

Genetic Variability Induced by Gamma Radiation in Cowpea [(*Vigna unguiculata* L. (Walp)] in Burkina Faso

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Abstract

Cowpea is a good source of protein, minerals, and vitamins in most developing countries in Africa. However, this legume production is still limited by various biotic and abiotic constraints. Beyond these constraints, the pressure of conventional breeding methods on natural variability exhausted a narrow genetic base of cowpea. Therefore, it is important to

explore other ways of broadening the genetic base of the crop. Gamma irradiation is an alternative to increase genetic variability and improve the genetic base of crops. This paper focuses on evaluating the level and structuring of agro-morphological variability induced by gamma radiation among M2 plants of cowpea genotype. So, Tiligre, Moussa local, and KVx-396-4-5-2D genotypes were treated with 150 Gy of gamma rays. The M2 generation was then screened for genetic variability. Thus, 38 M1 plants (either 38 x 10 seeds) by genotypes are advanced to the M2 generation based on the plant by line method. Four (04) agro-morphological parameters were used to assess phenotypical variability. Analysis of variance showed a highly significant difference between the families of plants except those of KVx-396-4-5-2D for the flowering time. The maximum variability was observed for the number of branches and the length of the peduncles with a very high coefficient of variation. A high broad-sense heritability was recorded for all measured traits (>75%) of Moussa Local; for plant height (81.1%) of KVx396-4-5-2D, for first flower date (80.2%), and peduncles length (78.6%) of Tiligre. Based on phenotypic characters, the principal component analysis (PCA) and the cluster analysis showed variation among genotypes irradiated, and they were grouped into four clusters in KVx-396-4-5-2D, six clusters in Moussa local and Tiligre populations. So, 150 Gy dose of γ -rays was effective to induce variability in morphological and phenological characters within cowpea genotypes.

Keywords: *Vigna unguiculata*, γ -rays, mutants, genetic diversity

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is a tropical grain legume which plays an important role in developing countries of Africa. It is an important source of protein, one that is rich in leucine, isoleucine, lysine, tyrosine, and phenylalanine (Elhardallou *et al.*, 2015). Cowpea grains contain vitamins and mineral elements (Zia-ul-haq *et al.*, 2012) which are useful in solving children's malnutrition in sub-Saharan Africa. This legume has antioxidant and anti-free radical properties and contributes to preventing degenerative diseases caused by free radicals (Zia-ul-haq *et al.*, 2013). In addition to human nutrition, cowpea fodder is an excellent source of protein for animal feed. On the other hand, cowpea can fix 52 to 56% of atmospheric nitrogen, thereby raising soil mineral nitrogen from 13 to 40% (Bado *et al.*, 2002). Indeed, cowpea has important food and agronomic values. However, its yield is still low in Burkina Faso due to various abiotic and biotic constraints among which are the drought and the parasitic weed *Striga gesnerioides*. Most cowpea breeding programs for resistance to this weed used conventional breeding methods exploiting only the natural variability

available. Selection within germplasm for a long time exhausted the genetic variability of the legume crop (Amri-Tilioune *et al.*, 2018). Adequate variability is not available to improve cowpea production because of the lower genetic pool (Batiemo *et al.*, 2018). Therefore, mutation breeding is an alternative solution to producing variant mutant form. Induced mutation can be efficiently used as an alternative to induce genetic variability (Malek *et al.*, 2014; Olasupo *et al.*, 2018). For quite a long time now, the use of mutagens has been proven to be beneficial in cowpea (Micke, 1993), and in groundnut (Gunasekaran & Paravadai, 2015). However, mutation breeding of cowpea and mutant cultivars in Burkina Faso is scarce. Therefore, this study was aimed at selecting a novel mutation in cowpea in the M2 generation of three genotypes populations (KVx-396-4-5-2D, Moussa local, Tiligre) in order to broaden the genetic base of cowpea for better improvement for biotic and abiotic stresses.

