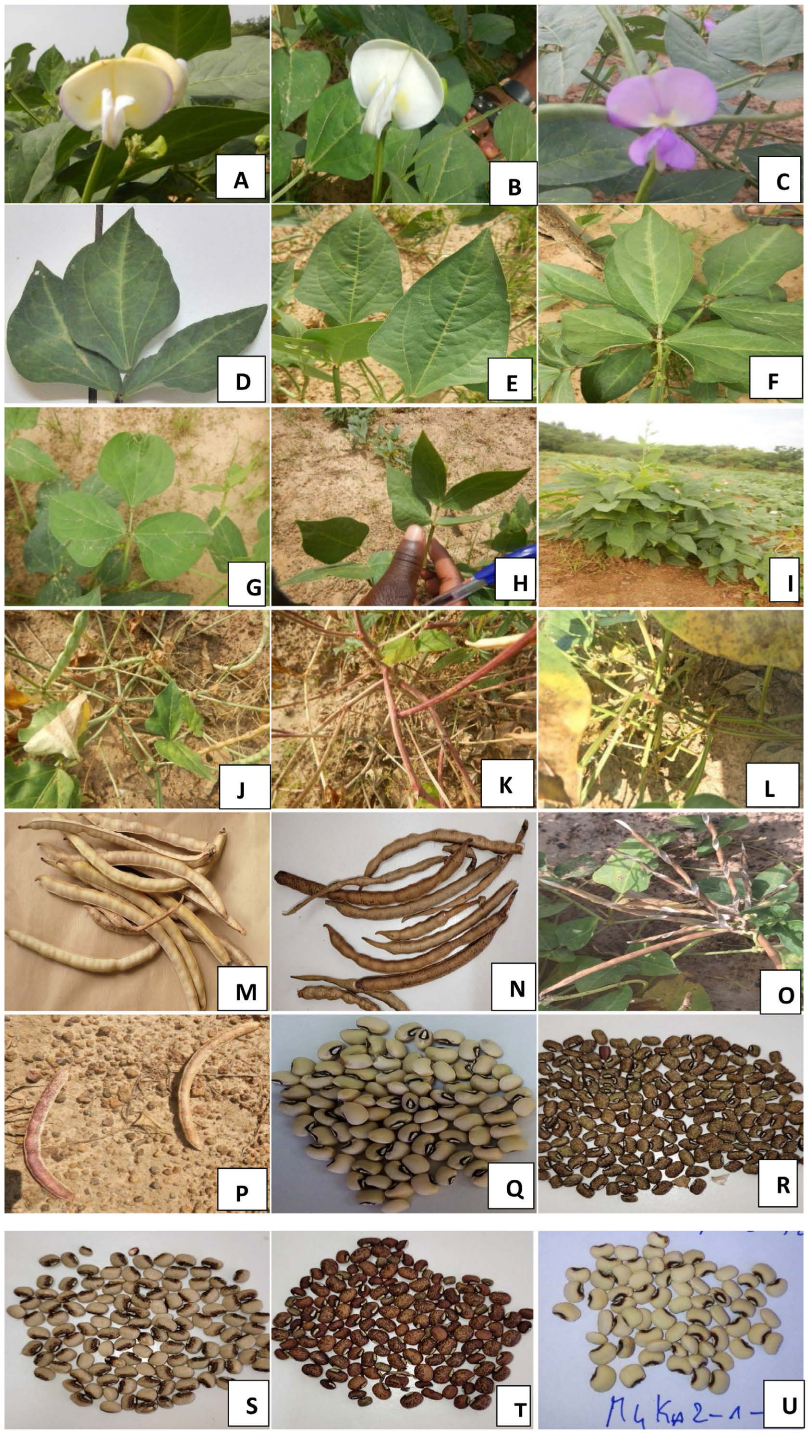


Figure 3. Effect of gamma rays on qualitative traits induced in cowpea M4 mutant lines. (A) Flower color of parent KVX396-4-5-2D; (B) White flower; (C) Purple flower; (D) Sub-lanceolate leaflet of parent KVX396-4-5-2D; (E) Lanceolate leaflet; (F) Oval leaflet; (G) Globular leaflet; ((H) (I)) Leaf anomalies in some Moussa local mutants; (J) Kvx396-4-5-2D parent branch pigmentation; (K) Reddish branch pigmentation; (L) Brownish pigmentation; (M) KVX396-4-5-2D yellow pod; (N) Brown pod; (O) Dehiscent pod; (P) Reddish and brown pods in Moussa local mutants; (Q) White seed of KVX396-4-5-2D; (R) Brown pods; (S) Whitish seed with maroon hilum; (T) Maroon seed; (U) Red hilum.

3.3. Descriptive Analysis of Recorded Morphological Traits

The coefficient of variation for mutant lines generated from KVX 396-4-5-2D and Moussa local varieties was high (>20%) for pod number, plant height, branch number, and peduncle length, moderate (10% - 20%) for the other parameters, except for days to flowering and days to grain maturity, which was low (<10%) (Table 4 and Table 5). ANOVA results showed significant differences between the mutant lines of both cowpea varieties and their parents for all the quantitative traits assessed. Flowering time ranged from 36 to 68 days after the sowing (DAS) for KVX 396-4-5-2D mutant lines, with a mean of 46 DAS compared to the parent's (Table 4). For the Moussa local mutant lines, flowering time ranged from 43 to 70 DAS, with similar mean flowering time (49 DAS) as the parent (Table 5). The minimum time of pod maturity for the KVX 396-4-5-2D mutant lines exhibited an earlier maturity of 59 DAS, which stood out of the parent of which the pod maturity was delayed of 70 DAS. The pod maturity time for the Moussa local parent was 71 DAS while that of the mutant lines ranged from 65 to 83 DAS. The highest branch numbers recorded for Moussa local mutant lines were 11 compared to seven for the parent. KVX 396-4-5-2D mutant lines showed branch numbers ranging from 1 to 9 branches whereas the parent was five branches. The means of leaflet diameter and length of KVX 396-4-5-2D mutant lines were 5.50 cm and 10.08 cm respectively, while those of the parent were 5.24 cm and 9.98 cm, respectively. The means of leaflet diameter (4.08 cm) and length (8.40 cm) of Moussa local mutant lines were higher than that of the parent (3.70 cm and 8.24 cm). The petiole length of the KVX 396-4-5-2D mutant lines ranged from 2 to 15 cm, with an average of 8 cm, similar the parent, whereas Moussa

Table 4. Results of analysis of variance of quantitative traits recorded from KVX 396-4-5-2D mutant lines at M4 generation.

Quantitative traits	Parent	Cowpea M4 mutant lines				
	Mean	Min.	Max.	SD	CV (%)	Mean
Days to 50% flowering	47.63	36.00	68	3.82	8.26	46.30***
Plant height (cm)	14.63	5.00	30.50	3.25	21.98	14.79***
Branch number	5.26	1	9	1.20	21.28	5.65***
Leaf diameter (cm)	5.24	2.70	9.40	0.75	13.66	5.50***
Leaf length (cm)	9.98	2.40	16	1.31	12.95	10.08***
Petiole length (cm)	7.79	2	15.50	1.40	17.33	8.09***
Days to grain maturity	69.89	59	86	4.10	6.02	68.09***
Peduncle length (cm)	16.33	1.50	32.50	4.35	26.59	16.34***
Pod number	27.53	2	93.00	15.51	51.71	29.99***
Pod length (cm)	14.27	4	19.50	2.04	14.14	14.42***
100-seed weight (g)	14.71	9	23.14	2.39	16.65	14.32***

Note: Min: Minimum; Max.: Maximum; SD: Standard deviation; CV: Coefficient of variation. ***Significant at $P < 0.001$ probability levels.

Table 5. Results of analysis of variance of quantitative traits recorded from Moussa local mutant lines at M4 generation.

