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Variability in Agro-Morphological and Morphometric Seed Traits of Some Mutant Cowpea Genotypes (*V. unguiculata* L. walp)

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ABSTRACT

An evaluation and morphometric seed studies were carried out in the screen-house of the College of Plant Science and Crop Production, and in the laboratory of Department of Plant Breeding and Seed Technology, FUNAAB respectively. Seeds of eight cowpea mutant genotypes were planted in plastic buckets filled with garden soil in the screen house, and arranged in a randomized complete block design with three replicates. Four buckets was assigned to each of the genotypes per replicate, to give a total of 96 buckets. Necessary cultural practices were carried out as required. Data were taken on both qualitative and quantitative traits for the screen-house study, these include; leaf colour, stem pigmentation, flower colour, plant height at six weeks, number of days to first flower and ripe pod, and seed related characters. Harvested seeds from the various genotypes were viewed using a digital morphometric seed reading metre to take various seed measurements such as, seed length, breadth, radius,circumference, testa length and seed angle. Data taken were analysed using analysis of variance (ANOVA) while their mean values were separated using Duncan's Multiple Range Test (DMRT). Phenotypic variance along with its other components and heritability were also computed. The level of relatedness was carried out using correlation coefficient analysis. The cowpea genotypes showed varying degree of phenotypes; green, yellow foliage; pigmented versus non pigmented stem; white to white with purple winged petal and complete purple flower colour. Results of analysis of variance for both the agronomic and seed characters showed high significant differences existed among the mutant genotypes for all the traits studied. IF-BR-Cr was ealiest (53days) in maturing while, IB-Cyt.Y (unstable leaf form) was the last to mature (71days). The highest 100seed weight was recorded for Rosa-1 (26.7g), followed by IF-BR-Cr (16.6g), while Tvu 6198 had the least (9.6g). Seed length and angle ranged between 6.8mm – 9.74mm and 82.30º- 97.4º respectively. The following traits had both low phenotypic and genotypic variance, days to first ripe pod, pod length, testa length, seed length and breadth. High heritability was recorded for all the traits except seed radius (2.71mm) and angle (1.93º). A positive correlation was observed among the traits studied. Variation for quantitative and qualitative traits among the mutant genotypes could be attributed to their genetic background. The mutant traits could serve as marker gene during conventional breeding programme, also IF-Br-Cr which was early maturing and gave relatively good seed yield is recommended for multi-location trial to determine the influence of the mutant trait on agronomic traits. Morphometric analysis increases the confidence and accuracy of morphological classification and description of cowpeas.
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Key words: cowpea, mutant genotypes, agro-morphological, morphometric analysis, heritability.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L) Walp.] is a warm-season leguminous crop that can be grown in the tropical region and dry savannas where the average annual rainfall is 60mm- 100mm (Wikipedia, 2016). It is a broadly adapted and highly variable crop, cultivated around the world primarily for seeds, but also as a vegetable (for leafy greens, green pods, fresh shelled green peas, shelled dried peas), as a cover crop and fodder (USDA, 2012). Cowpea adapts better to sandy soils and droughty conditions than soybeans (TJAI, 2010). This drought tolerant crop is commonly cultivated as a nutritious and highly palatable food source in the southern United States, Middle East, Africa, Asia, and throughout the Tropics and Subtropics. All parts of the cowpea plant that are used for food are nutritious, providing protein, vitamins (notably vitamin B) and minerals (Porbeni, 2009). Cowpea seeds are valued as a nutritional supplement to cereals and as an extender of animal proteins. The seeds contain 24% crude protein, 53% carbohydrates, and 2% fat (FAO, 2012). The protein in cowpea seeds is rich in the amino acids, lysine and tryptophan, compared to cereal grains. It is however, deficient in methionine and cystine when compared to animal proteins (IITA, 2012). Agro-morphological characters and how these traits affect yield are important aspects of any breeding work. Morphometric analysis however, helps in more detailed studies of pollens, seeds structure and its content USDA, 2015; Adetumbi *et al.*, 2012). Biodiversity studies with respect to variability in agro-morphological traits and their relationship with seed traits will serve as basis for crop

improvement by identifying these important traits. A thorough understanding of these mutant genotypes with novel traits is therefore necessary. The objectives of this study were to; evaluate some mutant cowpea genotypes for their morphological and agronomic differences, determine the association between some yield related traits and seed characters. Lastly, to determine the character(s) that contributes the highest variability.

