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GENETIC PURITY AND POLYMORPHIC MARKER EVALUATION OF COWPEA GENOTYPES

ÉVALUATION DE LA PURETÉ GÉNÉTIQUE ET DES MARQUEURS POLYMORPHES DES GÉNOTYPES DE NIÉBÉ

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ABSTRACT

Genetic purity and availability of polymorphic markers are fundamental pre-breeding requirements for accelerating crop improvement research. The objective of this study was to assess the purity of cowpea genotypes and evaluate the genotypes through morphological and molecular techniques. Fourteen cowpea genotypes were subjected to purity testing using 15 SSRs and converted-SNP markers. Genotypes including Bigbean, SpM028, Magic201, Alan Cash and IT82E-18 were identified as 100% pure at the loci tested. Field evaluation revealed high variability, with Bigbean, Alan cash, SpM028 and Padituya having large seed size and yield parameters. Cluster and molecular analyses revealed three and two groups, respectively: aiding in selection of divergent parents for heterosis maximisation. Polymorphic markers identified will be a useful resource in cowpea improvement, utilising the genotypes in the current study. The genotypes identified with large seed size and yield parameters, will serve as donors for cowpea improvement targeting these traits.

*Key Word*s: Genetic advance, heterosis, *Vigna unguiculata*

RÉSUMÉ

La pureté génétique et la disponibilité de marqueurs polymorphes sont des exigences fondamentales avant la sélection pour accélérer la recherche sur l'amélioration des cultures. L'objectif de cette étude était d'évaluer la pureté des génotypes de niébé et de les évaluer par des techniques morphologiques et moléculaires. Quatorze génotypes de niébé ont été soumis à des tests de pureté à l'aide de 15 SSR et de marqueurs SNP convertis. Des génotypes tels que Bigbean, SpM028, Magic201, Alan Cash et IT82E-18 ont été identifiés comme purs à 100 % aux loci testés. L'évaluation au champ a révélé une

forte variabilité, Bigbean, Alan Cash, SpM028 et Padituya présentant une taille de graine et des paramètres de rendement importants. Les analyses de cluster et moléculaires ont révélé respectivement trois et deux groupes, facilitant la sélection de parents divergents pour maximiser l'hétérosis. Les marqueurs polymorphes identifiés constitueront une ressource utile pour l'amélioration du niébé, en utilisant les génotypes de la présente étude. Les génotypes identifiés avec des paramètres de taille de graine et de rendement élevés serviront de donneurs pour l'amélioration du niébé ciblant ces caractères.

Mots Clés : Progrès génétique, hétérosis, *Vigna unguiculata*

INTRODUCTION

Genetic purity and availability of polymorphic markers, are fundamental pre-breeding requirements for accelerating crop improvement research. Selection of pure parents is crucial in crop improvement to prevent distortions in segregation ratios; and to provide certainty in selections. With the current DNA technology, identification and use of polymorphic markers among parent pairs, provides a means of fast advancement and confirmation of hybrids (Owusu *et al.*, 2022). Efficient crop improvement, through hybridisation among parent pairs, is hinged upon the purity of the parents involved in the cross.

The purity of a crop variety is the authenticity of the variety based on its agronomic characteristics and genetic makeup (Tu *et al.*, 2022; Kumar *et al.*, 2023). Agronomic or morphological characteristics, from plant architecture to seed are considered, including days to flowering, days to maturity, growth habit, leave shape, flower colour, pod pigmentations, plant height, pod colour, seed colour, and seed shape; among others are considered phenotypic characters (Guo and Zhu, 2014; Walter *et al.*, 2015; Fasoula *et al.*, 2020).

Agro-morphological techniques which are used for purity assessment of varieties, are experimenter-dependent and very subjective, time-consuming, laborious and cost-intensive; and in most cases not very reliable as certain features are masked by genetics in the present, only to appear in the next stage of cleaning (Pattanaik *et al.*, 2018). This could above all be misleading; hence, agro-morphological purity testing, when combined with other testing methods, for instance genotyping and sequencing, provide excellent identification of mixture and maintenance of purity of genotypes.

Hybridity testing plays a vital role in confirming the true status of hybrids (Direct cross (DC) F_1s, Backcross (BC_1s) or an intercross (IC_1) hybrid) generated. This is achieved with certainty and reliability, using polymorphic molecular markers; rather than the subjective comparison of phenotypic features of parents involved in the hybridisation (Owusu *et al.*, 2022). The presence of a single impure hybrid at the initial stages of population development, results in a cascading effect that will disrupt the efforts made.

Genetic purity, which deals with the shortfalls of phenotypic purity, involves DNA markers to access the genuineness of the innate of the variety (Bora *et al.*, 2016; Ben Romdhane *et al.*, 2018). Genetic purity testing is fast, reproducible and above all reliable. It has become the method of purity testing in several crops for seed and hybrid purity tests (Vieira *et al*., 2022). In varietal maintenance, purity testing is an important process in its development, and it was reported that a 1 % impurity in a hybrid seed reduces the yield of maize by 100 kg ha^{-1} (Mao *et al.,* 1998).

The marker system employed in genetic purity testing, is revolutionising the genetic purity testing process, with the advances in DNA technology. However, simple sequence repeats markers are the most used marker system in fingerprinting, marker-assisted breeding and purity testing of seeds and

hybrids (Sahoo and Samal, 2020). Simple sequence repeats (SSR) markers are crucial in breeding, despite the evolution of more advanced marker techniques, such as single nucleotide polymorphism (SNP) markers. This is because of their abundance in the genome, co-dominant nature, uniformity in distribution within the genome, the relatively cheaper cost in running at an average laboratory; and the less skill required for its utility (Santhy *et al*., 2023). In the cowpea genome, 49% of the genome has 12544 simple sequence repeats (Lonardi *et al.*, 2019); thus showing the abundance of SSRs in the cowpea genome.

Crop evaluations are important to ascertain the performance of lines and their characteristics prior to their usage in any breeding programme (Brown *et al*., 2020). This is so because it confirms the agronomic characteristics and provides a means of removing mixtures from the lot to ensure high purity of final breeding materials. It also results in unbiased estimates of crop parameters. Through evaluations, relationships among traits, are determined for associations that provide an avenue for selection. The aim of breeding programmes is often to achieve high yields; hence the direct and indirect effects of yield components on yield are fundamental.

Cowpea (*Vigna unguiculata*) is a legume native to Africa, due to the presence of wild species and archaeological data (Herniter *et al*., 2020). It is rich in nutrients, with prominence in its seed protein content of more than 25% (Owade e*t al*., 2020). It serves as food for humans and fodder for animals. Additionally, cowpea is important in improving soil fertility, through symbiotic nitrogen fixation (Kebede and Bekeko, 2020).

Despite the importance of the crop, its production is saddled with various constraints, which reduce its yield, quality and income to the farmer. Some of the major production constraints include low soil fertility, insect pests, pathogenic diseases‘ and nematodes proliferation; climate change-associated constraints, parasitic weeds and demand-related challenges (Roberts, 2010; Rubiales *et al.*, 2015; Awuku *et al.*, 2022). The objective of this study was to assess the purity of cowpea genotypes and evaluate markers through morphological and molecular techniques.

MATERIALS AND METHODS

Study site. The experiment was carried out at the CSIR-Savanna Agricultural Research Institute, Manga station in the Upper-East region of Ghana. Manga is located within latitude 11°01’N, longitude 00°16’W, and at an altitude of 249 m above sea level. The location is characterised by unimodal rainfall averaging, 141 mm mean annually (Asamoah and Ansah-Mensah, 2020).

Plant materials. The cowpea genotypes used in this study consisted of released varieties and landraces in Ghana; and advanced breeding lines from the University of California UCR-Riverside (Huynh *et al*., 2019) and International Institute of Tropical Agriculture (IITA) (Table 1).

Genetic purity testing. Genetic purity testing was carried out in a screen house, in 2021; with 20 plants per genotype. The genetic materials were planted in pots of dimensions 30 cm height and 25 cm diameter at the top. Two seeds were sown per a pot and later thinned to one seedling after two weeks. Leaf samples were taken from the two weeks old plants for purity testing. DNA was extracted on dried leaf samples, using the CTAB method (Doyle and Doyle, 1987).

