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PHENOTYPIC VARIABILITY IN COWPEA (*Vigna unguiculate* L. Walp) GENOTYPES ASSESSED WITH QUANTITATIVE AND QUALITATIVE CHARACTERS

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SUMMARY

A study on the assessment of morphological variability in cowpea was carried out at the Department of Crop Science and Technology, Federal University of Technology Owerri, Imo State, Nigeria. The aim of the study was to establish the phenotypic variation among six popular cowpea genotypes in Nigeria on the basis of quantitative and qualitative characters. The experimental design was randomized complete block design with five replications, and data were collected on qualitative and quantitative characters. Quantitative data were subjected to analysis of variance, and means were separated with Fisher's least significant difference at the 5% level of probability, whereas qualitative data were analyzed with descriptive statistics. Significant differences ($P < 0.05$) were observed for all the quantitative characters, except for leaf area and harvest index. Some qualitative characters, like stem and pod color, showed a reasonable level of variation among cultivars, whereas others, like leaf and seed color, had moderate variation. Seed size had the least variation. Hence, the quantitative and qualitative characters investigated proved useful in the appraisal of morphological variability in cowpea, which is very crucial in developing promising cowpea varieties. Expanding this study to involve more cowpea varieties and descriptors is recommended.

Keywords: Cowpea, crop improvement, diversity, germplasm, morphological characters

Key findings: The qualitative and quantitative characters used to appraise the six cowpea varieties revealed varying levels of morphological diversity. Quantitative characters discriminated the cowpea varieties more than qualitative characters. The study also revealed differences and similarities among the evaluated cowpea varieties, which is not only important in developing promising lines and in the grouping of genotypes, but also in the effective management and utilization of cowpea germplasm. This study has also strengthened the fact that morphological characters can be effectively used to characterize plants.

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INTRODUCTION

Traditionally, morphological characters are used to distinguish species. However, there are reports on the limitations of its use based on the environmental sensitivity of traits and the consequential effect of subjective characterization (William *et al*., 1990; Bhandari *et al*., 2017). The use of morphological descriptors to delimit species is the oldest and quickest way to identify or detect external differences (in shape, size, and color) in the forms and features of different plant organs, such as roots, stems, leaves, flowers, seeds, and fruits. Previous studies (Duminil and Michele, 2009; Dwari and Mondal, 2011) reported that the foremost characters used for the identification and description of plant taxa are morphological traits. Compared with other methods (biochemical, cytological, and molecular) for species delimitation, the use of morphological characters have the advantages of establishing high variation, easy accessibility and examination, and established descriptive terminology and does not require expensive technology (Szczepaniak and Ciéslak, 2011). Furthermore, morphological characters were reported to be the strongest determinant of the agronomic value of plants (Cholastova and Knotova, 2012).

Typically, phenotypic variability may be assessed with qualitative traits. The pattern of expression of these morphological characters is discrete or noncontinuous. Variation in qualitative traits falls into kinds or categories, and their expression is not influenced by the environment. Some scholars reported that qualitative traits are one of the most common non-tree-based approaches used for delimiting species over the years (Hlusko, 2004; Gaubert and Antunes, 2005; Gaubert *et al.*, 2005a). Quantitative characters, like qualitative traits, are also used to appraise morphological variability, but unlike qualitative traits they exhibit a continuous range of variation. They are called metric traits and hence cannot be analyzed on the basis of counts and ratios. Quantitative characters do not show a clear-cut difference between individuals in a population. Rather, they show a degree of variation among them. The variability observed in the field assessment of crops with quantitative characters has been reported to be due to genetic and environmental effects (Rohlf *et al*., 1990; Rohlf, 2000; Afolayan *et al.,* 2014).

