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ORIGINAL ARTICLE

Exploring phenotypic variation of diverse bambara groundnut (*Vigna subterranea* L) origin and development of mini-core collection for future breeding

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Abstract

Understanding the phenotypic variation and designing a mini-core collection is an efficient method to accelerate the genetic gain of bambara groundnut. A collection of 300 bambara groundnut landraces from 25 different countries of origin sourced from gene banks were used to analyze phenotypic variability among the landraces and develop a mini-core collection for future breeding. The landraces were evaluated in alpha lattice design with two replications for 2 years (2019 and 2020). The results showed highly significant differences ($p < 0.001$) among the bambara groundnut landraces for all the studied traits implying the selection of landraces with better agronomic traits could be achieved from the crop genetic pool. In addition, landrace x year interactions were significant for studied traits, except for shelling percentage and number of seeds per pod. The genotypic coefficient of variation values were high for most yield component traits, with the highest (65.39%) value obtained on seed dry weight. Furthermore, high heritability in conjunction with high genetic advance obtained in seed dry weight, pod dry weight, petiole length and plant height implies that these traits are majorly controlled by additive genetic action and could be improved through selection. Highly significant and positive correlations of yield were found with seed dry weight, pod dry weight, number of pod per plant, number of leaves, petiole length and plant height. A mini-core collection of 60 landraces (20%) was developed that represents the entire collection using Core Hunter algorithm. In general, the study provides insight into bambara groundnut germplasm that would enhance cultivar development and sustains the utilization of the crop. In addition, the mini-core collection established in the present study could be exploited for future bambara groundnut improvement efforts.

KEYWORDS

Bambara groundnut, core collection, legume, underused, variability

1 | INTRODUCTION

Bambara groundnut (*Vigna subterranea* [L.] Verdc.; $2x=2n=22$) is an underused African legume belonging to the family Fabaceae and subfamily Faboidea (Khan et al., 2020; Olanrewaju et al., 2022). It is an annual, herbaceous and self-pollinated plant (Bonny et al., 2019) mostly grown in the African continent (Uba et al., 2022). Bambara groundnut is a geocarpic crop, morphologically fits into the same niche as groundnut (peanut; *Arachis hypogaea* L.) and a close relative of cowpea (*Vigna unguiculata*) with the seed closer to chickpea (*Cicer arietinum*) based on its composition (Halimi et al., 2019; Mayes et al., 2019). It plays a very important socio-economic role in the semi-arid regions of the African continent (Paliwal et al., 2021). In addition, it is a cheap and rich source of protein – that can play an enormous role in improving the food and nutrition security of rural households. The biochemical analysis has revealed that bambara groundnut grain contains carbohydrate, fat, protein and minerals (zinc, iron, calcium and potassium), producing almost a balanced diet (Mubaiwa et al., 2018). Apart from being consumed at home, it is nowadays canned commercially (Makanda et al., 2009). It is adapted to a wide range of agro-ecological conditions and displays traits conferring drought tolerance (Collinson et al., 1996; Karikari & Tabona, 2004). Furthermore, it helps to fertilize the soil through its inherent ability to fix atmospheric nitrogen in its roots (Gbaguidi et al., 2018). These 'treasure trove' features of the crop have been attributed to its growing support by the scientific and consumer community that can greatly help to diversify the diets (Feldman et al., 2019; Massawe et al., 2016).

In spite of its nutritional, health and environmental relevance, bambara groundnut cultivation is principally dependent on landraces that are commonly low yielders (Alake & Ayo-Vaughan, 2016). The low productivity of this crop has been attributed to problems like unavailability of improved varieties, limited awareness on the importance of the crop among the growers and lack of introduction of high-yielding genotypes in the areas of its cultivation (Odongo et al., 2015; Uba et al., 2021). Furthermore, there is a paucity of information on the genetic variability of bambara groundnut landraces collected from different regions or countries of Africa. Most of the available reports on the genetic variability of the crop have been largely based on country-specific germplasm, using less than 150 landraces in the studies. The breeding techniques of bambara groundnut have not been well defined and there are no high-yielding varieties available for production by subsistence farmers, including women. This inability to develop high-yielding varieties that would adapt to different growing environment have been the major challenge for the adoption of bambara groundnut (Halimi et al., 2022; Mabhaudhi et al., 2016). In addition, it has been traded informally which may largely explain the low hectarage of the crop (Chibudu, 1995; Makanda et al., 2009). The increase in the global population has been estimated to be 80 million per year and is forecasted to reach 9.2 billion by 2050 (Godfray et al., 2010). This high population growth demands a rapid increase in the production and productivity of crops, including bambara groundnut, to improve food security challenges (Massawe et al., 2016). The development of bambara groundnut varieties with high yield potential requires the use of several landraces, which serve as a means of enhancing the intraspecific variation for various agronomic traits and their underlying derived characteristics. Therefore, the exploitation of landrace germplasm in the development of varieties that is high yielding remains the fundamental research gap that requires immediate attention of the scientific community.

Furthermore, effective phenotypic evaluation of a large population of bambara groundnut landraces is generally challenging, due to the limitation of establishing replicated multi-location trials because of high cost, tedious management and difficulty performing detailed assessment on many of the traits of interest. Hence, core collection development emerged as a fundamental approach in optimizing plant genetic resources management and utilization. However, much work has not been done, and information is limited on its application for the development of bambara groundnut core collection. Core collection strategy has been used in common bean (Upadhyaya & Ortiz, 2001), rice (Zhang et al., 2011), miracle plant (Tchokponhoué et al., 2020) and sugarcane (Shadmehr et al., 2017). A core collection is the minimum set of accessions representing maximum genetic diversity and collections of the core set, facilitating the proper characterization of the germplasm for better conservation and utilization (Li et al., 2005). Hence, there is a need for a smaller subset (core collection) that mirrors the entire bambara groundnut germplasm panel for convenient breeding efforts.

Genetic variability, germplasm assembly and evaluation, development of progeny, and maintenance are crucial components needed to design and release farmer-preferred varieties (Majola et al., 2021). The prospects of selecting bambara groundnut landraces with yield potentials have been reported by several researchers (Adeniji et al., 2022; Alake & Ayo-Vaughan, 2016; Gbaguidi et al., 2018; Khan et al., 2021) with a yield of 0.3 tons/ha under marginal conditions and up to 4.2 tons/ha with improved varieties under optimum conditions (Madamba, 1995; Makanda et al., 2009). Assessment and characterization of bambara groundnut germplasm are

vital for identifying of the best parents for its improvement (Khaliqi et al., 2021; Khan et al., 2021). In addition, it constitutes the first stage of investigation for collection of genetic resources and identification of desirable traits of interest (Shegro et al., 2013). Adequate knowledge of different landraces and their evaluation is necessary for landrace selection and improvement strategies (Ndiang et al., 2014). Therefore, the characterization of germplasm assists in identifying, differentiating and selecting important genotypes with their agronomic traits for crop improvement in a breeding programme (Paulos et al., 2022).