Material and Methods

Experimental Site

The present study was done at Kamboinse research station of the “Institut de l’Environnement et de Recherches Agricoles” (INERA) in Burkina Faso. The station is located at 12 Km from Ouagadougou on Kongoussi road (RN 22) with geographical coordinates of 12 ° 26 ' 59.4 ' north latitude: 1 ° 33 ' 08.0 ' west. In addition, it has an altitude of 296 m above sea level in the Centre Region in Burkina Faso.

Plant Material and Population Development

Seeds of three cowpea genotypes, Tiligre, Moussa local and KVx-396-4-5-2D, developed by INERA were used in the present study. One-year-old seeds (harvested in 2015) were treated with gamma-ray at dose 150 Gray (Gy) using a labeled Cobalt source (⁶⁰Co) in the laboratory of the International Atomic Energy Agency (IAEA) in Seibersdorf, Austria. This dose was chosen because germination assays indicated that 150 Gy corresponded to the optimal dose which induced 50% of seed germination of the three genotypes used. The treated seeds were planted to generate M1 plants in the field. Untreated seeds of the three genotypes were used as controls. The distance between and within rows was 80 cm x 15 cm. The seeds of individually harvested M1 plants were sown to produce M2 generation using the plant M1-line method. Planting was done in pots (10 L) filled with the mixture of sandy and loamy soil. One seed was planted per pot in the greenhouse. Planting was done on 19th October 2016. NPK fertilization (14-23-14) and phytosanitary treatments at K-Optimal (active ingredient) and Deltacal were applied.

Data Collection

Data collected on the individual plants were required for 50% flowering and included plant height (HP), peduncles length (LP), and branches number (NR). The first flower day (DPF) was also recorded. The first flower time corresponded to the number of dates from sowing to the appearance of the first flower. Plant height was measured from the soil level of the plant to apex. The average peduncle length randomly measured on three peduncles. Also, the number of primary branches was also counted.

Statistical Analysis

The data collected were subjected to the "linear model" and the student test using SAS version 8 software to estimate the level of variability within each genotype. The broad-sense heritability (H^2) was calculated according to the formula used by Dilnesaw *et al.* (2013) and was assessed according to the scale used by Nair *et al.* (2014) in order to estimate genetic and non-genetic factors. Genetic diversity analysis was performed using principal component analysis and hierarchical method for clustering the mutants.

Results and Discussion

Agro-morphological Variation

Results of Analysis of variance and value of genetic parameters were recorded in Table 1. A very highly significant difference for all measured traits in the M2 population of the three evaluated genotypes KVx396-4-5-2D, Moussa local, and Tiligre were observed. Results revealed a high coefficient of variation for the characters number of branches (27 to 61%) and the length of the peduncles (39 to 54%). Thus, a low value was recorded for the plant height (12 to 14%) and flowering date (6 to 12%). The value of genetic parameters differed from quantitative characters. So, Broad-sense heritability in M2 generation ranged from 34 to 82%. High broad sense heritability was recorded for branches number (81.4%), peduncle length (80.5%), plant height (76.6%), and first flowering date (76.8%) in the M2 population from Moussa local. In Tiligre population, a high value was observed for peduncle length (78.6%) and first flower date (80.2%); whereas it was moderate for the number of branches (54.2%) and plant height (54.6%). KVx396-4-5-2D recorded high heritability for plant height (81.1%), moderate heritability for the number of branches (55.7%), peduncle length (53.5%), and low heritability for first flower date (34.5%). According to the Student-Newman-Keuls test, the families of plants statistically different from their checks were determined for the four agronomic characters. On this basis, early and late flowering plants, tall and short, branched and long-peduncled plants were distinguished (Table 2). The results revealed a large range of morphological

and phenological variation with the ample diversity in flowering, plant height, peduncle length, and the number of branches. The maximum variation is observed for the number of branches and for the peduncle length. In Moussa local population, the four characters were highly heritable. Whereas, only peduncle length and first flower date of Tiligre plant and plant height of KVx396-4-5-2D were highly heritable.

