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Assessment of Genetic variability among ethidium bromide-derived genotypes of cowpea (*Vigna unguiculata* (L.) Walp) at M₇ generation

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Abstract – An induced mutation is a valuable tool for creating new genetic variability to complement existing germplasm and broaden crop genetic base. Thus, creating genetic diversity is needed for effective plant breeding programs. This study was aimed at evaluating the level of variation of phenotypic traits among ethidium bromide (EtBr)-derived genotypes of cowpea evaluated during the dry season of 2021-2022. Six cowpea genotypes obtained from the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko were used in the study. These were evaluated in a randomized complete block design (RCBD) in 3 replicates in the field. The traits measured were emergence percentage, number of leaves/plant, plant height, number of main branches/plant, number of peduncles/plant, peduncle length, pods/plant, pod length, seeds/pod, number of seeds/plant, 100-seed weight, and seed yield/plant. Upon statistical analysis, it was observed that G3 (IT98K-555-1) at a concentration of 0.5% for 16hrs was most suitable to broaden the genetic base of cowpea and can be selected for further breeding programs.

Keywords – Cowpea, Genetic Base, Genetic Diversity, Induced Mutation, Traits.

I. INTRODUCTION

The importance of selecting and evaluating varieties for quantitative traits and yield capacity in any breeding program has been previously pointed out by (1) and assessment of genetic diversity in cowpea genotypes would facilitate the development of cultivars for adaptation to specific production Several constraints. workers (2,3,4,5) have calculated the genotypic and phenotypic components of variance, heritability, and genetic advance for different yield characters in cowpea and other crops and have revealed that selection was effective for a population with broad genetic variability and character with high heritability. Yield, being a complex character and controlled by a large number of contributing characters and their interactions, which in turn are governed by a few numbers genes, also is influenced to a great extent by the environment. The correlation of characters also helps to simultaneously select more than one character of importance at a time. Still, the total correlation is insufficient to explain the true association between characters, as the yield is dependent on many components of characters. It would be more desirable to consider the relative magnitude of various characters in order to have a clearer picture of yield components for an effective selection program (6).

Cowpea (*Vigna unguiculata* L.) is an essential crop that is used as feed for animals and food for humans in Africa. It is a rich source of protein, fiber, fat, and oil. Consumers who cannot afford expensive protein rely on it as a low-cost supply of protein (7). However, cowpea (*Vigna unguiculata*) faces challenges related to its genetic base and yield potential. The narrow genetic base of cowpea can be attributed to several factors, including the impact of abiotic (such as drought, salinity, and nutrient deficiencies) and biotic (such as pests and diseases) stresses. Thus, the level of genetic diversity in cowpea is low and this necessitates breeding improvement on the crop through prompted mutation breeding.

Mutation induction has been the most frequently used method for developing mutant varieties because it is more efficient and faster than conventional breeding methods (hybridization majorly) and has led to its adoption (5). Therefore, this study was aimed at selecting and evaluating ethidium bromide (EtBr)-induced genotypes of cowpea for phenotypic variations and yield capacity for breeding programs.

II. MATERIALS AND METHODS

Experimental materials

Six (6) genotypes of cowpea were grown during the dry season of 2021/2022 to assess the genetic variability of cowpea genotype for yield and its component traits in Akungba-Akoko, Southwestern Nigeria. The seeds were derived from EtBr cowpea genotypes at M_7 generation. These genotypes were collected from the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Nigeria. The accessions corresponding treatments (forming the genotypes) are presented in Table 1.

Table 1. The list of EtBr-derived genotypes of cowpea at M_7 generation

S/N	Code	Accession	Treatment
1	G1	IT98K-555-1	Control
2	G2	IT98K-555-1	0.5%/2hrs
3	G3	IT98K-555-1	0.5%/16hrs
4	G4	IT98K-555-1	0.5%/32hrs
5	G5	IT98K-555-1	0.2%/32hrs
6	G6	IT98K-205-8	Check

G1 is the untreated parental line (Control) from which G2 - G5 were derived from using ethidium bromide (EtBr) at different concentrations and timing. G6 served as a check genotype for the experiment.