The quality of DNA was checked using the agarose gel-based technique (Stephan *et al*., 2012). Amplification was done in 10 µL PCR reaction volume in an ABI thermal cycler. The condition for amplification was denaturation at 94 °C for 30 sec; while annealing was done at X °C (depending on primer (Table 2)), for 30 sec and extension at 72 °C for 30 sec. The amplified products were size separated on a 6% horizontal polyacrylamide gel

TABLE 1. Pedigree profiles of cowpea genotypes used in the study

Genotype	Pedigree	Origin	Type of line
Bigbean	NA	Ghana	Landrace
Alan cash	NA	Ghana	Landrace
Supermagic028	MULTI-PARENT	UCR	Advanced variety
Kirkhouse benga 1	IT99k-573-2-1 X SARC1-57-2	Ghana	Released variety
Wang kae	IT99k-573-1-1 X SARC1-57-2	Ghana	Released variety
Padituya	SARC 3-122-2	Ghana	Released variety
Zayura Pali	ZAAYURA X SARC1-57-2	Ghana	Released variety
Pisnu	NA	Ghana	Landrace
Benga Raaga	NA	Burkina faso	Landrace
IT82E-18	NA	IITA	Advanced line
SARC-1-57-2	NA	Ghana	Released variety
Supermagic201	MULTI-PARENT	UCR	Advanced line
Difeele	ASETENAPA X SARC 1-57-2	Ghana	Released variety
Kubi	NA	Ghana	Advanced Breeding line

electrophoresis (hPAGE) system. Gel images were taken using a gel doc system for analysis.

Data analysis. Gel images were scored using GelJ software (Heras *et al.*, 2015), for clear and scorable bands in base pairs. The band scores were converted into the presence or absence of alleles, for all markers for each genotype. The presence and absence data were subjected to purity analysis, using Neighbour-Joining Cluster analysis; using the simple matching dissimilarity method in DARwin software (Perrier and Jacquemoud-Collet, 2006). The output from DARwin was visualised using Figtree software (Rambaut, 2016).

Parental evaluation

Field evaluation. The parental materials that were subjected to the purity testing, were subsequently used for the field evaluation at the CSIR-SARI Manga station, in the upper-east region of Ghana. The field was laid out in a randomised complete block design (RCBD), with three replications. Each genotype was allocated to a single-row plot of 2 m length, with a planting spacing of 40 cm by 60 cm; and at a population density of 420,000 plants per hectare.

Two seeds were planted per hill and thinned to one seedling at two weeks after sowing. There was no fertiliser applied; however, the recommended pesticide spraying regime for common cowpea pests was done, whenever necessary, after flowering. The chemical under the trade name Ema Star, was used to control insects at a rate of 1.5 ml L^{-1}. Weeding was done manually at two and four weeks after planting.

Qualitative data were taken on flower colour, growth type, pod tip pigmentation and pod position relative to the canopy. Quantitative data were taken on days to first flower appearance, days to 50% flowering, days to first pod physiological maturity, days to 50% pod maturity, plant height at flowering, number of peduncles per plant, pod length, number of seeds per pod, mean hundred seed weight, seed width, seed length, seed thickness, seed volume and grain yield. These were measured using standard cowpea descriptor procedures (Fery *et al.,* 1983).

Genetic evaluation. Leaf samples of two weeks old plants, for all the 14 genotypes,

TABLE 2. The DNA markers used in this study

Name	Forward	Reverse	Tm
CP239/240	CACCCCCGTACACACACAC	CACTTAAATTTTCACCAGGCATT	57
CP431/432	CCTCAACACCTTTTGGAGGA	CAAATGCACCTCCTGTGCTA	56
CP171/172	GTAGGGAGTTGGCCACGATA	CAACCGATGTAAAAAGTGGACA	56
CP171/172B	CATAGTAATATGGTATGTCAGTA	CAACCGATGTAAAAAGTGGACA	51
CP379/380	AAACATCAAAATTAAGGATAATCAATG	AGTAGCGTGGGTGGAATTTG	55
CP341/342	ACGAAACGATGTTAATGCTGATT	AAAAAGATTTGATGTGATCTATGATGTT	55
EX40	GAGCCGGAATACAGGATCATG	GATGCAGGCTATAACCGCGG	60
Y68	CATAAGCAAAAAACTCTAAACTCTG	GGCACTCCAATCAGAGCTG	57
C42-2B	CAGTTCCCTAATGGACAACC	CAAGCTCATCATCATCTCGATG	58
61RM2	GATTTGTTTGGTTTCCTTAAG	GGTTGATCTTGGAGGCATTTT	54
LRR8	CATTCATCCACTCTCTTCCC	CCTTTGGTCATTGAATACATG	55
SSR1	CCTAAGCTTTTCTCCAACTCCA	CAAGAAGGAGGCGAAGACTG	52
2_01258R	GCACCAGAGTATGCAATCCA		55
2_01258F1	GGGCGAAGGAGGAATTCACCATTAACG		
2_01258F2	GAAGAAAGGTGGGAGAAGGAGGAATTCACCATTATCA		
2_05766F	TGGCCATTGATCAAGTTTTT		55
2_05766R1	GAAGAAAGGTGGGAGCAATTCAAACACAGAAAACACC		
2_05766R2	ATCAACAATTCAAACACAGAAAACTCA		
2_29720F	TGTTACAAAAGAGCTTTGATGTCC		55
2_29720R1	GAAGAAAGGTGGGAGTGTAATGTTTCTCTCACCAGTC		
2_29720R2	CGAGCTGTAATGTTTCTCTCACCACTT		

were sampled into 96 well plates and sent to the Intertek Sweden laboratory, for genotyping on the SNP platform. In all, 2753 SNP markers were used to genotype the samples.

Statistical analysis. Data obtained from the field evaluation, were split into quantitative and qualitative. The quantitative data were subjected to analysis of variance (ANOVA), using R-statistical software and means separated by the Fischer's least significant difference (LSD) at $P<0.05$).

SNP data were subjected to cleaning up, to remove single nucleotide polymorphisms (SNPs) with heterozygous calls, missing calls and monomorphic SNPs. This resulted in a total of 1605 SNP markers, distributed across the 11 chromosomes of cowpea for further analysis. The informativeness of the SNP markers was assessed using the PowerMarker software 3.2.5 (Liu and Muse, 2005). This included polymorphic information content, gene diversity, heterozygosity, major allele frequency and the availability of the markers. A dissimilarity matrix was generated using the Jaccard method in DARwin (Perrier and Jacquemoud, 2009). Cluster analysis was carried out using DARwin software (Perrier and Jacquemoud-Collet, 2006), employing the Neighbour-Joining Clustering method under 50000 bootstrapping.

The filtered genotypic data were used to assess the structure of the population, using the Structure Software (Pritchard *et al.*, 2000). The run conditions for the admixture based model, were a burn-in-period of 200,000 and an MCMC of 400,000, at an iteration of 10 for an assumed population (K) range of 1 to 8. The results were used to estimate the Likelihood Number of Structure (maximum delta K), through the web based structure harvester (Earl and vonHoldt, 2012).

A final run was made for the K, at a single iteration with a MCMC of 800,000 and a burn-in-period of 400,000. Genotypes were assigned to a subpopulation at a probability threshold > 0.75. Genotypes, which did not meet this criterion, were grouped as admixture. The grouping for the phenotypic data, the Neighbour Joining Clustering from molecular data and the structure grouping, were ultimately compared.

RESULTS

Purity and polymorphic markers. The markers were highly informative in assessing the purity of the genotypes, as most of them produced single bands per genotype (Fig. 1). Among the 14 cowpea genotypes tested, five (Bigbean, superMagic028, superMagic201, Alan Cash and IT82E-18) showed 100% purity; being pure at all the loci tested. Difeele had two impure plants; while Benga raaga a landrace from Burkina Faso, had the highest level of impure plants (7). This was followed by Kirkhouse benga 1, Wang Kae and Kubi, with six impure plants each. The general view of the genotypes depicted by the Neighbour Joining Clustering (Fig. 2), clearly showing the impure genotypes clustering separately from the entire group of genotypes for a particular set.