Gamma radiation has induced the genetic variability in cowpea. 150 Gy dose of γ -rays was effective for inducing variability of morphological and phenological characters in cowpea. Induced variability by mutagen has been observed in many crops. Indeed, the agro-morphological diversity induced by gamma irradiation and high heritability were reported in Cowpea by Adekola and Oluleye (2007) and by Nair and Mehta (2014). In black gram, it was also reported by Usharani et al. (2015) and Usharani *et al.* (2016). The heritability level of the characters varied with the genotypes and also the environmental conditions of the experience. The high broad-sense heritability value indicated the predominance of additive gene action in the expression of these characters (Manggoel *et al.*, 2012). Their variation was due to the mutation of more than one locus (Amri-Tiliouine *et al.*, 2018). The variability of agro-morphological characters in cowpea after irradiation with gamma-ray are genetically controlled. So, these characters are less influenced by environmental change and could be used in the breeding program. However, the moderate and low heritability characters indicated that environmental effects and phenotypic selection will be less effective for such character. Increased genetic variability provides a great possibility for future selection. Mutagen would be an excellent tool for creating variability and for developing superior genotypes.

Table 1. Descriptive statistics and estimated genetic parameters in population M2 of KVx396-4-5-2D, Moussa local and Tiligre

Varieties	Characters	Mean	CV	Prob	GV	PV	H ² (%)
KVx396-4-5-2D	HP	21.73	12.66	0.0001	2.90	3.58	81.1
	NR	3.74	55.49	0.0001	0.26	0.46	55.7
	DPF	49.52	11.40	0.0347	0.80	2.31	34.5
	LP	9.15	44.49	0.0003	0.91	1.70	53.5
Tiligre	HP	28.57	12.50	0.0001	0.90	1.65	54.6
	NR	2.13	60.79	0.0001	0.12	0.22	54.2
	DPF	53.16	6.64	0.0001	2.98	3.71	80.2
	LP	7.13	39.40	0.0001	1.70	2.17	78.6
Moussa local	HP	28.04	13.23	0.0001	2.25	2.94	76.6
	NR	4.35	27.77	0.0001	0.32	0.39	81.4
	DPF	45.95	7.44	0.0001	1.93	2.52	76.8
	LP	8.43	53.01	0.0001	4.13	5.13	80.5

Legend: HP: Height of plants at flowering; NR: Number of branches at flowering; DPF: Date of the first flower; LP: peduncle length; CV: coefficient of variation; Prob: probability, GV; Genotypic Variance; PV: phenotypic Variance; H²: broad sense heritability

Table 2. Variation of Parameters in M2 population of KVx396-4-5-2D, Moussa local and Tiligre

Characters		Moussa local		Tiligre		KVx396-4-5-2D	
		number	percentage	number	percentage	number	percentage
HP	high	12	42.86	1	2.63	2	5.88
	normal	12	42.86	36	94.74	1	2.94
	Short	4	14.29	1	2.63	30	88.24
NR	high	0	0	5	13.16	28	82.35
	normal	1	3.57	30	78.95	5	14.71
	Low	27	96.43	2	5.26	0	0
LP	long	11	39.29	15	39.47	1	2.94
	normal	13	46.43	6	15.79	33	97.06
	Short	2	7.14	17	44.74	0	0
DPF	late	3	10.71	36	94.74	0	0
	normal	4	14.29	2	5.26	34	100
	early	21	75	0	0	0	0

Legend: HP, height of plants at flowering; NR, number of branches at flowering; DPF, date of the first flower; LP, peduncle length; CV, coefficient of variation; Prob., probability; GV, genotypic variance; PV, phenotypic variance; H², broad sense heritability

M2 Population Structure

The principal component analysis (PCA) revealed the relationship between the evaluated parameters and the similarities between the mutants. In the three populations, the main component analysis on quantitative data shows a high significance of Bartlett test ($p < 0.0001$) and the eigenvalues of the axes. Table 3 shows that 2 axes explain most of the variability. Most of the variability (70.5%) in KVx396-4-5-2D population was explained by the two axes (1 and 2). The first axis explains 47.5% of the variability defined by the variables related to the length of the peduncle and the date of the first flowering. The second axis which revealed 23% of the variability is related to the plant height and the number of primary branches. Therefore, axis 1 allowed helps in distinguishing the mutants of KVx396-4-5-2D as having a long stalk and as a late flowering. On the other hand, axis 2 has identified mutants that are of high size and ramified.