To date, morphological characters are indispensable tools in field experimentation. In fact, there are several reports on the use of qualitative and quantitative morphological characters to appraise genetic variation, differences, relationships, and taxonomic status among the populations of different plant species. These reports include the assessment of morphological variation in Irish (*Brassica oleracea*) species (El-Esawi *et al*., 2012), the evaluation of the quantitative and qualitative morphological characters of sunflower (*Helianthus annuus*) germplasm (Purwati and Herwati,

2016), and the assessment of the taxonomic status of the Palawan pangolin *Manis culionensis* (Pholidota) by using discrete morphological characters (Gaubert, and Antunes, 2005). Quantitative and qualitative differences in morphological traits between diploid fragaria species have been revealed (Sargent *et al*., 2004). Therefore, this study was performed to appraise the variation that exists in six popular genotypes of cowpea in Nigeria on the basis of qualitative and quantitative morphological characters.

MATERIALS AND METHODS

Six cultivars of cowpea collected from different areas of Nigeria were used for the study (Table 1). The experiment was conducted during September 2017 to October 2018 cropping season at the Teaching and Research Farm of the Department of Crop Science and Technology, Federal University of Technology, Owerri, Imo State, Nigeria. The size of the experimental field was 14 m × 14.5 m, and the experimental design was randomized complete block design with five replications. Each block was divided into six plots to give a total of 30 experimental units. A distance of 1 m was allowed between blocks and that of 0.5 m was allowed between plots. The size of each plot was 2 m × 2 m. Two seeds, which were later thinned down to one, were planted in a 4 cm hole with a spacing of 45 cm between plants. Each cowpea cultivar was randomly assigned to each block, and plots were labeled accordingly. Good agronomic practices, like supplying, weeding, and stalking, were applied for optimal crop production.

Table 1. Passport data of the cowpea cultivars used for the study.

Cultivars	Locality	State
Oloko beans	Obanliku	Cross River
Sokoto beans	Sokoto	Sokoto
Potasio brown beans	Odalu	Kogi
Potasio white beans	Afikpo	Ebonyi
Honey beans	Kwami	Gombe
Iron beans	Nafada	Gombe

DATA COLLECTION AND ANALYSIS

A total of 16 morphological traits covering germination, vegetative growth, flowering and maturity stages were used to evaluate the qualitative and quantitative characters of the six cowpea cultivars (Table 2). The details of the various methods used for data collection are displayed in Table 2. Data collected from qualitative morphological characters were rated visually and grouped on the basis of colors and sizes by using the standard shape and color charts of the Royal Horticultural Society Color Chart. The mean of each evaluated quantitative character was compared to test for significant difference by using the analysis of variance (ANOVA) in Genstat statistical package software (Discovery edition 3).

Table 2. Quantitative and qualitative characters evaluated, and method of data collection.

Characters	Methods	Description
Emergence	By counting	Derived by counting the number of days it took the seeds of each cultivar to emerge.
50% Emergence	By counting	Derived by counting the number of days it took half of the total seeds planted for each cultivar to emerge.
Branches at flowering	By counting	Derived by counting the number of branches on sample plants of each cultivar at flowering.
Plant height at flowering	By measurement	Measurements were taken from the base of sample plants from the soil level to the last point of leaf formation at the tip by using a meter rule.
Days to flower bud initiation	By counting	Derived by counting the number of days it took from sowing to the day the first flower bud appeared on sample plants of each cultivar.
Days to anthesis	By counting	Derived by counting the number of days it took from sowing to the day the first flower opened in sample plants of each cultivar.
Peduncle length at flowering	By measurement	Measurements were taken on the length of the peduncle of sample plants from each cultivar.
Pod length	By measurement	Pod length from sample plants from each cultivar was measured with meter rule.
Leaf area at flowering	By measurement/ calculation	Determined by multiplying the leaf length by leaf breadth by the correction factor (0.75), as observed by Agbogidi and Ofuoku (2005). Mathematically expressed as L × B × K (0.75)
Number of pods per plant	By counting	The number of pods of sample plants of each cultivar was counted at maturity.
100-seed weight	by weighing	100-seed weight of each cultivar was determined with a weighing balance.
Leaf color at maturity	visual observation	Standard color charts were used.
Stem color at maturity	Visual observation	-do-
Seed color at maturity	Visual observation	-do-
Pod color at maturity	Visual observation	-do-

Harvest index = $\frac{\text{Economical yield}}{\text{Biological yield}} \times \frac{100}{1}$

RESULTS

Table 3 shows the variability in qualitative morphological characters displayed by the investigated cowpea cultivars. The level of variation exhibited by each trait differed from the other. Some traits, like stem color and pod color, showed high levels of variation among cultivars. There were four types of stem and pod color. The obtained stem colors were light green, dark red, green, and light red, and their percentage distribution among

Table 3. Variability in the qualitative morphological characters of cowpea cultivars.