From the list of markers used for the purity testing, markers polymorphic among the lines were identified (Table 3). Of the 2753 SNP markers used for genotyping, the selected plants deemed pure from the SSR and initial screen house screening, 1931 SNPs were polymorphic among at least two pairs. The homozygosity of the 14 lines ranged from 99.76 to 100% for all 2753 SNPs. The two genotypes with homozygosity less than 100% were IT82E-18 (99.97%) and Pisnu (99.76%).

Field experimentation. Two growth types were recorded, semi-erect (11) and spreading (Bigbean, Alan cash and Benga raaga) (Fig. 3). Eight genotypes had the presence of pod tip pigmentation; while six did not. Two categories of pod position were recorded among the 14 genotypes, 12 of them were intermediate with pods close to the upper canopy; and two others (Bigbean and Alan

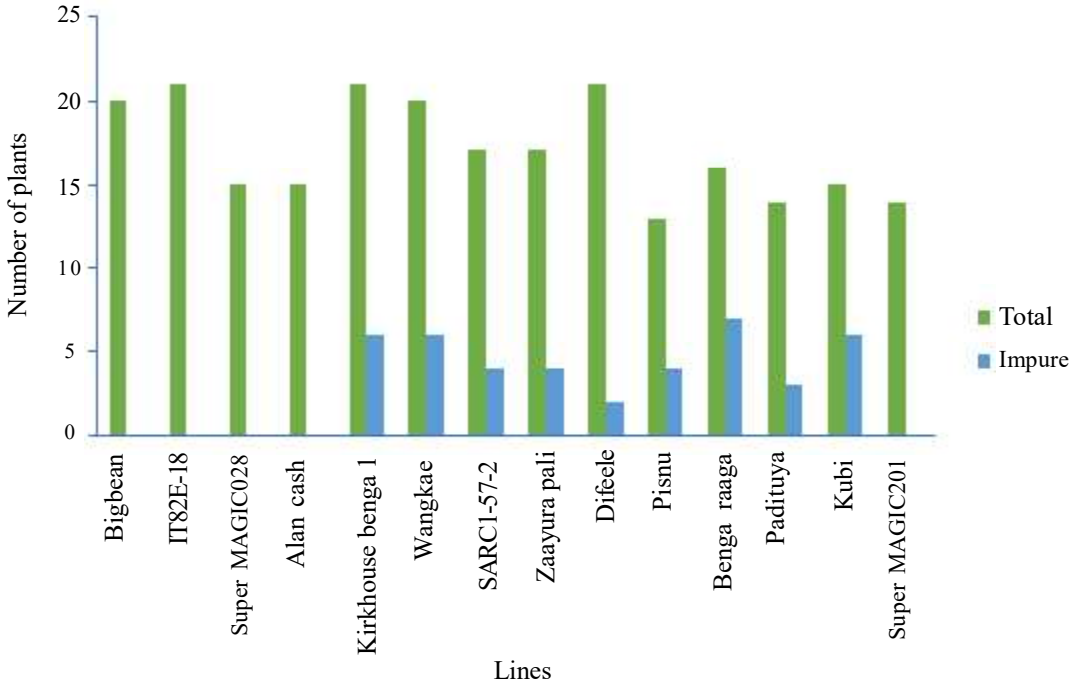


Figure 1. Variation in purity of cowpea genotypes as revealed by molecular markers.

cash) were within the canopy (Fig. 3). None of the genotypes had pod clearly above the canopy. Combined analysis of variance showed the presence of highly significant variations among the genotypes for all the 18 quantitative traits except canopy cover (Table 4).

The 14 cowpea genotypes were evaluated for 18 quantitative traits and four qualitative traits (Table 5, Fig. 3). There was variation in all qualitative traits; flower colour varied from white to purple with variation within the white, where some had purple pigmentation along the boundaries of the petals. Only one genotype (IT18E-82) had complete purple flower colour, while the remaining genotypes had a variation of white flowers.

The maturity related parameters (DFF, D50%F, DFPM and D50%PM) were significantly higher ($P<0.05$) for Bigbean and Alan cash than for Wankae, Kirkhouse benga, Difeele, Pisnu, Benga raaga, Zaayura pali, Soosima, IT82E-18, Kubi, SARC1-57-2, SpM028, MAGIC 201 and Padituya (Table 5). Bigbean and Alan cash flowered, on average ten days after all the other varieties had flowered and matured, on average 20 days after all other varieties had matured. Alan cash took 55 days to reach 50% flowering and 82 days to reach 50% pod maturity; while Bigbean also took 58 days to reach 50% flowering and 82 days to reach 50% pod maturity.

Seed length (SL), seed width (SW), seed thickness (ST) and seed volume (SV), also showed significant variation across genotypes (Table 5). A maximum of 11.610 mm was recorded for seed length, with a minimum of 7.837 mm (MAGIC 201) (Table 5). Seed width varied from a minimum of 5.657 mm (Difeele) to a maximum of 7.637 mm (Alan cash). Seed thickness (ST) also had a maximum value of 5.917 mm (spM028) and a minimum value of 3.544 mm (Pisnu). Seed volume (SV), which is the space occupied by the seed and is easily visualised by consumers, varied from 110.457 mm^3 for Difeele variety to 228.852 mm^3 for Bigbean variety.