MATERIALS AND METHODS

The experiments were carried out at the screen house and laboratory of the College of Plant Science and Crop Production (COLPLANT), and Department of Plant Breeding and Seed Technology respectively, both at the Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria. Seeds of each of the eight mutant cowpea genotypes used for the experiments were obtained from Genetic stock of the above named Department. Two seeds per genotype was planted in 30cm diameter plastic buckets filled with garden soil obtained from the Teaching and Research Farm of the University and established in the screen house during the first planting season (April- July, 2014). Four buckets was assigned to each of the genotypes randomly per replicate, to give a total of 96 buckets and 192 plant population. Four buckets each was assigned to a genotype using the randomized complete block experimental design in three replicates. Necessary cultural practices watering and insect pests control were carried out throughout the experimental period. The following quantitative data were taken; plant height at six weeks (cm), number of days from planting to first flower, days to first ripe pod, pod length (cm), number of seeds per pod, 100 seeds weight (g).

Fifteen seeds each of the eight cowpea genotypes harvested from the previous experiment were divided into three replicates, with each replicate having five seeds. Each seed was viewed with the aid of a digital microscope photo-camera with imaging software (Veto[tm]) installed, while the image of each seed was captured on the computer. The seed was placed under the USB microscope such that its testa axis was facing the lens of the camera under light, and adjusted until desired brightness and magnification was obtained. Captured image were later previewed and measurement on the various aspects of each seed taken (Plate 1) using the method of Daniel, *et al*., 2012. Seed aspects such as; seed length, breadth, testa-length, radius, and circumference taken was in millimeters (mm), while the seed angle was recorded in degree (º). The data obtained from the experiments were analyzed using the analysis of variance (ANOVA), while Duncan multiple range test (DMRT) was used to separate significance means. Inter-character correlation between the traits measured was done using Pearson correlation coefficient analysis. Phenotypic, genotypic and environmental variances of traits studied and their percentage heritability were also calculated (Oshinubi, 2015).

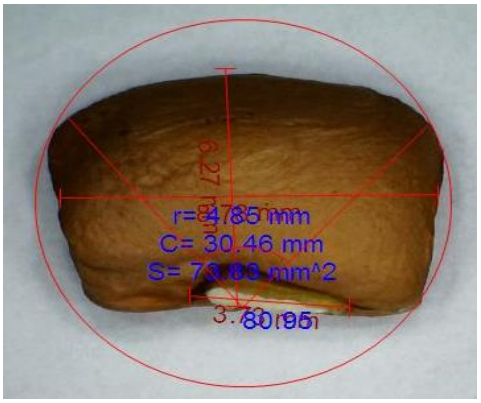

Plate 1: A cowpea seed with some measurements taken; r = seed radius, c = seed circumference, s = seed surface area

RESULTS

The cowpea genotypes showed varying degree of variation in their qualitative traits, with the foliage colour ranging from dark green to yellow. Flower colour however varied from solid white to purple, and mixture of white and purple (white standard petal with purple wing petal) Table 1. The result of analysis of variance for the various quantitative traits taken is presented on Table 2. Significant differences were observed among the mutant cowpea genotypes for all parameters taken. Block effect was only significant for days to first ripe pod (Table 2).

