Journal of Applied Life Sciences and Environment
https://jurnalalse.iuls.ro

USV 1842

Article
https://doi.org/10.46909/alse-592214
Vol. 59, Issue 2 (206) / 2026: 257-273

MID- AND BETTER-PARENT HETEROSIS FOR YIELD AND ITS COMPONENTS WITHIN ANTHOCYANIN PIGMENTATION CLASSES IN THE F_2 GENERATION OF A COWPEA CROSS

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Received: Jun. 12, 2026. Revised: Jul. 23, 2026. Accepted: Aug. 05, 2026. Published online: Sep. 03, 2026

ABSTRACT. This study evaluated generation mean performance, used single marker analysis (SMA) to determine associations between pigmentation and yield traits, and estimated mid- and better-parent heterosis in F_2 phenotypic classes from a cross between TVu1 and Ibadan BPC performed in an insect-proof screenhouse at the Department of Agricultural Technology, Federal College of Forestry, Ibadan, Nigeria, using a randomised complete block design with three replications. The parents, F_1, and 300 F_2 plants were grown in 10-L pots and spaced with 60 cm between rows and 45 cm within rows. Two seeds were sown per pot and thinned to one plant. Self-pollination was ensured using pollination bags. The following traits were measured: days to flowering, pod length, number of seeds per pod, 100-seed weight, yield, and pigmentation scores. SMA was performed using one-way analysis of variance, and heterosis was estimated using t-tests. Pigmentation classes were significantly associated with yield traits. Pigmented plants flowered earlier (47.56 days) and had longer pods (13.14 cm) and heavier seeds (11.05 g) than non-pigmented plants (53.10 days, 12.34 cm, and 10.71 g, respectively). Purple plants flowered earlier (44.10 days), produced more seeds per pod (11.41), and had a higher 100-seed weight (8.66 g) than white plants (46.29 days, 11.26 seeds, and 8.64 g, respectively). Immature pod pigmentation favoured earlier flowering (45.24 days),

Cite: Olunloyo, A.A.; Ogunjinmi, S.O.; Olla, N.O. Mid- and better-parent heterosis for yield and its components within anthocyanin pigmentation classes in the F_2 generation of a cowpea cross. *Journal of Applied Life Sciences and Environment* **2026**, *59* (2), 257-273. https://doi.org/10.46909/alse-592214

longer pods (13.33 cm), more seeds (12.00), and heavier seeds (10.15 g). Heterosis showed positive mid- and better-parent effects for 100-seed weight (35.05–45.68%). Pigmented classes showed superior performance for morphological marker selection in cowpea improvement.

Keywords: anthocyanin pigmentation; cowpea; F_2 generation; heterosis; morphological marker; single marker analysis; yield components.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is a diploid grain legume that is critical for food security in sub-Saharan Africa, Latin America, and Southeast Asia. In West Africa, Nigeria, where cowpea provides dietary protein, micronutrients, and income for smallholder farmers, accounts for the bulk of production (Matjeke *et al*., 2025). This crop fixes atmospheric nitrogen, tolerates drought, and adapts to marginal soils, making it indispensable in low-input systems. Despite these advantageous traits, yield remains low (300–800 kg ha^{-1}), far below the genetic potential of 1500–2000 kg ha^{-1}, attributable to biotic and abiotic stressors and limited genetically improved varieties.

Conventional breeding targets yield components, such as pod length (PL), seeds per pod (NSP), and 100-seed weight (100SW). However, seed yield (SY) is polygenic with low heritability and high genotype-by-environment interactions, making direct selection inefficient.

Exploiting heterosis offers an alternative, as F_1 hybrids outperform parental lines. In cowpea, significant positive heterosis exceeds 40% for SY and 100SW (Pathak *et al*., 2017), but its autogamous reproductive biology causes rapid inbreeding depression in F_2 and advanced generations, as favourable non-additive gene combinations disassociate.

Heterosis, or hybrid vigour, refers to the superiority of F_1 hybrids over the parental lines for yield and other agronomic traits (Shull, 1914). Although F_1 heterosis has been well-documented, estimating heterosis in the F_2 generation is critical because F_2 populations are the first segregating generation in which recombination and independent assortment generate novel genetic combinations. F_2 heterosis estimates in cowpea provide insight into whether the superior performance observed in F_1 hybrids is maintained or dissipated due to the fixation of homozygous loci and the breakdown of favourable epistatic interactions (Edematie *et al*., 2021). The F_2 generation acts as a bridge between hybrid performance and the development of stable, high-yielding inbred lines or open-pollinated varieties.

Qualitative pigmentation traits provide promising indirect selection tools. Anthocyanin variation in the node, flower, pod, and seed coat colour is simply inherited, and pigmented phenotypes are typically dominant. Node pigmentation is conditioned by a single dominant gene with pleiotropic effects on petal and seed coat coloration (Padi, 2003). Major-effect loci for the seed coat pattern localise to chromosomes 7, 9, and 10 and are regulated by transcription factor clusters (Herniter *et al*., 2019; Lo *et al*., 2019). These visually scorable traits require no laboratory infrastructure, making them ideal markers for resource-constrained programs.

Single marker analysis (SMA) partitions populations into phenotypic marker-based classes and identifies differences in quantitative traits.

Genomic regions harbouring pigmentation genes frequently co-localise with quantitative trait loci (QTLs) for phenology, pod development, and seed size (Mohammed *et al*., 2025; Sodo *et al*., 2025).

Flavonoids and anthocyanins in the seed indicate enhanced plant defence and rhizobial nodulation, functionally linking pigment biosynthesis to yield formation (Makoi *et al*., 2010).

Although high-density single nucleotide polymorphism (SNP) panels enable precise morphological marker selection (Ongom *et al*., 2024), genotyping costs remain prohibitive for many national programs.

