GENETIC CONTROL OF SEED SIZE IN COWPEA (*Vigna unguiculata* (L.) Walp)

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Abstract- Seed size contributes to yield and it is also a consumer trait of cowpea in Ghana and other parts of the world. Ghanaian consumers tend to prefer large seeded cowpea to small sizes although, Ghanaian varieties have smaller seeds compared to the imported types. This might be a contributing factor to high importation of cowpea to Ghana. To breed cowpeas with large grains, it is paramount to understand the inheritance of the trait. Generation mean analysis was performed to estimate gene actions on seed size of cowpea using CB27 and Gh3710 as the large and small seeded parents respectively. It was observed that eight genes control seed size and that small seed is partially dominant over large seed. The estimated broad sense heritability was 84% while the narrow sense was 35%. Both additive and non-additive genes with their interactions in the exception of additive - additive had significant effect on seed size inheritance in cowpea. The inheritance of seed size was observed to be complex, it was suggested that combinations of methods such as single seed descent and backcross methods with selection from early generations through advanced level would help improve seed size of cowpea.

Keywords- Additive Gene, Cowpea, Dominance, Generation Mean, Heritability, Seed size

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Introduction

Seed size is of interest to many people for the reason that 70-80% of the world's food calories and 60-70% protein consumed by man come from seeds [1]. The effort to increase the number of seeds or their sizes relates to increasing food production [2]. The genetic control of the final seed size is complex as a number of factors, although, discovered (mainly in *Arabidopsis thaliana*), the control of the trait is not well understood. According to Sundaresan [3] three different genes are involved in endosperm size control with *IKU1* acting upstream of *MINI3* and *IKU2*. A factor named GW2 was discovered in rice to induce cell proliferation in grains thereby increasing its size [4]. Li, et al [5] reported that "*DA1* plays a major role in setting final sizes of both seeds and organs by restricting the duration of proliferative growth". In addition to *AUXIN RESPONSE FACTOR2*, *APETALA2*, and *DA1*, Adamski, et al [6] reported *KLU* which acts independently of other maternal factors to influence seed coat cell proliferation. EOD3 and CYP78A9 gene families were reported to control seed size in Arabidopsis in both overlapping and distinct manner [7].

Each of the three major components of a seed namely; the seed coat, the endosperm (cotyledon) and the embryo contribute to its final size. The integument (seed coat) determines the maximum volume the seed can grow [7]. The size of the integument itself is specified by its cell number and size [2]. In most plants, the endosperm is the major contributor to the volume of the mature seed; however, in *Arabidopsis* and some other dicots, the endosperm is consumed, being replaced by the embryo, which then constitutes most towards the mature seed size [3].

Cowpea seed size which is an important component of seed yield is also an essential market trait as consumers in Ghana tend to prefer larger seeds to smaller ones [8]. Survey in some markets in Ghana has revealed that the varieties in cultivation in the country generally smaller seeds than imported ones. It will therefore be imperative to breed larger seeded cowpea. However, the mode of inheritance of the trait must first be understood before setting it as a breeding goal.

Inheritance of seed size of cowpea has been of interest to plant breeders for very long time. Drabo, et al [9] indicated that contradictory reports have been given on inheritance of seed size in cowpea since 1950. They also reported that cowpea seed weight (size) is governed by many genes acting mainly additively with small size partially dominant over large. Additive gene effect is desirable as progress can be made through selection. Yilwa [10] reported large seed to be dominant over small seed, continuing the contradiction

and therefore warranting the study of the trait among genotypes available to the individual for cowpea improvement purposes.

Cowpea seed is formed through the process of double fertilization as in angiosperms. The size of the seed formed following double fertilization is affected by maternal, paternal and positional effects jointly [11]. The positional effect is the contribution of the zygote to the seed size.

Relationship between genetic effects and the way traits are transmitted from parents to their progenies is important in crop improvement. Several genetic designs have been developed to measure the relative importance of additive, dominance and epistasis which are the main gene actions in the expression of quantitative traits like seed size. Generation mean analysis proposed by Mather and Jinks is a design favored by breeders and geneticists working with self-pollinated species [12].

Materials and Methods

Cowpea genotypes, CB27 and Gh3710 with respective seed weights of - and - were used for the experiment. CB27 was obtained from the University of California Riverside while Gh3710 was obtained from CSIR-PGRRI. Both genotypes flowered about the same number of days after planting (32 days). The parents were crossed and subsequently the generations, F_1, F_2, BC_1, BC_2 together with the parents used for generation mean analysis for seed size. The experiment was conducted at Ohawu [Latitude = 6*03'50.74812''N; Longitude = 0*54'49.85784''E] in the coastal savanna of Ghana in 2013.