The selection of genotypes with high yield, farmers preferred traits and wide or specific environmental adaptation is important for enhancing the production and productivity of bambara groundnut. Improvement of traits in bambara groundnut could be achieved through phenotypic selection with the valuation of different genetic parameter analyses. Muhammad et al. (2021) noted that the extent of genetic variation in the breeding program and the magnitude of the heritability of yield traits determine crop yield increase. Heritability, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and genetic advance (GA) are some of the most common tools that assist breeders in determining the genetic variability of the studied germplasm and designing the breeding strategy to improve certain traits. Therefore, understanding the level of genetic variability in bambara groundnut germplasm would help in designing and implementing a breeding programme for conservation and utilization of these landraces. Hence, this work aimed to investigate phenotypic variation in bambara groundnut population, determine the method of the germplasm improvement for each trait, investigate the association among traits and develop a mini-core collection for the germplasm for future breeding.

2 | MATERIALS AND METHODS

2.1 | Plant material and experimental site

A total of 300 landraces of bambara groundnut accessions were used in the study (Figure 1), with 290 accessions obtained from the gene bank of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, and 10 accessions from Crops and Soil Science Research Farm in Bunda, Malawi. The information about the country of origin for each accession was provided in Table S1. The study was conducted for 2 years (2019 and 2020) at the research and experimental farm of Jimma University, College of Agriculture and Veterinary Medicine at Ela-Dale research farm. The site is located in the Southwestern part of Ethiopia in Oromia Regional State 356 km away from Addis Ababa. The area is classified as a mid-altitude sub-humid agro-ecology (7°42 N latitude and 36°50 E longitude) with an altitude of 1710 masl. In addition, it receives an average annual rainfall of 1250 mm and has an average maximum temperature of 26.2°C. The soil of the experimental site contains 0.98% organic carbon, 0.04% total nitrogen, 30.6 ppm available phosphorus and 52.04 μS/cm electrical conductivity (Sintayehu et al., 2015). Furthermore, the average soil pH of the area ranges from 5 to 6.0, and the soil is reddish-brown clay and classified as Nitisol (BPEDORS, 2000).

2.2 | Experimental design and cultural practice

The experiment was laid out in an alpha lattice design with two replications. The field was disc-plowed to a depth of 0.5 m, harrowed and raked to achieve a good seedbed before sowing. The size of each experimental plot

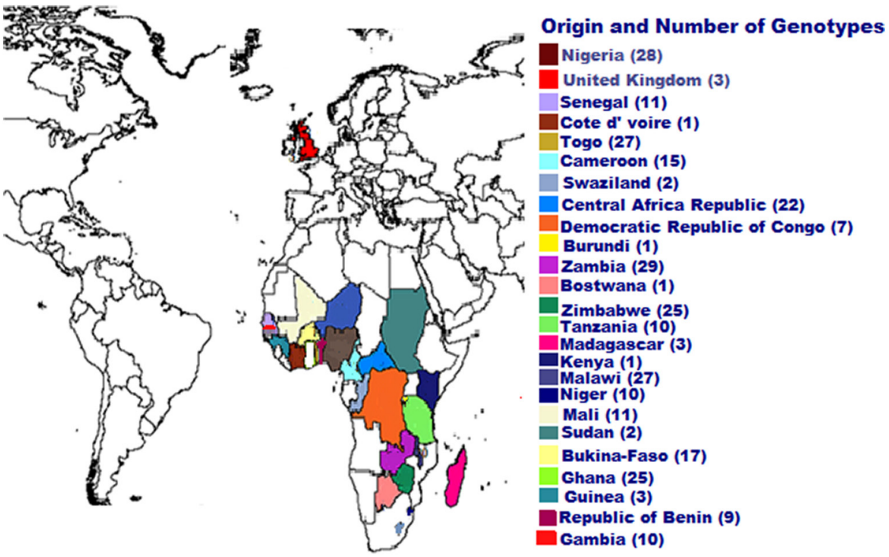

FIGURE 1 Geographical distribution of the 300 bambara groundnut accessions generated using DIVA-GIS software (version 1.4) environment http://www.diva-gis.org (Hijmans et al., 2001).

was 3 m long and 1.2 m wide. Two seeds were sowed per hill at a depth of 5 cm with inter and intra-row spacing of 0.5 x 0.3 m, respectively, and were later thinned to one seedling per hill at the true-leaf stage resulting in a plant population of 66,666 plants/ha. Fertilizer application at the rate of 60 kg DAP per hectare was applied 4 weeks after planting and three times hand weeding was carried out in the growing season to keep the trial clean until harvest. No pesticides were applied in the field. Other crop management practices were carried out as per the best practices recommended for bambara groundnut production.

2.3 | Data collection

Data were collected from 10 randomly sampled plants from the middle rows on 16 agro-morphological traits (Table 1) and were measured as per the descriptors established for bambara groundnut (IPGRI, IITA, BAMNET, 2000).

2.4 | Statistical analysis

2.4.1 | Phenotypic variation

All statistical analysis was performed for the phenotypic variability of the germplasm using R software (R Core Team, 2017). Descriptive statistics (minimum, maximum, mean, standard deviation) were analyzed to understand the overall variability of the germplasm. Bartlett's homogeneity test of error variance was conducted for each year and when the condition was met the analysis was estimated. The across years combined analysis of variance (ANOVA) for the genotypes and their interactions was performed for each trait and the level of significant differences was determined.

The ANOVA model used was

$$P_{\mathrm{ijk}} = \mu + G_i + Y_j + \mathrm{GY}_{\mathrm{ij}} + R_k\left(Y_j\right) + \varepsilon_{\mathrm{ijk}}$$

where the phenotypic response (P_{ijk}) is the function of the overall mean (μ), the fixed effect of the i^{th} genotypes (G_i), the effect of the j^{th} year (Y_j), the k^{th} replication (R_k) within the j^{th} year (Y_j), the genotype by year interaction ($\mathrm{GY}_{\mathrm{ij}}$) and the residual error ($\varepsilon_{\mathrm{ijk}}$).

2.4.2 | Genetic parameters

The estimate of genotypic variance, phenotypic variance, PCV, GCV and GA as percentage mean and broad-sense heritability was computed using the package 'Variability' in R software (Raj et al., 2020). The GCV, PCV and GA values were characterized as low (0%–10%), moderate (10%–20%) or high (>20%) while broad sense heritability values were characterized as low (0%–30%), moderate (30%–60%) and high (≥60%), as suggested by Khan et al. (2021).

