

Electronic Journal of Plant Breeding



Research Article

Heterotic response and inbreeding depression in seed traits and other related attributes of cowpea (*Vigna unguiculata* [L.] Walp.)

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Abstract

Cowpea is a vital source of plant protein in West Africa, yet its productivity remains low due to the use of unimproved cultivars. To address this, breeding programs aim to develop improved varieties. This study, conducted in 2022 at the Teaching and Research Farms of the University of Cape Coast, evaluated heterosis and inbreeding depression in cowpea involving 13 crosses generated through North Carolina Design II. The results showed that hybrids from UCC15-36 × UCC15-07, UCC15-36 × IT93K-693-2, UCC15-36 × Aluba Kpole, UCC15-25 × IT93K-693-2, UCC15-41 × Kubi, UCC15-03 × Kubi and UCC15-41 × Kubi expressed positive heterosis for seed traits, including length, width, thickness, and 100-seed weight, indicating strong yield potential compared to their parental lines. The magnitude of the inbreeding depression was high in plant height, and number of branches. The observed performance was likely influenced by dominance, additive × additive, and other non-additive gene effects, suggesting that selection for these traits would be more effective in later generations. Thus, both additive and non-additive effects can be exploited to enhance cowpea improvement.

Keywords: Cowpea, Heterosis, Inbreeding depression, Seed traits

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is a diploid leguminous plant ($2n = 22$) classified under the family *Fabaceae*. (Get *et al.*, 2021; Joshi *et al.*, 2022). It ranks among the earliest domesticated food crops, and valued both for its green pods and dry grains. Native to Africa, cowpea is a protein-rich annual legume (about 25%) and excellent nutritional value (Deepa-Priya *et al.*, 2018). Cowpea seeds are particularly rich in essential amino acids, particularly lysine and tryptophan, which are generally deficient in most cereal grains. Heterosis describes the enhanced performance of an F_1 hybrid compared to its parental lines, particularly in yield and other beneficial characteristics (Pathak *et al.*, 2017; Get *et al.*, 2021). Conversely,

inbreeding depression describes the decline in vigor, fertility and yield that results from inbreeding. For plant breeders, it is essential to understand both heterosis and the degree of inbreeding depression in subsequent crop generations. This knowledge helps in predicting the long-term success of a breeding program, choosing between maintaining hybrids or selecting stable lines which, prevent performance decline in subsequent generations and, ultimately, sustains genetic improvements over time (Gupta *et al.*, 2020).

Information on heterosis and inbreeding depression is also valuable for identifying promising crosses that offer the highest potential for isolating transgressive

segregants. This is especially important in self-pollinated crops like cowpea, where breeding success relies heavily on such genetic advancements (Joshi *et al.*, 2022).

A total of 14.4 million hectares of land were used to produce 8.1 million metric tons (MMT) of cowpea globally, with Africa accounting for 96.8% of the overall output (FAOSTAT, 2019). Nigeria, Niger, Burkina Faso and Mali are the top four producers of the crop in Africa, with Ghana coming in fifth position. Ghana produced 183,918 MT of cowpea of dried seeds in 2019 (FAOSTAT, 2019). Cowpea ranks as Ghana's second-leading food legume, following groundnut (Egbadzor *et al.*, 2013). Historically, the most widely cultivated variety in the country was Asontem, initially bred by International Institute of Tropical Agriculture (IITA) and later introduced in 1984 by Ghana's Council for Scientific and Industrial Research (CSIR)-Crop Research Institute (CRI). Despite its popularity, Asontem's red seed coat may limit its consumer preference. Another well-known variety, Nhyira, released by CRI, produces cream-colored seeds but with smaller grain size, which is less favored by consumers (Egbadzor *et al.*, 2013). Consumer preferences in Ghana tend to favor cowpea grains that are large with white or cream-colored testa. However, varieties that combine both traits are mainly imported and tend to be costly. Developing locally adapted cowpea varieties with large, white or cream seeds could enhance farmers' incomes and encourage greater adoption of cowpea cultivation, particularly across the dryland savannah areas of northern Ghana the coastal savannah, and the savannah-forest transition zone.

