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Diallel Analysis of Pod Traits in Cowpea (*Vigna unguiculata* L. Walp.)

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Abstract: Knowledge on the nature of gene involved in the genetic expression of pod traits is necessary for efficient breeding and yield improvement in cowpea. The present study was undertaken at Dang (soudano-guinean zone of Cameroon) to evaluated genetic variability of three pod parameters *viz.*, pod length, pod diameter and number of seeds per pod of 15 cowpea (*Vigna unguiculata*) diversity germplasm and investigate the genetic basis of these traits through a 6x6 half-diallel cross mating. The experimental design was a randomized complete block with three replicates. Analysis of results showed that, these genotypes presented a significant variability for these characters ($p < 0.05$). High significant differences were observed for mean squares of general combining (GCA) and specific combining ability (SCA) of these characters. Globally, the three traits studied were highly heritable ($h^2 = 0.62-0.97$), with the variance due to genetic interactions greater than environmental variance. Narrow sense heritability ranged from 0.52 to 0.88, confirming the presence of additive gene action. Pod length and pod diameter were controlled mainly by additive gene whereas dominant gene were predominantly involved in the genetic control of number of seeds per pod. These results suggested that, breeding for increased grain yield of cowpea can be quite successful through recurrent selection.

Keywords: half-diallel, pod traits, soudano-guinean zone, *Vigna unguiculata*, yield improvement.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp), an indigenous African annual legume, is a high protein (25%), fiber (6.3%) and low fat (1.9%) vegetable crop having good nutritional qualities [1]. Cowpeas are an important part of the staple diet in many developing countries since the earliest practice of agriculture [2]. Traditionally in Africa, cowpeas are consumed as boiled vegetables using fresh and rehydrated seeds or processed into flour to make other food products [3]. It has potential of becoming an industrial crop and widespread consumption of convenience foods containing significant amounts of cowpea substantially increased the demand and value of cowpea seed [4, 5]. Dry seeds for human consumption are the principal product of the plant, but leaves, fresh peas, and fresh green pods are consumed by many poor people who do not have access to broadly based diet [4, 6]. In west and central Africa, cowpea is second in importance after groundnuts, with Nigeria accounting for over 70% of the total world production [7]. In Cameroon it is the third most important legume food crop after groundnuts (*Arachis hypogaea*) and common beans (*Phaseolus vulgaris*) and is frequently intercropped with cereals [8].

In northern Cameroon, litle informations were avalable on genetic improvement particularly for pod traits of *V. unguiculata*. The cultivars exhibit not only lower productivity, but non synchronous flowering and fruiting, non-response to high doses of inputs like fertilizers *etc.*, non-suitability of the various cropping systems, lodging and shattering susceptible, long duration, complete or partial absence of genetic resistance to major insect pest and diseases like mosaic virus, rust, powdery mildew and bacterial blight which cause considerable damage and very poor harvest indices [6]. Indeed, for further utilization in hybrid development in cowpea, pod length and number of seeds per pod were identified as best selection criteria in cowpea breeding [9]. According to Othman *et al.* [10], genetic information is needed for efficient breeding procedures which could lead to development of improved and high yielding cowpea varieties suitable for different ecological zones and cropping systems. Proper understanding of genetic variability and heritability studies of plant traits is vital for effective use of germplasm in any breeding program [11]. In addition, for the improvement of any traits, knowledge on the nature of gene action governing the quantitative trait is essential [12]. The purpose of this study was to evaluate in *V. unguiculata* the varietal differences and assess through diallel analysis the genetic control for these traits.

MATERIALS AND METHODS

Experimental Site

The research was carried out during 2006 to 2007 at the University of Ngaoundéré campus (1113 m altitude, 7.28°N latitude and 13.34°E longitude), which is located at Dang, a village of Ngaoundéré in the Adamawa region, Cameroon. This region belongs to the high altitude Guinea savannah ecological zone [13]. The soil is ferruginous type, developed on basalt and has a brown reddish clay texture. The climate is characterized by two seasons with an average annual rainfall of 1480 mm that is fairly distributed over the rainy growing period (April to September). The average annual temperature is 22°C, while the annual hygrometry is about 70% [14].

Plant material and experimental design

Fifteen genetic diversity of cowpea germplasm including four improved varieties were sourced from the National Institute of Agricultural Research for Development (IRAD, Cameroon) namely Lori-niébé, VYA, BR1 and CRSP (Collaborative Research Support Program); ten registered genotypes originated from the International Institute for Tropical Agriculture (IITA, Nigeria): ITK-452-1, ITK-610, ITK-589-2, ITK-568-18, IT97K-573-1-1, IT97K-1042-3, IT99K-573-2-1, IT00K-126-3, IT99K-1122-2 and IT99K-494-6; and one local landrace from northern Cameroon: Local Adamaoua (Local AD), were used for the study.