Despite this potential, empirical studies evaluating pigmentation–yield associations in Nigerian F_2 germplasm are scarce. The practical utility of qualitative pigmentation scoring in early generation selection, particularly in heterotic classes, remains inadequately characterised.

The present study was justified by the need to enhance selection efficiency using low-cost morphological markers.

The objectives were to:

(i) evaluate the generation mean performance of the parents, F_1, and F_2 for days to flowering (DTF), PL, NSP, 100SW, and SY,

(ii) determine associations between pigmentation traits and yield components via SMA

(iii) estimate mid- and better-parent heterosis within each F_2 phenotypic class to assess the utility of pigmentation for indirect selection.

MATERIALS AND METHODS

Experimental site

The experiment was conducted in a screenhouse at the Department of Agricultural Technology, Federal College of Forestry, Ibadan, Oyo State, Nigeria (latitude 7° 23' N, longitude 3° 56' E, altitude approximately 200 m above sea level), during the 2024/2025 cropping season. Ibadan is situated in the derived savanna–forest transition agro-ecological zone of southwestern Nigeria, characterised by a bimodal rainfall pattern, mean annual precipitation of 1200–1500 mm, and mean daily temperature of 26–32°C. The screenhouse was enclosed with insect-proof netting (2-mm mesh) on all sides and covered with a transparent UV-stabilised polyethylene roof to attenuate direct solar radiation by approximately 30% while maintaining an ambient photoperiod. The temperature and relative humidity inside the screenhouse were monitored daily using a digital thermo-hygrometer (Model HTC-2, HTC Instruments, China) and ranged from 24.5 to 31.8°C and 62 to 89%, respectively, throughout the experimental period.

Plant material and genetic populations

Two morphologically different pure lines, namely TVu1 (P1, female) and Ibadan BPC (P4, male), were selected to develop a mapping population of 300 F_2 plants. The female parent, P1, is an early maturing, high-yielding landscape plant with pigmented nodes, purple flowers, pigmented immature pods, and red seed coats. The male parent, P4, is a late-maturing breeding line with non-pigmented (green) nodes, white flowers,

non-pigmented (green) immature pods, and cream-white seed coats. Crosses were made in June 2019, and F_1 seeds were collected and raised in September 2019, followed by self-pollination to generate the F_2 segregating population. Standard agronomic practices and need-based plant protection measures were adopted during mapping population development and the F_2 plant evaluation trial.

Crop management

Seeds were sown at a depth of 2.5 cm, with 2 seeds per pot thinned to one vigorous seedling at 7 days after emergence (DAE). Irrigation was applied manually in 3-day intervals using a watering can fitted with a fine rose to deliver approximately 500 mL per pot, maintaining the soil moisture at 60–70% of field capacity. A basal application of single superphosphate equivalent to 20 kg P ha^{-1} was incorporated into the potting mixture at sowing. No nitrogen fertiliser was applied to encourage biological nitrogen fixation.

Insect pests, principally *Aphis craccivora* (cowpea aphid) and *Maruca vitrata* (pod borer), were controlled using weekly sprays of cypermethrin (10% EC) at 1.5 mL L^{-1} and dimethoate (40% EC) at 2.0 mL L^{-1}, commencing at 21 DAE and continuing in 14-day intervals until pod maturity. A foliar fungicide (mancozeb 80% WP at 2.5 g L^{-1}) was applied prophylactically at flowering to prevent anthracnose (*Colletotrichum lindemuthianum*) and *Cercospora* leaf spot. Weeds were manually removed as needed.

Phenotyping of F_2 plants

Seeds of the parental lines (P1 and P4) and F_1 and F_2 generations were raised concurrently in the screenhouse to permit direct comparative evaluation. The evaluation was carried out in May 2020. The experiment was conducted with 350 entries [300 F_2 plants + 2 parents (10 times) + 30 F_1 plants]. The plants were raised in 10-L black polythene pots (30 cm diameter × 28 cm height) filled with 8 kg of topsoil sieved through a 5-mm mesh and mixed with well-decomposed poultry manure in a 3:1 (v:v) ratio. Pots were spaced with 60 cm between rows and 45 cm within rows to minimise inter-plant competition and facilitate routine management.

Each plant was tagged and visually observed for the presence or absence of pigmentation on the node, flower, fresh pod and seed coat, and data were recorded. Phenotyping observations were recorded on the following agronomic traits: DTF, PL (cm), NSP, 100SW (g), and SY (g) at the time of harvest.

Data collection

Qualitative (pigmentation) traits

Anthocyanin pigmentation was scored visually on individual F_2 plants at defined developmental stages following the International Board for Plant Genetic Resources cowpea descriptors with modifications. Node pigmentation was recorded at the primary trifoliate node stage (21 DAE) as pigmented (purple or violet streaks on the node) or not pigmented (green node). Flower colour was recorded at 50% anthesis as purple or white. Immature pod pigmentation was assessed on 10-day-old pods as pigmented (purple pigmentation on the pod wall) or green/not pigmented. The seed coat colour was determined on mature, air-dried seeds as red or cream-white. These discrete classifications were

used to partition the F_2 population into contrasting phenotypic classes for subsequent single-marker and heterosis analyses.

Quantitative (agronomic and yield) traits

The following traits were measured on individual plants: DTF, number of days from sowing to the appearance of the first fully opened flower; PL, length (cm) of 10 randomly harvested mature pods per plant, measured with a digital calliper to the nearest 0.01 cm; NSP, mean seed count from 10 dehisced mature pods; 100SW, weight (g) of 100 randomly counted seeds from each plant, determined using a digital balance (0.01 g precision); and SY, total weight (g) of all mature, oven-dried (65°C for 72 h) seeds per plant.

Statistical analyses

Generation mean analysis

Analysis of variance (ANOVA) for a randomised complete block design was performed on the quantitative traits using a general linear model to test the significance of differences among generations (P1, P4, F_1, and F_2). Means were separated using the least significant difference test at a 5% probability level. The standard error of the mean (± SE) was computed for each generation.