2.4.3 | Correlation, principal component and clustering analysis

The correlation, principal component and clustering analysis were all computed using R software (R Core Team, 2017). Spearman's correlation tests were used to compute the relationship among agro-morphological traits using the pairs. panels () function of the 'psych' package (Peterson et al., 2018). To assess the relationships among the accessions, a series of multivariate analyses were performed. The principal component analysis (PCA) was performed using 'FactoMineR' package (Lê et al., 2008). Only principal components (PCs) with eigenvalues >1.0 were selected to define the variation of the agro-morphological trait among accessions. Furthermore, hierarchical clustering on principal components (HCPC) was performed using 'FactoMineR' package to identify the phenotypic group of the germplasm. Finally, we compared the means of all the agro-morphological traits across the identified cluster using boxplots constructed with 'ggpubr' package and Kruskal–Wallis test (Kassambara, 2017).

2.4.4 | Development and assessment of mini-core collection

The mini-core collection was developed with the phenotypic data using the distance-based method of the Core Hunter approach. The Core Hunter phenotype data was developed with 'Core Hunter' package (De-Beukelaer et al., 2018) in R software using the phenotype () function implemented in the Core Hunter version 3. Afterward, the samplecore () function of the Core Hunter package was applied to the data previously generated from the analysis to develop the mini-core set of genotypes using the average-entry-to-nearest-entry distance optimization objective supported by the Gower distance measure. The mini-core collection developed was assessed with various criteria to check their quality by estimating the mean difference percentage (MD%) (Equation 1), variance difference percentage (VD%) (Equation 4), coincidence rate of range (CR%; Equation 3) and variable rate (VR%; Equation 4) between the entire collection and the mini-core collection according to Hu et al. (2000) and Kim et al. (2007).

$$\mathrm{MD}\,\% = \frac{1}{m}\sum_{i=1}^{m}\frac{\mathrm{Mei} - \mathrm{Mci}}{\mathrm{Mci}} \times 100 \quad (1)$$

TABLE 1 Descriptions of traits used for phenotypic assessment of bambara groundnut.

Character	Code	Phenotypic scale
Days to 50% emergence	DE	Number of days from sowing to the appearance of 50% of first true leaf on the soil surface
Days to 50% flowering	DF	Recorded as the number of days from the date of 50% seedling emergence to when 50% of the plants in the plot had flowered
Days to maturity	DM	Number of days when 95% of the plants in the plot matured
Number of leaves	NOL	Total number of leaves on the plant counted 2 weeks after flowering
Plant height (cm)	PH	Measured from the base of the plant at ground level to the highest point of the terminal leaflet 10 weeks after sowing
Petiole length (mm)	PTL	Measured from the base of the plant at ground level to the point where the terminal leaflet starts 10 weeks after sowing
Terminal leaflet length (mm)	TLL	Maximum length of the central leaflet of the plant measured with meter rule 10 weeks from sowing
Terminal leaflet width (mm)	TLW	Maximum width of the central leaflet of the plant measured with metered ruler 10 weeks from sowing
Pod length (mm)	PL	Length of dried pods measured with digital vernier caliper within 2 months of harvest
Pod width (mm):	PW	Width of dried pods measured with digital vernier caliper within 2 months of harvest
Number of pods per plant	NPP	Number of pods produce per plant counted during harvest
Number of seeds per pod	NOSP	The average number of seeds obtained from 50 randomly selected mature pods
Pod dry weight (g)	PDW	Data measured after drying of pods (12% moisture) at 37°C within 3 weeks of harvest
Shelling percentage (%)	SH	Measured within 2 months of harvest; the ratio of dry seed divided by dry pod weight (at 12% moisture content)
Seed dry weight (g)	SDW	Weight of dried seed taken after the pod is shelled and the seeds are dried
Yield (tons ha^{-1})	YLD	Yield per hectare was estimated on a plot basis and converted the plot yield of the seed dry weight to tons/ha

where MD% mean difference percentage, Mci is the mean of the mini-core collection for trait *i* and Mei is the mean of the entire collection for trait *i*. The means for each trait was tested for significant difference between the entire and mini-core collection using *t*-test.

$$\mathrm{VD}\,\% = \frac{1}{m}\sum_{i=1}^{m}\frac{\mathrm{Vei}-\mathrm{Vci}}{\mathrm{Vci}}\times 100 \quad (2)$$

where VD% is the variance difference percentage, Vci is the variance of the mini-core collection for trait *i* and Vei is the variance of the entire collection for trait *i*.

$$\mathrm{CR}\,\% = \frac{1}{m}\sum_{i=1}^{m}\frac{\mathrm{Rci}}{\mathrm{Rei}}\times 100 \quad (3)$$

where CR% is the coincidence rate of range, RCi is the range of the mini-core collection for trait *i* and Rei is the range of the entire collection for trait *i*.

$$\mathrm{VR}\,\% = \frac{1}{m}\sum_{i=1}^{m}\frac{\mathrm{CVci}}{\mathrm{CVei}}\times 100 \quad (4)$$

where VR% is the variable rate, CVci is the coefficient of variation of the mini-core collection for trait *i* and CVei is the coefficient of variation of the entire collection for trait *i*.

3 | RESULTS

3.1 | Analysis of variance and genetic variability among bambara groundnut landraces

The ANOVA revealed highly significant differences ($p < 0.01$) among bambara groundnut landraces for all the studied traits (Table 2). The result also showed that the mean square for years were significant for most of the traits, except for terminal leaf width, pod length, shelling percentage, pod dry weight, number of seeds per pod and seed dry weight. Furthermore, the landrace-by-year interaction revealed that all the studied traits were significant except for shelling percentage and number of seed per pod.

The variability exhibited wide ranges for all the agro-morphological traits among the bambara groundnut landraces (Table 3). Despite variations in the magnitude of the ranges, mean values of the landraces displayed considerable differences between the minimum and maximum

TABLE 2 Combined mean squares of analysis of variance for agro-morphological traits studied across the years.

Traits	Landrace df = 299	Year df = 1	Landrace*year df = 299	Residual df = 576
Days to 50% emergence (day)	4.01***	318.79***	3.32***	1.64
Days to 50% flowering (day)	18.67***	8796.67***	1.63***	7.66
Days to maturity (day)	572.87***	1391.05***	117.70***	61.95
Plant height (cm)	31.14***	215.40***	5.70***	2.22
Petiole length (mm)	1748.60***	2776.23***	344.07***	151.49
Terminal leaf length (mm)	125.45***	3196.12***	65.39***	35.75
Terminal leaf width (mm)	37.71***	38.8 ns	16.97***	10.58
Number of leaves	6771.96***	1,040,111.67***	2101.49***	956.1
Number of pods per plant	197.27***	17,297.68***	51.99***	22.70
Pod length (mm)	63.35***	115.85 ns	43.16**	32.71
Pod width (mm)	7.56***	31.19***	2.80***	1.72
Number of seeds per pod	0.02***	0.11 ns	0.01 ns	0.01
Shelling percentage (%)	0.023***	0.031 ns	0.01 ns	0.01
Pod dry weight (g)	699.36***	4.78 ns	137.68***	56.51
Seed dry weight (g)	12.65***	8.39 ns	2.21***	34.27
Yield (tons ha^{-1})	1.60***	10.21***	0.31***	0.15

ns, not significant.