Characters	Expression	Percentage %
Leaf color	Light green	33.33
	Green	33.33
	Dark green	33.33
Stem color	Light green	16.67
	Green	33.33
	Light red	33.33
	Dark red	16.67
Pod color	Light green	33.33
	Green	33.33
	Red	16.67
	Purple	16.67
Seed color	Milky	16.67
	White	66.68
	Red	16.67
Seed size	Small	50.00
	Large	50.00

the cowpea cultivars were as follows: light green, 16.67%; dark red, 16.67%; green, 33.33%; and light red, 33.33%. Similarly, the evaluated cowpea varieties had four types of pod colors, namely, light green, green, red, and purple. The percentage of the cowpea cultivars that had light green and green pod colors was 33.33% each, whereas those with red and purple pod colors were 16.67% each. The trait that showed the least variability among the six cowpea varieties was seed size. It resolved the cultivars into two types: small- and big-seeded. The percentage of the distribution that had small or big seeds was 50% for each. Furthermore, two traits showed moderate variation among the cultivars. They were leaf color and seed color. The leaf colors of the cowpea varieties were found to be of three types: light green, green, and dark green. Each leaf color constituted 33.33% of the distribution. The obtained number of the color of the seed was similar to that of the leaf. Invariably, there were three seed colors: white, milky, and red. The percentage distribution of the cowpea varieties to seed colors were as follows: light green, 16.67%: green, 66.66%; and dark red, 16.67%.

The ANOVA results for quantitative characters are shown in Table 4. Traits that showed significant differences ($P < 0.005$) were number of days to emergence, number of days to 50% emergence, plant height, number of branches, days to flower initiation, days to anthesis, peduncle length, pod length, number of pods per plant, and 100-seed weight. By contrast, leaf area and harvest index did not show any significant differences ($P > 0.005$) among the evaluated cowpea cultivars. A detailed study on the ANOVA result showed that some quantitative morphological characters clearly exposed the differences that existed among the evaluated cowpea varieties. Moreover, the ANOVA results for the assessment of variation based on quantitative morphological characters could be broken down into three phases: the germination phase,

Table 4. ANOVA for the quantitative morphological traits of cowpea cultivars.

Cultivars	Emergence	50% emergence	Plant height	Branches (#)	Leaf area (cm^2)	Days to flower initiation	Days to anthesis	Peduncle length (cm)	Pod length (cm)	Pods $plant^{-1}$	100 seed weight (g)	Harvest index
Honey beans	79.94*	4.00*	22.80*	5.45*	46.75	54.25*	56.50*	7.69*	12.84*	41.10*	124*	16.1
Iron beans	99.98*	3.02*	95.20*	7.75*	60.36	93.80*	95.50*	7.95*	6.89*	52.90*	407*	22.5
Oloko beans	92.30*	3.98*	132.00*	12.70*	50.02	44.45*	67.30*	7.04*	13.42*	53.40*	412*	13.9
Potasio brown	91.12*	3.00*	61.30*	13.15*	72.01	76.85*	79.80*	13.04*	8.72*	36.70*	178*	28.7
Potasio white	77.74*	2.98*	72.70*	10.90*	34.61	69.85*	72.70*	8.58*	9.19*	29.90*	246*	19.6
Sokoto	93.30*	3.00*	62.40*	12.35*	56.65	57.81*	62.40*	10.47*	9.77*	40.65*	369*	25.2
$LSD_{0.05}$	0.02	0.04	56.38	1.76	-	5.088	25.33	1.075	1.192	6.203	81.2	17.02

* significantly different at $P < 0.005$

Table 5. Statistical parameters for the quantitative traits of the evaluated cowpea cultivars.