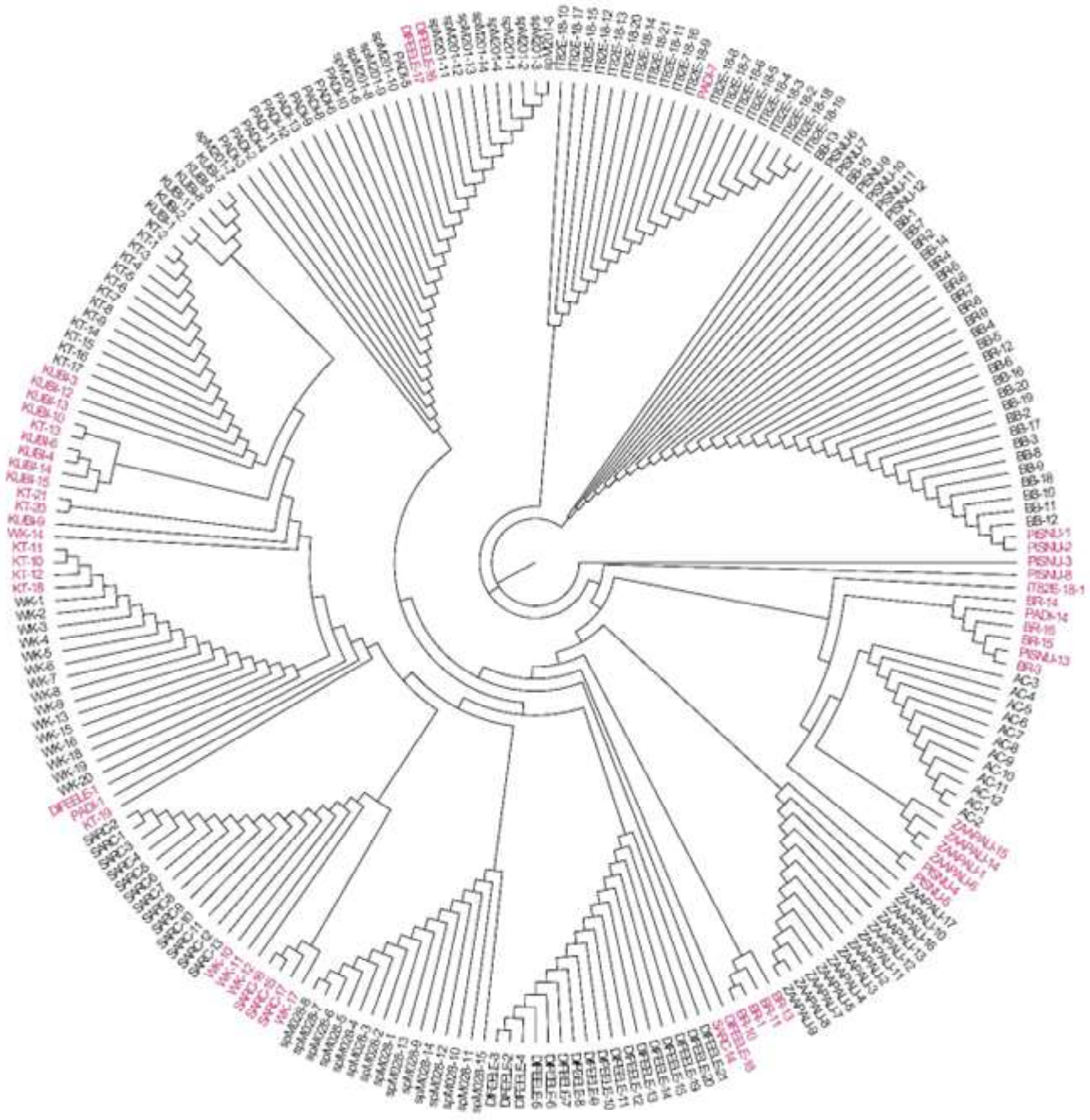


Figure 2. Unweighted Neighbour Joining Clustering of the 14 genotypes depicting impure/mixtures among each set. The coloured red genotypes were the impure/mixtures, BR-Benga raaga, BB-Bigbean, AC-Alan cash, Wk-Wangkae, KT-Kirkhouse Benga 1 and PADI-Padituya.

TABLE 3. Polymorphic markers among cowpea lines used in the present study

Genotypes	CP239/ 240	CP341/ 342	SNP2	CP431/ 432	C42-2B	CP171/ 172B	SSR1
Kirkhouse benga 1	180	225	150	200	280	175	150
Wangkae	180	225	125	250	280	175	150
IT82E-18	180	225	125	250	-	150	-
Bigbean	120	200	125	250	-	150	-
SPMAGIC028	180	200	125	200	-	175	-
Alan cash	120	225	150	250	-	150	-
Pisnu	120	200	125	250	-	150	-
Zaayura pali	120	200	150	250	-	150	-
SARC1-57-2	120	200	150	200	-	175	-
Benga raaga	120	200	125	250	-	150	-
Kubi	180	225	150	200	280	150	150
spMagic201	180	200	125	250	-	175	-
Difeela	180	200	150	250	-	175	-
Padituya	180	200	125	250	-	175	-

Genotypes, Bigbean, Alan cash, spM028 and MAGIC201, had seed volumes >200 mm^3 (Table 5). Of the four genotypes, those with >150 mm^3 were categorised as large; while those with <200 mm^3 were categorised as medium (Kubi, Padituya, SARC1-57-2 and Zaayura Pali); and those <150 mm^3 as small (Wang kae, Kirkhouse benga-1, Benga raaga, Difeele, IT82E-18 and Pisnu).

Mean 100-seed weight (MHSW) also varied significantly ($P<0.05$) across the genotypes, with Difeele having the lowest value of 15.822 g; and with genotype Bigbean recording the highest of 30.867 g (Table 5). Three genotypes, Alan cash, Bigbean and spM028, had the three highest seed weights. Based on seed weight, the genotypes were categorised as heavy, medium and light seeded based on the mean 100-seed weight.

Genotypes with MHSW < 20 g per 100 seeds, were categorised as light (Difeele, Pisnu, Wangkae, It82e-18, Kirkhouse Benga 1, Benga Raaga and Kubi). Those with MHSW > 20 g per 100 seeds, but less than 25 g per 100 seeds, were categorised as medium (Sarc1-57-2, Magic201, and Padituya). Genotypes with > 25 g per 100 seeds as heavy (Zaayura Pali, Alan cash, Bigbean and spM028).

Pod characteristics including pod length, number of seeds per pod, pod weight and number of pods per plant also varied significantly across the 14 genotypes (Table 5).

Decomposition of genetic components. Heritability estimates for the measured traits were high for most of the traits. The least was 0.235 which was for canopy cover and the highest heritability recorded was 0.971 which was for mean hundred seed weight (Table 6). The heritability for most of the traits were above 0.80 (80%), which is quite high. Seed length, seed width, seed thickness and seed volume recorded very high broad sense heritability, greater than 90% (0.90). Days to first flowering had the highest heritability of 0.899 (89.9%); while days to 50% pod maturity recorded a heritability of 87.1% (Table 6).

The genetic advance, which is the gain in selection for a given trait, varied from 0.223 for seed density to 420.607 for pods weight (Table 6). High gain was recorded for seed

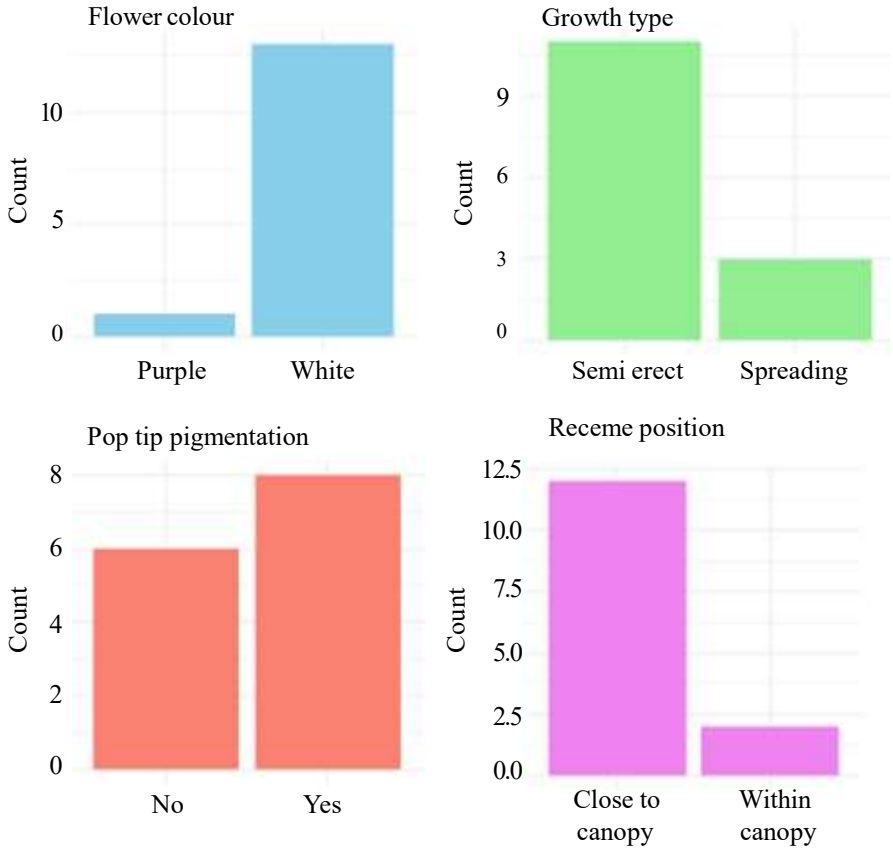


Figure 3. Variation in qualitative traits among the cowpea lines evaluated.