Quantitative traits	Parent	Cowpea M4 mutant lines				
	Mean	Min.	Max.	SD	CV (%)	Mean
Days to 50% flowering	48.50	43	70	3.12	6.28	49.67***
Plant height (cm)	10.83	4.50	21	2.46	20.4	12.05**
Branch number	7.83	2	11	1.48	20.7	7.15***
Leaf diameter (cm)	3.70	2.15	6.70	0.53	13.1	4.08***
Leaf length (cm)	8.24	4.70	10.75	1.10	13.1	8.41***
Petiole length (cm)	7.40	3.25	12.25	1.24	17.5	7.07***
Days to grain maturity	71	65	83	2.82	3.96	71.17***
Peduncle length (cm)	10.16	3.67	18.67	2.81	28.4	9.91***
Pod number	32.50	3	92	15.50	50.1	31.55***
Pod length (cm)	15.75	7.56	17.90	1.90	13.2	14.43**
100-seed weight (g)	16.11	1.32	22.77	3.08	20.6	14.93***

Note: Min: Minimum; Max.: Maximum; SD: Standard deviation; CV: Coefficient of variation. *, **, and *** are significant at $P < 0.05$, 0.01 and 0.001 probability levels, respectively.

local mutants varied from 3 cm to 12 cm, with an average of 7 cm. Average peduncle length was 16 cm for KVX 396-4-5-2D mutant lines and their parent, and 10 cm for Moussa local mutant lines and their parent. The maximum pod number for the KVX 396-4-5-2D and Moussa local mutant lines were higher than that of their parents, with 93 and 92 pods, respectively. The mean pod length was 14 cm for the KVX 396-4-5-2D mutant lines and parent with maximum length of 19 cm. Moussa local mutant lines and the parent had a mean pod length of 15 cm. KVX 396-4-5-2D mutant lines exhibited a maximum peduncle length of 32 cm, which is longer than that the parent (16 cm). Moussa local mutant lines showed maximum peduncle length ranged from 3 to 18 cm while that of parent had peduncle length of 10 cm. Hundred-seed weight for the KVX 396-4-5-2D mutant lines and the parent ranged from 9 to 23 g and 15 g, respectively, whereas those for Moussa local mutant lines were between 1 and 22 g, compared to the parent with a weight of 16 g.

3.4. Variation of Photosynthesis Parameters in Cowpea Mutant Lines

The result of analysis of variance showed a significant difference between the mutants of both varieties according to the photosynthetic parameters, except of the unregulated quantum yield of lost energy PhiNO of Moussa local mutant lines (Table 6). The quantum yield of photosystem II (Phi2), ranged approximately from 0.29 to 0.69 in the mutants of both varieties. The mean of PhiNPQ was 0.15 for KVX 396-4-5-2D mutant lines and 0.12 for the Moussa local mutant lines.

The chlorophyll content ranged from 4.56 to 70.34 with KVX 396-4-5-2D mutant lines and from 2.99 to 71.33 in the Moussa local mutant lines. The coefficient of variation was low to moderate with KVX 396-4-5-2D mutant lines (5.27% - 27.92%) whereas the coefficient of variation of Moussa local mutant lines was low to high (6.94% - 45.10%).

Table 6. Variation of the photosynthetic parameters in KVX 396-4-5-2D and Moussa local mutant lines at M4 generation.

Genotypes	Traits	Parent	Cowpea M4 mutant lines				
		Mean	Min.	Max.	SD	CV (%)	Mean
KVX 396-4-5-2D mutant lines	Phi2	0.58	0.29	0.69	0.04	5.27	0.56***
	PhiNO	0.29	0.09	0.33	0.02	7.00	0.28***
	PhiNPQ	0.11	0.02	0.60	0.05	27.92	0.15***
	Chlorophyll content	67.22	4.56	70.34	4.26	6.13	66.41*
Moussa local mutant lines	Phi2	0.57	0.27	0.69	0.05	6.94	0.58***
	PhiNO	0.27	0.13	0.34	0.28	9.90	0.28 ns
	PhiNPQ	0.14	0.02	0.54	0.06	45.10	0.12***
	Chlorophyll content	60.57	2.99	71.33	13.18	18.99	62.66*

Note: Phi2: Quantum yield of Photosystem II; PhiNPQ: Yield of non-photochemical quenching; PhiNO: Yield of non-regulatory energy dissipation; CV: Coefficient of variation. ns: Not significant; * and *** are significant at P < 0.05 and 0.001 probability levels, respectively.

3.5. Correlation between the Quantitative Variables

According to Pearson's coefficients strong positive and negative correlations were observed in the mutant lines of both varieties. In KVX 396-4-5-2D mutant lines, a strong and positive correlation was noted between peduncle length and leaf dimensions (leaflet diameter and length, and petiole length). Leaf diameter was highly correlated with the number of plant branches and all other quantitative traits, except the days to grain maturity, which was negatively and highly correlated with pod number and length (**Table 7**). The number of pods was highly and positively correlated with the pod length and 100-seed weight.

Table 7. Pearson's correlation coefficients of quantitative traits with respect to yield in KVX396-4-5-2D mutant lines.

	DaFl	PlHeight	NumBr	LeafDia	LeafLen	PetLen	DaMa	PdLen	NumPod	PodLen	100-SW
Days to 50% flowering	1										
Plant height	−0.096	1									
Branch number	−0.497***	0.218***	1								
Leaf diameter	−0.383***	0.037	0.419***	1							
Leaf length	−0.404***	0.094	0.365***	0.621***	1						
Petiole length	−0.338***	−0.078	0.317***	0.502***	0.427***	1					

Continued

Days to grain maturity	0.706***	0.006	−0.368***	−0.088	−0.085	−0.168***	1				
Pod length	−0.407***	0.058	0.461***	0.574***	0.497***	0.415***	−0.083	1			
Pod number	−0.507***	0.067	0.440***	0.435***	0.446***	0.315***	−0.239***	0.598***	1		
Pod length	−0.325***	0.121*	0.344***	0.361***	0.392***	0.311***	−0.195***	0.457***	0.476***	1	
100-seed weight	−0.198***	0.071	0.272***	0.221***	0.261***	0.291***	−0.022	0.288***	0.146**	0.296***	1

Note: DaFl: Days to flowering; PlHeight: Plant height; NumBr: Branch number; LeafDia: Leaflet diameter; LeafLen: Leaflet length; Petlen: Petiole length; DaMa: Days to grain maturity; PdLen: Peduncle length; NumPod: Pod number; Podlen: Pod length; 100-SW: One seed weight. *, ** and *** are significant at P < 0.05, 0.01 and 0.001 probability levels, respectively.

In the Moussa local mutants, only the correlation between cowpea plant height and mean of pod length was negative (Table 8). The correlations between days to grain maturity, days to flowering, branch number per plant, hundred-seed weight, peduncle length and pod number were positive and highly significant (Table 8).

Table 8. Pearson's correlation coefficients of quantitative traits with respect to yield in Moussa local mutant lines.

	DaFl	PlHeight	NumBr	LeafDia	LeafLen	PetLen	DaMa	PdLen	NumPod	PodLen	100-SW
Days to 50% flowering	1										
Plant height	0.102	1									
Branch number	−0.137	0.041	1								
Leaf diameter	−0.167	0.126	0.579***	1							
Leaf length	−0.085	0.185	0.390**	0.636***	1						
Petiole Length	−0.159	0.018	0.091	0.377**	0.589***	1					
Days to grain maturity	0.605***	0.136	−0.091	0.046	−0.014	0.145	1				
Pod length	−0.160	−0.170	−0.091	0.039	0.043	0.250*	0.133	1			
Pod number	0.021	−0.024	−0.092	0.085	0.057	0.183	0.387**	0.629***	1		
Pod length	−0.153	−0.344**	−0.010	−0.152	−0.068	0.121	0.036	0.446***	0.452***	1	
100-seed weight	−0.060	−0.096	0.427***	0.361**	0.232	0.265*	0.127	−0.050	−0.117	0.016	1

Note: DaFl: Days to 50% flowering; PlHeight: Plant height; NumBr: Branch number; LeafDia: Leaflet diameter; LeafLen: Leaflet length; Petlen: Petiole length; DaMa: Days to grain maturity; PdLen: Peduncle length; NumPod: Pod number; Podlen: Pod length; 100-SW: 100-seed weight. *, ** and *** are significant at P < 0.05, 0.01 and 0.001 probability levels, respectively.

3.6. Clustering Analysis of Cowpea M4 Mutant Lines

3.6.1. Principal Components Analysis

Principal components analysis (PCA) was performed to better understand and identify the parameters that explained the source of variation between mutant lines. This graph examines the relationships between the quantitative variables collected. In the correlation circle, the size of the vectors reflects the quality of the variable representations.