A preliminary field trial was conducted during 2006 growing season to evaluate the genetic variability of these materials for pod traits. The experimental design was a randomized complete block design (RCBD) with three replications. Cowpea plants were grown in an experimental area of 300 m^2 (30.0 m length × 10 m width). The plot unit consisted of one row of 15 m length with an inter-row spacing of 70 cm. Three seeds were sown with an intra-row spacing of 25 cm and later thinned to one plant per hill. A safety and protection distance of 2 m surrounded the experimental field. At flowering stage, experimental plots were sprayed with a standard insecticide formulation, cypermethrin + dimethoate, at the rate of 30 g + 250 g a.i. L^{-1} to control pod borers and other pests. At maturity, ten representative plants per treatment in each replication were selected randomly, tagged and observations were recorded on these plants for different characters *viz*., pod length, pod diameter and number of seeds per pod. The characters studied were assessed as described by Ajayi *et al*. [15] and Manggoel *et al.* [16].

Crossings

Six genotypes (IT97K-1042-3, IT99K-573-2-1, IT00K-126-3, IT99K-1122-2, Local AD and IT99K-494-6) were selected as parents for diallel crossing on the basis of their genetic variability for pod traits. These genotypes which were chosen were planted in pots for diallel analysis. At anthesis, plant-to-plant pollination of all possible crosses except reciprocals were made in 15 crosses combinations following the 6 × 6 diallel crossing pattern. Each cross was tagged for easy identification, and at maturity, the F$_1$ seeds were harvested separately. The six parental lines and the 15 F$_1$ hybrids obtained were planted in a RCBD with three replications during the 2007 rainy season. Plot unit size, spacing and treatments were as described above.

Statistical Analysis genetic analysis

The means of generations for each combination or those of the pure lines were subjected to analysis of variance (ANOVA) using STATGRAPHICS PLUS 5.0 software. The genotypic means were compared using Least Significant Difference at 5% level of probability (LSD 5%).The diallel analysis was done using DIAL 98 microcomputer package [17]. Griffing [18] method 2 (excluding reciprocal F$_1$ crosses) and model 1(fixed effects) were used to estimate the general combining ability (GCA) of the lines and the specific combining ability (SCA) of crosses. GCA and SCA estimates of parents and hybrids, respectively, were obtained as:

$$AGCi= Xi–X;\ ASCi= Xij–Xi–Xj + X$$

Where X is the general mean of the population, Xi is the mean of the hybrids from parent i, Xj is the mean of the hybrids from parent j, and Xij is the value of the hybrid from parents i and j.

It supplemented by the analysis of variance by Walters and Morton [19]. With this approach, the components of variation were partitioned into the additive effects (a) and the dominance effects (b) which were further sub-divided into b$_1$, b$_2$ and b$_3$. The genetic parameters were estimated as per [20]. Heritability in broad sense (h^2) was measured as the proportion of genetic variance (δ^2g) in the phenotypic variance between the parents (δ^2p), while heritability in narrow sense (h$_{2n}$) was calculated as the proportion of additive variance (δ^2_A) in the phenotypic variance between the parents (δ^2p) [21]. The simple additive-dominance model was tested by the regression of the covariance values between the parents and their offspring in the rth array (Wr) against variance values of the rth array (Vr). The correlation between parental values (Pr) and recessive factor (Wr+Vr) indicated the gene action for each trait [18].

RESULTS

Genetic variability for pod traits

The results of analysis of variance revealed that, the genotypes exhibited highly significant ($P<0.05$) differences for all traits studied (Table 1). The values ranged from 13.07 to 21.49 cm (mean = 15.60 ± 0.35 cm) for pod length, 6.58 to 9.88 mm (mean = 8.07±0.43 mm) for pod diameter and from 9.00 to 15.86 (mean = 11.45 ± 0.45) for number of seeds per pod. IT97K-573-1-1, IT00K-126-3 and IT99K-1122-2 were the parents which had high values for pod length, pod diameter and number of seeds per pod respectively.