TABLE 4. Combine analysis of variance for measured cowpea traits

	Df	DFF	D50%F	DFPM	D50%PM	PH	NP
Block	2	0.167	2.952	5.31	6.595	10.132	26.681
Genotype	13	63.958***	106.082***	73.804***	159.766***	123.208***	71.318**
Residual	6	2.321	4.978	14.156	5.364	26.399	16.764
		NPP	Canopy	SL	SW	ST	SV
Block	2	138.29	174.372*	0.2946*	0.24435**	0.08148*	832.4**
Genotype	13	420.35***	61.264	3.8218***	1.05919***	1.2822***	6011.5***
Residual	6	58.42	31.88	0.0631	0.03415	0.01616	108.6
		SD	PL	NSP	MHSW	GYP	PW
Block	2	0.005182	1.1966	0.8602	7.799***	64503*	93647*
Genotype	13	0.046081***	8.7692***	9.4068***	81.07***	114907***	207682***
Residual	6	0.003168	0.4895	0.5613	0.789	15117	26866

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

DFF = Days to first flowering, D50%F = Days to fifty percent flowering, DFPM = Days to first pod maturity, D50%PM = Days to fifty percent pod maturity, PH = Plant height, GYP = Grain yield per plot, MHSW = Mean hundred seed weight, NPP = Number of pods per plant, NP = Number of peduncles, PW = Pod weight, PL = Pod length, NSP = Number of seeds per pod, SD = Seed density, SL = Seed length, SW = Seed Width, ST = Seed thickness, SV = Seed volume

TABLE 5. Performance of the 14 cowpea genotypes grown under rain fed conditions in 2021 season in Ghana

Genotypes	Canopy	DFF	D50%F	DFPM	D50%PM	PH	GYP	MHSW	NPP
Alan cash	54.200	49	55	67	82	41.867	650.267	29.078	26
Benga raaga	43.800	44	47	67	75	21.000	811.800	17.622	58
Bigbean	54.133	51	58	71	82	35.533	831.633	30.867	35
Difeele	63.200	40	44	60	70	49.800	312.533	15.822	27
IT82E-18	52.800	36	38	53	59	33.333	204.333	17.056	13
Kirkhouse benga 1	50.067	37	39	57	62	37.467	294.167	17.489	15
Kubi	50.400	38	40	61	66	33.867	263.833	19.578	12
MAGIC201	53.833	38	40	59	63	33.000	340.733	22.689	20
Padituya	56.733	40	42	62	65	41.733	364.833	24.356	13
Pisnu	57.467	42	44	63	69	36.933	499.800	16.489	23
SARC1-57-2	51.733	38	41	58	62	38.333	469.733	21.467	22
SpM028	55.667	38	40	57	67	37.200	385.000	28.433	26
Wangakae	49.467	37	40	55	61	31.400	385.467	16.756	18
Zaayura pali	55.533	40	42	59	66	35.867	330.133	25.289	19
CV (%)	10.553	4	5	6	3	14.178	28.015	4.104	33
LSD (0.05)	9.476	3	4	6	4	8.623	206.353	1.491	13
	PW	PL	NSP	SD	SL	SW	ST	SV	
Alan cash	932.833	18.017	14	1.291	9.722	7.637	5.793	225.290	
Benga raaga	1016.933	13.437	12	1.546	8.383	6.262	4.146	114.081	
Bigbean	1101.000	18.750	14	1.349	10.282	7.617	5.580	228.852	
Difeele	398.700	16.953	13	1.438	7.837	5.657	4.740	110.457	
IT82E-18	266.767	18.784	14	1.221	8.543	6.431	4.855	139.751	
Kirkhouse benga 1	373.233	16.730	14	1.403	8.128	6.479	4.520	124.662	
Kubi	353.300	17.217	13	1.230	9.385	6.288	5.157	159.670	
MAGIC201	469.733	20.107	10	1.071	11.610	7.361	4.744	212.743	
Padituya	479.900	18.910	14	1.292	10.400	6.713	5.154	188.648	
Pisnu	659.200	16.530	13	1.483	9.223	6.491	3.544	111.203	

TABLE 5. Contd.

Genotypes	Canopy	DFF	D50%F	DFPM	D50%PM	PH	GYP	MHSW	NPP
SARC1-57-2	648.533	18.797	12	1.185	10.483	6.600	5.003	181.615	
SpM028	484.667	19.177	10	1.330	10.454	6.595	5.917	213.954	
Wangkae	455.667	16.763	16	1.334	8.132	5.801	5.095	126.328	
Zaayura pali	404.833	15.960	10	1.336	9.189	6.929	5.677	189.415	
CV (%)	28.523	3.9796	6	4.257	2.669	2.786	2.545	6.270	
LSD (0.05)	275.095	1.1742	1	0.094	0.422	0.310	0.213	17.489	

DFF = Days to first flowering, D50%F = Days to fifty percent flowering, DFPM = Days to first pod maturity, D50%PM = Days to fifty percent pod maturity, PH = Plant height, GYP = Grain yield per plot, MHSW = Mean hundred seed weight, NPP = Number of pods per plant, PW = Pod weight, PL = Pod length, NSP = Number of seeds per pod, SD = Seed density, SL = Seed length, SW = Seed Width, ST = Seed thickness, SV = Seed volume, CV = Coefficient of variation, LSD = Least significant difference

volume (88.856), and except for seed density, all traits had genetic advances >1. The genetic advance, as a percentage of the mean (GAM), also varied across measured traits, from 5.84% for canopy cover to 73.19% for pod weight (Table 6). Among the seed size-related traits, seed volume was the highest (53.526 %). The rest, including seed length and width; and seed thickness recorded a GAM of 23.90, 17.311 and 26.292%, respectively (Table 6). Grain yield per plot also had a high GAM (70.983%).

Phenotypic and molecular cluster analysis. The Neighbour-Joining Cluster analysis classified the 14 cowpea genotypes into three clusters (Fig. 4). Cluster 1 was made up of 6 genotypes (Wangkae, Zaayura pali, Difeele, Kirkhouse benga 1, IT82E-18 and Kubi). Individuals within this cluster were characterised by lower values for most traits studied, with a range of 1.327 for seed density, to 372.4167 g for pod weight.

Cluster 2 consisted of seven genotypes (Alan cash, Bigbean, Padituya, spM028, Benga raaga, Pisnu and SARC1-57-2) (Fig. 4). These are characterised by higher values for traits measured with a range of 1.354 for seed density to 760.438 g for pod weight. In this Cluster, Alan cash and Bigbean were separated from the rest, affirming their superiority in almost all traits measured. Cluster 3 consisted of only one genotype (MAGIC201).

Clustering based on the genotypic data of 1605 polymorphic SNP markers, also put the 14 genotypes into three clades or clusters (Fig. 5). Cluster 1 consisted of seven genotypes (Alan cash, Bigbean, IT82E-18, SARC1-57-2, Zaayura pali, Kirkhouse benga 1 and Difeele), which were typified by medium to large-seeded genotypes. Cluster 2 consisted of six genotypes (Wang kae, Padituya, Kubi, Pisnu, MAGIC201, and Benga raaga), also typified by small-seeded genotypes. Cluster 3 comprised of only genotype Spm028.

The molecular dissimilarity among pairs of genotypes ranged from 0.01 to 0.66, with Alan Cash and Big bean being the most similar with dissimilarity of 0.01 between them (Table 7).

TABLE 6. Components of variance, heritability and genetic advance of cowpea genotypes

Traits	$\bar{X}$+SEM	δ^2_e	δ^2_e	δ^2_e	CV_E	CV_G	CV_P	H_b	GA	GAM
DFF	40.595 ± 0.880	2.3205	20.5458	22.8663	3.753	11.166	11.779	0.899	8.851	21.803
D50%F	43.548 1.288	4.978	33.7015	38.6795	5.124	13.331	14.282	0.871	11.163	25.634
DFPM	60.595 2.172	14.156	19.8828	34.0385	6.209	7.359	9.628	0.584	7.020	11.586
D50%PM	67.762 1.337	5.3645	51.467	56.8315	3.418	10.587	11.125	0.906	14.064	20.755
PH	36.238 2.966	26.399	32.2695	58.6685	14.179	15.676	21.137	0.55	8.679	23.949
NPP	23.210 4.413	58.418	120.643	179.0615	32.930	47.323	57.653	0.674	18.573	80.019
Canopy	53.502 3.260	31.880	9.7947	41.6746	10.553	5.850	12.066	0.235	3.126	5.842
SL	9.412 0.145	0.063	1.2529	1.316	2.669	11.892	12.188	0.952	2.250	23.904
SW	6.633 0.107	0.034	0.3417	0.3758	2.786	8.813	9.242	0.909	1.148	17.311
ST	4.995 0.073	0.016	0.422	0.4382	2.545	13.006	13.253	0.963	1.313	26.292
SV	166.191 6.016	108.584	1967.627	2076.211	6.270	26.691	27.418	0.948	88.956	53.526
SD	1.322 0.032	0.003	0.0143	0.0175	4.257	9.045	10.006	0.817	0.223	16.845
PL	17.581 0.404	0.490	2.7599	3.2494	3.980	9.450	10.253	0.849	3.154	17.940
NSP	12.874 0.433	0.561	2.9485	3.5098	5.819	13.338	14.552	0.840	3.242	25.184
MHSW	21.642 0.513	0.790	26.760	27.5492	4.104	23.903	24.253	0.971	10.503	48.530
PW	574.664 94.633	26866.42	60271.72	87138.14	28.523	42.721	51.368	0.692	420.607	73.191
GYP	438.876 70.986	15117.02	33263.18	48380.2	28.015	41.557	50.118	0.688	311.528	70.983