The creation of superior cowpea cultivars with consumer-desired seed traits is paramount to achieving food security, improving economic livelihoods and saving foreign

exchange for Ghana. Some cowpea varieties developed and released by the Ghanaian system (Catalogue of Crop Varieties Released & Registered in Ghana, 2019) possess grain characteristics preferred by consumers. These could be employed in breeding programmes to develop consumer-preferred market-driven cowpea varieties to enhance grain production. This research was undertaken to develop improved cowpea genotypes characterized by large white grains using selected parental lines with the potential for enhancing cowpea seed size. The present study estimated the extent of heterosis and inbreeding depression for seed size and its related traits in cowpea.

MATERIALS AND METHODS

Study site: The research was carried out during the 2022 planting season at the Teaching and Research Farms of the School of Agriculture, College of Agriculture and Natural Sciences, University of Cape Coast. The University of Cape Coast lies in the coastal savannah ecological zone at Latitude 5° 7' 8" N and Longitude 1° 17' 19" W (<https://mapcarta.com/W330376417>).

Experimental design: A total of 13 crosses were made from eight parents, employing the North Carolina mating design II and marker-assisted backcrossing, were grown along with the parents (Aluba Kpole, Kubi, IT93K-693-2, UCC15-03, UCC15-07, UCC15-25, UCC15-36 and UCC15-41). The F1 population was made up of 97 plants (Table 1). Potted setups were used to conduct the experiment in a screen house, following a completely randomized block design with three replications. Plant spacing was maintained at 60 cm between rows and 30 cm within rows. Standard crop management techniques and appropriate plant health management techniques were applied in a timely manner to ensure successful crop growth.

Table 1. F₁ hybrids evaluated for seed and seed related traits

S.No.	Name of the cross	Number of plants
1	UCC15-03 × Aluba Kpole	06
2	UCC15-03 × UCC15-07	10
3	UCC15-03 × IT93K-693-2	05
4	UCC15-03 × Kubi	10
5	UCC15-25 × IT93K-693-2	05
6	UCC15-25 × Aluba Kpole	05
7	UCC15-25 × UCC15-07	06
8	UCC15-36 × Aluba Kpole	15
9	UCC15-36 × UCC15-07	13
10	UCC15-36 × IT93K-693-2	09
11	UCC15-41 × Kubi	03
12	UCC15-41 × Aluba Kpole	05
13	UCC15-41 × UCC15-07	05
Total		97

Field evaluation involving the F_2 (645) progenies were conducted at the St. Joseph's College of Education Farms in the Ahafo Region of Ghana from October to December 2022. Plants were established at a spacing of 60 cm within rows and 100 cm between rows, with one plant per hill. Weeding was performed twice, during the second and sixth weeks after planting. Insect pest control was managed using Lambda Super 2.5 EC (lambda-cyhalothrin, Kumark Company, Kumasi), applied with a CP-15 knapsack sprayer at a dosage of 0.02 kg active ingredient per hectare, diluted in 150 liters of water. Spraying was carried out at three critical phenological stages: flower bud initiation, 50% flowering, and 50% podding, in line with national cowpea pest control guidelines. Each plant was treated as an independent entry, and data were collected individually on vegetative, reproductive, yield, and seed-related traits.

Data collection: Data were collected from five randomly chosen F_1 and F_2 plants for trait evaluation, including plant height, number of branches, days to flowering, days to pod maturity, number of pods per peduncle, seed length, seed width, seed thickness, and 100-seed weight. The plant height was assessed 45 days after sowing (DAS) using a standard meter rule, measuring vertically relative to the soil baseline of the pot to the apex of the main stem (the highest leaf tip). The count of primary branches on the main stem was recorded during the eighth week after planting.

Days to flowering was determined by counting the number of days from sowing until the first flower fully opened, while days to maturity referred to the duration from sowing until approximately 90% of the pods had reached physiological maturity. At harvest, the number of pods per peduncle was determined by counting all mature, harvestable pods borne on a single peduncle.