With KVX 396-4-5-2D mutant lines, PCA revealed that the first three dimensions contributed to 62.25% of the total variation. Dimension 1 contributed 39.36% and was positively and strongly correlated to the number of branches (0.69), leaf diameter (0.73), peduncle length (0.76), pod number (0.73), leaf length (0.71), petiole length (0.62) and pod length (0.63) (**Figure 4**). Dimension 2 explained 12.87% of the variation and was highly correlated to days to grain maturity (0.83) and days to 50% flowering (0.55). Dimension 3 contributed 10.02% of the total variation and highly correlated to plant height which was the major contributor of this axis. Thus, axis 1 contributed to plant productivity while the axis 2 was related to the cycle length of the crop genotype.

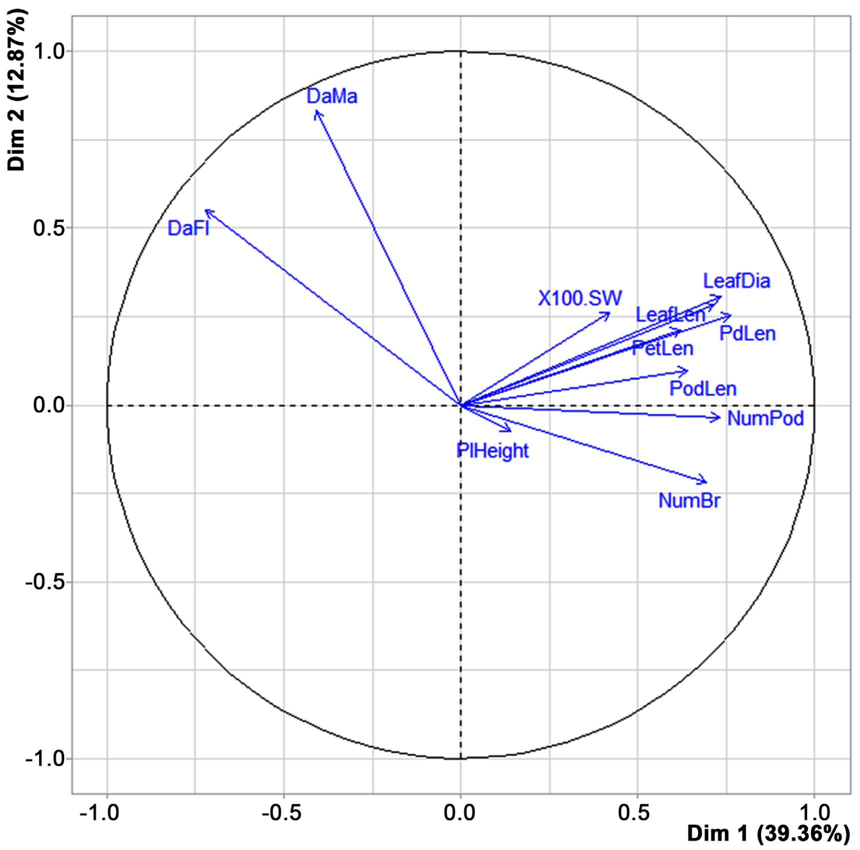


Figure 4. Principal components analysis showing associations between traits in KVX 396-4-5-2D mutant lines at M4 generation.

With Moussa local mutant lines, PCA showed that the first three dimensions explained 60.9% of the data variance (dimension 1: 24.4%, dimension 2: 20.6%, dimension3: 15.9%). Dimension 1 is positively correlated with branch number (0.65), leaf diameter (0.84), leaf length (0.81) and petiole length (0.66), while dimension 2 is positively correlated with peduncle length (0.81), pod number (0.83) and pod length (0.72) whereas dimension 3 is positively correlated with days to 50% flowering (0.81) and days to grain maturity (0.83). Axis 2 and axis 3 represent the productivity and cycle length of the mutants, respectively (**Figure 5**).

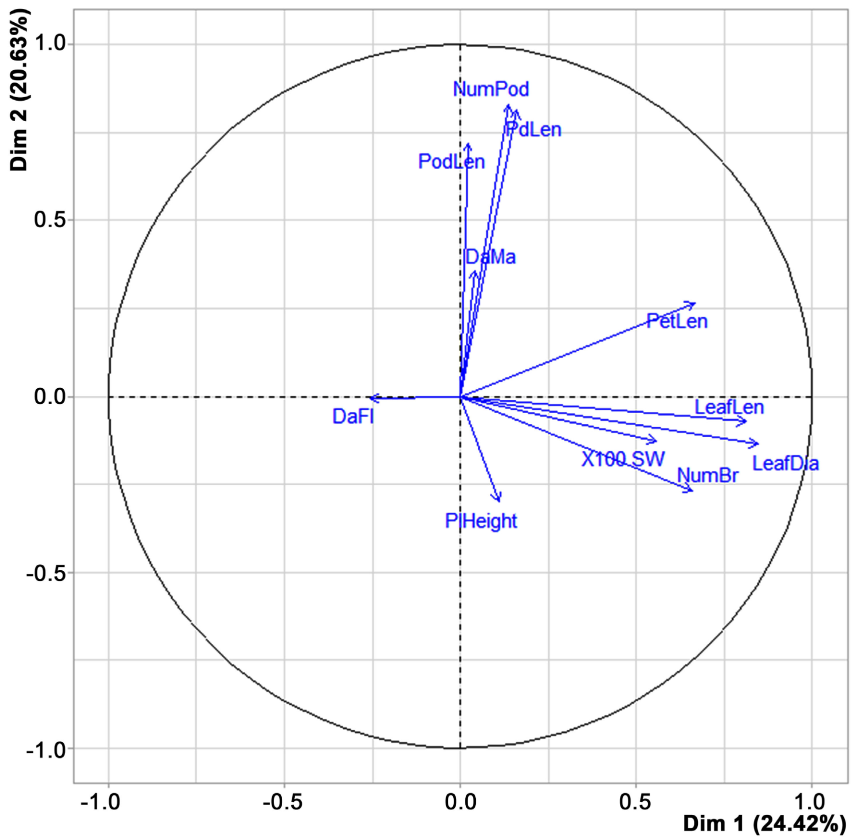


Figure 5. Principal components analysis showing associations between traits in Moussa local mutant lines at M4 generation.

3.6.2. Hierarchical Classification of Cowpea M4 Mutant Lines

Hierarchical ascending classification was used to cluster cowpea mutant lines and their parents. A dendrogram was constructed based on the average population linkage for each variety. This classification revealed three clusters in KVX 396-4-5-2D mutant lines (**Figure 6**). Cluster 1 contained the highest number of genotypes (198), including the parent and Clusters 2 and 3 composed of 93 and 92 mutant lines, respectively. Moussa local mutant lines were classified into six clusters (**Figure 7**). Cluster 1 contained the highest number of genotypes including the parent (30), followed by Cluster 2 with 12 lines. Clusters 3, 4, 5, and 6 involved fewer mutant numbers (6, 5, 8, and 4, respectively).

The characteristics of each cluster for the quantitative traits are presented in **Table 9**, along with the corresponding test values (V-tests). Test values for KVX 396-4-5-2D mutant lines showed positive values for days to flowering and days to grain maturity, and negative values for the other variables in Cluster 1. The mean values for days to flowering and days to grain maturity for this cluster were 50 and 71 DAS, respectively, which were higher than the overall means of 46 and 68 DAS. Cluster 2 showed only negative V-test values for pod and branch numbers, peduncle length, plant height, and leaflet diameter. In Cluster 3, the V-tests were positive for yield components and leaf dimensions and negative for days to flowering and days to grain maturity. This cluster had the highest 100-seed