DFF = Days to first flowering, D50%F = Days to fifty percent flowering, DFPM = Days to first pod maturity, D50%PM = Days to fifty percent pod maturity, PH = Plant height, GYP = Grain yield per plot, MHSW = Mean hundred seed weight, NPP = Number of pods per plant, PW = Pod weight, PL = Pod length, NSP = Number of seeds per pod, SD = Seed density, SL = Seed length, SW = Seed Width, ST = Seed thickness, SV = Seed volume, = Environmental variance, = Genotypic variance, = Phenotypic variance, = Environmental Coefficient of Variance, = Genotypic coefficient of variance, = Phenotypic Coefficient of Variance, = Heritability (Broad Sense), GA = Genetic Advance GAM: Genetic Advance as a percentage of the mean

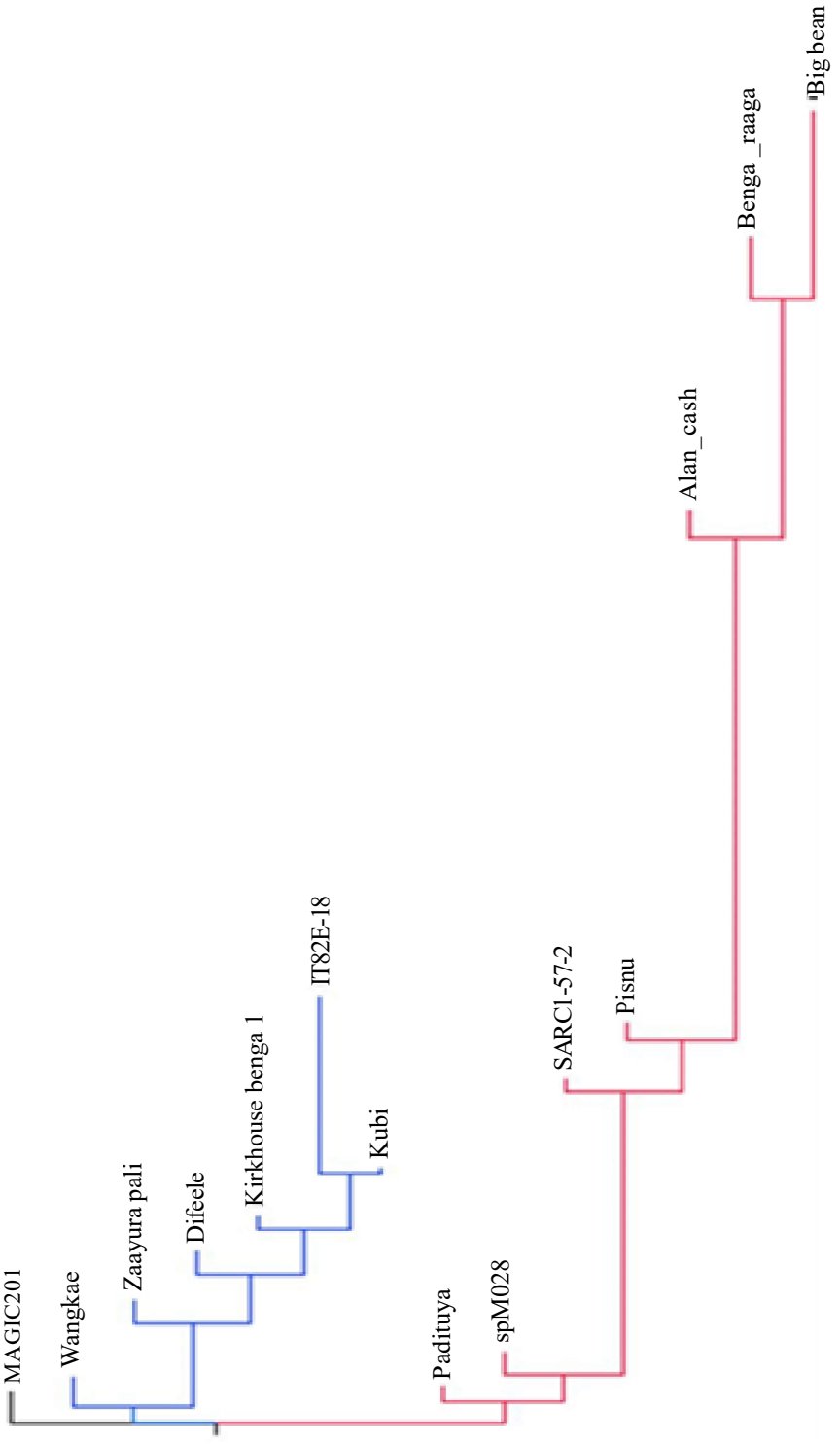


Figure 4. Clustering of the 14 cowpea genotypes based on quantitative field data.

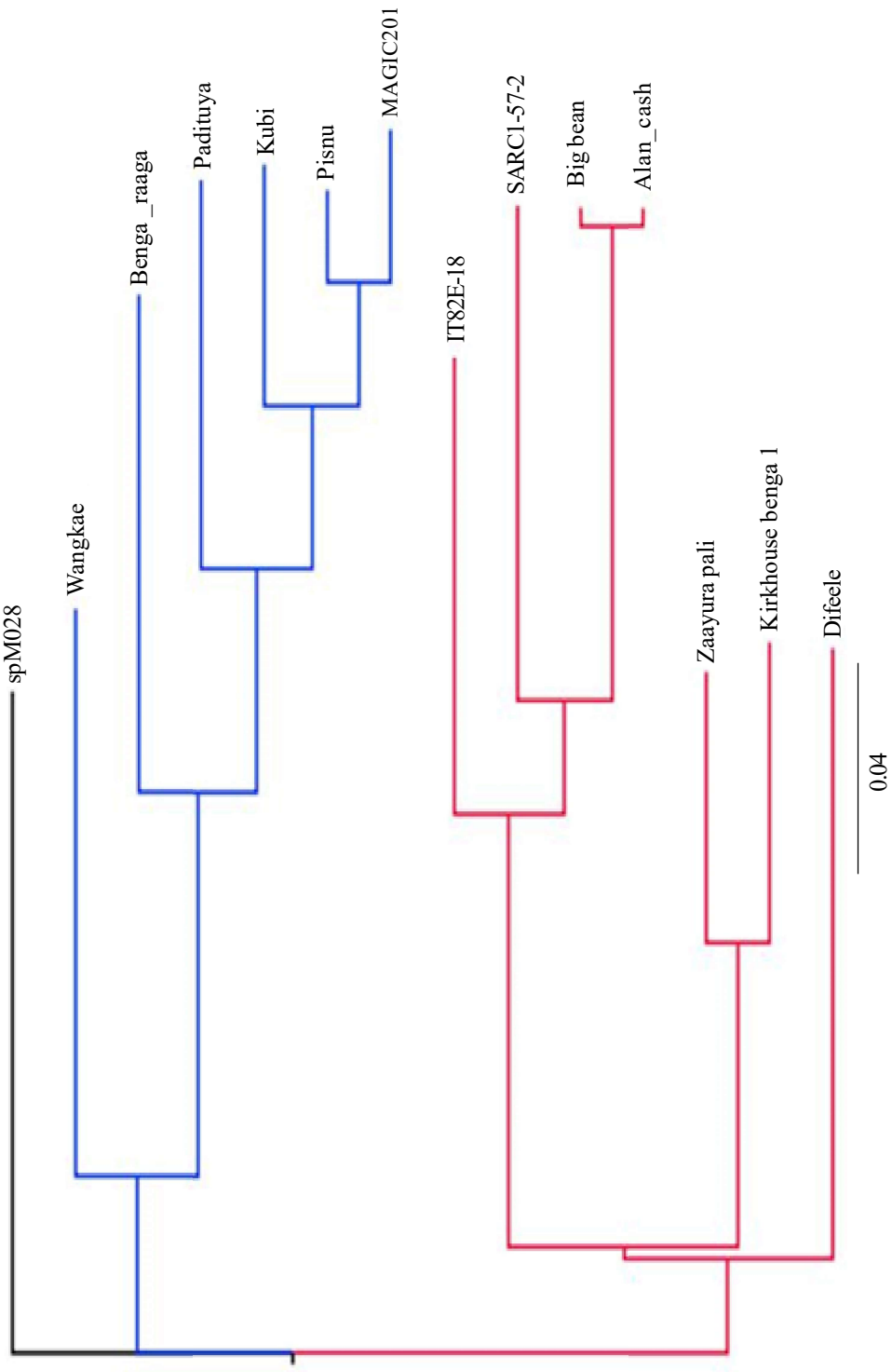


Figure 5. Clustering of the 14 cowpea genotypes based on the 1605 polymorphic SNP markers.