Table 3 shows the mean performance of the mutant cowpea genotypes with respect to their quantitative traits. Rosa-2 had the highest plant height at 6 weeks after planting (76.50cm), while IB-Cyt-Y (normal leaf form) had the lowest plant height (36.00cm). IF-BR-Cr and IB-Cyt.Y (unstable leaf form) recorded the shortest (42.5days, 52.5days) and longest (60.86days, 71.0days) number of days to first flower and ripe pod respectively among the genotypes studied. The longest pod among the genotypes was IB-Cyt.Y unstable leaf form (15.12cm), while Ife BPC had the shortest pod (12.33cm). The least number of seeds per pod was recorded for Rosa-1 (8 seeds), while Rosa-2 had the best pod filling (13.5 seeds). The highest and least 100 seed weight was recorded for Rosa-1 and TVu 6198 (26.65g and 9.60g) respectively. Analysis of variance for seed characters is shown on Table 4. All the parameters taken showed high significant differences amongst the mutant genotypes, except seed angle which was not significant.

Table 5 shows the mean performance for seed characters of the mutant genotypes. There were significant differences for all the parameters taken among the mutant genotypes except seed radius which showed no significance difference. Heritability values of some agronomic traits taken revealed that all the traits were highly heritable; however, seed radius and angle were not and could be easily influence by environmental factors (Table 6).

Table 1: Cowpea mutants used for the study and their morphological traits.

Genotypes	Leaf colour	Stem pigmentation	Flower colour	Seed coat colour	Mutant trait
Ife-BPC	Green	Non-pigmented	White with purple keel	Brown	Branched peduncle
Ife Brown	Green	Non-pigmented	White with purple keel	Brown	Non
Rosa-1	Green	Pigmented	Purple	Black with mottled white	Rosa flower form
IF-BR-Cr	Dark green	Non-pigmented	White with purple keel	Brown	Crinkled leaf form
Rosa- 2	Green	Non-pigmented	White with purple keel	Brown	Rosa flower form
TVu 6198	Green	Non-pigmented	White	White	Non
IB-Cyt.Y. (normal leaf form)	Yellow	Pigmented	Purple	Red	Yellow leaf colour
IB-Cyt.Y (unstable leaf form)	Yellow	Pigmented	Purple	Brown	Yellow leaf colour, varied leaflet number.

Table 2: Analysis of variance for some quantitative traits of the mutant cowpea genotypes studied.

Source of Variation	Degree of freedom	plant height (cm)	days from sowing to first flower	days to first ripe pod	Pod length (cm)	No. of seeds per pod	100 seeds weight (g)
Block	2	0.28	1.88	7.31**	0.14	0.99	3.02
Genotypes	7	547.7**	153.80**	142.51**	4.41**	12.14**	146.64**
Error	14	1.42	1.63	0.89	0.24	2.10	1.84

* Significant at 5% probability level, ** highly significant at 1% probability level.

Table 3: Mean performance of some agronomic traits of the mutant cowpea genotypes stud.

Genotypes	Plant height at six weeks (cm)	Days of sowing to first flower	Days to first ripe pod	Pod length (cm)	No. of seeds per pod	100 seeds weight (g)
Rosa-2	76.50^{a}	51.00^{d}	63.10^{c}	14.40^{a}	13.50^{a}	12.80^{bc}
IF-BR-Cr	72.50^{b}	42.50^{e}	52.50^{de}	14.55^{a}	10.00^{bc}	16.60^{b}
Ife Brown	64.00^{c}	43.50^{e}	54.00^{d}	12.90^{b}	10.00^{bc}	13.00^{bc}
TVu 6198	60.50^{d}	43.50^{e}	54.50^{d}	13.10^{b}	13.70^{a}	9.60^{d}
Rosa-1	59.00^{d}	51.50^{d}	66.00^{b}	12.53^{b}	8.00^{c}	26.65^{a}
Ife-BPC	51.00^{e}	54.50^{c}	64.99^{c}	12.33^{b}	9.00^{bc}	10.14^{c}
IB-Cyt.Y (normal leaf form)	45.50^{f}	57.50^{b}	65.86^{b}	15.11^{a}	10.80^{b}	13.90^{bc}
IB-Cyt.Y (unstable leaf form)	36.00^{g}	60.86^{a}	71.00^{a}	15.12^{a}	10.00^{bc}	9.80^{d}