As for the local Moussa population, the principal component analysis (PCA) showed most of the variability (70.3%) explained by the two first axes. The contribution of axis 1 of 44.5% is related to the length variables of the stalk, the date of the first flowering, and the number of primary branches; while axis 2 (25.8%) is related to the height of the plants. Axis 1 distinguishes the mutants characterized by a high number of primary branches, a long stalk, and late flowering and axis 2 identified the high-size mutants.

The contribution of the two axes to the variability of the population of Tiligre is 62.5%. The first axis explains 36.2% of the variability. It is defined

by the length of the peduncle and the date of the first flowering. This axis characterizes mutants with a long stalk and late flowering. Axis 2 represented by the number of primary branches and the plant height explains 26.3% of the variability. This axis identifies the mutants of high size and ramified. The clouds of the individuals projected on the F1 axis and the F2 axis, shows variability within the irradiated populations of KVx396-4-5-2D (Figure 1a) of Moussa local (Figure 2a) and of Tiligre (Figure 3a). Using similarity, the hierarchical analysis allows the grouping of the mutants. So, the KVx396-4-5-2D mutant population was classified into four clusters (Figure 1b), whereas local Moussa (Figure 2b) and Tiligre (Figure 3b) are grouped into six clusters. The cluster organizes individuals as endpoints that have joined a new point, with drawn lines that represent membership. Longer lines represent a greater distance between clusters. Morphological and phenological characteristics and number of families vary with clusters and with genotypes (Table 4). In KVx396-4-5-2D population, cluster 3 is the largest one with 172 families. It is followed by cluster 2 with 152 families including control, and then cluster 4 with 61 families. The smallest group identified was cluster 1 with 48 families. The mutant families included in clusters 1, 3, and 4 were characterized with small size, a short stalk, and late flowering. In contrast, the mutants of cluster 1 exhibited a high number of branching and those of Group 4 shows a very late flowering.

In Moussa local population, cluster 1 was the largest groups with 150 families, followed by cluster 2 with 100 families, cluster 6 with 83 families, and clusters 4 with 59 families. Cluster 3 included control and cluster 5 was the smallest registered, with 50 and 3 families respectively. The mutant families of groups 1, 2, 4, 5, and 6 showed high-sized families with a small number of branches. In addition, Group 1 and 2 had a short peduncle and a slightly delayed flowering, while Group 4 has a long peduncle and a slightly early flowering. Families with a very long peduncle and a slightly delayed flowering were in Group 5, while families with short peduncle and late flowering were in Group 6.

Finally, in Tiligre population, the six groups registered 68, 70, 68, 64, 75 and 76 families respectively. Group 4 brought together families similar to the witness. Except for Group 3, groups 1, 2, 5, and 6 had high mean values for plant height. Group 1, 2, and 3 exhibited the longer peduncle, while 5 and 6 had the shorter peduncle. The high number of branches is only recorded in clusters 1, 3, and 5. All groups exhibited late flowering.

The results of the cluster analysis confirm the presence of genetic differences between mutant families. In Moussa local population, in particular, mutant families in cluster 1 with high number of branching could be useful for improving cowpea yield, families in cluster 4 and 5 would be suitable for harvesting by machines, and the cluster 4 would be also useful

for the improvement of early flowering. In Tiligre population, cluster 1 and 2 would be suitable for machine harvesting and the families in clusters 1, 3, and 5 would be useful for agronomic performance. The selected mutants show useful agronomic traits capable of conferring on them the selective advantage for increased yield. The agronomical advantage of higher branching was reported by Adekola *et al.* (2007). Kumara (2011) reported a positive correlation between higher branching and seed yields. A positive correlation between plant height and yield component was also reported by Kumara (2011). Early flowering mutants may be interesting for the areas with short rainfall duration as they show better tolerance to drought. Long peduncles will be interesting for easy harvesting by manual or mechanical means (Olasupo *et al.*, 2018). It could be a strategy to reduce the cost of cultivation. Overall, gamma-ray mutation induction is useful for creating a new source of variability. The 150 Gy of gamma rays on KVx396-4-5-2D, Moussa local, and Tiligre is effective in generating significant variability for most of the quantitative traits studied. The high induced genetic variability is useful for the enhancement of Cowpea reproduction.