Seed traits, including length, width, thickness, and the weight of 100 seeds, were assessed after sun-drying the harvested seeds to about 12% moisture content. Measurements for seed length, width, and thickness were taken from five healthy, mature seeds using a stainless steel digital caliper (range: 0–150 mm). The length of the seed was measured from base to tip along the seed's curve. The width was measured from the hilum to the opposite side and thickness was taken perpendicularly to the length axis. For 100-seed weight, one hundred healthy seeds from each line were weighed using a digital balance (GENERIC SF400), and the weight was recorded in grams.

The mean values were used to estimate the heterosis over the mid-parent as per the formula suggested by Ashokbhai (2015), viz:

$$\text{Heterosis (\%)} = \frac{(\text{OP} - \text{MiPP})}{\text{MiPP}} \times 100$$

Where, OP = Offspring performance, MiPP = Mid-parent performance.

Inbreeding depression was computed for all the characters for that heterosis was estimated by using the following formula:

$$\text{Inbreeding depression (\%)} = \frac{(F_1 - F_2)}{F_1} \times 100$$

Where, F_1 and F_2 are the measurements for the first and second filial generations, respectively.

The significance of heterosis and inbreeding depression was evaluated by comparing treatment means using the Least Significant Difference (LSD) test at a 5% probability level.

RESULTS AND DISCUSSION

Heterosis: The current study evaluated heterosis among the progenies raised from the crosses to identify the best cross-combinations that yielded a high degree of beneficial heterosis for potential application in future breeding programmes. Heterosis occurs when the F_1 generation of genetically distinct individuals exhibits either increased or decreased vigour in terms of size, yield, among others, when compared to the better and mid-parent value (Ashokbhai, 2015). Thus, the F_1 hybrid's superiority over its parents in terms of yield and other attributes is expressed as the heterosis. The conventional heterosis spectrum of variation for four seed characters and five contributing characteristics for four crosses are presented in **Table 2**. Each character had a different quantity of heterotic crosses, and each character's heterosis varied in both direction and intensity from cross to cross. The findings showed that the genetic makeup of the parental lines affected the type of gene action in specific hybrids, as evidenced by significantly high and/or low heterosis.

High magnitudes of heterosis and inbreeding depression in plant height as well as number of branches were recorded for hybrids of UCC15-03 × Kubi, UCC15-41 × Kubi, UCC15-03 × Aluba Kpole, UCC15-03 × UCC15-07 and UCC15-03 × IT93-693-2. The five groups of hybrids exhibited significant vigour over the mid-parent value and increased in performance resulting from inbreeding. Specifically, heterosis was high in plant height as well as number of branches among the hybrid of UCC15-03 × Kubi, UCC15-41 × Kubi, UCC15-03 × Aluba Kpole, UCC15-03 × UCC15-07, UCC15-25 × IT93k-693-2, UCC15-41 × Aluba Kpole, UCC15-36 × IT93k-693-2 and UCC15-03 × IT93-693-2. The increase in plant height and number of branches is an indication that the hybrids could produce a greater number of pods and seeds, resulting in higher yields (Hall, 2004). Progeny derived from these hybrids may exhibit enhanced adaptability across diverse environments, as tall and well-branched plants tend to be more tolerant to environmental stresses

Table 2. Percent heterosis for seed and contributing traits

Hybrids	Plant height	Number of branches	Days to pod maturity	Pods per peduncle	Days to flowering	Seed length	Seed width	Seed thickness	100 Seed weight
UCC15-03 × Aluba Kpole	145.00	3.00	-10.00	-5.00	-18.00	-22.00	-21.00	-22.00	-48.00
UCC15-03 × UCC15-07	36.00	44.00	-8.00	0.00	-6.00	-7.00	-15.00	-12.00	-11.00
UCC15-03 × IT93K-693-2	32.00	17.00	-13.00	-11.00	-23.00	4.00	1.00	-3.00	17.00
UCC15-03 × Kubi	11.00	64.00	-43.00	-5.00	-21.00	6.00	-4.00	2.00	5.00
UCC15-25 × IT93K-693-2	39.00	38.00	-13.00	-11.00	-25.00	5.00	1.00	2.00	34.00
UCC15-25 × Aluba Kpole	-8.00	-6.00	-4.00	-20.00	-13.00	-19.00	-18.00	-14.00	-27.00
UCC15-25 × UCC15-07	-24.00	20.00	-9.00	-10.00	-8.00	-19.00	-21.00	-16.00	-35.00
UCC15-36 × Aluba Kpole	7.00	-5.00	-12.00	-10.00	-19.00	-13.00	-1.00	-16.00	-27.00
UCC15-41 × Kubi	82.00	67.00	-24.00	-5.00	-22.00	1.00	0.00	5.00	10.00
UCC15-41 × Aluba Kpole	274.00	50.00	-7.00	0.00	-16.00	-1.00	-5.00	-14.00	-46.00
UCC15-36 × IT93K-693-2	36.00	6.00	-12.00	-11.00	-21.00	23.00	20.00	11.00	75.00
UCC15-36 × UCC15-07	-49.00	33.00	-8.00	-20.00	-11.00	13.00	16.00	13.00	65.00