Location of the study

The study was carried out at the Experimental Field of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Nigeria. The location for the study lies between Latitude 7° 28'N and Longitude 5° 44'E, Altitude 423 m above sea level.

Experimental layout

The experiment was laid out in a randomized complete block design (RCBD) and replicated three times. The spacing within and between ridges were 30 and 60 cm, respectively. Three seeds were planted per hill and the seedlings were thinned to one two weeks after emergence.

Agronomic practices

Agronomic management practices included preventive weeding which was carried out as when required. Supplementary irrigation was done by manual watering. Animal pests and rodents were controlled by chicken gauze and an insecticide (cypermetrine) was applied to control insects' invasion on the field.

Data collection

Data on emergence percentage were collected at 10 days after planting. Data on other quantitative traits of five randomly selected plants per genotype per replicate were collected, and their means calculated. Data such as plant height, number of main branches, and number of leaves were collected at week 5 after planting. Other data such as the number of peduncle, peduncle length, number of pods per plant, pod length, number of seeds per pod, number of seeds per plant, 100-seed weight, and seed yield per plant were collected at maturity.

Statistical analyses

The data recorded were subjected to analysis of variance (ANOVA) using SPSS version 20. Estimates of genetic parameters were done according to procedures in (8).

The coefficient of variation (CV) is a relative measure of variability or dispersion expressed as a percentage. A higher CV indicates a greater relative variability, while a lower CV suggests a more homogeneous dataset. It is calculated as;

$$CV \% = \frac{\sqrt{MSe} \times 100\%}{x}$$

Where the Mean square error is divided by the Mean and multiplied by 100 to express it as a percentage.

Estimation of genetic parameters

The following were estimated: $GV = \frac{MSg - MSe}{r}$

PV = GV + MSe

$$GCV = \frac{\sqrt{Vg}}{X} \times 100$$
$$PCV = \frac{\sqrt{VP}}{X} \times 100$$

 $\mathrm{H}^{2} \mathrm{B} = \frac{vg}{vp} \times 100$

GA = $h^2 \times I \times \sqrt{Vp}$; where k = 2.06 at 5% selection difference

$$\text{GAM} = \frac{GA}{X} \times 100.$$

Where:

x = Grand mean

r = replicates

MSe = Mean Square error

MSg = Mean Square genotype

GV = Genetic variance

PV = Phenotypic variance

GCV = Genetic coefficient of variation

PCV = Phenotypic coefficient of variation

 $H^2 B =$ Heritability in broad sense

GA = Genetic advance

GAM = Genetic advance as percent of mean

Genotypic and phenotypic coefficient of variation (GCV and PCV)

The value classification was according to (9) as shown below;

0% - 10% low variability

10% - 20% moderate variability

Above 20% high variability

Heritability

Broad sense heritability was done as the ratio of GV to PV in percentage and classified according to (10) as:

0% - 30% low

30% - 60% moderate

Above 60% high

Genetic Advance (GA)

Genetic Advance was calculated according to (10) Where I = selection differential (2.06) at 5% selection intensity.

Genetic advance as percentage over mean (GAM)

It was categorized according to (1) 0% - 10% low 10% - 20% moderate Above 20% high

III. RESULTS

The mean square values from ANOVA for quantitative traits of EtBr-induced genotypes of cowpea at M_7 generation are represented in Table 2. Mean square values for all traits were

significant among genotypes. Replication was also significant for all traits except for plant height, pod length, seed per plant, and 100-seed weight. The most variable trait was number of seeds per plant (CV = 249.55%) while the least variable trait was plant height (CV = 12.39%).