**Significant at ($p < 0.01$); ***Significant at ($p < 0.001$).

values for most of the studied traits. Days to seedling emergence varied from 10 and 20 days while days to 50% flowering are ranged from 48 and 75 days. The minimum and maximum values for days to maturity varied between 118 and 185 days. Plant height (10.51 to 28.0 cm), petiole length (53.75 to 191.00 mm), terminal leaf length (43.10 to 80.80 mm), terminal leaf width (10.29 to 48.40 mm), number of leaves (27.60 to 356.25), number of pods per plant (2.58 to 96.80), pod length (15.77 to 51.90 mm), pod width (6.80 to 18.00 mm), shelling percentage (0.10% to 0.75%), pod dry weight (3.26 to 113.26 g), seed dry weight (1.33 to 86.52 g) and yield (0.09 to 4.43 tons ha^{-1}) displayed varied differences between the minimum and maximum values. The highest value on standard deviation was obtained for number of leaves (59.79) while the least was for the number of seeds per pod (0.11). However, days to 50% emergence (12.87 ± 0.91), days to 50% flowering (59.33 ± 1.77), days to maturity (153.95 ± 5.42), plant height (18.18 ± 1.19), petiole length (105.56 ± 9.27), terminal leaf length (105.56 ± 9.27), terminal leaf width (64.25 ± 4.04), number of leaves (129.7 ± 22.92), number of pods per plant (14.72 ± 3.61), pod length (23.48 ± 3.28), pod width (12.64 ± 0.84), number of seeds per pod (1.31 ± 0.05), shelling percentage (23.48 ± 3.28), pod dry weight (21.28 ± 5.87), seed dry weight (15.01 ± 4.35) and yield (0.93 ± 0.28) obtained these averaged values with standard error of means (SEM) for various traits in the germplasm.

3.2 | Analysis of genetic parameters

The output of genetic components analysis for the agro-morphology traits studied is shown in Table 4. The phenotypic variance is higher than the corresponding genotypic variance in all the studied traits. The phenotypic variance ranged from 0.01 for number of seeds per pod to 2694.41 for number of leaves. A similar trend was also obtained in genotypic variance. Slight differences were observed between the PCV and GCV for most of the studied traits. The PCV ranged from 5.74% for days to flowering to 80.19% for yield while the GCV value ranged from 2.59% for days to flowering to 65.39% for seed dry weight. Higher PCV values were obtained on yield (80.12%), seed dry weight (80.02%), pod dry weight (72.40%), number of pods per plant (58.25%), number of leaves (40.02%), shelling percentage (36.45%), pod length (27.90%) and petiole length (22.21%) while other traits showed low to moderate PCV. The GCV with higher values is seed dry weight (65.39%), yield (63.84%), pod dry weight (58.31%), number of pods per plant (43.63%), shelling percentage (20.60%) and number of leaves (28.42%) while moderate to low GCV was obtained for other traits. High heritability was recorded on yield (63.38%), seed dry weight (66.76%), pod dry weight (64.86%), petiole length (63.78%), plant height (67.41%) and days to maturity (60.62%) while terminal leaf width (32.58%), number of leaves (50.44), number

TABLE 3 Descriptive statistics of the agro-morphological traits studied.

Traits	Maximum	Minimum	Mean ± SEM	Standard deviation
Days to 50% emergence (day)	20.00	10.00	12.87 ± 0.91	1.73
Days to 50% flowering (day)	75.00	48.00	59.33 ± 1.77	4.36
Days to maturity (day)	185.00	118.00	153.95 ± 5.42	14.3
Plant height (cm)	28.03	10.51	18.18 ± 1.19	3.23
Petiole length (mm)	191.00	53.75	105.56 ± 9.27	24.58
Terminal leaf length (mm)	85.80	43.10	64.25 ± 4.04	8.33
Terminal leaf width (mm)	48.40	10.29	20.13 ± 2.06	4.37
Number of leaves	356.25	27.60	129.7 ± 22.92	59.79
Number of pods per plant	96.80	2.58	14.72 ± 3.61	9.37
Pod length (mm)	51.90	15.77	23.48 ± 3.28	6.57
Pod width (mm)	18.00	6.80	12.64 ± 0.84	1.86
Number of seeds per pod	1.80	1.01	1.31 ± 0.05	0.11
Shelling percentage (%)	0.75	0.10	0.3 ± 0.04	0.29
Pod dry weight (g)	113.26	3.26	21.28 ± 5.87	16.1
Seed dry weight (g)	86.52	1.33	15.01 ± 4.35	10.89
Yield (tons ha^{-1})	4.43	0.09	0.93 ± 0.28	0.67

of pod per plant (56.10%), pod width (39.76%), number of seed per pod (38.65) and shelling percentage (31.93%) had moderate heritability. Terminal leaf length (30.42%), pod length (27.90%), days to flowering (20.35%) and days to emergence (16.73%) had low heritability. The GA results showed that yield (104.69%), seed dry weight (110.05%), pod dry weight (96.74%), number of pods per plant (67.32%), number of leaves (41.59%), petiole length (30.49%), plant height (24.52%) and shelling percentage (23.99%) showed high GA. Moderate GA was showed for pod width (12.03%), terminal leaf width (14.56%) and days to maturity (11.57%) while pod length (9.14%), terminal leaf length (7.90%), days to 50% emergence (4.37%) and days to 50% flowering (2.41%) had low GA.

3.3 | Principal component analysis

The PCA identified four PCs with eigenvalues greater than 1 (>1) which accounted for 76.70% of the total variation for assessed phenotypic traits (Table 5). The PC1 accounted for 49.04% of the total variation and traits responsible for the germplasm discrimination along this axis were pod dry weight, yield, seed dry weight, petiole length, plant height, number of pods per plant, days to maturity and number of leaves. The PC2 associated with shelling percentage, pod width, days to 50% flowering and terminal leaf length accounted for 12.39% of the total variation. Number of seeds per pod, pod length and days to 50% flowering correlated with PC3 and accounted for 8.3% of the total variation while days to 50% emergence contributed most to PC4 explaining 6.7% variability that existed in the germplasm.