Table-1: Genetic variability of fifteen cowpea genotypes for pod traits

Lines	Pod length (cm)	Pod diameter (mm)	Number of seeds per pod
Lori-niébé	13.80 ± 1.53^{def}	8.13 ± 0.41^{de}	9.93 ± 1.71^{ef}
VYA	13.75 ± 1.93^{def}	6.58 ± 0.42^{j}	10.87 ± 1.51^{cdef}
BR$_1$	14.13 ± 0.99^{def}	8.07 ± 0.74^{ef}	10.47 ± 2.41^{def}
CRSP	13.5 ± 0.69^{ef}	8.38 ± 0.32^{cde}	11.06 ± 1.75^{cde}
ITK-452-1	15.91 ± 1.63^{cde}	7.71 ± 0.43^{efg}	10.66 ± 1.92^{def}
ITK-610	15.39 ± 1.38^{def}	6.96 ± 0.34^{ij}	10.80 ± 1.56^{cdef}
ITK-589-2	16.15 ± 1.15^{cde}	8.23 ± 0.67^{cde}	10.73 ± 2.54^{def}
ITK-568-18	15.51 ± 1.17^{def}	7.55 ± 0.23^{fgh}	12.86 ± 0.74^{bc}
IT97K-573-1-1	21.49 ± 1.39^{a}	8.62 ± 0.26^{bc}	13.33± 1.34^{b}
IT97K-1042-3	16.43 ± 1.19^{cd}	8.24 ± 0.47^{cde}	12.27 ± 1.66^{bc}
IT99K-573-2-1	19.36 ± 1.86^{ab}	8.88 ± 0.68^{bc}	12.00 ± 1.81^{bcd}
IT00K-126-3	18.54 ± 1.71^{bc}	9.88 ± 0.74^{a}	10.93 ± 1.94^{cdef}
IT99K-1122-2	13.27 ± 0.83^{f}	9.08 ± 0.72^{b}	15.86 ± 1.12^{a}
Local Adamaoua	13.07 ± 0.89^{f}	7.68 ± 0.38^{efgh}	9.00 ± 1.06^{f}
IT99K-494-6	13.63 ± 1.25^{def}	7.14 ± 0.33^{ghij}	11.00 ± 2.03^{cdef}
Mean	15.60 ± 0.35	8.07 ± 0.43	11.45 ± 0.45
LSD (5%)	2.84	0.65	2.06

Means with the same subscript within the same column do not differ ($p > 0.05$); LSD (0.05): least significant difference at 5% level.

Genetic analysis

ANOVA based on the method of Walters and Morton [19] showed that, both additive (a) and dominance (b) effects were all significant ($p < 0.01$) for pod length, pod diameter and number of seeds per pod (Table 2). Within the dominance components (b_1, b_2 and b_3), the mean dominance effects (b_1), the additional dominance effects due to the parents (b_2) and the residual dominance effects (b_3) were also strongly significant ($p < 0.01$) for these traits.

Table-2: Mean squares for ANOVA of 6 x 6 half-diallel for pod traits in cowpea

		Mean square for pod traits		
Source	Df	Pod length	Pod diameter	Number of seeds/Pod
Replications	2	0,60^{ns}	0.22^{ns}	0.41^{ns}
a	5	115.33**	18.67**	83.61***
b	15	232.61**	19.56**	130.33***
b_1	1	21.36***	1.24**	16.19***
b_2	5	3.73*	3.72**	15.06**
b_3	9	207.94***	14.59**	99.08**
Error	40	8.73	1.78	8.25

Df: Degree of freedom; a = Additive effects of genes; b = dominant effects of genes; b_1 = mean dominance effects; b_2 = additional dominance deviation due to the parents, b_3 = residual dominance effects, ns: not significant; * indicates significance at 5%, ** and *** indicates significance at 1%.

For each pod trait, the genetic parameters (average degree of dominance, product of additive by dominance, correlation between the degree of dominance and parental value) as well as the broad and narrow sense heritability values were presented in Table 3. For these characters, broad sense heritability ranged from 0.62 (pod length) to 0.97 (number of seeds per pod) while narrow sense heritability ranged from 0.52 (Number of seeds per pod) to 0.88 (pod length). The average degree of dominance $(H_1/D)^{1/2}$ was greater than one only for the number of seeds per pod. The parents tested had a moderate proportion of dominant genes for all traits. Product of additive by dominance (F) was negative for pod length and less than unity for pod diameter. The correlation between parental values (Pr) and recessive factor (Wr+Vr) was positive and significant only for pod length.