Table 2. Mean square values of quantitative traits of EtBr-derived genotypes of cowpea at M7 generation

Source of		EM	PH						PDL			100-SW	SDYPL
variation	DF	(%)	(cm)	NL	NMB	PD	PEDL	PDP	(cm)	SPP	SDPL	(g)	(g)
Genotype	5	470.92*	58.99*	4781.74*	386.4*	31.41*	52.77*	39.62*	115.83*	101.17*	6814.54*	22.48*	155.93*
			0.03										
Replication	2	80.29	ns	82.03*	229.19*	2.66*	0.65*	1.49*	9.77 ns	0.31 ns	100.7*	0.37 ns	1.55*
Error	10	127.1	3.02	41.14	199.31	5.02	2.44	3.46	95.58	5.46	438.67	4.53	9.19
CV (%)		19.92	12.39	12.49	99.84	33.14	24.26	51.38	79.09	27.62	249.55	15.84	67.07

*: Significant at $P \le 0.05$; ns: Not significant. DF: Degree of freedom; CV: Coefficient of variation.

Emergence percentage (EM%), number of leaves (NL), plant height (PH), number of main branches (NMB), number of peduncles (PD), peduncle length (PEDL), pods per plant (PDP), pod length (PDL), seeds per pod (SPP), number of seeds per plant (SDPL), 100-seed weight(SW), and seed yield per plant (SDYPL).

The mean performance of EtBr-derived genotypes of cowpea at M_7 generation is represented in Table 3. The emergence percentage of the genotypes varied greatly, with genotype G5 showing the highest percentage (75%) and genotype G3 the lowest (37.50%). The plant height varied from 12.47 cm in genotype G3 to 17.45 cm in genotype G1. The number of leaves ranged from 22 in genotype G6 to 73.67 in genotype G5, while the number of branches ranged from 6.00 in genotype G6 to 19.33 in genotype G5. The number of peduncle varied from 5.20 in genotype G4 to 9.10 in genotype G2, while peduncle length ranged from 4.83 in genotype G6 to 9.43 in genotype G2.

Table 3. Mean performance of EtBr-derived genotypes of	f cowpea at M7 Generation
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Treatment	EM (%)	PH (cm)	NL	NMB	PD	PEDL (cm)	PDP
G1	58.33±11.02 ^{abc}	$17.45 \pm 0.18^{\circ}$	58.33±1.67°	18.00 ± 5.03^{b}	7.64 ± 0.54^{b}	5.83 ± 0.54^{b}	5.56 ± 0.44^d
G2	$56.25{\pm}3.60^{abc}$	12.70 ± 0.52^{a}	62.50± 0.29 ^c	17.50 ± 2.59^{b}	9.10 ± 0.52^{d}	$9.43{\pm}0.10^{d}$	$2.84{\pm}~0.09^{bc}$
G3	37.50 ± 7.22^{a}	$12.47{\pm}0.14^{a}$	45.33 ± 2.33^{b}	$12.33{\pm}3.84^{ab}$	$6.01{\pm}0.33^{ab}$	$8.08{\pm}0.39^{\rm c}$	5.50 ± 0.50^d
G4	$62.50{\pm}~0.00^{bc}$	$12.95{\pm}0.28^a$	$46.33{\pm}1.86^{\text{b}}$	11.67 ± 2.73^{ab}	5.20 ± 0.20^{a}	$5.08{\pm}~0.06^{ab}$	1.61 ± 0.20^{a}
G5	75.00± 7.22 ^c	15.24 ± 0.33^{b}	73.67 ± 2.72^d	19.33 ± 4.09^{b}	$6.92{\pm}~0.08^{bc}$	$5.35{\pm}0.18^{ab}$	$3.72 \pm 0.49^{\circ}$
G6	$50.00{\pm}~0.00^{ab}$	$13.33{\pm}0.00^a$	22.00 ± 1.16^{a}	$6.00 \pm 0.58^{\mathrm{a}}$	5.67 ± 0.44^{ab}	4.83 ± 0.17^{a}	2.50 ± 0.29^{ab}
Grand mean	56.59± 3.52	14.02 ± 0.44	51.36 ± 4.00	14.14 ± 1.63	6.76 ± 0.36	6.44 ± 0.43	3.62 ± 0.38