Means with the same alphabet are not significantly different, at 5% probability level

Table 4: Analysis of variance of the seed characters of the mutant cowpea genotypes

Source of Variation	D. F	Seed length (mm)	Seed breadth (mm)	Seed testa length (mm)	Seed radius (mm)	Seed circumference (mm)	Seed angle (º)
Block	2	0.05	0.22*	0.03	1954.34	3.37	260819.94
Genotypes	7	3.15**	1.90**	0.29**	2000.22	589.85**	252100.97
Error	14	0.24	0.05	0.02	1996.90	10.81	256959.64

* Significant at 5% probability level, ** highly significant at 1% probability level.

Table 5: Mean performance of the seed morphometric characters of the mutant cowpea genotypes

Genotypes	Seed length (mm)	Seed breadth (mm)	Seed testa length (mm)	Seed radius (mm)	Seed circumference (mm)	Seed angle (◦)
Rosa-2	8.24^{b}	5.40^{b}	3.32^{b}	4.45^{a}	27.62^{bcd}	82.30^{a}
IF-BR-Cr	6.80^{c}	4.01^{c}	3.10^{bc}	3.64^{a}	22.32^{d}	86.90^{a}
Ife Brown	8.77^{b}	5.94^{a}	3.70^{a}	4.77^{a}	30.19^{bc}	83.70^{a}
TVu 6198	8.20^{b}	4.31^{c}	2.89^{d}	4.20^{a}	29.74^{bc}	93.0^{a}
Rosa- 1	9.63^{a}	6.10^{a}	3.63^{a}	4.99^{a}	31.66^{bc}	88.40^{a}
Ife- BPC	8.00^{b}	5.83^{a}	3.70^{a}	4.29^{a}	25.95^{cd}	97.4^{a}
IB-Cyt.Y (normal leaf form)	9.74^{a}	6.70^{a}	3.20^{bc}	7.42^{a}	31.98^{b}	89.8^{a}
IB-Cyt.Y. (unstable leaf form)	9.61^{a}	9.61^{a}	3.20^{bc}	4.42^{a}	67.11^{a}	89.8^{a}

Means with the same letter are not significantly different using DMRT at 5% probability level

Table 6: Estimates of phenotypic, genotypic and environmental variances of some traits and broad sense heritability among the mutant cowpea genotypes studied.

Traits/ morphometric parameters	Phenotypic variance	Genotypic variance	Environmental variance	Heritability (%)
Plant height (cm)	182.57	182.57	0.47	99.74
Days from sowing to first flower (cm)	51.27	50.72	0.54	98.94
Days to first ripe pod (cm)	47.50	47.20	0.30	99.37
Pod length (cm)	1.48	1.40	0.08	94.71
100 seeds weight (g)	89.80	89.19	0.61	99.32
Seed length (mm)	1.05	0.97	0.08	92.72
Seed breadth (mm)	0.63	0.62	0.02	97.62
Testa length (mm)	0.09	0.09	0.01	94.31
Seed radius (mm)	666.74	14.44	652.30	2.71
Seed circumference (mm)	196.63	193.03	3.60	98.17
Seed angle (◦)	84033.66	-1619.56	85653.21	-1.93

Inter character association among the agronomic traits and seed characters studied is presented on Table 7. Number of seeds per pod was positively correlated with all agronomic traits measured, except number of days to first flower (r= -0.22). A negative correlation was however observed between all agronomic traits studied and seed circumference, except number of days to first lower (r= -0.61) and pod length (r= -0.34). Seed circumference was also negatively correlated with testa length (r= -0.12) and seed radius (r= -0.03). A highly significant correlation was observed between 100 seed weight and pod length (r= -0.59**), and most of the seed characters taken such as; seed length, seed breadth, seed angle and testa length (r= 0.41*, 0.56**, 0.56**, and 0.65**) respectively.

Table 7: Inter-character association of some agronomic and seed characters of eight mutant cowpea genotypes used in the study.