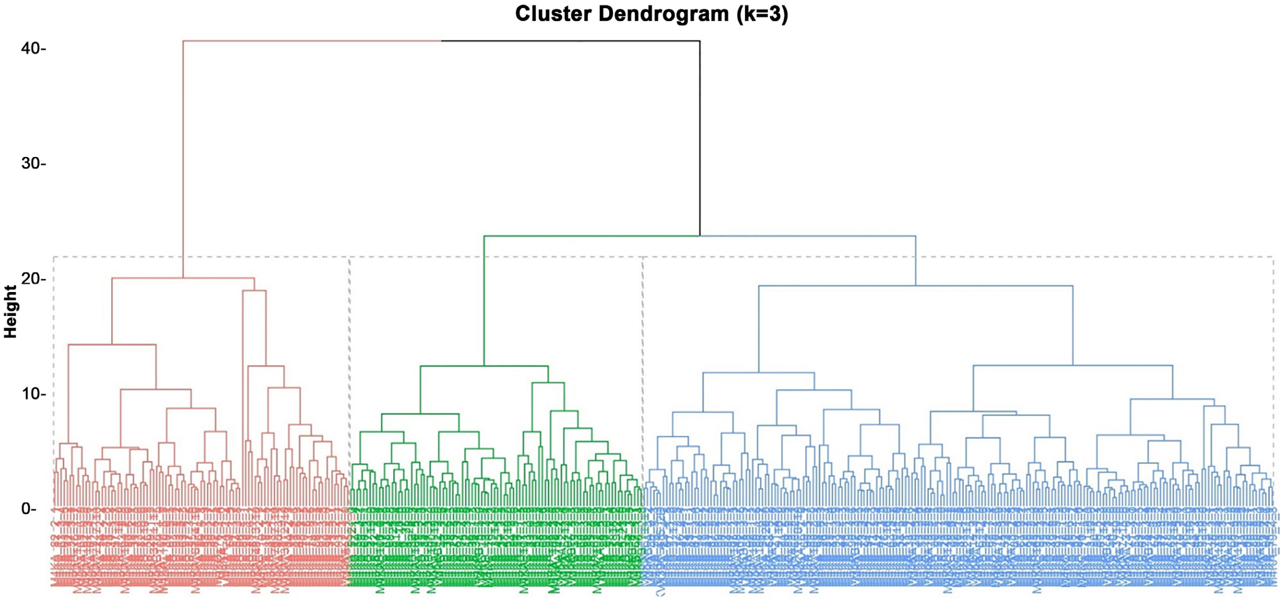


Figure 6. Dendrogram of cowpea KVX 396-4-5-2D mutant lines based on quantitative traits at M4 generation.

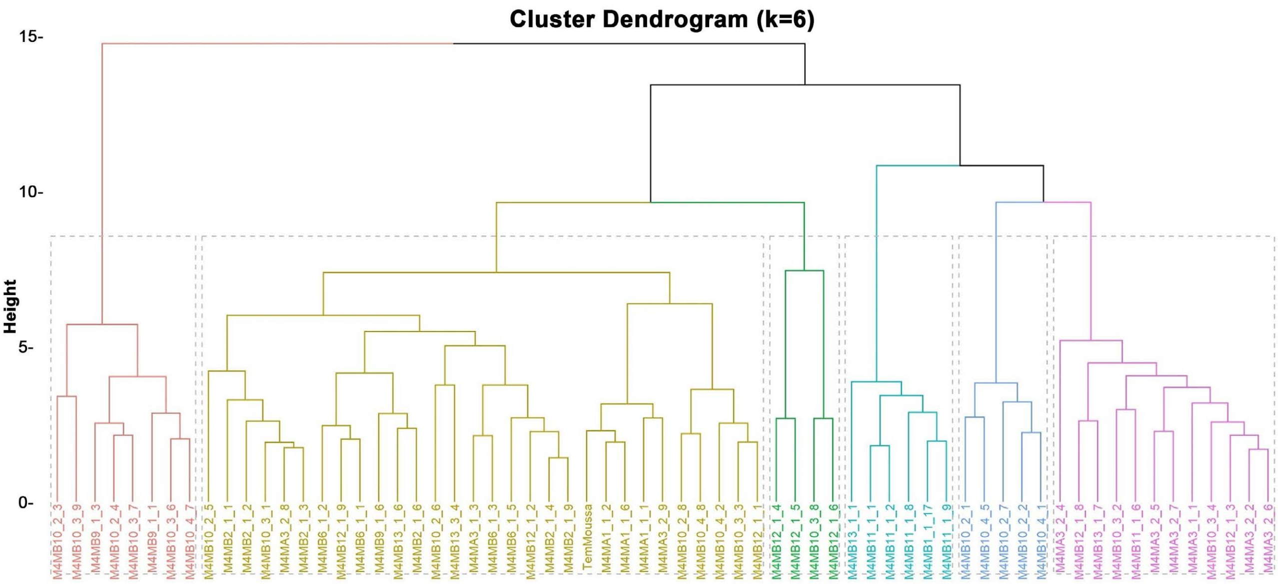


Figure 7. Dendrogram of cowpea Moussa local mutant lines based on quantitative traits at M4 generation.

weight (14 g) and pod number (40 pods/plant) compared to the two other clusters. For the Moussa local mutant lines, the test values in Moussa local 1 were all negative for 100-seed weight, plant height, branch number, and leaf size (Table 10). The average weight of hundred-seeds in this cluster was 13.75 g and the plant height was 11 cm. Cluster 2 showed positive V-tests for days to grain maturity, flowering date, and plant height. They were negative for the branch number and 100-seed weight. The average height of the individuals in this cluster was 14 cm, compared to the overall average of 12 cm. Cluster 3 showed negative V-tests for the yield components. In Cluster 4, pod length had a positive V-test, with an average of 15 pods greater than the overall average. In this cluster, V-tests of petiole and leaflet lengths were also positive. Cluster 5 showed all positive V-tests for yield components. Cluster 6 consisted of mutant lines with the highest 100-seed weight of 17 g and a positive V-test result. Leaf size and the branch number also showed positive V-tests. The pod length in this cluster, with an average of 13 cm, had a negative V-test result. The clustering diagram using K-means clustering showed a distribution of KVX 396-4-5-2D mutant lines in the three clusters (**Figure 8**) and a distribution of Moussa local mutant lines in six clusters (**Figure 9**). The distribution of these different mutant lines supports the hierarchical classification, which divided the mutant population into three and six groups for KVX 396-4-5-2D and Moussa local, respectively.

Table 9. Characteristic means of three cluster groups of cowpea KVX 396-4-5-2D mutant variables at M4 generation.

Variables	Cluster 1		Cluster 2		Cluster 3		Overall mean
	V-test	Cluster mean	V-test	Cluster mean	V-test	Cluster mean	
Days to 50% flowering	12.23	50.84			−10.06	44.38	46.51
Days to maturity	8.42	71.65			−6.97	66.72	68.35
100-seed weight	−5.79	13.02			3.28	14.71	14.28
Pod Length	−8.31	12.72			7.11	15.23	14.38
Branch number	−9.29	4.65	−2.15	5.52	9.47	6.22	5.62
Pod number	−9.47	16.28	−3.90	27.31	11.45	39.90	29.97
Petiole length	−9.53	6.92			7.71	8.62	8.07
Pod length	−9.59	12.52	−2.94	15.78	10.53	18.85	16.34
Leaf diameter	−9.65	4.856	−2.08	5.44	9.69	5.90	5.50
Leaf length	−10.07	8.85			9.23	10.74	10.07
Plant height			−3.79	14.35	3.62	15.36	14.81

Table 10. Characteristic means of six cluster groups of cowpea Moussa local mutant variables at M4 generation.

Variables	Cluster 1		Cluster 2		Cluster 3		Cluster 4		Cluster 5		Cluster 6		Overall mean
	V-test	Cluster mean	V-test	Cluster mean	V-test	Cluster mean	V-test	Cluster mean	V-test	Cluster mean	V-test	Cluster mean	
Days to 50% flowering			3.76	52.06			−3.79	48.32					49.6
Days to maturity			4.31	74.37	−3.68	68.53	−2.32	70.33					71.22
100-seed weight	−2.07	13.75	−2.60	13.29							5.06	17.69	15.08
Plant height	−2.44	10.95	4.25	14.08			−2.38	11.42					12.05
Petiole length	−4.50	5.90					2.97	7.47					7.06
Leaf diameter	−5.39	3.38									3.93	4.44	4.05
Leaf length	−6.29	6.88					2.06	8.64			2.12	8.77	8.37
Peduncle length					−4.24	6.98	2.33	10.83	4.11	13.72			9.97
Pod number					−3.42	18.85			5.21	58.24			32.09
Pod length					−2.15	13.64	2.87	15.08	3.67	16.37	−2.59	13.73	14.49
Branch number	−3.35	6.06	−2.27	6.37					1.96	8.14	3.4	8.14	7.20