Single marker analysis (SMA)

The F_2 population was stratified into two discrete phenotypic classes for each of the four qualitative pigmentation traits (node, flower, immature pod, and seed coat).

The mean values of each quantitative trait were computed for the contrasting classes (e.g., pigmented vs. non-pigmented node). The significance of the mean differences between phenotypic classes was tested using one-way ANOVA (F-test), with significance set at $p \leq 0.05$. SMA was conducted to infer the association between qualitative marker loci and polygenic yield component traits.

Heterosis estimation

Mid- and better-parent heterosis were estimated for each quantitative trait within each F_2 phenotypic class. The mid-parent value (MPV) was computed as the arithmetic mean of the two parental means (P1 and P4), and the better-parent value (BPV) was the mean of the superior parent for the trait under consideration (P1 for earliness, yield, and yield components; P4 for PL, NSP, and 100-SW as the higher-performing parent). The percent heterosis was calculated as follows (*Equation 1* and *Equation 2*):

$$\text{Mid-parent heterosis (\%)} = \frac{\text{F}_2\text{class mean} - \text{MPV}}{\text{MPV}} \times 100 \quad (1)$$

$$\text{Better-parent heterosis (\%)} = \frac{\text{F}_2\text{class mean} - \text{BPV}}{\text{BPV}} \times 100 \quad (2)$$

where the F_2 class mean is the mean of the F_2 plants belonging to a specific phenotypic class (e.g., pigmented node and purple flower).

The significance of the heterotic effects was determined using the t-test as outlined by Gomez and Gomez (1984), and the standard error was derived from the pooled error mean square from ANOVA at the 5% level. The genetic segregation of the two phenotypic classes in the F_2 was tested for goodness-of-fit to the expected Mendelian ratios using the Chi-square (χ^2) test (Snedecor and Cochran, 1989). All statistical computations were performed using R software (v4.3.1).

RESULTS AND DISCUSSION

Qualitative variation in anthocyanin pigmentation

The two parental lines contrasted each other for all qualitative pigmentation traits (*Table 1*). P1 was characterised by pigmented nodes, purple flowers, pigmented immature pods, and red seed coats. Conversely, P4 exhibited non-pigmented nodes, white flowers, non-pigmented (green) immature pods, and cream-white seed coats. The F_1 generation uniformly expressed the pigmented phenotypes of maternal parent P1, exhibiting pigmented nodes, purple flowers, pigmented immature pods, and red seed coats. The complete dominance of the pigmented alleles over their non-pigmented counterparts is consistent with the established genetic architecture of anthocyanin expression in cowpea, in which pigmented phenotypes are typically dominant to non-pigmented or green alternatives (Padi, 2003; Sangwan and Lodhi, 1998). The absence of phenotypic variation in the F_1 generation confirms the homozygosity of the parental lines for the contrasting alleles at each pigmentation locus and validates the genetic purity of the parental stocks used in the hybridisation program. In the F_2 generation, all four pigmentation traits segregated in a manner consistent with monogenic dominant–recessive inheritance. The observed phenotypic ratios were as follows: 3 pigmented (227 plants): 1 not pigmented (73 plants) for node pigmentation, 3 purple (220 plants): 1 white (80 plants) for flower colour, 3 pigmented (221 plants): 1 not pigmented (79 plants) for immature pod pigmentation, and 3 red (220 plants): 1 cream-white (80 plants) for seed coat colour (*Table 1*). As expected, this conforms to Mendelian segregation for a single gene controlling each trait. These results corroborate earlier findings by Sangwan and Lodhi (1998), who demonstrated the monogenic inheritance of flower and pod colour in cowpea, and by Padi (2003), who established that leaf node pigmentation is conditioned by a single dominant gene with pleiotropic effects on petal and seed coat coloration. The conformity to the 3:1 ratio indicates that the observed segregation was not significantly distorted by gametic selection, differential viability, or environmental modification, confirming the reliability of these traits as discrete genetic markers in early segregating generations. The 3:1 segregation observed across all four pigmentation traits suggests that the loci governing node pigmentation, flower colour, immature pod pigmentation, and seed coat colour are either independently inherited or functionally linked in the genomic regions that regulate the anthocyanin biosynthetic pathway. The discrete classification of the F_2 population into contrasting phenotypic classes based on these qualitative traits provided the framework for the SMA employed in this study. Following the classical marker–trait association concept described by Sax (1923), the F_2 population was partitioned into contrasting classes (e.g., pigmented versus non-pigmented nodes) to determine the mean differences in quantitative yield components. Because anthocyanin pigmentation traits are simply inherited, highly heritable, and visually scorable without laboratory infrastructure, they represent ideal morphological markers for breeding

programs operating under resource constraints (Mohammed *et al.*, 2025; Sodo *et al.*, 2025). The 3:1 segregation ratios obtained in this study validate the deployment of these traits as preliminary screening tools, enabling breeders to reduce the population size and focus resources on the most promising progeny before advanced yield testing. Furthermore, the consistent dominance of pigmentation across all four traits suggests that selection for pigmented phenotypes in early segregating generations can serve as a proxy for favourably linked QTLs governing agronomic performance, contributing to accelerated genetic gain in Nigerian cowpea germplasm improvement (Makoi *et al*., 2010).