Traits	Days from sowing to first flower	Days to first ripe pod	Pod length (cm)	No. of seed per pod	Seed Length (mm)	Seed breadth (mm)	Seed Testa-length (mm)	Seed Radius (mm)	Seed circumference (mm)	Seed angle (º)	100 seeds weight (g)
Plant height (cm)	-0.76**	-0.27	-0.14	0.32	0.61**	-0.38	0.01	-0.22	-0.72**	-0.14	0.11
Days from sowing to first flower		0.32	0.13	-0.22	0.62**	0.55**	0.14	0.25	0.61**	0.10	-0.22
Days to first ripe pod			0.73**	0.57**	0.15	0.47*	0.58**	-0.82**	-0.12	0.36	0.11
Pod length (cm)				0.60**	0.01	-0.49*	-0.80**	0.19	0.34	-0.21	-0.59**
No. of seed per pod					-0.16	-0.41*	-0.50**	-0.01	-0.13	-0.10	-0.30
Seed Length (mm)						0.67**	0.26	0.13	0.51*	-0.14	0.41*
Seed Breadth (mm)							0.70**	0.24	0.12	0.10	0.56**
Testa-length (mm)								-0.22	-0.12	0.18	0.65**
Seed Radius (mm)									-0.03	-0.04	-0.10
Seed Circum-ference (mm)										-0.12	-0.11
Seed Angle (º)											0.81**
100 seeds weight (g)											

* Significant at 5% probability level, ** highly significant at 1% probability level, ns-not significant

DISCUSSION

Large variation were observed in the morphological and agronomic traits of the mutant cowpea genotypes studied. Differences in foliage colour of plants often reflect the level of chlorophyll concentration in the leaves. Several mutations affecting foliage colour have been reported in cowpea (Kirchhoff *et al.*, 1989; Fawole, 1997, Porbeni and Fawole, 2013). The yelllow foliage colour of IB-Cyt.Y (both stable and unstble leaf form) which is under cytoplasmic control (Porbeni, 2009), negatively affected their plant height (slow growth), number of days from sowing to first flower and ripe pods. The postitions of the male and female reproductive organs are also important determinant of compatiblity. Flower form in cowpea generally is papillonaceous. Rosa-1 and 2 with mutant flower form, however had rosaceous flower form. This mutation makes the stigma longer than the stamen and most times pollens are are shed without falling on the stigmatic surface of the plant. The effect of this is seen in the number of days to ripe pods. The positive correlation observed between most of the agronomic traits studied, except plant height and 100-seed weight, could be as a result of the genetic background of the genptypes. Osuagwu *et al.,* 2013 reported a positive association between pod length and the number of seeds per pod. Selection based on 300 seed weight have been reported to be more reliable and effective for yield related trait in soybean, due to its strong genetic association with seed yield (Adebisi, 1994).

According to Dell' Aquila (2004), quantitative descriptors for seed differences was effective in physiological studies, and in cultivar and genotype description. Daniel et al., 2012 reported that morphometric measurements are good quantitative descriptors to better define the cultivar identities and uniqueness even before planting the seeds. Stokefeld *et al.,* 1999 and Adetumbi *et al*., 2012, demonstrated that it is possible to use digital image analysis to study and classify seed, based on the shape, structure and seed content analysis. The high significant correlation observed for most of the parameters studied except seed angle, suggested that selection during breeding programme of any of the seed traits will lead to significantly increase of the other parameters. An increase in seed length during selection however, will lead to a decrease in seed angle. Variations in both quantitative and qualitative traits among the mutant genotypes could be attributed to the differences in their genetic background. Morphological traits such as stem pigmentation, flower colour and the mutant characters could be used as genetic marker during breeding programme. IF-BR-Cr which was found to be early

maturing and yielded relatively high seed weight could be tested across different environments to ascertain the stability of the mutant trait and its effect on agronomic traits. Morphometric analysis increases the confidence and accuracy of morphological classifications in the taxonomic description of cowpeas.

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