Meansfollowed by the same superscript within a column are not significantly different from one another at $P \leq 0.05$ using DMRT. Emergence percentage (EM%), number of leaves (NL), plant height (PH), number of main branches (NMB), number of peduncle (PD), peduncle length (PEDL), peduncle per plant (PDP) Furthermore, the number of pods per plant ranged from 1.61 in genotype G4 to 5.56 in genotype G1, with pod length varying from 7.50 cm in genotype G6 to 16.02 cm in genotype G3. The number of seeds per pod varied from 4.89 in genotype G6 to 11.97 in genotype G3, while the number of seeds per plant ranged from 9.93 in genotype G4 to 65.80 in genotype G3. Additionally, the weight of 100 seeds varied from 12.00 g in genotype G5 to 15.20 g in genotype G3, and seed yield per plant ranged from 1.41 g in genotype G4 to 10.02 g in genotype G3.

Table 3 cont'd.

Treatment	PDL (cm)	SPP	SDPL	100-SW (g)	SDYPL (g)
G1	13.25 ± 1.85^{ab}	8.47 ± 0.29^{b}	$46.81 \pm 2.49^{\circ}$	12.93 ± 0.47^{ab}	$6.08 \pm 0.53^{\circ}$
G2	11.75 ± 2.63^{ab}	9.70 ± 0.64^{b}	27.62 ± 2.73^{b}	14.00 ± 0.29^{bc}	3.85 ± 0.30^{b}
G3	16.02 ± 2.07^{b}	11.97 ± 0.26^{d}	65.80 ± 6.10^{d}	15.20 ± 0.20^{cd}	10.02 ± 1.03^{d}
G4	12.67 ± 0.17^{ab}	6.07 ± 0.52^{a}	9.93 ± 2.04^{a}	14.17 ± 0.17^{cd}	1.41 ± 0.31^{a}
G5	12.97 ± 1.69^{ab}	9.65 ± 0.35^{b}	38.13 ± 5.79^{bc}	12.00 ± 0.58^{a}	4.28 ± 0.51^{b}
G6	$7.50 \pm 0.29^{\mathrm{a}}$	4.89 ± 0.11^{a}	12.17 ± 1.17^{a}	12.33 ± 0.33^{a}	1.49 ± 0.11^{a}
GM	12.36 ± 0.85	$8.46{\pm}~0.59$	33.08 ± 4.90	13.44 ± 0.29	4.52 ± 0.74

Means followed by the same superscript within a column are not significantly different from one another at $P \leq 0.05$ using DMRT. Grand mean (GM), pod length (PDL), no of seeds per pod (SPP), number of seeds per plant (SDPL), weight of 100 seeds per pod (SW) and seed yield per plant (SDYPL).

The study reports estimates of various quantitative traits of EtBr-derived genotypes of cowpea as shown in Table 4. It include Genotypic coefficient of variation (GCV), Phenotypic coefficient of variation (PCV), Heritability in broad sense (H² B), Genetic advance (GA), and Genetic advance as per mean (GAM). The results show that emergence percentage had a PCV of 27.47% which is higher than GCV of 18.92%, with moderate heritability and high genetic advance as per mean. Plant height had a high PCV of 33.21%, which is higher than GCV of 30.81%, high heritability and high genetic advance as per mean. Number of leaves had a high PCV of 78.39% and moderate heritability, with high genetic advance as per mean. Number of main branches had a high PCV of 114.4%, moderate heritability, and high genetic advance as per mean. Other traits such as number of peduncle per plant, pod length, number of seeds per pod, number of seeds per plant, and weight of 100 seeds also showed high PCV values, with moderate to high heritability, and high genetic advance as per mean. Overall, the results suggest that breeding work has produced promising results for improving the quantitative traits of EtBr-derived genotypes of cowpea.