The experiment was conducted on field under rain-fed condition. The experiment was laid in a randomized complete block design with 10 replications for the parents and F_1 populations while the Backcrosses and F_2 were replicated 30 and 60 times respectively. The between row spacing was 100 cm and the within row 50 cm, while between block was 200 cm.

One hundred seed mass per plant was weighed with a digital scale in grams.

Results

Differences in Seed Weight of Various Generations

Analysis of variance showed significant differences between 100 seed weight of the various generations of plants used as treatments. Boxplot of the seed weight is shown in [Fig-1].

The means and standard errors (SE) of the various generations are shown in [Table-1].

Table 1- Average of 100 seed weight (g) and standard errors for various cowpea generations with a matrix showing LSDs (5%) with non-significant pairs indicated with asterisks (*)

Generation	Mean ± SE	LSD					
1 (BCP$_1$)	16.79 ± 0.2340	-					
2 (BCP$_2$)	13.52 ± 0.2340	0.63	-				
3 (F$_1$)	15.92 ± 0.4243	0.97*	0.97	-			
4 (F$_2$)	15.98 ± 0.1892	0.63	0.63	0.97*	-		
5 [CB27 (P$_1$)]	22.58 ± 0.4243	0.97	0.97	1.1	0.97	-	
6 [Gh3710 (P$_2$)]	11.20 ± 0.4243	0.97	0.97	1.1	0.97	1.1	-
	Generation	1	2	3	4	5	6

Note: The number of observations for P$_1$ P$_2$ and F$_1$ = 10; BCP1 and BCP2 = 30 and F$_2$ = 60

Matrix pairing of the generations is also shown in the Table and the generations with significant differences in 100 seed weight indicated with asterisks. There were only two non-significant pairs of generations; F_1 against F_2 and BC_1.
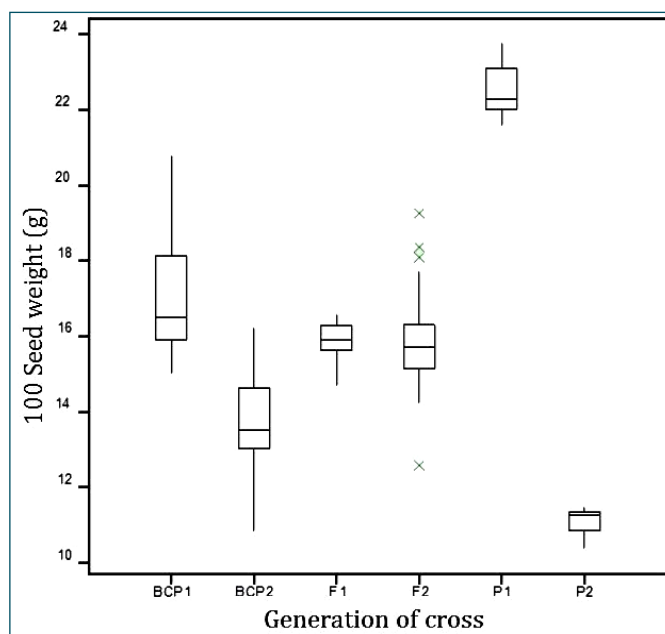

Fig. 1- Boxplot of 100 seed weight (g) of different generation of cowpea

Heritability of 100 Seed Weight in the Broad Sense

Broad sense heritability ($H_b{}^2$), of 100 seed weight of cowpea was estimated by the formula of Ito & Brewbaker [13] as shown in [Eq-1].

$$H_b{}^2 = (VF_2 - VE) / VF_2: \qquad (1)$$

where,

$H_b{}^2$ = Broad sense heritability

VE = Error variance = $\frac{1}{4}(VP_1 + VP_2 + 2VF_1)$ = 0.39255

VF_2 = Variance of F_2 family = 2.4722

VP_1 = Variance of parent 1 = 0.3686

VP_2 = Variance of parent 2 = 0.2084

VF_1 = Variance of F_1 family = 0.4966

$H_b{}^2$ = 0.84

The estimated broad sense heritability for 100 seed weight is therefore 84%.