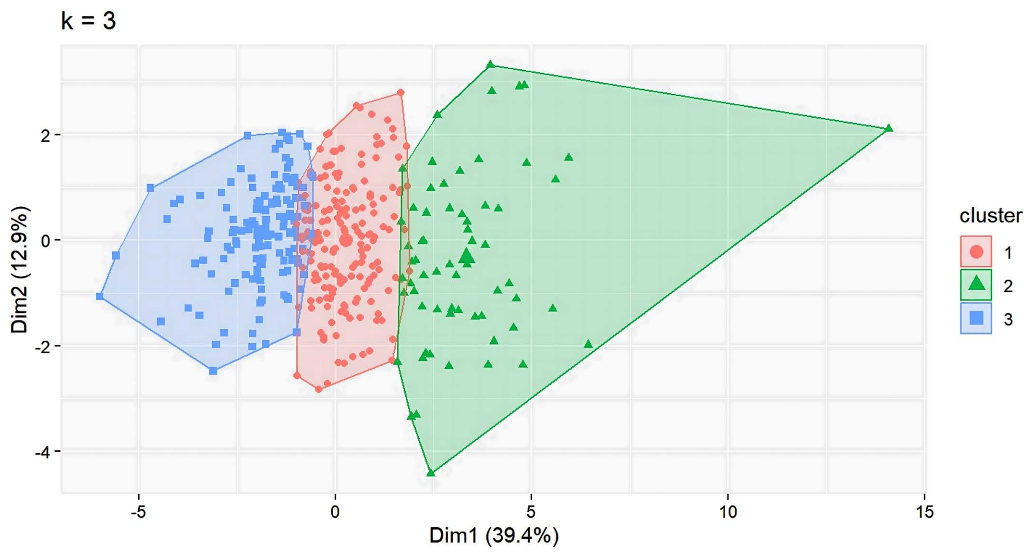


Figure 8. Clustering diagram of cowpea KVX 396-4-5-2D mutant lines at M4 generation.

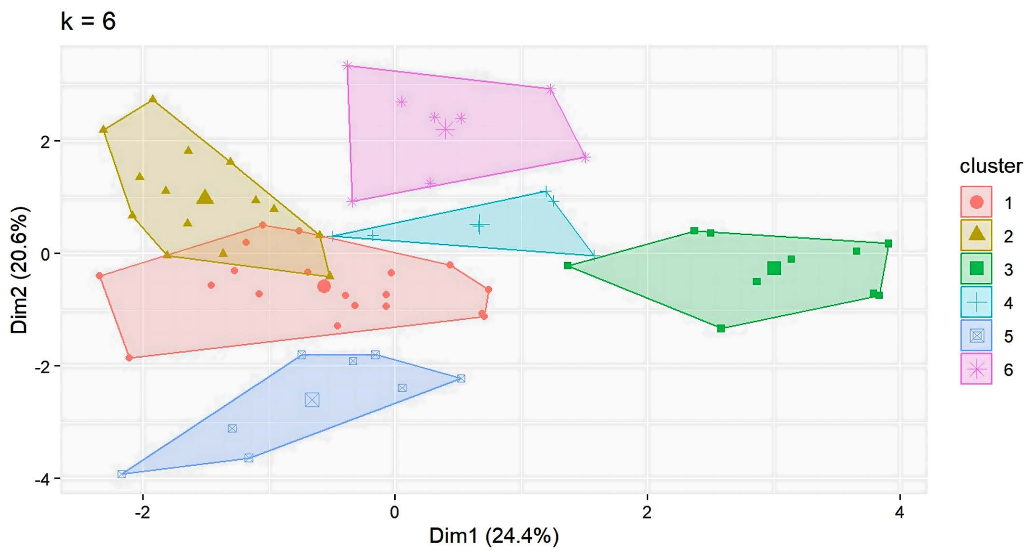


Figure 9. Clustering diagram of cowpea Moussa local mutant lines at M4 generation.

4. Discussion

Induced mutagenesis is an established means for diversifying gene pools in crop varieties. However, in addition to having positive effects, electromagnetic irradiation can simultaneously affect seed germination rates, seedling survival and growth rates, and fertility of plants [19]. The radio-sensitivity test showed that plant height and germination percentage decreased significantly with increasing gamma irradiation doses. This decreasing of germination percentage was noted by [20] in gamma-irradiated cowpea studies. Indeed, high irradiation doses have a high impact on the genome of cowpea varieties, causing chromosomal aberrations, lethality, and sterility [21] [22]. Therefore, these anomalies could be responsible for the observed reduction in plant height [20]. LD_{50} determined in this study were 223 Gy and 230 Gy for Moussa local and KVX 396-4-5-2D, respectively. These optimal doses for both Cowpea varieties are almost similar but weaker than those determined by [19] (300 Gy), [23] (800 Gy) and [24] (500 Gy) for other Cowpea varieties. The LD_{50} may depend on the plant tissue, seed moisture content, and seed characteristics of each genotype [25].

Gamma rays induced morphological variations in the mutants of both Cowpea varieties. Some mutant lines exhibited morphological traits such as the shape of the median leaflet, branch pigmentation, flower characteristics, seed traits, hilum color, pod color, and growth habits different to those of the parents. These changes were likely due to the induced mutation. Similarly, oval, sub-globular and lanceolate leaflets were noted, differing from the sub-lanceolate form of the parents. This change in leaf morphology has been observed by some authors [26]-[28]. [29] indicated that the mutation affects the genes controlling the shape of cowpea leaves. Some KVX 396-4-5-2D mutants showed reddish and brownish branch pigmentation, differing from the greenish pigmentation of the parent. Similar results were reported by [27]. The semi-erect habit was dominant in KVX 396-4-5-2D mutant lines while the creeping habit was dominant in Moussa local mutant lines. The induction of erect growth habit is, generally growth-determining. This trait enables cowpea varieties to be grown at higher plant densities [30]. In addition, this type of growth habit makes harvesting less arduous and facilitates farming mechanization. The appearance of growth habit is due to a change in the monogenic gene-controlling growth habit of cowpea [31]. The mutation induced changes in flower, seed, pod, and hilum coloration in KVX 396-4-5-2D mutant lines. For Moussa local mutant lines, only a change in pod color was observed. These variations in flower color were recorded by [32] on mung bean and [27] on cowpea. These results indicate that gamma rays induce damage to chromosomal material which exhibited new phenotypic characters [33]. These mutations affect floral color at high irradiation doses. Variety seed color is a desired selection trait driven by consumers' preferences. Seed color mutation occurred only in few KVX 396-4-5-2D mutant lines, resulting in brown, maroon, and whitish colors that were different from the white color of the parent. These different colors of seeds were observed in brown pods of some mutants while the yellow pods exclusively showed white seeds. Several authors have used mutagenesis to induce seed and pod color changes in cowpeas [12] [27] [28] [32]. Seed color has been studied in many crops and has often been linked to loci that control anthocyanin biosynthesis [34]. These color changes are due to macro-mutations governed by a few major genes or oligo-genes [35]. The results also showed that mutants with pod dehiscence were only found in KVX 396-4-5-2D mutant lines. Pod dehiscence is very common in wild species, enabling them to spread naturally. Mutants with this trait are not agronomically beneficial as it leads to grain yield loss. However, wild species are endowed with resistance genes against some biotic and abiotic factors [36]. Mutations linked to pod dehiscence are caused by a change in the twisting force of the pod walls or the structural strength of the pod dehiscence slit zone [37]. Gamma irradiation caused changes in the dominant NSH gene controlling pod indehiscence in cowpea [38]. These dehiscent pod mutants are a source of information that could help breeders understand the mechanism of dehiscence. Dehiscence of legumes is often positively correlated with aridity [39]. Breeders should consider this trait in the context of climate change.

According to quantitative parameters, the mutation induced earlier flowering and grain maturity in some KVX 396-4-5-2D mutants. This earliness helps to avoid late drought and to minimize insect and disease infestations in a context of climate change. Previous studies have used EMS [40] and gamma rays [41] to select early cowpea varieties. [42] reported that genetic control of earliness in cowpea is governed by complementary and duplicated epistasis, showing that mutagenesis has improved the yield component (number of pods, pod length, and seed weight) of some mutant lines whereas other mutants showed reduced yield components compared to the parents. Yield increasing resulting from induced mutagenesis has been noted in [12] [27] [35] [43]. However, mutagenesis can also induce a decrease in yield components of mutant lines compared to the parent [28] [44]. This could be due to genetic alterations at the chromosomal and molecular levels caused by irradiation, which could result in either an improvement or a reduction in yield. The 280 Gy dose induced a reduction in plant height in some KVX 396-4-5-2D mutants which would help to control lodging. Similarly, [45] showed that reduced-size mutants induced by gamma irradiation had a high nitrogen-fixing capacity. [26] [45] and [27] also generated cowpea mutant lines with reduced plant height compared to the parental varieties. The 230 Gy dose in KVX 396-4-5-2D induced an increase of peduncle length. Thus, increased peduncle length favors mechanized pod harvesting. The mutation also induced variability in the leaf size. The diameter of the median leaflet increased in Moussa local mutants with doses of 170 and 220 Gy. In the KVX 396-4-5-2D mutant lines, average diameters up to 9 cm and lengths up to 15 cm were recorded. These wide, long leaflets have also been noted by [26] as having enhanced photosynthetic activity. Light penetration can be deeper in the leaf canopy, implying a high leaf area index.