3.4 | Relationship among traits

The Pearson correlation coefficient among the studied agro-morphological traits for the bambara groundnut germplasm is presented in Table 6. The strongest correlation was found between seed dry weight and pod dry weight ($r=0.99$); yield and seed dry weight ($r=0.99$). The correlation analysis revealed that yield had a positive, significant strong relationship with seed dry weight ($r=0.99$), pod dry weight ($r=0.98$), number of pods per plant ($r=0.91$), number of leaves ($r=0.70$), petiole length ($r=0.77$) and plant height ($r=0.75$) while moderate significant correlation was obtained with number of seeds per pod ($r=0.40$), terminal leaf width ($r=0.45$), days to maturity ($r=0.53$) and shelling percentage ($r=-0.53$). In addition, yield showed a low significant correlation with terminal leaf length ($r=0.25$), pod length ($r=0.39$) and pod width ($r=0.38$) while low non-significant correlation was observed on days to 50% flowering (0.01) and days to 50% emergence (−0.01).

3.5 | Clustering of landraces and characterization of the phenotypic groups

The HCPC analysis using the 16 agro-morphological traits grouped landraces into three different phenotypic

TABLE 4 Variance components, heritability and genetic advance of the agro-morphological traits.

Traits	σ_g^2	σ_p^2	GCV (%)	PCV (%)	H^2_{bs} (%)	GA (%)
Days to 50% emergence	0.45	2.67	5.19	12.69	16.73	4.37
Days to 50% flowering	2.36	11.59	2.59	5.74	20.35	2.41
Days to maturity	123.21	203.23	7.20	9.26	60.62	11.56
Plant height	6.94	10.30	14.5	17.66	67.41	24.52
Petiole length	382.8	600.18	18.97	23.21	63.78	30.49
Terminal leaf length	19.95	65.59	6.95	12.60	30.42	7.90
Terminal leaf width	6.21	19.07	12.38	21.69	32.58	14.56
Number of leaves	1359.18	2694.41	28.42	40.02	50.44	41.59
Number of pods per plant	41.25	73.52	43.63	58.25	56.10	67.32
Pod length	6.82	42.89	11.12	27.90	15.91	9.14
Pod width	1.37	3.45	9.25	14.68	39.76	12.03
Number of seeds per pod	0.003	0.009	4.32	7.04	38.65	5.46
Shelling percentage	0.004	0.01	20.60	36.45	31.93	23.99
Pod dry weight	153.98	237.41	58.31	72.40	64.86	96.74
Seed dry weight	96.35	144.33	65.39	80.02	66.76	110.05
Yield	0.35	0.55	63.84	80.19	63.38	104.69

Abbreviations: σ_g^2, genotypic variance; σ_p^2, phenotypic variance; GA, genetic advance (GA) as percentage mean; GCV, genotypic coefficient of variation; H^2_{bs}, broad sense heritability; PCV, phenotypic coefficient of variation.

TABLE 5 Principal component analysis showing the contributions of each trait to the variation in the germplasm.

Descriptor	PC1	PC2	PC3	PC4
Days to 50% emergence	0.05	0.43	0.07	0.78
Days to 50% flowering	0.15	0.52	−0.55	0.37
Days to maturity	0.85	0.10	−0.22	0.16
Plant height	0.90	0.21	0.03	−0.15
Petiole length	0.91	0.14	−0.04	−0.11
Terminal leaf length	0.45	0.46	0.40	−0.20
Terminal leaf width	0.61	0.30	0.05	−0.20
Number of leaves	0.79	−0.01	−0.29	−0.21
Number of pods per plant	0.86	−0.36	−0.16	0.02
Pod length	0.52	0.31	0.43	0.05
Pod width	0.57	0.54	0.09	−0.13
Number of seeds per pod	0.40	−0.20	0.70	0.27
Shelling percentage	−0.40	0.61	−0.10	−0.27
Pod dry weight	0.95	−0.18	−0.08	0.05
Seed dry weight	0.93	−0.29	−0.06	0.10
Yield	0.93	−0.29	−0.03	0.07
Eigen value	7.85	1.98	1.32	1.12
Proportion	49.04	12.39	8.3	6.97
Cumulative	49.04	61.43	69.73	76.70

groups or clusters (Figure 2). The different number of landraces varied among each group with cluster 1 (50.1% of total landraces) possessing the highest number followed by cluster 2 (26%) while the least is cluster 3 (23% of total landraces). Cluster 1 is characterized with lowest on all the studied traits, except shell weight (shelling percentage). The cluster 2 landraces had longer days to emergence, longer days to flowering, longer terminal leaf length, highest shelling percentage and wider pod width. The landraces in cluster 3 are characterized by longer days to maturity, taller plant height, taller petiole length, broad terminal leaf, high number of leaves, high

TABLE 6 Correlation coefficients among agro-morphological traits of bambara groundnut landraces.

	DE	DF	DM	PH	PTL	TLL	TLW	NOL	NPP	PL	PW	NOSP	SH	PDW	SDW
DF	0.27**	1													
DM	0.14*	0.34**	1												
PH	0.03	0.17**	0.75**	1											
PTL	0.02	0.20**	0.77**	0.95**	1										
TLL	0.11*	0.07	0.31**	0.57**	0.48**	1									
TLW	0.08	0.11*	0.46**	0.62**	0.60**	0.41**	1								
NOL	−0.11*	0.16**	0.69**	0.68**	0.70**	0.24**	0.45**	1							
NPP	−0.07	−0.00	0.70**	0.67**	0.71**	0.15**	0.38**	0.71**	1						
PL	0.13*	0.07	0.37**	0.45**	0.43**	0.34**	0.29**	0.36**	0.30**	1					
PW	0.17**	0.20**	0.48**	0.61**	0.54**	0.45**	0.44**	0.40**	0.26**	0.46**	1				
NOSP	0.07	−0.19**	0.25**	0.30**	0.30**	0.22**	0.16**	0.10*	0.29**	0.42**	0.11*	1			
SH	0.07	0.14*	−0.31	−0.21**	−0.26**	−0.05	−0.09	−0.18**	0.46*	0.03	0.07	−0.33**	1		
PDW	0.02	0.09	0.77**	0.78**	0.81**	0.27**	0.48**	0.74**	0.91**	0.43**	0.45**	0.37**	0.40**	1	
SDW	0.00	0.04	0.74**	0.74**	0.77**	0.24**	0.44**	0.70**	0.92**	0.38**	0.38**	0.40**	−0.53**	0.99**	1
YLD	−0.01	0.01	0.53**	0.75**	0.77**	0.25**	0.45**	0.70**	0.91**	0.39**	0.38**	0.40**	−0.53**	0.98**	0.99**

Abbreviations: DE, days to 50% emergence; DF, days to 50% flowering; DM, days to maturity; NOL, number of leaves; NOL, number of pods per plant; NOSP, number of seeds per pod; PDW, pod dry weight; PH, plant height; PL, pod length; PTL, petiole length; PW, pod width; SDW, seed dry weight; SH, shelling percentage; TLL, terminal leaf length; TLW, terminal leaf width; YLD, yield.