Table 3. Eigenvectors-eigenvalues and accumulated variation of the first four principal components (PC)

Varieties	Characters	F1	F2	F3	F4
Moussa	HP	0.19567	0.90969	0.23162	-0.2838
	NR	0.50099	-0.3627	0.76084	-0.1964
	LP	0.61613	0.1412	-0.1418	0.7618
	DPF	-0.5754	0.14472	0.58939	0.54825
KVx396-4-5-2D	HP	0.32938	0.8753	0.31105	-0.1691
	NR	0.40553	-0.4346	0.80391	0.01888
	LP	0.61562	0.02019	-0.3166	0.72138
	DPF	-0.59	0.211	0.39592	0.67132
Tiligre	HP	0.15813	0.70135	-0.6944	0.03003
	NR	0.3559	0.58467	0.65798	-0.3139
	LP	0.69072	-0.1173	0.06948	0.71015
	DPF	-0.6093	0.39052	0.28289	0.62947

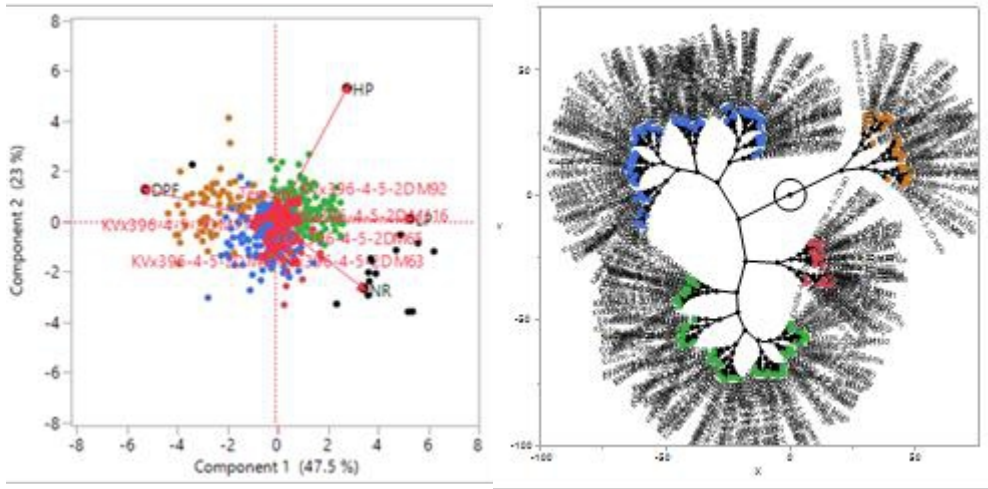


Figure 1. Representation of genotype-trait biplot (a) and cluster constellation plot of KVx396-4-5-2D