Narrow Sense Heritability

The method of Warner [14] was used to estimate narrow-sense heritability (h^2ns) [Eq-2]

$$h^2ns = [2VF_2 - (VBCP_1 + VBCP_2)] / VF_2 \qquad (2)$$

where,

VF_2, $VBCP_1$ and $VBCP_2$ are the variances of the F_2, CB27/F_1 and Gh3710/F_1 respectively.

VF_2 = 2.472

$VBCP_1$ = 2.54

$VBCP_2$ = 1.539

h^2ns = 0.35

The estimated narrow sense heritability for 100 seed weight is therefore, 35%.

Number of Effective Factors (Genes)

The number of effective factors controlling seed weight was estimated using the method of Burton [15], shown in [Eq-3]

$$k = [0.25(0.75 - h + h^2) D^2] / (VF_2 - VF_1) \qquad (3)$$

where,

D = P_1 – P_2: = 11.38

h = (F_1 - P_2) / P_1 – P_2 = 0.4148

k = minimum number of effective factors\

VF_2 = Variance of F_2 population = 2.47222

V^2F_1 = Variance of F_1 population = 0.4966

P_1 = mean of parent 1 = 22.58

P_2 = mean of parent 2. = 22.58

Number of effective factors controlling seed mass = 8

The Degree of Dominance

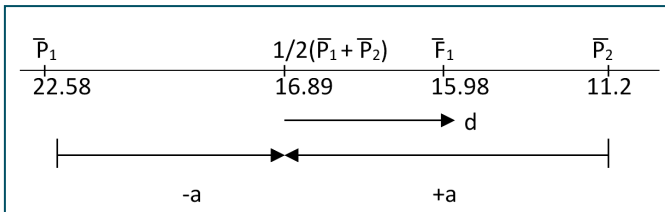The degree of dominance is the deviation from the mid-parent value towards one of them as illustrated [Fig-2].

Fig. 2- Diagrammatic representation of the genotypic values based on Falconer [16]

d = F1 – ½ (P1 +P2)

= 15.98 – ½ (22.58 + 11.2)

= - 0.91 P2 is dominant over P1 making d negative

a = ½ (P1 – P2)

= 5.69

Degree of dominance = d/a = 0.1599 = 16%

The mean value, that is, the F1 value is closer to the smaller seeded variety than to the larger which means that the former is the dominant variety.

Gene Action Controlling 100 Seed Weight

Generation mean analysis was used to estimate genetic control of seed weight in cowpea

The GMA model is stated below:

Y = m + αa + βd + α²aa + 2αβad +β²dd

α and β are the coefficients for a and d, respectively.

Y = the observed mean

m = mean = mean of the F2

a = pooled additive effects

d = pooled dominance effects

aa = additive x additive gene interaction effects

ad = additive x dominance gene interaction effects

dd = dominance x dominance gene interaction effects

The mode of inheritance of 100 seed weight in cowpea was estimated by generation mean analysis with six generations (P_1, P_2, F_1, F_2, BCP_1 and BCP_2) of CB27 and Gh3710. Following significant differences in 100 seed weight of the various generations, their means and variances were used to perform generation mean. Vital

results from regression analysis from SAS are presented in [Table-2]. The generation mean analysis indicated that there were additive and dominance gene effects and their interactions in the inheritance of the trait.

Table 2- Analysis of variance and parameter estimates for genetic control of 100 seed weight of cowpea obtained through generation mean analysis with CB27 and Gh3710.

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Type I SS	Type II SS
Intercept	1	18.747	1.722	10.89	<.0001	100712	487.836
Rep	1	0.008	0.012	0.65	0.5151	0.743	1.753
a	1	-5.678	0.217	-26.21	<.0001	3196.69	2828.02
d	1	-9.706	3.469	-2.8	0.0058	39.062	32.234
aa	1	-1.99	1.682	-1.18	0.2387	36.671	5.763
ad	1	4.775	0.674	7.09	<.0001	200.316	206.906
dd	1	6.61	1.917	3.45	0.0007	48.964	48.964

Note: (a) additive; (d) dominance; (aa) additive-additive; (ad) additive-dominance; (dd) dominance-dominance gene effects.

Discussion

Significant differences were observed between the various generations of plants from the cross of CB27 and Gh3710 in terms of 100 seed weight. The means of F_1, F_2 and the backcrosses fell between the two parental means of 11.2g and 22.58g, showing no transgressive segregates. This result is similar to that of Lopez, et al [17], although, comparably each of their generations had smaller weight with their small and large seeds having 100 seed weight of 4.82g and 15.14g respectively. Lucas, et al [18] on the other hand used slightly heavier genotypes (11.6g and 26.41g) as the parents in QTL studies for seed size.