Quantitative traits	Min	Max	Range	Mean	SD	CV
Emergence	77.74	99.98	22.24	89.06	7.78	60.55
50% emergence	2.98	4.00	1.02	3.33	0.46	0.21
Plant height	22.80	132.00	109.20	74.40	33.49	1121.91
Number of branches	5.45	13.15	7.70	10.33	2.84	8.07
Leaf area	34.61	72.01	37.4	53.40	11.64	135.67
Days to flower initiation	44.45	93.80	49.35	66.16	16.20	12.69
Days to anthesis	56.50	95.80	39.30	72.36	12.69	161.21
Peduncle length	7.04	13.04	6.38	9.21	2.05	4.20
Pod length	6.89	13.42	6.53	10.13	2.29	5.27
Pods per plant	29.90	53.40	23.50	42.44	8.41	20.78
100-seed weight	124.00	412.00	288	289.33	113.17	12807.89
Harvest index	13.90	28.70	14.80	21.00	5.09	25.92

the vegetative phase, and maturity (which encompassed flowering, fruiting, and harvesting). The quantitative morphological characters that were used to appraise the variation among the cowpea varieties based on germination stage were percentage emergence and number of days to 50% emergence. These traits showed statistically that clear differences existed among the cowpea varieties. Similarly, all the characters, except for leaf area, used to assess the vegetative phase (plant height, number of branches, leaf area, and peduncle length) showed that clear statistical differences existed among the cowpea varieties. The maturity phase characters (number of days to flower bud initiation, number of days to anthesis, pod length, number of pods per plant, 100 seed-weight, and harvest index), had similar results as the germination phase characters in that all showed significant statistical differences among the cowpea cultivars. However, as could also be deduced from the ANOVA (Table 4) results, the characters that most differentiated the cowpea varieties were number of days to emergence, number of days to 50% emergence, number of branches per plant, and pod length. Other quantitative morphological characters that also had a reasonable level of variation among cowpea cultivars were the number of days to flower bud initiation, number of pods per plant, and 100-seed weight.

The summary of the basic statistics, ranges, means, standard deviations, and coefficients of variation is shown in Table 5. These statistical tools were used for measuring the variation displayed by the quantitative characters across the evaluated cowpea cultivars. These results showed that 100-seed weight had the highest variability among the traits used to discriminate the cowpea varieties. It had a range of 288.00, mean of 289.33, standard deviation of 113.17, and coefficient of variation of 12807.89%. Another character that showed high variability among the cowpea varieties was plant height. The range, mean, standard deviation, and coefficient of variation values of this trait were 109.20, 74.40, 33.49, and 1121.91%, respectively. Days to anthesis and leaf area were two other characters that had a high level of variation among cowpea varieties. The range, mean, standard deviation, and coefficient of variation recorded for days to anthesis were 39.30, 72.36, 12.69, and 161.21%, respectively. Leaf area had a range of 37.40, a mean of 53.40, a standard deviation of 11.64, and a coefficient of variation of 135.67%. On the other hand, some traits had low values for range, mean, standard deviation, and coefficient of variation. These characters included days to 50% emergence, peduncle length, pods per plant, and number of branches per plant. In fact, the lowest value was recorded for days to 50% emergence. This character had a range of 1.02, mean of 3.33, standard deviation of 0.46, and coefficient of variation 0.21%. However, moderate variability was obtained for several other characters, such as days to emergence and days to flower initiation. The results clearly showed that number of days to emergence had a range of 22.24, mean of 89.06, standard deviation of 7.78, and coefficient of variation of 60.55, whereas the range, mean, standard deviation, and coefficient of variation

of the number of days to flower bud initiation were 49.35, 66.16, 16.20, and 12.69% respectively.