like drought, heat, and pest pressure, making them better suited for varied cultivation conditions. (Hall, 2004). They could also be more competitive with weeds, reducing the need for herbicides and other weed control measures (Osipitan, 2024). Similar results were reported by Balakrishna, *et al.* (2017) in okro and Mudhalvan *et al.* (2021) in cotton.

There was moderate to severe negative heterosis in the progenies of all the hybrids or crosses for days to flowering and days to pod maturity. The negative heterosis values for days to flowering and pod maturity indicate that the hybrid flowered earlier and the pods matured earlier compared to the mid-parent. These traits thus allowed the hybrids to flower and produce pods earlier than the parents, which should lead to improved adaptation to shorter growing seasons (Hall, 2004; Owusu *et al.*, 2018) and potentially for multiple harvests (Owusu *et al.*, 2018). In cowpea breeding, earlier flowering and maturity are desirable traits, as they can increase yield potential, improve drought tolerance, enhance adaptability to diverse environments and allow for more efficient crop rotation (Mwale *et al.*, 2017; Owusu *et al.*, 2018; Nkomo *et al.*, 2021; Ogbache *et al.*, 2023).

The progenies of UCC15-25×Aluba Kpole recorded negative heterosis for all the nine traits studied. With the exception of number of branches, the progenies of UCC15-25 ×UCC15-07 and UCC15-36×UCC15-07, recorded negative heterotic values for the other eight traits. Negative heterosis was recorded for all the seed characteristics in the hybrid populations of UCC15-03 × UCC15-07, UCC15-25 × UCC15-07, UCC15-03 × Aluba Kpole, UCC15-25 × Aluba Kpole, UCC15-41 × Aluba Kpole and UCC15-36 × Aluba Kpole indicating a reduction in the seed characteristics of the hybrid over the mid-parent value. The hybrids thus had lower values for seed length, seed width, seed thickness and 100-

seed weight compared to their parental lines. Such seed characteristics can lead to decreased seedling emergence and establishment, reduced seedling vigor and lower drought tolerance. The smaller seeds and lower 100-seed weight could also be an indication of reduced grain yield and lower harvest index. However, smaller seeds coupled with lower 100-seed weight could also be an indication of increased number of seeds per pod or plant, improved seed dispersal and planting efficiency and reduced seed costs.

Positive heterosis was recorded in the hybrids of UCC15-36 × UCC15-07, UCC15-36 × IT93K-693-2, UCC15-25 × IT93K-693-2 and UCC15-41 × Kubi for all the seed characteristics, indicating an improvement in the hybrid seed characteristics over the mid-parent values. The progenies of UCC15-03 × Kubi and UCC15-36 × IT93K-693-2, recorded positive heterosis in three three seed traits *viz.*, seed length, thickness and 100 seed weight, and seed length, width and 100 seed weight, respectively. Positive heterosis over the mid-parent for seed length, width, thickness and 100-seed weight in cowpea breeding implies that the hybrid population exhibits improved seed characteristics compared to the mid-parent value. The hybrids therefore produced larger seeds, which may have better germination rates and seedling vigour (Hall, 2004) and be more attractive to farmers (Egbadzor *et al.*, 2013) and consumers. The positive heterosis for 100-seed weight indicates that the hybrid produced heavier seeds, indicating an improvement in seed quality as well. These seeds may be more nutritious and possess energy reserves, which can lead to better seedling establishment and growth (Egbadzor *et al.*, 2013).