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Trait	Grand mean	GV	PV	GCV (%)	PCV (%)	H ² B (%)	GA	GAM (%)
EM (%)	56.59	114.61	241.71	18.92	27.47	47.42	18.08	31.95
PH (cm)	14.02	18.66	21.68	30.81	33.21	86.07	8.25	58.85
NL	51.36	1580.20	1621.34	77.39	78.39	97.46	80.46	156.66
NMB	14.14	62.36	261.67	55.83	114.40	23.83	7.99	56.51
PD	6.76	8.79	13.81	43.86	54.97	63.65	4.89	72.34
PEDL (cm)	6.44	16.78	19.22	63.61	68.08	87.31	7.86	122.05
PDP	3.62	12.05	15.51	95.89	108.79	77.69	6.33	174.86
PDL (cm)	12.36	6.75	102.33	21.02	81.84	6.59	1.46	11.81
SPP	8.46	31.9	37.36	66.76	72.25	85.39	10.70	126.48
SDPL	33.08	2125.29	2563.96	139.36	153.07	82.91	86.58	261.73
100-SW (g)	13.44	5.98	10.51	18.19	24.12	56.89	3.81	28.35
SDYPL (g)	4.52	48.91	58.10	154.75	168.64	84.18	13.19	291.81

Table 4: Genetic parameters for 12 quantitative traits of EtBr-derived genotypes of cowpea at M7 generation

Emergence percentage (EM%), number of leaves (NL), plant height (PH), number of main branches (NMB), number of peduncles (PD), peduncle length (PEDL), pods per plant (PDP), pod length (PDL), seeds per pod (SPP), number of seeds per plant (SDPL), 100-seed weight (SW), and seed yield per plant (SDYPL). genetic variance (GV), phenotypic variance (PV), genetic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (H² B), genetic advance (GA), genetic advance as percent of mean (GAM)

As shown in Table 5, the estimates of correlation coefficient between all combinations of traits among the studied genotypes revealed that the number of pods per plant was highly significant and positively correlated with number of seed per plant (0.934), and seed yield per plant (0.893). The number of seeds per pod was highly positively correlated with number of

seeds per plant (0.843), and seed yield per plant (0.835). Number of seeds per plant was highly positively correlated with seed yield per plant (0.985).

		Table 5. Correlation analysis of 12 quantitative traits of EtBr-derived genotypes of cowpea at M7 generation										
	EM	PH						PDL			100SW	
TRAITS	(%)	(cm)	NL	NMB	PD	PEDL	PDP	(cm)	SPP	SDPL	(g)	SDYPL
EM	1	0.42	0.55	0.646	0.14	-0.304	-0.374	-0.319	-0.172	-0.401	-0.595	-0.508
PH		1	0.408	0.384	0.22	-0.388	0.447	-0.41	-0.044	0.199	-0.519	0.73
NL			1	0.767	0.559	0.283	0.212	0.367	0.571	0.293	-0.106	0.2
NMB				1	0.54	0.236	0.111	0.232	0.444	0.178	-0.223	0.94
PD					1	0.519	0.233	0.092	0.379	0.214	-0.54	0.161
PEDL						1	0.197	0.26	0.674	0.404	0.552	0.462
PDP							1	0.504	0.64	0.934**	0.128	0.893**
PDL								1	0.66	0.637	0.378	0.635
SPP									1	0.843**	0.368	0.835**
SDPL										1	0.311	0.985**
100SW											1	0.455
SDYPL												1

**: Significant at $P \leq 0.01$; *: Significant at $P \leq 0.05$.

Emergence percentage (EM %), number of leaves (NL), plant height (PH), number of main branches (NMB), number of peduncle (PD), peduncle length (PEDL), peduncle per plant (PDP), pod length (PDL), no of seeds per pod (SPP), number of seeds per plant (SDPL), weight of 100 seeds per pod (SW) and seed yield per plant (SDYPL).