*Significance at 0.05; **Significance at 0.01.

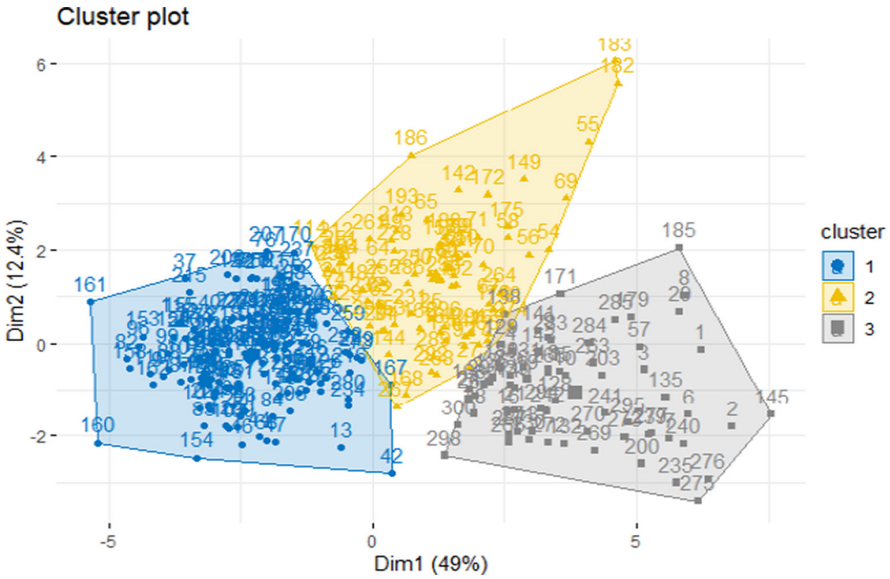

FIGURE 2 Hierarchical clustering on principal component analysis showing the number of phenotypic groups identified in the population.

number of pods, higher pod dry weight, higher seed weight, higher yield and lowest on shelling percentage (Figure 3).

3.6 | Mini-core collection

The min-core collection had good representativeness to the entire collection with the Core Hunter algorithm returning 60 landraces (20%) as a mini-core collection (Table S2) out of the 300 genotypes used for the entire collection. The CR% was high in all the studied traits and varied from 84.65% to 100% with traits like days to 50% emergence, days to maturity, terminal leaf width, number of leaves, pod width, shelling percentage and yield recording CR% of 100% (Table 7). The VR% ranges from 83.49% for yield in tons ha^{-1} to 160.16% for pod length while VD% showed the highest percentage on pod length (66.26%) and the least on days to maturity (3.53%). The MD% result revealed that all the traits had a low difference (<20%) between the entire collection and the mini-core collection. Number of seeds per pod (0.57%) had the lowest MD % while the highest was obtained on seed dry weight (19.64%). The t-test result performed between the entire collection and the core collection using the means were not significant for all the studied traits.

4 | DISCUSSION

4.1 | Variation of quantitative traits

The wide range of phenotypic values obtained for the studied agro-morphological traits indicated the presence of substantial variability in the studied bambara groundnut landraces from diverse origins. This revealed the ability to establish a successful selection strategy for bambara groundnut genetic resource conservation program which could be exploited for a future breeding programme. Several authors have reported the potential of bambara groundnut landraces for breeding purposes (Alake & Ayo-Vaughan, 2016; Gbaguidi et al., 2018; Khan et al., 2021; Khan et al., 2022; Ntundu et al., 2006). The average obtained for most of the quantitative traits was similar to what has been reported by others in the previous studies on this crop. The flowering period among different landraces ranges from 48 to 76 days indicating the difference in the genetic make-up of the genotypes. A similar observation has been reported by Massawe et al. (2005) for a flowering period of 64 to 76 days while Ouedraogo et al. (2008) noted a period of 32 to 53 days among the studied genotypes. The yield of bambara groundnut in this study ranges from 0.09 to 4.43 tons ha^{-1} revealing the possibility of selecting potential landrace for yield and other important agronomic traits. Gbaguidi et al. (2018) reported yields ranging from 146.6 to 2678.6 kg ha^{-1} whereas Khan et al. (2021) reported yields from 588.98 to 2991.77 kg ha^{-1} among bambara groundnut genotypes. The average yield of the entire germplasm is 0.93 tons/ha which is similar to 0.65–0.85 tons ha^{-1} noted by Olukolu et al. (2012). This is an indication that several bambara groundnut landraces perform poorly because of a lack of improved varieties. Some landraces performed above 3.5 tons ha^{-1} is an indication that high-yielding landraces could be selected from the current germplasm panel, which would boost the productivity of bambara groundnut. Several authors have used descriptive statistics analysis to describe the existence of phenotypic variability in bambara groundnut, which is in line with the present study (Bonny et al., 2019; Gbaguidi et al., 2018; Khan et al., 2020; Khan et al., 2022; Ntundu et al., 2006).