The positive significant heterosis recorded in some hybrid characteristics of the current study could be caused by genetic, breeding, genomic, physiological

and environmental factors. Genetic factors such as dominance complementation (dominant alleles from parents combining for improved traits), over dominance (heterozygous genotypes exhibiting superior performance), epistasis (synergistic interactions between genes) (Natesan, *et al.*, 2024) and polygenic inheritance (multiple genes contributing to trait improvement) could result into positive heterosis over the average parental values in hybrids (positive heterosis) (Hübner & Kantar, 2021; Aswini *et al.*, 2023). The effective parental selection (choosing parents with diverse desirable traits), strategic hybridization (optimized crossing schemes (North Carolina Design II), and marker-assisted selection (MAS) could also result in the hybrids with positive significant heterosis for selected characters over the average parental values (Hallauer *et al.*, 2010; Acquaaah, 2012; Pauly *et al.*, 2016). Genomic factors such as increased genetic diversity (Combining diverse parental genomes) and increased heterozygosity leading to hybrid vigor, could have contributed to hybrids having improved trait as compared to their mid-parent (Sandeep *et al.*, 2014).

On the other hand, several factors could have contributed to reduced hybrid characteristics, leading to negative heterosis. These include inbreeding depression (Pathak *et al.*, 2017), genetic drag (Hübner & Kantar, 2021), epistasis (Mathew *et al.*, 2018; Olatoye, 2019) and genetic instability (Aswini *et al.*, 2023). Environmental factors, such as drought and heat stress (Boukar *et al.*, 2018), pests and diseases (Horn *et al.*, 2015) and nutrient deficiencies (Boukar *et al.*, 2018) which occurred during the study, could also have accounted for reduction in hybrid characteristics especially seed size and 100-seed weight. These environmental challenges could result into hormonal imbalance (Cardona-Ayala *et al.*, 2020), photosynthesis limitations (Taiz *et al.*, 2015) and assimilate

partitioning (Carvalho *et al.*, 2019), which could also have contributed to the reduction in hybrid characteristics. However, these factors were not examined in the current study.

Overall, the hybrids of UCC15-03 × Kubi and UCC15-41 × Kubi showed improvement in almost all the characteristics studied, particularly seed size characteristics over the mid-parent values. Therefore, they were superior cross combinations which could be used for better transgressive segregants in subsequent segregating generations based on the nature and amount of heterosis.

Inbreeding depression: In the current study, the estimation of inbreeding depression (Table 3) was done to determine the type of gene actions involved in the expression of the seed size and its contributing characteristics in cowpea, since a knowledge of inbreeding depression is essential for designing efficient cowpea improvement strategies and choosing parents to produce quality segregants to increase crop vigour. Generally, the magnitude of the inbreeding depression was high for plant height and number of branches (Table 3). However, there was moderate beneficial magnitude of inbreeding depression for most of the characters studied. Significant ($p < 0.05$) beneficial magnitude of inbreeding depression for days to flowering, days to pod maturity and seed length was recorded in all the four hybrid populations. Hence, the progenies were earlier in flowering and pod maturity compared to the parental lines. Beneficial magnitude was also recorded for seed width and thickness in the hybrids of UCC15-03 × Kubi, UCC15-03 × UCC15-07, UCC15-25 × UCC15-07, UCC15-25 × Aluba Kpole, UCC15-03 × Aluba Kpole, UCC15-36 × Aluba Kpole and, UCC15-03 × IT93K-693-2 indicating the improvement in seed width and thickness in the hybrids. The beneficial (negative)