Measuring chlorophyll fluorescence helps to highlight the process of energy use by the plant and the dissipation of excess energy through photosystem II [46]. The Phi2 quantum yield of Photosystem II measures its efficiency, that is, the fraction of light energy captured by PSII that is directly used for photosynthesis. Therefore, Phi2 is an indicator of the photosynthetic performance [47]. The chlorophyll fluorescence parameters (Phi2, PhiNPQ, and PhiNO) varied significantly in the mutant lines. Similar results were recorded for *Eleusine coracana* (L.) Gaertn. irradiated with gamma rays [48]. The low Phi2 values shown by some mutants indicate a reduction in photosynthetic performance, whereas the high values show an improvement. Under fluctuating light intensity conditions, PhiNPQ enables the identification of mutants with a high photoprotective capacity against excess energy [49]. High PhiNPQ values indicate a high photoprotective capacity [50]. The loss of unregulated PhiNO energy is a loss for the plants. Mutants with low PhiNO values are of interest in breeding programs. Variations in fluorescence parameters could be due to the different leaf characteristics (chemical and physical composition of the leaves) of the mutants, as well as to environmental conditions. Biotic and abiotic factors therefore can lead to a drop in Phi2. Therefore, chlorophyll fluorescence is used for early detection of deficiency or pathogen attack [51] [52].

Correlations between the traits of mutant lines are significant in breeding programs. The agronomic value lies in determine which traits are highly correlated before selection [53]. This study showed a strong positive correlation between branch number and hundred-seed weight in mutants derived from both varieties. This correlation indicates that the increasing number of branches leads to the improvement of yield components. Similar trait associations have been found in a mutant cowpea population treated with EMS [54]. In addition, strong links between yield components (number of pods, pod length, and hundred-seed weight) and leaf size were noted with KVX 396-4-5-2D mutants. This suggests that lines with large leaves would contribute to increase seed weight. However, these correlations were not observed in the Moussa local mutants. According to [55], mutagens also alter correlations between certain traits.

Principal component analysis and hierarchical ascending classification are two techniques frequently used to cluster genotypes according to their similarity which can be used to select desired traits in mutant populations [56]. Principal component analysis allowed to identify some traits leading to phenotypic variability among the mutant lines. Pod and peduncle length, number of pods and branches, and leaf dimensions were therefore correlated with axis 1, which explained 39% of the variation in the KVX 396-4-5-2D mutants. Thus, the selection for these traits could occur within mutant genotypes with a desirable high average performance. With Moussa local mutants, 100-seed weight and leaf size contributed predominantly to axis 1 and pod number and pod length to axis 2. These traits are well-suited for the selection of forage and grain yields. Based on these results, the first component was the major contributor to diversity in the mutant lines, as emphasized by [48], who found that the first axis accounted for traits associated with phenotypic variation. These highly variable traits can be selected in breeding programs to achieve a high level of trait segregation [43]. The hierarchical classification resulted in three clusters in KVX 396-4-5-2D mutant lines and six clusters in Moussa local mutant lines showing a high variability among the mutants. The positive test values (V-tests) recorded within the different clusters showed that the average of the traits in that cluster was higher than the overall average of the trait in the total lines. Similarly, negative V-tests indicate that the average value of the trait in the cluster is lower than the overall mean [57]. In Cluster 3 of KVX 396-4-5-2D mutant lines, V-tests for the date to flowering and the date to grain maturity were negative and those for hundred-seed weight and pod number were positive. Thus, this cluster includes both early- and high-yielding mutant lines. Moussa local mutant lines in Cluster 4 showed some early grain maturity while in Cluster 6, high foliage and 100-seed weight were observed. Within Cluster 6, the selection should be made for the dual use of cowpea for forage and yield.

5. Conclusion

Radio-sensitivity tests determined the LD_{50} for each cowpea variety. The gamma-ray irradiation induced genetic variability in both cowpea varieties, resulting in induced variation in qualitative and quantitative cowpea traits, such as growth habit, leaf size and seed color. These variations enable the selection of mutant lines that meet consumer preferences. Gamma rays improved the seed weight, pod length, pod number, and photosynthetic efficiency, thereby contributing to improved cowpea productivity. Pearson's correlation analysis revealed a strong and positive correlation between pod number and branch number. Principal component analysis indicated that pod number and leaf size accounted for most of the phenotypic variation among the mutant lines. Hierarchical clustering showed heterogeneity in the distribution of the mutant lines from both varieties. By clustering the mutant lines based on their similarities and differences, genotypes of interest with earliness, high hundred-seed-weight and high potential forage production were identified. These mutant lines will be further advanced to stabilize genotypic traits, which can be utilized for cowpea improvement.

Acknowledgements

The authors are thankful to the International Atomic Energy Agency (IAEA, RAF5076 & RAF5083) for its assistance in irradiating of cowpea seeds with gamma rays and its contribution to laboratory and human capacity building. They are grateful to Sustainable Intensification Innovation Lab (SIIL) for its financial support of screening experiments. They also acknowledge "Institut de l'Environnement et de Recherches Agricoles (INERA)" of Burkina Faso for facilitating the implementation of experiments at Kamboinsé Research Station. Dr. Boubacar Amadou KOUNTCHE (CIRAD-Burkina Faso) is warmly thanked by the authors for his corrections to the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Sombié, P., Compaoré, M., Coulibaly, A., Ouédraogo, J., Tignégré, J. and Kiendrébéogo, M. (2018) Antioxidant and Phytochemical Studies of 31 Cowpeas (*Vigna unguiculata* (L. Walp.)) Genotypes from Burkina Faso. *Foods*, **7**, Article 143. https://doi.org/10.3390/foods7090143

[2] Horn, L.N. and Shimelis, H. (2020) Production Constraints and Breeding Approaches for Cowpea Improvement for Drought Prone Agro-Ecologies in Sub-Saharan Africa. *Annals of Agricultural Sciences*, **65**, 83-91. https://doi.org/10.1016/j.aoas.2020.03.002

[3] Nkomo, G.V., Sedibe, M.M. and Mofokeng, M.A. (2021) Production Constraints and Improvement Strategies of Cowpea (*Vigna unguiculata* L. Walp.) Genotypes for Drought Tolerance. *International Journal of Agronomy*, **2021**, Article ID: 5536417. https://doi.org/10.1155/2021/5536417

[4] FAOSTAT (2021) Cowpeas, Dry. https://www.fao.org/faostat/en/#data/QCL

[5] Abebe, B.K. and Alemayehu, M.T. (2022) A Review of the Nutritional Use of Cowpea (*Vigna unguiculata* L. Walp) for Human and Animal Diets. *Journal of Agriculture and Food Research*, **10**, Article ID: 100383. https://doi.org/10.1016/j.jafr.2022.100383

[6] ZinmanKé, C., Antoine, B., Jean-Baptiste, T., Zakaria, K., Benoit Joseph, B., Zakaria, D., *et al.* (2020) Évaluation des performances agronomiques de douze (12) variétés de niébé vert [*Vigna unguiculata* (L.) walp.] au Burkina Faso. *Journal of Applied Biosciences*, **153**, 15745-15755. https://doi.org/10.35759/jabs.153.2

[7] Raina, A., Laskar, R.A., Tantray, Y.R., Khursheed, S., Wani, M.R. and Khan, S. (2020) Characterization of Induced High Yielding Cowpea Mutant Lines Using Physiological, Biochemical and Molecular Markers. *Scientific Reports*, **10**, Article No. 3687. https://doi.org/10.1038/s41598-020-60601-6