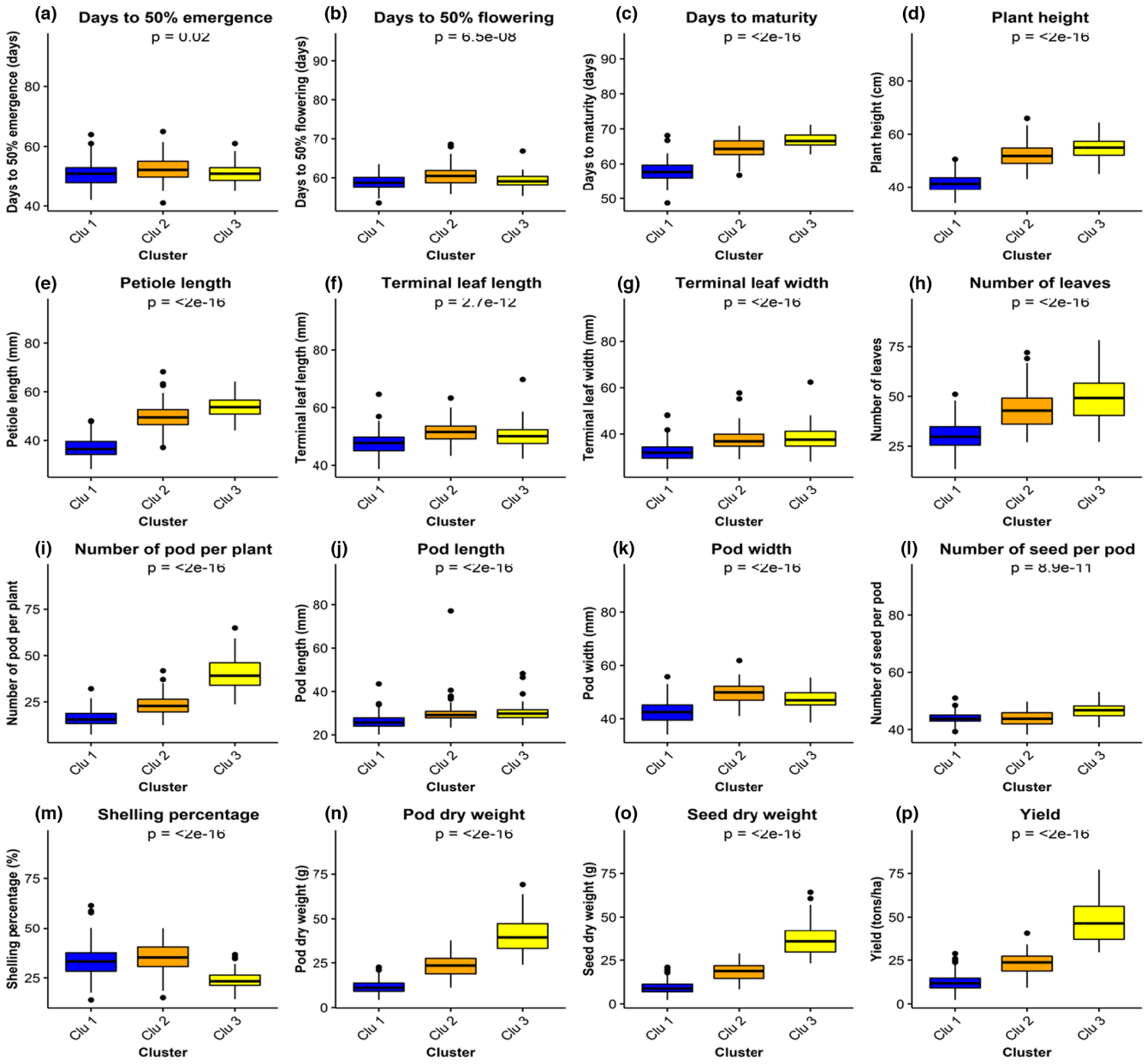

FIGURE 3 Boxplot showing the variations in bambara groundnut performance based on the identified cluster using hierarchical clustering on principal component analysis.

The phenotypic variations among the landraces were further confirmed by the ANOVA that showed significant differences ($p < 0.01$) among the bambara groundnut landraces for all the studied agro-morphological traits. This signifies that developing bambara groundnut landraces with better performance could be achieved using this genetic pool. The presence of variability in germplasm is vital for genetic studies and the selection of desirable genotypes for improvement. Alake (2017) and Khan et al. (2021) observed significant variations among the studied bambara groundnut accessions. In addition, landrace x year interaction revealed that all traits were significant except for shelling percentage and number of seeds per pod. This implies that bambara groundnut landrace was highly variable or inconsistent in their performance across the years. Thereby making the selection process in bambara groundnut process difficult and slowing the process of cultivar development and increasing the costs associated with the activity. The influence of genotype x year has been reported which confirms the result of this study (Alake, 2017; Alake et al., 2015; Jonah et al., 2012). However, significant landrace x year effects for most of the studied traits have confirmed the need to test these landraces in multiple environments over different years to identify landraces that could be used for a breeding programme. Adeniji et al. (2022) pointed out

TABLE 7 Percentage of trait differences between the entire collections and the developed mini-core collection.

Traits	CR (%)	VR (%)	VD (%)	MD (%)	Mean comparison (entire vs. Core), *p*
Days to 50% emergence	100.00	131.94	43.87	1.15	0.99
Days to 50% flowering	95.90	138.08	48.22	0.64	0.99
Days to maturity	100.00	98.79	3.53	2.97	0.99
Plant height	99.52	95.14	3.25	6.42	0.98
Petiole length	84.65	89.25	7.89	7.29	0.98
Terminal leaf length	96.35	132.98	46.19	2.46	0.99
Terminal leaf width	100.00	129.17	47.97	6.83	0.98
Number of leaves	100.00	107.13	33.12	12.39	0.97
Number of pods per plant	99.51	99.21	29.41	16.65	0.97
Pod length	99.49	160.16	66.26	6.97	0.98
Pod width	100.00	109.32	21.76	3.30	0.99
Number of seeds per pod	94.29	122.04	33.62	0.57	0.99
Shelling percentage	100.00	123.75	32.10	1.97	0.99
Pod dry weight	90.70	84.49	13.26	19.31	0.96
Seed dry weight	94.19	84.86	14.75	19.64	0.96
Yield	100.00	83.84	12.06	19.38	0.96

Abbreviations: CR%: coincidence rate for the trait between the entire collection and mini-core collection; MD%: mean difference percentage for the trait between the entire collection and mini-core collection; VD%: variance difference percentage for the trait between the entire collection and mini-core collection; VR%: variable rate percentage for the trait between the entire collection and mini-core collection.

that the measurement of phenotypic traits across environments or years is imperative for identifying phenotypically stable and unstable traits

4.2 | Breeding strategy for the bambara groundnut germplasm

The estimation of genetic parameters of plant traits in breeding populations aids in determining a breeding strategy that could be used to develop productive and resilient genotypes with acceptable end-user qualities. The relative variability of genotypic variances is best expressed as GCV, which determines the extent of variation available for selection (Alake & Ayo-Vaughan, 2016). The PCV result in this study are higher than the corresponding GCV in all the studied traits, implying environmental influence on the expression of the traits. Several authors have reported the influence of the environment on the growth and yield of bambara groundnut (Alake & Ayo-Vaughan, 2016; Ibrahim et al., 2022; Khaliqi et al., 2021; Khan et al., 2021). Slight differences were observed between PCV and GCV for most of the studied traits. The relatively small difference observed between the PCV and GCV may be associated with the genetic differences of these characters. Khan et al. (2020) reported that a higher difference between PCV and GCV values obtained for any traits indicates great effects of the environment on the traits while a smaller difference indicates a strong and significant result of genotypes on detectable expression. High GCV values found for most of the yield component traits (seeds dry weight, yield, pod dry weight, shelling percentage and number of pods per plant) and few vegetative traits (number of leaves) revealed that yield component traits show higher variability than vegetative traits. High GCV values suggest good prospects for improvement in the traits from selection. This finding concurs with the report of Alake and Ayo-Vaughan (2016) who noted that reproductive characters show higher variability than vegetative characters. The valuation of heritability provides the appropriate criterion for predicting the effectiveness of phenotypic selection and assists in designing suitable breeding strategies (Jonah et al., 2010). High heritability estimates obtained for yield, seed dry weight, pod dry weight, petiole length, plant height and days to maturity are indication of strong genetic control and the possibility of effective selection for these traits. A similar observation of high heritability has been previously reported on bambara groundnut by several authors (Alake & Ayo-Vaughan, 2016; Jonah et al., 2012; Khan et al., 2020). High heritability in conjunction with high GA, gives better clues than individual characteristics (Khaliqi et al., 2021). The efficiency of crop improvement is dependent on the extent of GCV, heritability and GA (Bhasker et al., 2017). Moderate to high

heritability together with high GA and high GCV were obtained for seed dry weight, pod dry weight and number of pods per plant that implies that these traits in the bambara groundnut germplasm could be improved through direct selection, because the traits are mainly controlled by additive gene actions and would respond positively to selection pressure. Research has shown that high heritability together with high GA values can be subjected to direct selection (Khaliqi et al., 2021).