Table 3. Percent inbreeding depression for seed and contributing traits

Hybrids	Plant height	Number of branches	Days to pod maturity	Pods per peduncle	Days to flowering	Seed length	Seed width	Seed thickness	100 Seed weight
UCC15-03 × ALUBA KPOLE	62.26	30.96	-16.32	2.26	-7.73	-21.15	-15.46	-15.27	-60.23
UCC15-03 × UCC15-07	55.55	29.40	-16.36	8.51	-9.37	-9.94	-16.18	-14.56	-12.90
UCC15-03 × IT93K-693-2	-20.57	40.79	-10.01	-6.77	-22.46	7.45	-0.87	-2.74	17.60
UCC15-25 × IT93K-693-2	-12.85	34.17	-10.16	-5.24	-18.95	9.21	-37.38	1.89	0.156
UCC15-25 × ALUBA KPOLE	-34.00	-14.20	-6.80	-11.31	-7.97	-9.63	-15.69	-9.18	-17.58
UCC15-25 × UCC15-07	-5.13	19.52	-16.14	-0.56	-8.72	-20.05	-19.44	-15.79	-53.19
UCC15-36 × ALUBA KPOLE	-1.81	0.183	-19.41	0.93	-9.87	-8.04	-8.63	-9.85	-13.61
UCC15-41 × KUBI	54.96	61.35	-38.33	7.78	-12.25	-1.029	3.21	4.44	11.03
UCC15-03 × Kubi	22.71	40.54	-72.79	2.80	-11.69	-0.19	-4.99	-2.30	3.65
UCC15-03 × UCC15-07	-26.36	21.20	-15.49	-6.98	-10.59	15.30	8.97	0.80	31.68
UCC15-03 × IT93K-693-2	70.73	28.37	-9.99	9.34	-2.54	12.63	0.96	-5.36	27.78
UCC15-25 × IT93K-693-2	-46.62	55.92	-9.36	-13.30	-12.97	18.61	13.46	12.13	38.10
UCC15-41 × UCC15-07	-12.32	34.65	-21.87	0.65	-9.67	8.89	3.18	-2.61	2.49

inbreeding depression recorded in these traits could be due to overdominance (Roff, 2002), epistasis (Awad and Roze, 2020), genetic drift (Lynch, 1991), gene interaction (Lynch, 1991) and/or homozygosity (Awad and Roze, 2020). Cowpea is self-pollinating with high homozygosity, which results in the low and/or no inbreeding depression in most of the traits, especially seed characteristics. The hybrids viz., UCC15-41 × Kubi, UCC15-03 × Aluba Kpole, UCC15-03 × UCC-15-07 and UCC15-03 × IT93K-693-2 were found to have high significant heterosis over the mid-parent with high inbreeding depression in plant height and number of branches. Such results could stem from dominance, epistatic interactions (additive × additive), or non-additive genetic effects, therefore, selection would be most effective at later generations (Patel *et al.*, 2013; Pathak *et al.*, 2017). Negative heterosis and inbreeding depression for days to flowering and days to pod maturity were recorded in all the hybrids of the four crosses. Negative heterosis (weak vigour) and gain in all the seed characteristics due to inbreeding were recorded in the hybrids of UCC15-03 × UCC15-07, UCC15-25 × UCC15-07, UCC15-25 × Aluba Kpole, UCC15-303 × Aluba Kpole and UCC15-36 × Aluba Kpole. These findings are in agreement with findings reported by Sharma *et al.* (2010), Yadav *et al.* (2010) and Patel *et al.* (2013) in cowpea.

All the hybrids had reduced days to flowering and days to pod maturity, facilitating earlier flowering and better suitability to brief growing periods thus, making them potentials for multiple harvests. Hybrids of UCC15-36 × UCC15-07, UCC15-36 × IT93K-693-2, UCC15-36, Aluba Kpole, UCC15-25 × IT93K-693-2, UCC15-41 × Kubi, UCC15-03 × Kubi and UCC15-41 × Kubi recorded positive heterosis for seed length, width and thickness, and 100-seed weight suggesting that these hybrids have the potential for much higher yields when measured against their parents. The hybrids therefore performed better than the mid parent in terms of seed characteristics. The magnitude of the inbreeding depression was high in plant height, and number of branches. The high heterosis levels in most of the traits, suggest that further breeding and selection could refine these hybrids even more. It is recommended that the specific alleles or genetic markers underlying the improved seed traits be identified and utilized to achieve further genetic improvement of these hybrids.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Department of Molecular Biology and Biotechnology, University of Cape Coast, as well as the Samuel and Emelia Brew-Butler SGS/GRASSAG Research Grant (UCC) for their partial support of this work.

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