Generation mean performance of days to flowering, pod length, and number of seeds/pod

The parental lines showed variation in DTF. P1 flowered significantly earlier than P4 at 41.17 ± 1.17 days, whereas P4 flowered at 61.83 ± 1.72 days. The F_1 generation recorded 42.97 ± 2.09 days, indicating dominance for earliness, and the F_2 generation exhibited an intermediate mean of 49.49 ± 1.59 days *(Table 2)*. For PL, the parental means were comparable: P1 (13.13 ± 0.78 cm) and P4 (13.85 ± 0.31 cm). The F_1 mean was 13.84 ± 1.33 cm, like the parents, but the F_2 mean declined to 11.28 ± 2.25 cm. For NSP, P1, P4, F_1, and F_2 averaged 12.00 ± 1.41, 10.00 ± 0.89, 11.67 ± 1.58, and 10.23 ± 2.17 seeds, respectively. The generation mean patterns revealed distinct gene action for these yield-related traits. The DTF for F_1 was close to that of the early flowering parent (P1), suggesting partial to complete dominance for earliness, an agronomically desirable trait in cowpea breeding for drought escape (Fatokun *et al*., 2002). The mean DTF for F_2 was intermediate between the parents, indicating segregation of both early and late alleles, with additive genetic effects contributing to the population mean. For PL, the reduction in F_2 compared to the parents and F_1 suggests the influence of inbreeding depression or gene recombination with negative effects on pod elongation in the segregating generation. NSP showed a decline from F_1 to F_2, approaching that of the lower-yielding parent (P4), consistent with the self-pollinating nature of cowpea, in which the heterozygous advantage is lost in advanced generations (Shull, 1914). These patterns highlight the importance of rigorous selection in early generations to fix desirable alleles for yield components.

Generation mean performance of 100-seed weight and seed yield

The 100SW was 7.36 ± 1.32 g for P1 and 6.65 ± 0.46 g for P4. The F_1 generation showed hybrid vigour with a mean of 12.76 ± 1.58 g, exceeding that of both parents. The F_2 generation maintained an elevated mean of 9.48 ± 2.69 g, intermediate between the F_1 and parental means. SY was 31.36 ± 1.32 g for P1, 21.58 ± 2.49 g for P4, 36.84 ± 1.33 g for F_1 (exceeding the better parent), and 24.50 ± 4.35 g for F_2 *(Table 3)*. The F_1 generation exhibited hybrid vigour for both 100SW and SY, with F_1 exceeding the better parent by approximately 73.60% for 100SW and 17.50% for SY. This heterosis in F_1 aligns with findings by Raut *et al*. (2017) and Shirisha *et al*. (2022), who reported significant mid- and better-parent heterosis for test weight and

grain yield in cowpea hybrids. The decline in F_2 means nearing the parental range reflects the dissolution of heterozygous gene combinations due to segregation and recombination, a well-documented phenomenon in self-pollinated legumes in which the commercial exploitation of F_1 hybrid vigour is constrained by the inability to fix heterosis (Shull, 1914). The transgressive performance of F_2 for 100SW suggests non-additive gene action, including dominance and overdominance effects, which may be targeted by biparental mating or reciprocal recurrent selection to accumulate favourable alleles in homozygous lines (Fatokun *et al*., 2002).

Single marker analysis (SMA) for yield traits using node pigmentation

SMA using node pigmentation as the phenotypic class revealed significant associations ($p < 0.05$) with DTF, PL, and 100SW but non-significant associations with NSP and SY. Plants with non-pigmented nodes flowered later (53.10 days) than those with pigmented nodes (47.56 days) *(Table 4)*.

For PL, plants with non-pigmented nodes averaged 12.34 cm, compared to 13.14 cm for those with pigmented nodes.

For 100SW, plants with non-pigmented nodes averaged 10.71 g, compared to 11.05 g for those with pigmented nodes.

Table 1 – Variation in anthocyanin pigmentation (qualitative traits) of the cowpea lines studied

Generation	Node	Flower	Immature pod	Seed coat
Ibadan BPC	Not pigmented	White	Not Pigmented	Cream-white
TVu1	Pigmented	Purple	Pigmented	Red
F_1	Pigmented	Purple	Pigmented	Red
F_2	3 Pigmented (227): 1 Not pigmented (73)	3 Purple (220): 1 White (80)	3 Pigmented (221): 1 Not pigmented (79)	3 Red (220): 1 Cream-white (80)
χ^2	0.06	0.36	0.28	0.36

Table 2 – Generation mean performance of days to flowering (DTF), pod length (PL), and number of seeds per pod (NSP) traits in a cowpea cross

Cross	Generation	DTF (days)	PL (cm)	NSP
TVu1 (P1) × IbBPC (P4)	P1	41.17 ± 1.17	13.13 ± 0.78	12.00 ± 1.41
	P4	61.83 ± 1.72	13.85 ± 0.31	10.00 ± 0.89
	F_1	42.97 ± 2.09	13.84 ± 1.33	11.67 ± 1.58
	F_2	49.49 ± 1.59	11.28 ± 2.25	10.23 ± 2.17

Table 3 – Generation mean performance of 100-seed weight and seed yield in a cowpea cross

Cross	Generation	100-Seed weight	Seed yield
TVu1 (P1) × IbBPC (P4)	P1	7.36 ± 1.32	31.36 ± 1.32
	P4	6.65 ± 0.46	21.58 ± 2.49
	F_1	12.76 ± 1.58	36.84 ± 1.33
	F_2	9.48 ± 2.69	24.50 ± 4.35

The significant association of node pigmentation with DTF, PL, and 100SW suggests that the locus controlling node pigmentation is either linked to QTLs affecting these yield traits or pleiotropic effects. This finding is consistent with the established genomic architecture of cowpea, in which pigmentation loci on chromosomes 7 (*C*), 9 (*H*), and 10 (*W*) reside in genomic regions harbouring multiple trait associations (Herniter *et al*., 2019). The direction of the effect of pigmented nodes, which were associated with earlier flowering and marginally longer pods, indicates that the pigmentation allele is linked to earliness and pod elongation genes.

However, the lack of a significant association with SY and NSP suggests that these complex polygenic traits are not directly governed by the node pigmentation locus, indicating that yield is controlled by multiple minor genes distributed across the genome (Acquaah, 2007). These results demonstrate the utility of morphological markers as proxies for linked-yield QTLs in resource-limited breeding programs.