[8] Mekonnen, T.W., Gerrano, A.S., Mbuma, N.W. and Labuschagne, M.T. (2022) Breeding of Vegetable Cowpea for Nutrition and Climate Resilience in Sub-Saharan Africa: Progress, Opportunities, and Challenges. *Plants*, **11**, Article 1583. https://doi.org/10.3390/plants11121583

[9] Badiane, F.A., Diouf, M. and Diouf, D. (2014) Cowpea. In: Singh, M., Bisht, I. and Dutta, M., Eds., *Broadening the Genetic Base of Grain Legumes*, Springer, 95-114. https://doi.org/10.1007/978-81-322-2023-7_5

[10] Boukar, O., Belko, N., Chamarthi, S., Togola, A., Batieno, J., Owusu, E., *et al.* (2018) Cowpea (*Vigna unguiculata*): Genetics, Genomics and Breeding. *Plant Breeding*, **138**, 415-424. https://doi.org/10.1111/pbr.12589

[11] Gnankambary, K., Batieno, T.B.J., Sawadogo, N., Sawadogo, M., Tignegre, J.B., Yonli, D., *et al.* (2019) Genetic Variability Induced by γ Radiation in Cowpea [(*Vigna unguiculata* L. (walp)] in Burkina Faso. *European Scientific Journal*, **15**, 153-164. https://doi.org/10.19044/esj.2019.v15n15p153

[12] Horn, L.N., Ghebrehiwot, H.M. and Shimelis, H.A. (2016) Selection of Novel Cowpea Genotypes Derived through γ Irradiation. *Frontiers in Plant Science*, **7**, Article 262. https://doi.org/10.3389/fpls.2016.00262

[13] Nikièma, M.P., Yonli, D., Rabefiraisana, H.J., Ali, A., Ouédraogo, N., Traoré, H., Yanogo, H.Y.A., Dao, K., Sawadogo, M., Jankuloski, L., Ivan, I. and Abdelbagi, M.A.G. (2020) Induced Resistance to Striga Hermonthica in Sorghum by γ Irradiation. *American Journal of Plant Sciences*, **11**, 1545-1561. https://doi.org/10.20944/preprints202007.0727.v1

[14] Sanou, A., Yonli, D., Nofou, O., Honore, K., Souleymane, D., Karim, T., Irenée, S. and Hamidou, T. (2022) Criblage Des Lignées de Riz Mutant Vis-à-Vis Du Striga Hermonthica. *Science et Technique*, *Sciences Naturelles et Appliquées*, *Hors-Serie*, **6**, 194-204.

[15] Gnankambary, K., Sawadogo, N., Diéni, Z., Batieno, T.B.J., Tignegré, J.B.D.S., Sawadogo, M., *et al.* (2020) Assessment of Cowpea (*Vigna unguiculata* (L.) Walp.) Mutant Lines for Drought Tolerance. *International Journal of Agronomy*, **2020**, Article ID: 8823498. https://doi.org/10.1155/2020/8823498

[16] Thiombiano, A. and Kampmann, D. (2010) Atlas de La Biodiversité de l'Afrique de l'Ouest, Tome II: Burkina Faso. Projet BIOTA Afrique.

[17] Kuhlgert, S., Austic, G., Zegarac, R., Osei-Bonsu, I., Hoh, D., Chilvers, M.I., *et al.* (2016) MultispeQ *β*: A Tool for Large-Scale Plant Phenotyping Connected to the Open PhotosynQ Network. *Royal Society Open Science*, **3**, Article ID: 160592. https://doi.org/10.1098/rsos.160592

[18] R Core Team (2019) R: A Language and Environment for Statistical Computing [Computer Software]. R Foundation for Statistical Computing. https://www.R-project.org

[19] Kang, R., Seo, E., Kim, G., Park, A., Kim, W.J., Kang, S., *et al.* (2020) Radio Sensitivity of Cowpea Plants after *γ*-Ray and Proton-Beam Irradiation. *Plant Breeding and Biotechnology*, **8**, 281-292. https://doi.org/10.9787/pbb.2020.8.3.281

[20] Horn, L. and Shimelis, H. (2013) Radio-Sensitivity of Selected Cowpea (*Vigna unguiculata*) Genotypes to Varying *γ* Irradiation Doses. *Scientific Research and Essays*, **8**, 1991-1997.

[21] Mudibu, J., K. C. Nkongolo, K., Kalonji-Mbuyi, A. and Kizungu, R.V. (2012) Effect of *γ* Irradiation on Morpho-Agronomic Characteristics of Soybeans (*Glycine max* L.). *American Journal of Plant Sciences*, **3**, 331-337. https://doi.org/10.4236/ajps.2012.33039

[22] Roy, U., Basak, D. and Nath, S. (2019) Mutagenic Sensitivity Analysis of *γ* Irradiations in Cowpea (*Vigna unguiculata* L. Walp). *Emergent Life Sciences Research*, **5**, 12-16. https://doi.org/10.31783/elsr.2019.521216

[23] Raina, A., Khursheed, S. and Khan, S. (2018) Optimisation of Mutagen Doses for *γ* Rays and Sodium Azide in Cowpea Genotypes. *Trends in Biochemical Sciences*, **11**, 2386-2389.

[24] Vasudevan, S., Dhanarajan, A., Kasim, Y., Sankar, V., Govindasamy, B. and Gurunathan, S. (2023) Evaluation of Morphophysiological, Biochemical and Antioxidant Activity of Green Gram (*Vigna radiata* (L.) R. Wilczek) in Responses to *γ* Irradiation. *Plant Science Today*, **10**, 289-301. https://doi.org/10.14719/pst.2141

[25] Riviello-Flores, M.D.L.L., Cadena-Iñiguez, J., Ruiz-Posadas, L.D.M., Arévalo-Galarza, M.D.L., Castillo-Juárez, I., Soto Hernández, M., *et al.* (2022) Use of *γ* Radiation for the Genetic Improvement of Underutilized Plant Varieties. *Plants*, **11**, Article 1161. https://doi.org/10.3390/plants11091161

[26] Adekola, O.F. and Oluleye, F. (2007) Induction of Genetic Variation in Cowpea (*Vigna unguiculata* L. Walp.) by *γ* Irradiation. *Asian Journal of Plant Sciences*, **6**, 869-873. https://doi.org/10.3923/ajps.2007.869.873

[27] Diouf, M., Diallo, S., Abaye Badiane, F., Diack, O. and Diouf, D. (2021) Development of New Cowpea (*Vigna unguiculata*) Mutant Genotypes, Analysis of Their Agromorphological Variation, Genetic Diversity and Population Structure. *BIOCELL*, **45**, 345-362. https://doi.org/10.32604/biocell.2021.013706

[28] Opoku Gyamfi, M., Eleblu, J.S.Y., Sarfoa, L.G., Asante, I.K., Opoku-Agyemang, F. and Danquah, E.Y. (2022) Induced Variations of Ethyl Methane Sulfonate Mutagenized Cowpea (*Vigna unguiculata* L. Walp) Plants. *Frontiers in Plant Science*, **13**, Article 952247. https://doi.org/10.3389/fpls.2022.952247

[29] Fawole, I. (2001) Genetic Analysis of Mutations at Loci Controlling Leaf Form in Cowpea (*Vigna unguiculata* [L.] Walp.). *Journal of Heredity*, **92**, 43-50. https://doi.org/10.1093/jhered/92.1.43

[30] Massey, P. and Nautiyal, M. (2020) Studies on Induction of Genetic Variation through Seed Mutation in Cowpea (*Vigna unguiculata* L. Walp.) by *γ* Irradiation. *International Journal of Chemical Studies*, **8**, 796-800. https://doi.org/10.22271/chemi.2020.v8.i1l.8362

[31] Lachyan, T.S., Desai, S.S. and Dalvi, V.V. (2016) Inheritance Study of Qualitative and Quantitative Characters in Cowpea Varieties (*Vigna unguiculata* (L.) Walp). *Electronic Journal of Plant Breeding*, **7**, 708-713. https://doi.org/10.5958/0975-928x.2016.00091.0

[32] Wani, M., Dar, A., Tak, A., Amin, I., Shah, N., *et al.* (2018) Chemo-Induced Pod and Seed Mutants in Mungbean (*Vigna radiata* L. Wilczek). *SAARC Journal of Agriculture*, **15**, 57-67. https://doi.org/10.3329/sja.v15i2.35161