The broad sense heritability estimated for 100 seed weight in this research was 84%. This value is very close to 83.3% reported [19]. Drabo, et al [9] also reported heritability estimate of 85% while Manggoel, et al [20] reported 86.84%. Omoigui, et al [21] and Tchiagam, et al [22], however, reported broad sense heritability as high as 98.9% and 90% respectively for the trait. Egbadzor, et al [23], however, used association mapping with SNP markers and reported 96% broad sense heritability for 100 seed weight in cowpea.

The low narrow sense heritability observed may have been caused by the significant additive dominance and dominance dominance epistatic effects. However, such low narrow sense heritability has been reported: Adeyanju, et al [24] for example reported 15 and 25% from two different crosses. Relatively higher narrow sense heritability for 100 seed weight of 67% was reported for cowpea by Tchiagam, et al [22]. These reports suggest that breeding methodology to improve seed size in cowpea may be influenced by the genotype and environment in which the breeding is to take place.

Findings in this experiment confirm that of Drabo, et al [9] that small seed size is partially dominant over larger seed. Partial dominance genetic variance was evident in that the F_1 of CB27/Gh3710 resembled Gh3710 more than CB27 in terms of seed size. This means that small seed size is partially dominant over large seed. Dominance gene effect was observed to be important in seed size inheritance in lentil as well [25]. However, the finding in this experiment was contrary to the observation of Lopez, et al [17] who stated that large seed in cowpea is rather partially dominant over the small. These contradictory findings may be due to the genotypes used in the various studies. It was also observed that seed from none of the

generations namely: P_2, F_1, F_2, BCP_1 or BCP_2 equaled the weight of P_1 (CB27). Similarly, Fatokun, et al [19] had no F_2 seeds as heavy as the heavy parent used in their experiment.

The number of gene pairs estimated to control seed weight was eight, the exact number reported by Drabo, et al [9] in spite of different genotypes used in the separate trials. Lopez, et al [17], however, estimated five factors. The genetic effect of these genes, being it additive, dominant or their interactions would determine progress that could be made in breeding.

Additive and non-additive gene effects with their interactions were shown to significantly control seed size in cowpea in the exception of additive - additive (aa) in the current experiment. This means that (a), (d), (ad) as well as (dd) were significant. However, the magnitude of the significant fixable gene action (a) has smaller magnitude compared with the non-fixable (d), (ad) and (dd)) [26]. Gene interaction observed was "duplicate" because (d) was negative while (dd) was positive [25]. The negative (d) also shows that small seed is dominant over large seed. The presence of two or more of the eight factors estimated to control seed size in this experiment would act in duplicate manner [27] to affect the trait. Although, (aa) gene effect was not significant, the associated negative sign indicates the dispersion of seed weight genes in both parents (CB27 and Gh3710).

Significant (a) gene effect is an assurance that gains can be made with selection in the long term [28] and in this case for seed size in cowpea. Seeds of the backcross generations in this experiment are indication of the practicality of this principle by being significantly larger or smaller than the F_1s. The backcross to the larger seeded parent (CB27) resulted in larger seeds while the F_1 crossed to the small seeded parent (Gh3710) were also smaller than the F_1.

Both fixable and non-fixable gene effects are important and therefore make breeding for cowpea seed size complex. The presence of significant (a) gene effect would make selection from early generation effective while selection at advanced generations would also be effective because of significant (ad) and (dd). The implication is that selection for seed size for cowpea should commenced from the segregation population through advanced level where varieties are at evaluation stage.

Conclusion

It was observed in the experiment that eight genes control 100 seed weight in cowpea using the genotypes CB27 and Gh3710 as parents in generation mean analysis. Small seed size in partially dominant over large seed. Additive, dominant, additive - dominant and dominant - dominant gene actions were found to be significant. The inheritance of seed weight is complex and suggesting that a combination of different approaches to improve the trait is a panacea to succeed contrary to the use of a single approach. Such methods as combination of single seed descent and backcrosses with selection from early generation through to advanced state may be used to make progress.

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Conflict of Interest: None declared.

References

[1] Lu J., Zhanga C., Baulcombe D.C. and Chen Z.J. (2012) *Proceedings of the National Academy of Sciences*, 109(14), 5529-5534.

[2] Herridge R.P., Day R.C., Baldwin S. and Macknight R.C. (2011) *Plant Methods*, 7(1), 3.

[3] Sundaresan V. (2005) *Proceedings of the National Academy of Sciences,* 102(50), 17887-17888.