DISCUSSION

Analyzing the diversity in the germplasm of crops is very important in crop improvement programs. The establishment of variability in crops is very essential in the efficient management and utilization of crop germplasm. It exposes characters that are responsible for similarities and differences that exist within and among crop varieties. This has helped in the selection of desirable traits for the development of new varieties of crops and in the selection of lines with good agronomic properties as new crop varieties (pure line selection). Diversity analysis in crops has helped in the establishment of systematic relationships among crop varieties and in the simple clustering of crops into varieties (Beuselinck and Steiner, 1992; Karp and Edwards, 1995, Engels and Visser, 2003). Furthermore, the use of morphological characters in the study of genetic variability remains relevant because it provides a simple way of appraising variation that exists in a population of plant species while evaluating their performance under normal crop-growing conditions (Fufal *et al.,* 2005). In this study, qualitative and quantitative morphological characters were used to assess the morphological variation that existed in six cowpea cultivars. The evaluated morphological traits showed different levels of variation among genotypes. This result is consistent with the report of Sargent *et al*. (2004).

The result of this study showed morphological differences among the six cowpea cultivars for almost all the traits evaluated. Quantitative morphological characters, except for leaf area and harvest index, showed significant differences ($P < 0.005$) across genotypes. Invariably, these cowpea genotypes expressively differed from each other in relation to these quantitative characters that showed significant differences. This result can be used for the characterization of other cowpea cultivars. The coefficient of variation, which was used to measure genetic variability among the cowpea cultivars, showed a very high level of genetic variation for some traits, like number of days to emergence (60.55%), plant height (1121.91%), leaf area (135.67%), number of days to anthesis (161.21%), and 100-seed weight (12807.89%). It also showed that some traits had low variability, such as number of days to 50% emergence (0.21%), number of branches (8.07%), number of days to flowering (12.69%), peduncle length (4.20%), pod length (5.27%), and number of pods per plant (20.78%). However, the high variability obtained for more traits indicated that these genotypes exhibited reasonable genetic differences among each other with respect to these characters. Correspondingly, these two results (ANOVA and coefficient of variation) were in perfect agreement, both showing that the investigated cowpea cultivars had distinct morphological differences. This result has some implications in selection for crop improvement (Adebisi *et al*., 2001). Some workers reported distinct differences among the crop genotypes that were evaluated in different studies (Makane *et al*., 2011; Sargent *et al*., 2004).

Unlike that observed for quantitative characters, the level of variability obtained for the qualitative characters of the investigated cowpea cultivars were relatively low. Some traits, like seed size, scarcely delimited the cowpea cultivars. There were only two types of seed size: small and big. Neither of the two seed sizes dominated the distribution; rather, each constituted 50% of the distribution. This result has some genetic implication for selection for crop improvement (Hahn, 1997). By contrast, stem color and pod color showed very clear differences among the cultivars that can be exploited in crop breeding programs. The genotypes had four distinct stem colors (light green, dark red, green, and light red). Similarly, pods also had four different colors (light green, green, red, and purple). A previous study reported that most investigated qualitative morphological characters, except for one or two traits, showed reasonable differences among genotypes (Purwati and Herwati, 2016; Tan and Tan, 2011). Furthermore, the low variation obtained for some traits could be attributed to the low number of qualitative flowering traits used in discriminating among cowpea cultivars (El-Esawi, 2012).

The foregoing clearly showed that the quantitative and qualitative morphological characters used in the present study helped to establish the varietal similarities and differences that exist among the evaluated cowpea varieties. The morphological differences among the six cowpea cultivars were established with quantitative and qualitative characters that could be used to discriminate among the genotypes, whereas the traits that could not delimit the genotypes helped to buttress the similarities that exist amongst the cultivars. Invariably, the findings of this study can be used in the establishment of the general relationship that exists among the investigated cowpea cultivars (Engels and Visser, 2003) and in the simple clustering of the evaluated genotypes into groups (Karp and Edwards, 1995).

CONCLUSION

This study clearly demonstrated that quantitative and qualitative characters can be used to establish the morphological variations that exist in cowpea germplasm. Specifically, 10 quantitative and two qualitative characters significantly discriminated the six cowpea cultivars. Invariably, these morphological characters can be used for further characterization of cowpea germplasm, which is crucial in developing new or promising cowpea varieties. In summary, the investigated quantitative and qualitative characters proved to be useful in the appraisal of morphological variability in cowpea. However, a more detailed study wherein more morphological descriptors and varieties of cowpea will be used for the experiment should be carried out.

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