Single marker analysis (SMA) using flower colour

Using flower colour as a phenotypic marker, significant associations ($p < 0.05$) were detected with DTF, NSP, and 100SW, while PL and SY showed non-significant F-tests *(Table 5)*. Purple-flowered plants flowered earlier (44.10 days) than white-flowered plants (46.29 days). For NSP, purple-flowered plants averaged 11.41 seeds per pod compared to 11.26 for white-flowered plants. For 100SW, purple-flowered plants averaged 8.66 g versus 8.64 g for white-flowered plants. Neither PL (12.62 cm vs. 12.57 cm) nor SY (28.30 g vs. 28.51 g) significantly differed among classes. Flower colour in cowpea, which is determined by anthocyanin accumulation in floral tissues, is controlled by the same biosynthetic pathway that affects seed coat and vegetative pigmentation (Herniter *et al*., 2019; Saunders, 1960).

The significant association between purple flower colour and earlier flowering suggests genetic linkage between the flower colour locus and earliness genes or, alternatively, pleiotropic effects of anthocyanin regulatory genes on the floral development rate.

The marginal differences in NSP and 100SW between flower colour classes, although statistically significant, are of small magnitude and reflect genetic drag rather than functional pleiotropy. The absence of association with PL and SY indicates that flower colour is not a reliable morphological marker for these traits.

Previous mapping studies have reported tight linkage between flower and seed coat colour loci, suggesting that these qualitative markers co-segregate and are proxies for linked genomic regions affecting quantitative traits (Herniter *et al*., 2019).

Single marker analysis (SMA) using immature pod pigmentation

Immature pod pigmentation showed significant associations ($p < 0.05$) with DTF, PL, NSP, and 100SW but not SY. Green (non-pigmented) pods were associated with later flowering (52.02 days) compared to pigmented pods (45.24 days) *(Table 6)*.

For PL, green pods averaged 12.57 cm compared to 13.33 cm for pigmented

pods. For NSP, green pods averaged 11.12 seeds compared to 12.00 for pigmented pods. For 100SW, green pods averaged 9.91 g compared 10.15 g for pigmented pods. SY showed no significant differences (27.39 g vs. 28.42 g). Immature pod pigmentation emerged as the most broadly associated morphological marker among the three evaluated pigmentation traits, showing significant associations with four of the five yield components.

The direction of effects consistently favoured pigmented pods: earlier flowering, longer pods, more seeds per pod, and a higher 100SW. This pattern suggests that the locus controlling immature pod pigmentation resides in a genomic region with linkage to yield-related QTLs or that anthocyanin regulatory genes in the pod have pleiotropic effects on multiple developmental processes.

The phenylpropanoid pathway, which produces anthocyanins in cowpea pods, is regulated by an MYB-bHLH-WD40 transcription factor complex (Liu *et al*., 2015; Petroni and Tonelli, 2011), and genes in this pathway have been implicated in stress responses and developmental regulation (Chalker-Scott, 1999).

Despite associations with yield components, the lack of association with total SY reflects the complex polygenic nature of yield and the compensatory interactions among yield components (Acquaah, 2007).

Better- and mid-parent heterosis in the F_2 phenotypic class for days to flowering

Better- and mid-parent heterosis for DTF varied significantly across phenotypic classes. All better-parent heterosis values were negative and significant ($p < 0.05$), ranging from −12.47% (white seed) to −28.33% (pigmented pod), indicating earliness compared to the better parent *(Table 7)*. Mid-parent heterosis was significant and negative for pigmented nodes (−9.06%), pigmented pods (−14.28%), and red seeds (−11.73%) but not significant for non-pigmented nodes (1.13%), purple flowers (−10.18%), white flowers (−1.66%), non-pigmented pods (1.53%), and white seeds (4.70%).

The consistently negative better-parent heterosis for DTF across all phenotypic classes indicates that F_2 plants flowered earlier than the better parent (P1), representing desirable heterobeltiosis for earliness.

The magnitude of this effect was greatest in the pigmented classes (pigmented pods, pigmented nodes, and red seeds), suggesting that the anthocyanin pigmentation allele is associated with enhanced earliness expression in hybrid combinations.

This aligns with the SMA results (*Table* 3, *Table 4* and *Table 5*) showing that pigmented phenotypes are associated with earlier flowering. The non-significant mid-parent heterosis in non-pigmented and white-flowered classes suggests that these phenotypic categories carry more late-flowering alleles, diluting the earliness effect. The exploitation of earliness through heterosis is agronomically valuable in cowpea, enabling drought escape and adaptation to short growing seasons (Fatokun *et al*., 2002).

However, as cowpea is a highly self-pollinated crop, fixing heterosis for earliness requires selection in advanced generations to identify transgressive

segregants that combine early maturity with high yield (Shirisha *et al*., 2022).

Better- and mid-parent heterosis in the F_2 phenotypic class for pod length

Both mid- and better-parent heterosis for PL were negative across all phenotypic classes *(Table 8)*.

Mid-parent heterosis ranged from −10.81% (white flower) to −14.97% (purple flower) ($p < 0.05$). Better-parent heterosis ranged from −13.44% (white flower) to −17.48% (purple flower), with all classes significant except white seed (−16.73%).

The universal negative heterosis for PL indicates that F_2 plants produced shorter pods than both mid- and better-parent values, representing undesirable heterotic effects for this trait.

This pattern contrasts with those found by Raut *et al*. (2017) and Shirisha *et al*. (2022), who reported positive heterosis for PL in some cowpea hybrids. However, this pattern agrees with observations that the direction and magnitude of heterosis are highly cross-dependent.

The consistent reduction in PL across all pigmentation classes suggests that the genetic factors reducing PL are broadly distributed in the genome and not specific to pigmentation-linked regions.