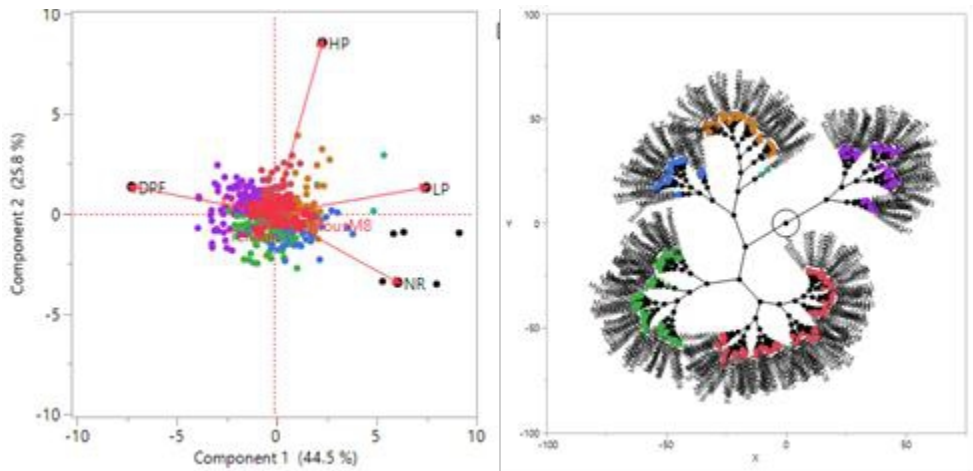


Figure 2. Representation of genotype-trait biplot (a) and cluster constellation plot of Moussa mutants

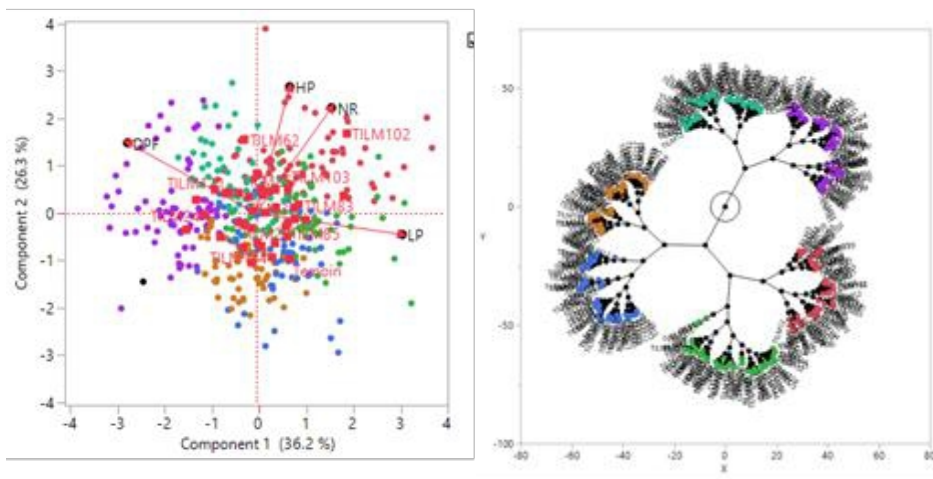


Figure 3. Representation of genotype-trait biplot (a) and cluster constellation plot of Tiligre mutants (b)

Table 4. Mean values of clusters formed by hierarchical clustering

	Cluster	Count	HP	NR	LP	DPF
Kvx396-4-5-2D	1	48	28.7917	5.6875	8.3021	49.5
	2	152	33.2621	4.0263	11.4489	46.6447
	3	172	27.218	3.1802	8.2195	47.8895
	4	61	28.8197	2.5246	4.2158	61.1639
Moussa local	1	150	30.4407	4.2933	7.6566	45.4733
	2	100	23.8961	3.97	6.9317	44.51
	3	50	26.6334	6.62	9.7799	44.3
	4	59	29.8983	4.9322	14.5537	43.5254
	5	3	31.6667	4.6667	37.1333	44.6667
	6	83	28.1773	3.0964	4.1707	51.3133
Tiligre	1	68	30.6821	3.3382	8.4449	52.2059
	2	70	31.3071	1.7	10.4545	50.7429
	3	68	24.0603	2.3824	8.9265	52.6176
	4	64	26.0839	1.4531	5.6591	50.0938b
	5	75	31.4667	2.0267	5.4478	53.7467
	6	76	27.5329	1.6184	4.2368	58.8553

Conclusion

Genetic variability was observed in all agronomic measured characters of M2 generation after 150 Gy dose of gamma ray treatment. The extent of this variability depends on the genotype's response to gamma irradiation. Cluster analysis results confirm the presence of genetic dissimilarities among the mutant families. The identified mutant families could be used in the future breeding program in cowpea particularly in enhancement programs for Striga resistance and drought tolerance research.

Conflict of Interests

The authors have not declared any conflict of interests.

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