Table-3: Genetic components estimates and heritability values for cowpea pod traits based on a 6 x 6 half-diallel

Genetic parameter	Pod traits		Number of seeds/Pod
	Pod length	Pod diameter	
$(H_1/D)^{1/2}$	0.73	0.80	1.17
Kd	0.39	0.62	0.59
F	-2.44	0.47	2.41
h^2	0.62	0.88	0.97
h_{2n}	0.88	0.65	0.52
r (Pr,Wr+Vr)	0.86*	-0.09^{ns}	0.18^{ns}

$(H_1/D)^{1/2}$: Average degree of dominance; kd: Proportion of dominant genes; F: Product of additive by dominance; h^2: Broad sense heritability; h_{2n}: Narrow sense heritability. r (Pr, Wr+Vr): Correlation between the degree of dominance of the parents (Wr+Vr) and the parental value (Pr); ns: not significant; * significant at the 5% level.

The highly significant difference in mean squares implied that there is discernable evidence of inherent genetic variability among the cowpea accessions for pod traits. The significance of combining abilities for three traits shows the importance of both additive and dominance effects for these characters. For pod length and pod diameter, the ratio δ^2GCA/δ^2SCA is greater than unity.

Table-4: ANOVA for combining ability of pod traits in 6 x 6 half-diallel cross of cowpea

Source	Df	Pod length	Pod diameter	Number of seeds/Pod
GCA	5	171.57***	12.09**	60.01***
SCA	9	34.83**	3.82^{ns}	58.18**
Error	28	9.72***	8.17**	11.74***
$\boldsymbol{\delta}^2$GCA/ $\boldsymbol{\delta}^2$SCA		2.21	1.41	0.48

Df: Degree of freedom; GCA: Variation due to general combining ability; SCA: variation due to specific combining ability; Error: error variation or interaction between the replication and genotypes; δ^2GCA: variance of general combining ability; δ^2SCA: variance of specific combining ability; ns: not significant; **and *** indicates significance at $P \leq 0.01$ respectively.

The estimates of GCA effects of the parents (Table 5) revealed that, IT99K-573-2-1 showed highly positive and significant values for pod length (2.21) and pod diameter (0.45) whereas IT99K-1122-2 exhibited highly positive and significant values for number of seed (1.92).

Table-5: Predicted general combining ability effects (GCA) for pod traits of six cowpea cultivars on a 6 x 6 half-diallel

GCA effect of pod traits			
Parents	Pod length	Pod diameter	Number of seeds/Pod
IT97K-1042-3	0.37*	-0.61*	-0.73*
IT99K-573-2-1	2.21**	0.45**	0.17**
IT00K-126-3	1.71**	0.30*	-0.14*
IT99K-1122-2	-1.36	0.40**	1.92**
IT99K-494-6	-1.16*	-0.42*	-0.70*
Local AD	-1.77	-0.12	-0.51
SE	0.29	0.21	0.35

SE: Standard error; *significant at P = 0.05 and **significant at P = 0.01.

Among fifteen crosses combinations (Table 6), the hybrids IT99K-573-2-1 x IT99K-494-6 (high x low general combiners) IT00K-126-3 x Local AD (moderate x moderate general combiners) and IT00K-126-3 x IT99K-1122-2 (moderate x low general combiners) for pod length; IT00K-126-3 x IT99K-494-6 (high x high general combiners) for pod diameter and IT99K-494-6 x Local AD cross combination (low x low general combiners) for number of seed were identified as good specific combiners.

Table-6: Estimation of specific combining ability (SCA) of fifteen cowpea crosses in 6 x 6 half dialer

Crosses	Pod length	Pod diameter	Number of seeds/Pod
IT97K-1042-3 x IT99K-573-2	0.62*	0.10*	0.72*
IT97K-1042-3 x IT00K-126-3	0.18*	0.24**	-0.03
IT97K-1042-3 x IT99K-1122-2	0.75*	0.10*	-0.03
IT97K-1042-3 x Local AD	-0.11	-0.17	-1.97
IT97K-1042-3 x IT99K-494-6	-1.44	-0.26	1.25**
IT99K-573-2-1 x IT00K-126-3	-0.89	-0.04	1.18**
IT99K-573-2-1 x IT99K-1122-2	-1.76	-0.02	-1.32
IT99K-573-2-1 x Local AD	0.82*	-0.05	-0.36
IT99K-573-2-1 x IT99K-494-6	1.21**	0.01	0.30*
IT00K-126-3 x IT99K-1122-2	1.07**	-0.68	0.05
IT126-3 x Local AD	0.10*	-0.02	-0.36
IT00K-126-3 x IT99K-494-6	-0.46	0.49**	-0.84
IT99K-1122-2 x IT99K-494-6	0.72*	0.06	-1.34
IT99K-1122-2 x Local AD	-0.78	0.54**	2.58**
IT99K-494-6 x Local AD	-0.03	-0.30	0.64*
SE	0.48	0.37	0.52

SE: Standard error; *significant at P = 0.05 and **significant at P = 0.01.