The non-significant better-parent heterosis for white seed (−16.73%) reflects greater variability within this class.

The negative heterosis for PL in this cross indicates that selection for longer pods should focus on parental combinations with complementary additive effects rather than heterotic recovery in F_2 populations.

Table 4 – Single marker analysis for yield traits using node pigmentation as the phenotypic class in an F_2 population

Traits	Not pigmented: Pigmented	F-Test
Days to flowering	53.10: 47.56	0.38*
Pod length	12.34: 13.14	0.12*
No. seeds/pod	10.74: 11.84	0.04 ns
100 seed weight	10.71: 11.05	0.44*
Seed yield	25.69: 26.71	1.21 ns

* = significant ($p < 0.05$); ns = not significant

Table 5 – Single marker analysis for yield traits using flower colour as the phenotypic class in an F_2 population

Traits	Purple: White	F-Test
Days to flowering	44.10: 46.29	0.23*
Pod length	12.62: 12.57	1.12 ns
No. seeds/pod	11.41: 11.26	0.95*
100 seed weight	8.66: 8.64	0.95*
Seed yield	28.30: 28.51	1.29 ns

* = significant ($p < 0.05$); ns = not significant

Table 6 – Single marker analysis for yield traits using immature pod pigmentation as the phenotypic class in an F_2 population

Traits	Green: Pigmented	F-Test
Days to flowering	52.02: 45.24	0.13*
Pod length	12.57: 13.33	0.61*
No. seeds/pod	11.12: 12.00	0.66*
100 seed weight	9.91: 10.15	0.87*
Seed yield	27.39: 28.42	1.17 ns

* = significant ($p < 0.05$); ns = not significant

Table 7 – Better- and mid-parent heterosis in days to flowering based on F_2 phenotypic (pigmentation) classes of a cowpea cross

Phenotypic class	Mid-parent heterosis	Better-parent heterosis
Pigmented node	−9.06*	−23.97*
Non-pigmented node	1.13 ns	−15.45*
Purple flower	−10.18 ns	−24.91*
White flower	−1.66 ns	−17.79*
Pigmented pod	−14.28*	−28.33*
Non-pigmented pod	1.53 ns	−15.11*
White seed	4.70 ns	−12.47*
Red seed	−11.73*	−26.20*

* = significant ($p < 0.05$); ns = not significant

Table 8 – Better- and mid-parent heterosis in pod length based on the F_2 phenotypic (pigmentation) class of a cowpea cross

Phenotypic class	Mid-parent heterosis	Better-parent heterosis
Pigmented node	−14.08*	−16.61*
Non-pigmented node	−11.25*	−13.88*
Purple flower	−14.97*	−17.48*
White flower	−10.81*	−13.44*
Pigmented pod	−13.48*	−16.04*
Non-pigmented pod	−13.08*	−15.65*
White seed	−14.19*	−16.73 ns
Red seed	−12.87*	−15.45*

* = significant ($p < 0.05$); ns = not significant

Better- and mid-parent heterosis in the F_2 phenotypic class for number of seeds per pod

Mid-parent heterosis for NSP was negative but not significant across all phenotypic classes, ranging from −3.16% (white flower) to −7.33% (purple flower) *(Table 9)*. In contrast, better-parent heterosis was negative and significant ($p < 0.05$) for all classes, ranging from −11.23% (white flower) to −15.05% (purple flower). The pattern of non-significant mid-parent but significant negative better-parent heterosis for NSP indicates that although the F_2 population mean did not significantly deviate from

the mid-parent value, individual phenotypic classes performed below the better parent (P1). This suggests that the genetic potential for a high NSP is not fully recovered in the F_2 generation, likely due to the breakdown of favourable dominant and epistatic gene combinations present in F_1 (Shull, 1914). The purple-flowered class showed the largest negative deviation, indicating that this phenotypic class carries allele combinations that reduce seed set per pod.

Similar variable heterosis for NSP has been reported in cowpea depending on the parental genotype (Raut *et al*., 2017; Shirisha *et al*., 2022).

Better- and mid-parent heterosis in the F_2 phenotypic class for 100-seed weight

Both mid- and better-parent heterosis for 100SW were positive and highly significant ($p < 0.05$) across all phenotypic classes (*Table 10*). Mid-parent heterosis ranged from 35.05% (white seed) to 45.68% (white flower), and better-parent heterosis ranged from 29.15% (white seed) to 39.33% (white flower). The highest heterosis was observed in white-flowered and red-seeded classes. The consistently high positive heterosis for 100SW represents the most promising heterotic response observed in this study. The magnitude of mid- (35–46%) and better-parent heterosis (29–39%) exceeds typical values reported among cowpea hybrids (Raut *et al*., 2017; Shirisha *et al*., 2022). The uniformity of this response across all pigmentation classes suggests that genes that enhance seed size are widely distributed and effectively combined in this cross, independent of the pigmentation background. The elevated 100SW in F_2 compared to the parents confirms that this heterotic advantage is partially maintained in the segregating generation. In cowpea, a large seed size is preferred by consumers, enhancing its marketability and nutritional value (Herniter *et al*., 2019), and the identification of heterosis for this trait provides a strong basis for selecting large-seeded transgressive segregants in advanced generations.

Better- and mid-parent heterosis in the F_2 phenotypic class for seed yield

Mid-parent heterosis for SY was negative and not significant across all phenotypic classes, ranging from −1.90% (white flower) to −6.65% (purple flower). Better-parent heterosis was negative and significant ($p < 0.05$) for all classes, ranging from −17.73% (white flower) to −21.71% (purple flower) *(Table 11)*. The negative better-parent heterosis for SY across all phenotypic classes showed that the F_2 generation failed to maintain the observed F_1 hybrid vigour. This decline is characteristic of self-pollinated crops in which heterozygosity is lost through segregation, leading to the dissipation of non-additive genetic effects that drive F_1 performance (Shull, 1914). The non-significant mid-parent heterosis suggests that the F_2 population mean is similar to the average parental performance, with no residual heterotic advantage. The purple-flowered and white-seeded classes showed the largest negative deviations, indicating that these phenotypic categories are associated with yield-reducing allele combinations in this cross.