IV. DISCUSSION

Genetic variability

The nature and extent of genetic diversity are essential for crop improvement programs. Insight into the type and extent of genetic variation is crucial in planning efficient breeding programs to improve the yield potential of genotypes. Information on the association of plant traits with seed yield is very important in selecting the desired genotype for breeders. Ajayi and Adesoye, 2013 (4) reported that diversity of genetic material is important for the success of crop improvement methods. Therefore, identifying genotypes with more desirable traits, using them in breeding programs, and establishing appropriate selection criteria will help successful breeding programs (5).

In the present study, ANOVA showed significant differences among genotypes for all traits. This indicates the presence of sufficient degree of variability between genotypes. This is consistent with the results of (11). However, a more comprehensive understanding of the genetic factors that control these traits, as well as the environmental factors that interact with genotype to influence reproductive development and crop yield, is desired. Information on the relationship between genetic variants and genotypes will help breeders develop better breeding techniques to address the low-yield problem in cowpea. Previous studies on heritability in cowpea and various crops have shown that various genetic parameters of heritability degree and personality vary with environment. Data on the variable nature and degree of heritability of the reproductive segments of cowpeas in wet savannahs are limited.

Genetic parameters

Genetic parameter estimates indicate the relative importance of different types of genetic effects in influencing overall changes in plant traits. Genotypic and phenotypic coefficients of variation (GCV and PCV) and heritability (H²B) with genetic advance (GA) are very important parameters in trait improvement (12). Higher heritability estimates associated with GAM indicated additive genetic effects on the traits observed in the study. The estimates revealed that PCV was higher than GCV for all traits. A low GCV/PCV ratio suggests that the trait is more influenced by environmental factors than genetic factors, while a high GCV/PCV ratio suggests that the trait is more influenced by genetic factors than environmental factors (13). However, it is important to also note that the interpretation of GCV/PCV ratios is not always straightforward and depends on other factors, such as heritability and genetic advance, as well as the overall breeding objectives and goals. Therefore, it is recommended to interpret GCV/PCV ratios in conjunction with other genetic parameters and breeding goals to make informed decisions regarding trait selection and breeding (14).

In the present study, number of main branches and pod length were both determined primarily by environmental factors thus leading to low heritability. On the other hand, the remaining traits were all determined by genetic influences rather than environmental factors thus, leading to between moderate to high heritability. Selection based on these latter traits can be effective in improving cowpea yields or creating new cultivars. Pod length has the lowest heritability (6.59%) and selection based on this trait is not suitable for further breeding programs. This was followed by number of main branches (23.83%), moderate emergence percentage (47.42%) and moderate 100-seed weight (56.89%). Leaf number (97.46%) had the highest heritability and also had a high genetic advance value of 80.46%. Therefore, selection for this trait is more effective as high heritability and high genetic advance provide the most effective conditions for selection (15).

Correlation

Ajayi et al, 2014 (5), hypothesized that correlation is a measure of the degree of association between traits, and that selection of trait selection leads to improvement of all positive correlated traits and regression of negative correlated traits. The high positive correlations observed between the number of pods per plant, the number of seeds per plant, and the seed yield per plant suggest that they are interdependent and should be considered together when breeding for improved seed yield in crop such as cowpea (16). The strong positive correlation between these traits also indicates that selection for one trait may result in indirect improvement of the other traits (17). Therefore, selection of the number of pods per plant makes a positive contribution to seed yield, and selection of seeds per plant also makes a positive contribution to seed yield.

V. CONCLUSION

Analysis of variance from this study showed significant differences between genotypes for all traits. Cowpea genotypes displayed a high degree of variability as a result of ethidium bromide treatment. Thus, we further confirm the fact that ethidium bromide can induce genetic diversity and expand the genetic base of cowpea.

This study identified genotype G3 (IT988K-555-1) at a concentration of 0.5% for 16 hours. This was the most suitable for further genetic studies as it showed the consistent highest values for pod length and number of seeds per pod, numbers of seeds per plant, 100-seed weight and seed yield per plant.

This genotype can be used for breeding programs based on observed quantitative traits. Also, genotype G6 (IT98K-205-8), which was used as check, scored low for most of the observed traits, suggesting that the genotype should be improved upon.

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