[33] Sakamoto, K., Nishi, M., Ishiji, K., Takatori, Y. and Chiwata, R. (2019) Induction of Flower-Colour Mutation by Synchrotron-Light Irradiation in Spray Chrysanthemum. *Acta Horticulturae*, **1237**, 73-78. https://doi.org/10.17660/actahortic.2019.1237.9

[34] Herniter, I.A., Muñoz-Amatriaín, M., Lo, S., Guo, Y. and Close, T.J. (2018) Identification of Candidate Genes Controlling Black Seed Coat and Pod Tip Color in Cowpea (*Vigna unguiculata* [L.] Walp). *G3 Genes|Genomes|Genetics*, **8**, 3347-3355. https://doi.org/10.1534/g3.118.200521

[35] Gnanamurthy, S. and Dhanavel, D. (2014) Effect of EMS on Induced Morphological Mutants and Chromosomal Variation in Cowpea (*Vigna unguiculata* (L.) Walp). *International Letters of Natural Sciences*, **22**, 33-43. https://doi.org/10.56431/p-i0xny2

[36] Vining, K.J., Hummer, K.E., Bassil, N.V., Lange, B.M., Khoury, C.K. and Carver, D. (2020) Crop Wild Relatives as Germplasm Resource for Cultivar Improvement in Mint (*Mentha* L.). *Frontiers in Plant Science*, **11**, Article 1217. https://doi.org/10.3389/fpls.2020.01217

[37] Parker, T.A., Lo, S. and Gepts, P. (2021) Pod Shattering in Grain Legumes: Emerging Genetic and Environment-Related Patterns. *The Plant Cell*, **33**, 179-199. https://doi.org/10.1093/plcell/koaa025

[38] Girish, G., Viswanatha, K.P. and Manjunath, A. (2010) Arrangement of Genes on Chromosome in Cowpea *Vigna unguiculata* (L.) Walp. *Environment and Ecology*, **28**, 1031-1045.

[39] Parker, T.A., Berny Mier y Teran, J.C., Palkovic, A., Jernstedt, J. and Gepts, P. (2019) Pod Indehiscence Is a Domestication and Aridity Resilience Trait in Common Bean. *New Phytologist*, **225**, 558-570. https://doi.org/10.1111/nph.16164

[40] Gnanamurthy, S. and Dhanavel, D. (2014) Effect of EMS on Induced Morphological Mutants and Chromosomal Variation in Cowpea (*Vigna unguiculata* (L.) Walp). *International Letters of Natural Sciences*, **22**, 33-43. https://doi.org/10.56431/p-i0xny2

[41] Dorvlo, I.K., Amenorpe, G., Amoatey, H.M., Amiteye, S., Kutufam, J.T., Afutu, E., *et al.* (2022) Improvement in Cowpea Variety Videza for Traits of Extra Earliness and Higher Seed Yield. *Heliyon*, **8**, e12059. https://doi.org/10.1016/j.heliyon.2022.e12059

[42] Owusu, E.Y., Kusi, F., Kena, A.W., Akromah, R., Attamah, P., Awuku, F.J., *et al.* (2022) Genetic Control of Earliness in Cowpea (*Vigna unguiculata* (L) Walp). *Heliyon*, **8**, e09852. https://doi.org/10.1016/j.heliyon.2022.e09852

[43] Raina, A. and Khan, S. (2023) Field Assessment of Yield and Its Contributing Traits in Cowpea Treated with Lower, Intermediate, and Higher Doses of γ Rays and Sodium Azide. *Frontiers in Plant Science*, **14**, Article 1188077. https://doi.org/10.3389/fpls.2023.1188077

[44] Kumar, V.A., Vairam, N. and Amutha, R. (2010) Effect of Physical Mutagen on Expression of Characters in Arid Legume Pulse Cowpea (*Vigna unguiculata* (L.) Walp.). *Electronic Journal of Plant Breeding*, **1**, 908-914.

[45] Anjana, G. and Thimmaiah, S.K. (2002) Evaluation of Dwarf Mutant of Cowpea

(*Vigna unguiculata* L. Walp.) Developed through γ Irradiation for Nitrogen Fixation Characters. *Journal of nuclear agriculture and biology*, **31**, 94-98.

[46] Sreeharsha, R.V., Mudalkar, S., Sengupta, D., Unnikrishnan, D.K. and Reddy, A.R. (2018) Mitigation of Drought-Induced Oxidative Damage by Enhanced Carbon Assimilation and an Efficient Antioxidative Metabolism under High CO_2 Environment in Pigeonpea (*Cajanus cajan* L.). *Photosynthesis Research*, **139**, 425-439. https://doi.org/10.1007/s11120-018-0586-9

[47] Maxwell, K. and Johnson, G.N. (2000) Chlorophyll Fluorescence—A Practical Guide. *Journal of Experimental Botany*, **51**, 659-668. https://doi.org/10.1093/jexbot/51.345.659

[48] Sellapillaibanumathi, L., Dhanarajan, A., Raina, A. and Ganesan, A. (2021) Effects of γ Radiations on Morphological and Physiological Traits of Finger Millet (*Eleusine coracana* (L.) Gaertn.). *Plant Science Today*, **9**, 89-95. https://doi.org/10.14719/pst.1142

[49] Ruban, A.V. (2016) Nonphotochemical Chlorophyll Fluorescence Quenching: Mechanism and Effectiveness in Protecting Plants from Photodamage. *Plant Physiology*, **170**, 1903-1916. https://doi.org/10.1104/pp.15.01935

[50] Fernández-Calleja, M., Monteagudo, A., Casas, A.M., Boutin, C., Pin, P.A., Morales, F., *et al.* (2020) Rapid On-Site Phenotyping via Field Fluorimeter Detects Differences in Photosynthetic Performance in a Hybrid—Parent Barley Germplasm Set. *Sensors*, **20**, Article 1486. https://doi.org/10.3390/s20051486

[51] Jin, C., Zha, T., Bourque, C.P.-., Liu, P., Jia, X., Tian, Y., *et al.* (2022) Key Stress Indicators from Chlorophyll Fluorescence in Five Desert Plant Species. *Ecological Indicators*, **145**, Article ID: 109679. https://doi.org/10.1016/j.ecolind.2022.109679

[52] Kalaji, H.M., Jajoo, A., Oukarroum, A., Brestic, M., Zivcak, M., Samborska, I.A., *et al.* (2016) Chlorophyll a Fluorescence as a Tool to Monitor Physiological Status of Plants under Abiotic Stress Conditions. *Acta Physiologiae Plantarum*, **38**, Article No. 102. https://doi.org/10.1007/s11738-016-2113-y

[53] Sharma, R., Chaudhary, L., Kumar, M., Yadav, R., Devi, U., Amit, *et al.* (2022) Phenotypic Diversity Analysis of *Lens culinaris* Medik. Accessions for Selection of Superior Genotypes. *Sustainability*, **14**, Article 5982. https://doi.org/10.3390/su14105982

[54] Raina, A., Laskar, R.A., Wani, M.R., Jan, B.L., Ali, S. and Khan, S. (2022) γ Rays and Sodium Azide Induced Genetic Variability in High-Yielding and Biofortified Mutant Lines in Cowpea [*Vigna unguiculata* (L.) Walp.]. *Frontiers in Plant Science*, **13**, Article 911049. https://doi.org/10.3389/fpls.2022.911049

[55] Shin, J.M., Kim, B.K., Seo, S.G., Jeon, S.B., Kim, J.S., Jun, B., Kang, S.Y., Lee, J.S., Chung, M.N. and Kim, S.H. (2011) Mutation Breeding of Sweet Potato by γ-Ray Radiation. *African Journal of Agricultural Research*, *Academic Journals*, **6**, 1447-1454. https://doi.org/10.5897/AJAR10.936.

[56] Mohammadi, S.A. and Prasanna, B.M. (2003) Analysis of Genetic Diversity in Crop Plants—Salient Statistical Tools and Considerations. *Crop Science*, **43**, 1235-1248. https://doi.org/10.2135/cropsci2003.1235

[57] Nikièma, M.P., Yonli, D., Yanogo, H.Y.A., Ouédraogo, N., Traoré, H., Sawadogo, M. and Hingane, A.J. (2023) Induced Mutagenesis for Enhancing Genetic Variability and Agronomic Performance in Sorghum Varieties for Burkina Faso. *Annals of Plant Sciences*, 12, 5895-5911. http://dx.doi.org/10.21746/aps.2023.12.7.3