These findings align with the complex quantitative inheritance of yield

in cowpea, which is influenced by multiple genes with additive, dominant, and epistatic effects (Acquaah, 2007; Fatokun *et al*., 2002). Although the F_1 heterosis for yield is impressive, sustainable genetic improvement requires selection in advanced generations to pyramid favourable additive alleles and develop stable high-yielding pure lines, as commercial hybrid production is not feasible in this highly self-pollinated species (Shirisha *et al*., 2022).

Table 9 – Better- and mid-parent heterosis in the number of seeds per pod based on the F_2 phenotypic (pigmentation) class of a cowpea cross

Phenotypic class	Mid-parent heterosis	Better-parent heterosis
Pigmented Node	−5.89 ns	−13.73*
Non-Pigmented Node	−4.62 ns	−12.57*
Purple Flower	−7.33 ns	−15.05*
White Flower	−3.16 ns	−11.23*
Pigmented Pod	−4.71 ns	−12.65*
Non-Pigmented Pod	−6.36 ns	−14.17*
White Seed	−6.95 ns	−14.7*
Red Seed	−5.24 ns	−13.13*

* = significant ($p < 0.05$); ns = not significant

Table 10 – Better- and mid-parent heterosis in 100-seed weight based on the F_2 phenotypic (pigmentation) class of a cowpea cross

Phenotypic class	Mid-parent heterosis	Better-parent heterosis
Pigmented Node	40.58*	34.44*
Non-Pigmented Node	41.61*	35.44*
Purple Flower	39.28*	33.21*
White Flower	45.68*	39.33*
Pigmented Pod	40.69*	34.55*
Non-Pigmented Pod	41.5*	35.33*
White Seed	35.05*	29.15*
Red Seed	43.83*	37.56*

* = significant ($p < 0.05$); ns = not significant

Table 11 – Better- and mid-parent heterosis in seed yield based on the F_2 phenotypic (pigmentation) class of a cowpea cross

Phenotypic class	Mid.het	Bet.het
Pigmented Node	−5.30 ns	−20.58*
Non-Pigmented Node	−3.00 ns	−18.65*
Purple Flower	−6.65 ns	−21.71*
White Flower	−1.90 ns	−17.73*
Pigmented Pod	−4.63 ns	−20.02*
Non-Pigmented Pod	−4.68 ns	−20.06*
White Seed	−5.98 ns	−21.15*
Red Seed	−4.15 ns	−19.62*

* = significant ($p<0.05$); ns = not significant

CONCLUSIONS AND RECOMMENDATIONS

The present study demonstrated that anthocyanin pigmentation traits in the P1 × P4 cowpea cross are consistently associated with agronomic performance in the F_2 generation. SMA revealed that pigmented phenotypic classes, particularly those with pigmented immature pods and nodes, outperformed non-pigmented classes for DTF, PL, NSP, and 100SW. Heterosis analysis within phenotypic classes showed that pigmented classes exhibited transgressive earliness (negative mid-parent heterosis), whereas non-pigmented classes clustered around the mid-parent mean. However, PL and SY showed uniformly negative better-parent heterosis across all classes, and 100SW displayed strong positive heterosis regardless of the pigmentation status. These findings suggest that pigmentation loci are either genetically linked to or pleiotropically influence QTLs related to yield components, particularly those governing phenology and pod development, but do not protect against the yield breakdown observed in the F_2 generation.

Based on these findings, node and immature pod pigmentation can be a cost effective, visual morphological markers for the preliminary enrichment of early maturing, longer podded progeny in early segregating generations ($F_2 – F_3$).

However, selection for complex polygenic traits, such as SY, may not solely rely on pigmentation class, given the consistent failure of all phenotypic classes to recover the better-parent yield performance. Breeders can integrate these qualitative markers as preliminary field screens, followed by quantitative progeny evaluation and advanced-generation testing. Future studies should employ molecular markers and QTL mapping to validate the linkage observed between pigmentation loci and yield component regions, enabling the development of morphological marker selection protocols that combine the efficiency of visual pigmentation scoring and the precision of genomic tools for cowpea improvement.

Author contributions: Conceptualisation: AAO; Methodology: AAO, SOO, NOO; Formal analysis: AAO, SOO, NOO; Investigation: AAO, SOO, NOO; Resources: AAO, SOO; Data curation: AAO, SOO, NOO; Writing - original draft: AAO; Writing - review and editing: AAO, SOO, NOO; Visualisation: AAO, SOO, NOO; Supervision: AAO, SOO, NOO; Project administration: AAO, SOO, NOO. All authors have read and approved the published version of the manuscript.

Funding: There was no external funding for this study.

Data availability statement: The data presented in this study are available on request from the corresponding author.

Conflicts of interest: The authors declare no conflict of interest.

REFERENCES

Acquaah, G. Principles of Plant Genetics and Breeding; Wiley-Blackwell: Oxford, UK, 2007.

Chalker-Scott, L. Environmental Significance of Anthocyanins in Plant Stress Responses. *Photochemistry and Photobiology* **1999**, *70*, 1-9. https://doi.org/10.1111/j.1751-1097.1999.tb01944.x

Edematie, V.E.; Fatokun, C.; Boukar, O.; Adetimirin, V.O.; Kumar, P.L.