DISCUSSION

Significant differences among the fifteen cowpea lines for pod traits suggested large variability in the material. Genetic diversity for these traits indicated the possibility of their improvement through genotypic selection. Variability among cowpea varieties for these traits has previously been reported by Manggoel *et al.* [16]; Umaharan *et al.* [22]; Ubi *et al.* [23]; Omoigui *et al.* [24] and Nwosu *et al.* [25]. It suggested these traits maybe under genetic control rather than environmental influence. The presence of sufficient variability for a character could be considered valuable for further biometrical assessments. The genetic variation can be used in breeding programs to improve the potential of seed yield and earliness.

The significance of additive (a) and dominance (b) components showed the presence of both additive and dominance in the expression of these traits. The significance of the dominance deviation (b_1) for these parameters indicated that, the dominance was unidirectional effect in the direction of the best parent [19]. The significant the additional dominance deviation due to the parents (b_2) item illustrated an asymmetrical distribution of dominant genes among the parents, reflecting that some parents harbored considerably dominant genes than others. Dominant and recessive loci are not harmoniously distributed among the parents. The significance of the residual dominance (b_3) for these characteristics confirmed the presence of specific dominance or combining ability in some crosses.

Globally, high estimates of heritability in broad sense indicated that, the large proportion of the total variance was due to the genotype; hence selection would be effective for these characters. Narrow sense heritability values for traits studied confirmed the presence of additive gene action. In cowpea, Nwosu *et al.* [25] and Idahosa *et al.* [26] obtained an important value of broad sense heritability for pod traits. Martinez *et al.* [27] reported that full dominance in a negative sense was observed for pod length. Additive x dominance and additive x additive interaction effects were noted for pod length by Umaharan *et al.* [22]. The correlation between parental value (Pr) and recessive factor (Wr+Vr) for pod length suggested that, it may be therefore be concluded that the breeding value of a parent is closely associated with frequency of additive alleles in that parent for this trait.

The ratio between GCA and SCA was less than unity for except for number of seeds per pod indicating that, non-additive gene action was responsible for the genetic expression of this character. Hence, this result demonstrated that, this parameter can be enhanced through exploitation of heterosis. For pod length and pod diameter, the ratio δ^2GCA/δ^2SCA which was greater than one revealed that, further evidence for presence of additive gene action. Therefore, improvement of these traits can be achieved through direct selection. This observation was an agreement with information which was reported by Kwaye *et al.* [9] for pod traits on cowpea. In contrast, previous study reported by Kumar and Sangwan [28], Rangiah [29] and Nagaraj *et al.* [30] showed that, these parameters were controlled mainly by non-additive gene.

High GCA effects for pod traits are attributable to additive or additive x additive gene interaction, which represent the fixable genetic components of heritable variance. Parents IT97K-1042-3, IT99K-573-2-1, IT00K-126-3 and IT99K-1122-2 with high GCA effects may be used in a multiple crossing program for isolating desirable lines in cowpea. High GCA effects of the parent along with high *per se* performance can be considered as best combiner for hybridization and selection in the breeding program for traits governed by additive and additive x additive gene [21]. In Vigna unguiculata, *Singh et al.* [12], Ushakumari *et al.* [31] and Patel *et al.* [32] reported an important GCA effects for pod traits.

The variation among crosses implied that hybrid vigor depends on the choice of the complementary parents. Combinations with high/low, moderate/moderate, high/high low/low general combiners showing high SCA effects, may produce transgressive forms. High SCA effects result from gene dispersion and genetic interaction between favorable alleles from both combiners. *Singh et al.* [12], Ushakumari *et al.* [31] and Patel *et al.* [32] Recorded highly positive and significant SCA effects in hybrids F_1 cowpea. According to Patel *et al.* [32], high SCA effects due to high x high combiners reflect additive x additive type of gene interaction and superiority of favorable genes contributed by their parents, while those involving high x low or low x low indicated the interaction of additive x dominance and dominance x dominance, respectively.

CONCLUSION

Cowpea genotypes were highly variable for pod traits. These characteristics could be genetically improved and were controlled by additive and non-additive genes. Recurrent selection might be a useful breeding strategy. Improved methods to predict genetic gain and evaluate these quantitative traits without the environmental influence are also needed.

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