4.3 | Partitioning of phenotypic groups and relationship among traits

The PCA which indicated that only few eigenvalues explained the total genetic variation found in the population further confirmed that considerable genetic differences existed in the studied germplasm. Alake and Alake (2016) used a few eigenvalues to elucidate diversity among bambara groundnut landrace with nutritional and agronomic traits. The PC1 accounted for 49.04% of total variation with pod dry weight, yield, seed dry weight, petiole length, plant height, number of pods per plant, days to maturity and number of leaves having more loadings. This implies that vegetative and yield component traits could be used to explain the diversity of bambara groundnut germplasm. Alake and Ayo-Vaughan (2016) and Ntundu et al. (2006) have reported that vegetative and yield component characters had strong discriminatory power or can be used for the characterization of bambara groundnut landraces because of their loadings on PCs. The HCPC grouped the landraces into three clusters based on their individual quantitative traits expressions and their genetic similarity. Cluster 3 landrace characterized by desirable traits like higher number of pods, higher pod dry weight, higher seed weight, higher yield and lowest shelling percentage should be evaluated at different environments for years in other to select landraces adapted for yield improvement. Osundare et al. (2022) noted that accessions which clustered in the same group have genetic relationship in the population. In addition, genotypes within this group that are from different genetic origins with high intra-cluster distance showing potentials on yield component traits could be crossed to produce hybrids. Rajendran et al. (2018) reported that hybridization of maize genotypes of the same cluster having high intra-cluster distance will obtain heterosis and variation. Cluster 1 landraces with the lowest on early maturity suggest the presence of an early maturing gene pool in the group. Therefore, a hybridization programme with clusters 3 and 1 would lead to the development of early maturing genotypes with improved yield. Cluster analysis has been used for the identification and selection of the best parent for hybridization by characterizing and grouping genotypes according to their genetic similarity and morphological characteristics in several crops like bambara groundnut (Adeniji et al., 2022; Khaliqi et al., 2021; Khan et al., 2020; Ntundu et al., 2006), common bean (Nadeem et al., 2020) and African yam bean (Olomitutu et al., 2022). The selection of a genotype requires the manipulation of many characters, which are correlated. In a breeding program, yield is a major character and the nature of its relationship with others characters is a useful guide for the improvement of bambara groundnut. A strong positive association between yield in tons ha^{-1} on seed dry weight, pod dry weight, number of pods per plant, number of leaves, petiole length and plant height indicates selection of these traits would contribute to yield improvement in bambara groundnut. A similar finding has been previously reported on bambara groundnut by Khan et al. (2020). In addition, Nandkangre et al. (2022) in a study conducted in Burkina Faso noted that the number of leaves and most yield component traits had a positive significant effect on yield of bambara groundnut. Seed weight exhibited a moderate negative correlation with a shelling percentage indicating that high shell weight would lead to low bambara groundnut seed weight. Hence, less shell weight is desirable for increased seed weight per plant.

4.4 | Mini-core collection

Phenotypic traits or molecular markers or a combination of both have been used to generate core collections for breeding purpose (Kumar et al., 2016; Zhang et al., 2009). Tchokponhoué et al. (2020) used phenotypic traits to produce a core collection for miracle plants while Upadhyaya and Ortiz (2001) used common beans. The bambara groundnut agro-morphological traits used were able to generate the mini-core collection that capture the entire range of trait variability that existed in the entire collection which is important in guiding the management, utilization and conservation strategy of the germplasm. The mini-core collection produced in terms of size is 20% of the entire collections evaluated. The proportion of recommended size needed for a good core collection should range from 10% to 30% of the entire collection (Bhattacharjee et al., 2007; Tchokponhoué et al., 2020). The mini-core collection in our analysis of CR% in all the studied traits exhibited greater than 80%. This implies that the mini-core collection is good representativeness of the entire collection. A mini-core collection with a CR% above 80% has been recommended as a proper collection for breeding

purposes (Hu et al., 2000). The high values obtained on VR% and VD% indicate the mini-collections in this study incorporate the main portion of the diversity of the entire collection of bambara groundnut germplasm. Mahmoodi et al. (2019) reported that how much diversity of the entire collection maintained in the core collection is illustrated by the high values of VR% and VD%. The MD% was less than 20% for all the studied traits and the entire collection was not significantly different from the mini-core collections implying the effectiveness of the mini-core collection as representative of the entire collection. The combined statistics (CR%, VR%, VD% and MD%) revealed that the mini-core collections are adequate to represent the entire collection and could be used as a reliable working breeding material for future bambara groundnut improvement. These findings are in agreement with several authors who reported the potential of core collection in conservation and utilization of crops for future breeding programmes in common bean (Upadhyaya & Ortiz, 2001), rice (Zhang et al., 2011), miracle plant (Tchokponhoué et al., 2020) and sugarcane (Shadmehr et al., 2017).

5 | CONCLUSION

This current research has shown the possibility of identifying high-yielding bambara groundnut landraces from the current germplasm panel. All the analyses have demonstrated that considerable variation existed among the landraces that would be used for future breeding. Thus, improvement of the germplasm for seed dry weight, pod dry weight and number of pods per plant could be achieved through selection process because these traits are controlled by additive gene action. Association analysis revealed that selection of these traits (seed dry weight, pod dry weight, number of pods per plant, number of leaves and plant height) would contribute to yield improvement of bambara groundnut. Landraces in Cluster 3 were characterized with desirable traits like higher number of pods, higher pod dry weight, higher seed weight, higher yield and lowest shelling weight and hence could serve as a donor for high-yielding landraces. The mini-core collection developed can be used for future breeding to select high-potential landraces after evaluation across years and locations. This study will promote the breeding strategy and conservation of bambara groundnut for future improvement.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

All relevant data are within the paper and its Supporting Information files.

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SUPPORTING INFORMATION

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