TABLE 7. Molecular dissimilarity among the 14 cowpea genotypes

Genotypes	SARC1-57-2	Difeele	Zaayura pali	Kubi	IT82E-18	Padituya	Pisnu	Benga raaga	MAGIC 201	Bigbean	Wangkae	SpM028	Alan_ cash
Difeele	0.429												
Zaayura_pali	0.435	0.324											
Kubi	0.648	0.495	0.503										
IT82E-18	0.313	0.414	0.400	0.572									
Padituya	0.615	0.490	0.485	0.216	0.579								
Pisnu	0.649	0.481	0.484	0.109	0.587	0.197							
Benga_raaga	0.547	0.468	0.498	0.284	0.496	0.339	0.274						
MAGIC201	0.649	0.494	0.497	0.152	0.593	0.225	0.066	0.304					
Bigbean	0.265	0.439	0.411	0.647	0.270	0.631	0.655	0.572	0.658				
Wangkae	0.494	0.425	0.335	0.413	0.486	0.409	0.397	0.441	0.414	0.508			
SpM028	0.444	0.394	0.427	0.474	0.417	0.478	0.481	0.458	0.492	0.452	0.430		
Alan_cash	0.262	0.438	0.410	0.646	0.270	0.633	0.654	0.571	0.658	0.009	0.510	0.453	
Kirkhouse benga_1	0.452	0.338	0.152	0.500	0.443	0.485	0.482	0.525	0.501	0.429	0.327	0.395	0.428

TABLE 8. Comparative grouping of cowpea genotypes based on molecular and quantitative phenotypic data

Genotypes	Probability threshold		Structure groups	NJ_MD groups	NJ_QPD groups
Benga_raaga	0.307	0.693	Admixture	Cluster 2	Cluster 3
Wangkae	0.562	0.438	Admixture	Cluster 2	Cluster 2
SpM028	0.695	0.305	Admixture	Cluster 1	Cluster 3
SARC1-57-2	1.000	0.000	Subpopulation 1	Cluster 3	Cluster 3
Difeele	0.754	0.246	Subpopulation 1	Cluster 3	Cluster 2
Zaayura_pali	0.806	0.194	Subpopulation 1	Cluster 3	Cluster 2
IT82E-18	0.998	0.002	Subpopulation 1	Cluster 3	Cluster 2
Bigbean	1.000	0.000	Subpopulation 1	Cluster 3	Cluster 3
Alan_cash	1.000	0.000	Subpopulation 1	Cluster 3	Cluster 3
Kirkhouse_benga 1	0.781	0.219	Subpopulation 1	Cluster 3	Cluster 2
Kubi	0.000	1.000	Subpopulation 2	Cluster 2	Cluster 2
Padituya	0.131	0.869	Subpopulation 2	Cluster 2	Cluster 3
Pisnu	0.000	1.000	Subpopulation 2	Cluster 2	Cluster 3
MAGIC201	0.000	1.000	Subpopulation 2	Cluster 2	Cluster 1

NJ_MD = Neighbour Joining using molecular data, NJ_QPD = Neighbour Joining using quantitative phenotypic data

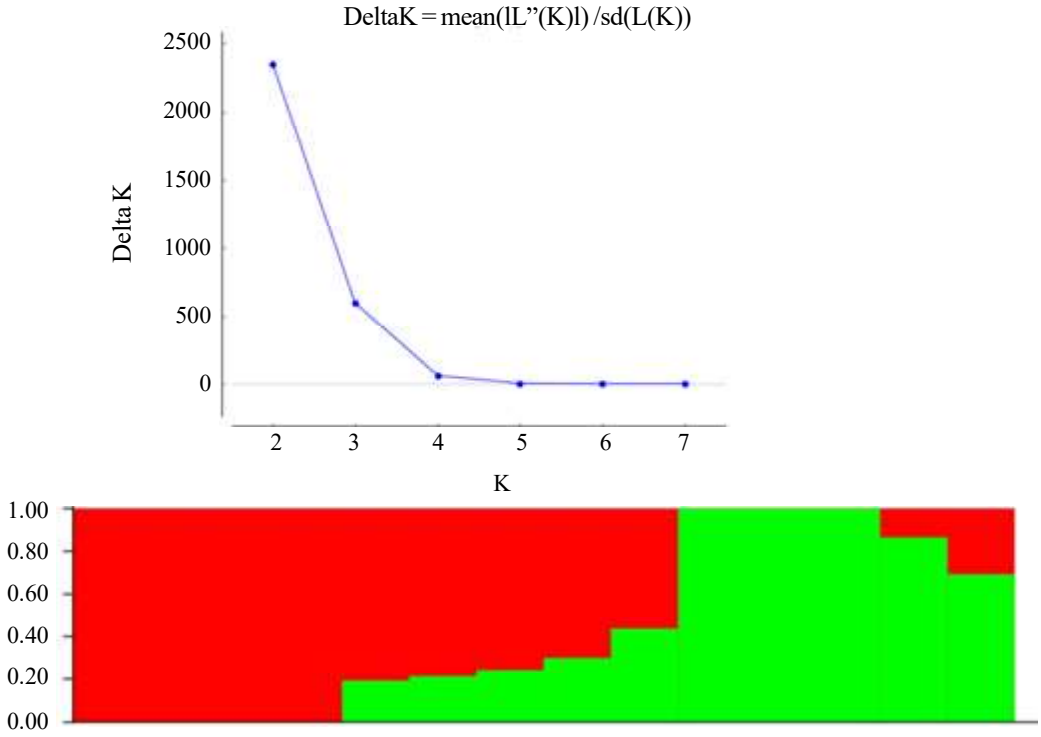


Figure 6. Structural analysis of 14 cowpea genotypes using data from 1605 informative SNPs. The diagram above shows the maximum delta K from the Evanno method; while the one below shows the two subpopulations obtained. Red and green represent subpopulations 1 and 2, respectively.

The least similar genotypes were Alan Cash and MAGIC201. There were slight differences between the two-clustering using morphological and molecular data (Table 8).

Population structure. The 1605 SNP markers used were highly informative, with no heterozygous calls, nor missing data (Fig. 6). Major allele frequencies ranged from a minimum of 0.50, to a maximum of 0.93; and averaged 0.67. Gene diversity recorded a range of 0.13 to 0.50, averaging 0.41 across the markers. The polymorphic nature of the SNP markers recorded as polymorphic information content (PIC), ranged from 0.12 to 0.38, and averaged 0.32.

The Admixture-Based Structure analysis revealed a maximum delta K value of 2, indicating a two-population structure among the 14 cowpea genotypes (Fig. 6). At the 75% threshold probability, 50% (7 genotypes) of the genotypes were grouped into subpopulation 1, and 29% (4 genotypes) grouped into subpopulation 2; with the remaining 21 % (3 genotypes) not meeting the threshold. Subpopulation 2 had a higher fixation index of 0.833, with a corresponding lower expected heterozygosity value of 0.100. Subpopulation 1 recorded a lower fixation index of 0.399, with a higher expected heterozygosity of 0.303. The allele frequency divergence between the two subpopulations was 0.427.