[4] Song X., Huang W., Shi M., Zhu M. and Lin H. (2007) *Nature Genetics*, 39(5), 623-630.

[5] Li Y., Zheng L., Corke F., Smith C. and Bevan M.W. (2008) *Genes Dev.*, 22(10), 1331-1336.

[6] Adamski N.M., Anastasiou E., Eriksson S., O'Neill C.M., Lenhard M. (2009) *Proceedings of the National Academy of Sciences*, 106(47), 20115-20120.

[7] Fang W., Wang Z., Cui R., Li J., Li Y. (2012) *The Plant Journal*, 70(6), 929-939.

[8] Langyintuo A.S., Lowenberg-DeBoer J., Faye M., Lambert D., Ibrod G., Moussa B., Kergna A., Kushwaha S., Musa S. and Ntoukam G. (2003) *Field Crops Research*, 82(2) 215-231.

[9] Drabo I., Redden R., Smithson J.B. and Aggarwal V.D. (1984) *Euphytica*, 33, 929-934.

[10] Yilwa V. (2012 *Genetics of Cowpea - Inheritance of Qualitative and Quantitative Traits*, Lambert Academic Publishing, 175-178.

[11] House C., Roth C., Hunt J. and Kover P.X. (2010) *Proceedings of the Royal Society B: Biological Sciences*, 277(1695), 2885-2893.

[12] Piepho H.P and Mohring J. (2010) *Crop Science*, 50, 1674-1680.

[13] .Ito G.M. and Brewbaker J.L. (1991) *J. Amer. Soc. Hort. Sci.*, 116, 1072-1077.

[14] Warner J.N. (1952) *Agron. J.*, 44, 427-430.

[15] Burton W.G. (1951) Agronomy Journal, 43, 409-417.

[16] Falconer D.S. and Mackay T.F.C. (1996) *Introduction to Quantitative Genetics*, Pearson Education ltd, England.

[17] Lopes F.C.C., Gomes R.L.F. and Filho F.R.F. (2003) *Scientia Agricola*, 60(2), 315-318.

[18] Lucas M.R., Huynh B.L., Vinholes P.S., Cisse N., Drabo I., Ehlers J.D., Roberts P.A., Close T.J. (2013) *Frontiers in Plant Sciences*, 4(95), doi: 10.3389/fpls.2013.00095.

[19] Fatokun C.A., Menancio-Hautea D.I., Danesh D. and Young N.D. (1992) *Genetics*, 132, 841-846.

[20] Manggoel W., Uguru M.I., Ndam O.N. and Dasbak M.A. (2012) *Journal of Plant Breeding and Crop Science*, 4(5), 80-86.

[21] Omoigui L.O., Ishiyaku M.F., Kamara A.Y., Alabi S.O., Mohammed S.G. (2006) *African Journal of Biotechnology*, 5(13), 1191-1195.

[22] Tchiagam J.B.N., Bell J.M., Ngakeu D.F., Njintang N.Y. and Youmbi E. (2011) *Agric. Biol. J. N. Am.*, 2(4), 698-707.

[23] Egbadzor K.F., Yeboah M., Danquah E.R., Ofori K and Offei S.K. (2013) *Int. J. Plant Breed. Genet.*, 7(2), 115-123.

[24] Adeyanju A.O., Ishiyaku M.F., Echekwu C.A. and Olarewaju J.D. (2012) *African Journal of Biotechnology*, 11(46), 10473-10483.

[25] Khodambashi M., Bitaraf N. and Hoshmand S. (2012) *J. Agr. Sci. Tech.*, 14, 609-616.

[26] Hakizimana F., Ibrahim A.M.H., Langham M.A.C., Rudd J.C. and Scott D. (2004) *Euphytica*, 139, 133-139.

[27] Suzuki D.T., Griffiths A.J.F., Miller J.H. and Lewontin R.C. (1989) *An Introduction to Genetic Analysis*, W.H. Freeman and Company, New York. P83.

[28] Acquaah G. (2007) *Principles of Plants Genetics and Breeding*, Blackwell Publishing.

[29] Fatokun C.A. (2002) *Proceedings of the World Cowpea Conference III*, IITA, Ibadan, Nigeria.

[30] Park S.O., Coyne D.P., Jung G., Skroch P.W. Arnaud-Santana E., Steadman J.R., Ariyarathne H.M. and Nienhuis J. (2000) *J. Amer. Soc. Hort. Sci.*, 125(4), 466-475.