Inheritance of Pod Length and Other Yield Components in Two Cowpea and Yard-Long Bean Crosses. *Agronomy* **2021**, *11*, 682. https://doi.org/10.3390/agronomy11040682

Fatokun, C.A.; Tarawali, S.A.; Singh, B.B.; Kormawa, P.M.; Tamò, M. Challenges and Opportunities for Enhancing Sustainable Cowpea Production. *Proceedings of the World Cowpea Conference III, International Institute of Tropical Agriculture* (IITA), Ibadan, Nigeria, 4–8 September 2000; IITA: Ibadan, Nigeria, 2002.

Herniter, I.A.; Lo, R.; Muñoz-Amatriaín, M.; Lo, S.; Guo, Y.-N.; Huynh, B.-L.; Lucas, M.; Jia, Z.; Roberts, P.A.; Lonardi, S.; Close, T.J. Seed Coat Pattern QTL and Development in Cowpea (Vigna unguiculata [L.] Walp.). *Frontiers in Plant Science* **2019**, *10*, 1346. https://doi.org/10.3389/fpls.2019.01346

Liu, Y.; Tikunov, Y.; Schouten, R.E.; Marcelis, L.F.M.; Visser, R.G.F.; Bovy, A. Anthocyanin Biosynthesis and Degradation Mechanisms in Solanaceous Vegetables: A Review. *Frontiers in Chemistry* **2018**, *6*, 52. https://doi.org/10.3389/fchem.2018.00052

Lo, S.; Muñoz-Amatriaín, M.; Boukar, O.; Herniter, I.; Cisse, N.; Guo, Y.-N.; Roberts, P.A.; Xu, S.; Fatokun, C.; Close, T.J. Identification of QTL Controlling Domestication-Related Traits in Cowpea (Vigna unguiculata L. Walp.). *Scientific Reports* **2018**, *8*, 6261. https://doi.org/10.1038/s41598-018-24349-4

Makoi, J.H.J.R.; Belane, A.K.; Chimphango, S.B.M.; Dakora, F.D. Seed Flavonoids and Anthocyanins as Markers of Enhanced Plant Defence in Nodulated Cowpea (Vigna unguiculata L. Walp.). *Field Crops Research* **2010**, *118*, 21-27.

Matjeke, M.B.; Labuschagne, M.T.; Gerrano, A.S.; Minnaar-Ontong, A.; Mbuma, N.W. Heritability and Expression of Yield and Yield Components in Cowpea, an Underutilized Crop in Africa. *Frontiers in Sustainable Food Systems* **2025**, *9*, 1588245. https://doi.org/10.3389/fsufs.2025.1588245

Mohammed, S.B.; Ongom, P.O.; Belko, N.; Umar, M.L.; Muñoz-Amatriaín, M.; Huynh, B.-L.; Togola, A.; Ishiyaku, M.F.; Boukar, O. Quantitative Trait Loci for Phenology, Yield, and Phosphorus Use Efficiency in Cowpea. *Genes* **2025**, *16*, 64. https://doi.org/10.3390/genes16010064

Ongom, P.O.; Fatokun, C.; Togola, A.; Garcia-Oliveira, A.L.; Ng, E.H.; Kilian, A.; Lonardi, S.; Close, T.J.; Boukar, O. A Mid-Density Single-Nucleotide Polymorphism Panel for Molecular Applications in Cowpea (Vigna unguiculata (L.) Walp.). *International Journal of Genomics* **2024**, *2024*, 9912987. https://doi.org/10.1155/2024/9912987

Padi, F.K. Genetic Analyses of Pigmentation in Cowpea. *Pakistan Journal of Biological Sciences* **2003**, *6*, 1655-1659.

Pathak, A.R.; Naik, M.R.; Joshi, H.K. Heterosis, Inbreeding Depression and Heritability for Yield and Yield Components in Cowpea. *Electronic Journal of Plant Breeding* **2017**, *8*, 72. https://doi.org/10.5958/0975-928X.2017.00010.2

Petroni, K.; Tonelli, C. Recent Advances on the Regulation of Anthocyanin Synthesis in Reproductive Organs. *Plant Science* **2011**, *181*, 219-229. https://doi.org/10.1016/j.plantsci.2011.05.009

Raut, D.M.; Gaikwad, N.B.; Potdar, M.B. Heterosis Studies in Cowpea (Vigna unguiculata (L.) Walp.). *International*

Journal of Current Microbiology and Applied Sciences **2017**, *6*, 1587-1593. https://doi.org/10.20546/ijcmas.2017.607.191

Sangwan, R.S.; Lodhi, G.P. Inheritance of Flower and Pod Colour in Cowpea (Vigna unguiculata L. Walp.). *Euphytica* **1998**, *102*, 191-193. https://doi.org/10.1023/A:1018369311372

Saunders, A.R. Inheritance in the Cowpea (Vigna sinensis). II: Seed Coat Colour Pattern; Flower, Plant, and Pod Colour. *South African Journal of Agricultural Science* **1960**, *3*, 141-162.

Sax, K. The Association of Size Differences with Seed Coat Pattern and Pigmentation in Phaseolus vulgaris. *Genetics* **1923**, *8*, 552-560.

Shirisha, K.; Sandhyakishore, N.; Sameer Kumar, C.V.; Pallavi, M. Estimation of Heterosis for Yield and Yield Contributing Characters in Cowpea (Vigna unguiculata L.). *Biological Forum – An International Journal* **2022**, *14*, 550-556.

Shull, G.H. Duplicate Genes for Capsule-Form in Bursa bursa-pastoris. *Zeitschrift für Vererbungslehre* **1914**, *12*, 97-149. https://doi.org/10.1007/BF01837282

Sodo, A.M.I.; Ongom, P.O.; Fatokun, C.; Olasanmi, B.; Dieng, I.; Boukar, O. Quantitative Trait Loci Mapping for Yield and Related Traits in Cowpea. *Genes* **2025**, *16*, 247. https://doi.org/10.3390/genes16030247

Academic Editor: Dr. Iulian GABUR