The Admixture Model grouped the genotypes into two subpopulations; while the Neighbour- Joining using molecular and phenotypic data, grouped them into three clusters (Table 8). The admixture-based grouping and the NJ grouping using molecular data, showed concordance. However, the grouping based on quantitative phenotypic data differed from those of the two groupings.

DISCUSSION

Genetic purity assessment. The markers used revealed great diversity within each set of the 14 genotypes, thus allowing for the identification of mixtures within a set (Table 3). Five (Bigbean, superMagic028, superMagic201, Alan Cash and IT82E-18) out of the 14 cowpea genotypes, showed 100% purity at all loci tested. This implies that there were no mixtures within this set and, thus provides the best form of parental materials required (Sendekie, 2020). However, the remaining 9 cowpea lines had some mixtures, hence requiring thorough cleaning before their utility in any breeding programme, to avoid mixture multiplication from cycle to cycle, which will affect the agronomic performance and subsequent yield.

Mao *et al.* (1998) opined that a 1% impurity in a hybrid seed has a corresponding yield reduction of 100 kg ha^{-1}; hence purity testing is vital in any breeding programme. Kirkhouse Benga and Wangkae, which were highly popular varieties, had some mixtures and hence, will require cleaning before seed multiplication and availability to farmers to maintain their performance. The possible source of mixtures in these advanced and released lines are numerous; however, the common ones are mechanical mixtures due to handling errors from seed preparation to planting, harvesting and storage, developmental variations associated with differences in the region of the development of variety and region of cultivation, and mutations (Kumar *et al*., 2023; Manjit *et al*., 2023). Simple sequence repeats converted SNP and SNP polymorphic markers identified form a great resource for hybridity testing, purity testing and quantitative trait loci analysis, through hybridisation using these genotypes.

Genetic purity assessment of parental lines forms an integral part of any breeding programme, hence maintaining the integrity and sustainability of the programmes (Alemu *et al*., 2021; Owusu *et al*., 2022; Sundareswaran *et al*., 2023). It thus ensures productivity and quality delivery of the product of the breeding programme. Traditionally, purity testing is by the grown-out-test (GOT), which is laborious, expensive, space and time-

constraining as plants need to be allowed to full maturity for certain traits to be taken (Alam *et al.*, 2015; Ahmar *et al.*, 2020).

Field evaluation. Field evaluation revealed significant variation among the 14 cowpea genotypes for all the traits measured, with a wide range of coefficient of variation across traits (Table 5). This was an indication of the existence of high diversity among the genotypes for selection for any of the traits (Said and Hefny, 2021). Bigbean, having the highest grain yield per plot, as well as the highest 100-seed weight, followed by Alan cash and Spm028; showed that they can be used for crop improvement programmes targeting grain yield. Their mean 100-seed weight, was higher than hitherto recorded national (CCVRRG, 2019), and regional (RCWA, 2021) catalogue. However, the growth habit of Bigbean and Alan Cash is a serious draw-back to their commercial use, coupled with their late maturity, photoperiod sensitivity and susceptibility to aphid and *striga gesnerioides* (Ehlers and Hall, 1997; Owusu *et al.*, 2022). Hence, this can be used as a donor in cowpea improvement programmes. Categorisation of seeds size has been done by Traore *et al.* (2022), through seed weight; however, what consumers see is the volume of the seeds, while in terms of yield, it is the weight. Hence, in the present study, seed size categorisation was based on seed volume and mean 100-seed weight. Bigbean, Alan cash and SpM028 were classified as large and heavy seeded, based on volume and seed weight, respectively. Thus, this makes these three most suited as donors for cowpea improvement targeting large and heavy seeds for improved yields.

Decomposition of genetic components. A higher phenotypic coefficient of variation observed, than genotypic coefficient of variation for all traits, indicates the less influence of the environment on these traits (Table 6). This was evident in the low coefficient of environmental variation for most of the traits. The high PCV and GCV observed for grain yield, pod weight, mean 100-seed weight, number of pods per plant and seed volume, is consistent with earlier reports (Aliyu and Makinde, 2016; Nguyen *et al.,* 2019; Owusu *et al.*, 2021).

The decomposition of the genetic components of variation for the traits, gives more details on the inherited genetic abilities for the traits and the effects of environment on it; and hence, giving vital information on breeding and selection (Owusu *et al*., 2020). Heritability estimates are important parameters for selection and crop improvement (Toghiani and Toghiani, 2012); whereby a high heritability is necessary for making good gains from selection. According to Li *et al.* (2001), heritability greater than 0.70 (70%) is key for making progress in trait selection.

In the present study, 11 traits had heritability values >70% (81.7 - 97.1%); which points to the fact that progress in selection for these traits, will be high. Owusu *et al.* (2021), observed high heritability for days to first flowering, days to 90% pod maturity, plant height at maturity, number of pods per plant, pod weight, grain yield and 100-seed weight; when they studied the genetic variability, heritability and correlation analysis among maturity and yield traits in cowpea. Similarly, the present study recorded very high heritability values for days to first flowering, days to 90% pod maturity, plant height at maturity, number of pods per plant, pod weight, grain yield and 100-seed weight. This indicates that these traits can easily be passed on through hybridisation and selection, resulting in positive progress in selection.

A high genetic advance (1.143-420.607) was observed for majority of the traits measured, except for seed density (0.223) (Table 6). The same was observed for genetic advance as a percentage of mean (5.842-80.019). Hence, a high heritability, combined with a high genetic advance and a high GAM,

implies that improvement for these traits through selection is achievable.

Population structure and cluster. The high number of polymorphic markers obtained for the SNP genotyping (Table 3), provides a means for utility in further breeding activities, involving these genotypes. An average PIC and GD of 0.32 and 0.41, respectively; shows the informative status of the markers used in differentiating the genotypes in the present study. The PIC reported in this study is higher than the average of 0.17 reported by Bagoin *et al.* (2023), though lower than the 0.58 reported by Dagnon *et al.* (2022); and 0.36 reported by Chen *et al.* (2017).

The differences observed in genetic differentiation parameters, are likely to be due to one or a combination of the following: marker system used, number of markers, nature of germplasm, and the method of estimation. Cluster analysis provides details on similarity and reveals the structure among a set of genotypes (Modak, 2024). Both morphological (NJ_QPD) and molecular (NJ_MD) cluster analysis revealed the same number of groupings (three cluster) among the genotypes for the present study. However, the admixture based grouping put the genotypes into two subpopulations, with an average allele frequency divergence of 0.427; hence, the two groups were very divergent.

The grouping based on the admixture model and the NJ_MD (Table 8), showed similar patterns of assignment of genotypes into groups with the same genotypes in the groups. This was so because the admixture based clustering and the NJ_MD, utilised the same molecular data; hence very little to no discrepancies were expected. However, there was lack of concordance between the NJ_QPD and the other two clustering methods (Table 8); perhaps because the NJ_QDP utilises quantitative phenotypic data, which is influenced largely by environmental effects, hence, affecting the complete expression of the genetic make of the genotypes (Singh *et al.*, 1991; Agre *et al.*, 2019).

Therefore, the reliability of molecular data in grouping is superior to that of quantitative morphological data (Zou and Zhang, 2016). Based on the molecular grouping, the genotypes had two population structures, namely subpopulation 1 and 2. That notwithstanding, the perfect view of the diversity and structure of the genotypes is appreciated by considering both phenotype and genotype structure of the genotypes.

CONCLUSION

Purity testing, polymorphic marker surveys, and parental line evaluation are essential pre-breeding activities ensuring reliability in breeding programmes. High genetic diversity among 14 cowpea genotypes indicates sufficient variation for selection gains. Identified polymorphic markers will aid purity testing, hybridity testing, and QTL studies in cowpea breeding.

Bigbean, Alan Cash, and SpM028, with large and heavy seeds, can serve as donor parents for improving seed size and yield-related traits. High heritability and genetic advance offer strong potential for cultivar development. Admixture-based grouping of genotypes into two clusters enables the selection of highly diverse parents for hybridisation, generating significant variability for selection. This research provides critical insights for cowpea improvement.

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