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Genetics and Breeding

Variability and interrelationships among seed metric, gravimetric and hydration attributes of Cowpea (*Vigna unguiculata* [L.] Walp) seeds

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Abstract. *Seed dimensional and gravimetric attributes, together with hydration properties, influence seed quality, germination, processing, and cooking outcomes. The objectives of this study were to assess the variability and associations among seed physical, gravimetric, and hydration properties, to identify grouping characters for selection, and to evaluate the contribution of these properties to seed weight. The laboratory experiment used a completely randomized design with four replications. Data were analyzed using R-Core software. Results showed significant differences in physical, gravimetric and hydration properties among cowpea breeding lines, with CP22 displaying superior performance due to its high geometric mean diameter. Significant responses (P<0.05) for geometric mean diameter (GMD) and arithmetic mean diameter (AMD) indicated non-uniform seed size, showing the relevance of these traits for efficient milling and selection of dehydrators. The observed associations between seed sphericity and aspect ratio, as well as between GMD and AMD, are critical for the optimization of seed processing equipment. CP22, CP17, and CP1 exhibited the GMDs, while GP22, GP24, and GP5 had the highest arithmetic mean diameters. The leading cowpea breeding line for true density also demonstrated uniform seed bulk density. A substantial difference between seed bulk density and true density suggests the presence of numerous air spaces, which is pertinent for the design of equipment for sorting, grading, and processing cowpea seeds. GMD, AMD, and sphericity (SPH) exhibited strong discriminatory power for characterization, conservation, and documentation. The complementarity between seed gravimetric and physical attributes was reflected in the high proportion of significant correlation coefficients among seed physical, hydration, and imbibition properties. Seed gravimetric, physical, and hydration properties were grouped into two major clusters, with overlapping characters within and between clusters as revealed by the correlation network, indicating interdependence between gravimetric and physical attributes of cowpea seeds.*

Keywords: cowpea breeding lines, gravimetric properties, physical properties, hydration properties, interdependence

Introduction

Cowpea is a popular and important staple food legume in tropical and subtropical climates. Over a hundred (100) cowpea varieties have been released in Nigeria through the combined efforts of the International Institute of Tropical Agriculture, National Agricultural Research System and development partners. Cowpea is a member of the family Fabaceae, which is the most widely distributed, extensively grown, and traded legume in sub-Saharan Africa (Horn, et al. 2022). The cowpea seed cotyledons contain parenchyma cells filled with starch granules and protein bodies. They are typically kidney-shaped but can also have rough or glabrous seed coats, and they vary in size and weight (Doe-Addo et al., 2024**)**. The morphology of cowpea seeds is important to farmers, consumers and industrial processors for breeding programs designed to improve acceptability. The seed coat (testa) has a range of colours, including white, red, brown, black and a speckled pattern. The seed coat colour, structure and thickness vary among varieties and influence water absorption and insect resistance. Cowpea seeds have a high proportion of protein, digestible and non-digestible carbohydrates, potassium, and amino acids, with low lipid and sodium content, and polyphenols with antioxidant activity (Okunola et al., 2023; Doe-Addo et al., 2024). The shape of cowpea seeds can be influenced by the space within a pod. Cowpea hydration capacity refers to the ability of a quiescent seed to imbibe water, which is an index for germination, cooking and other food processing applications. The seed hydration capacity depends on variety, seed size and weight, composition and storage conditions (Okunola et al., 2023). Seed hydration characteristics affect seed quality,

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germination, and cooking. Higher seed hydration capacity is a pointer to better water absorption, germinate faster and more uniformly, and cook faster, which is important for gelatinization of starch and protein alteration. The swelling capacity of a seed is the increase in its volume upon soaking. Cowpea seeds can swell upon hydration, with swelling capacity and swelling index as measurable parameters. Imbibition is a critical physiological process; after imbibition, the enzymes are activated to commence the breakdown of starch into sugars consumed by the embryo (Maurya and Kumar, 2018). The seed size is shaped by genetic variability, environmental components, photosynthetic capacity, assimilation of photoassimilates in sinks, and cultivar differences (Horn et al., 2015). Cowpea seed size is important for seed germination; larger seeds may require more water during germination than smaller ones.

Seed physical attributes help optimize processing outputs and reduce inefficiencies associated with processing, seed storage and storage equipment. The efficiency of agricultural machines, mechanizing threshing, cleaning, and grading, can cut operating expenses. For farmers, this means enhanced productivity as seeds that are consistent in size and shape can be processed faster and with less waste. The design of storage facilities (silos, bins, and hoppers) depends largely on the seed's physical characteristics. Varietal differences in seed physical properties should not be glossed over in the design and construction of processing and storage equipment, as selecting the right variety can lead to better storage outcomes and prolonged seed viability for both processors and farmers. The seed sphericity value measures how closely the seed shape resembles a sphere. High seed sphericity values indicate a more spherical shape, which benefits processors by allowing smoother flow through processing equipment. The seed bulk density determines the seed weight, while seed porosity measures the space within a given volume of seed. These seed metrics are important for consumers as they can directly influence the cooking effectiveness and nutrient digestion. The ratio of the void spaces (pore spaces) to the total volume of the bulk seed sample is measured by seed porosity. This is important in designing postharvest equipment (drying, storage, and aeration systems), thereby aiding farmers and processors in maximizing postharvest efficiencies. A high porosity value suggests greater air spaces between seeds, influencing water absorption and gas exchange during germination, which can be particularly beneficial for consumers who are interested in ready-to-cook varieties. On the other hand, seeds with low porosity have few air spaces, suggesting reduced water absorption and increased clumping, which can affect handling and processing.

Seed germination and cooking duration are determined by shape, seed coat thickness and seed size. A higher aspect ratio (longer and/or wider seeds) may indicate a more elongated shape, while a lower aspect ratio (thinner seeds) suggests a more flattened or spherical seed shape. Elongated cowpea seeds have a higher length-to-seed-width ratio. In commercial cowpea production, seed size uniformity and shape are critical factors in seed germination potential. Deformed seeds are the least preferred. The seed shape influences seed germination, purchaser preferences and cooking duration, while the seed aspect ratio determines the relationship between seed dimensions. The ratio of seed length to its width describes its shape; this is the ratio of the longest to the shortest dimension of the seed, often used by breeders to determine specific seed shapes (round, elongated, and oval). A ratio of 1:1 (length: width) indicates perfectly round or spherical, while a ratio greater than 1 is more elongated (Juan et al., 2021). Seed sphericity is important for selecting appropriate machinery and optimizing operational parameters to achieve a uniform, high-quality seed lot. It improves the design, efficiency, and performance of processing equipment by affecting a seed lot's flow characteristics, separation dynamics as well as susceptibility to mechanical damage. Plant breeders have documented information on genetic studies, mode of inheritance and genetic control, distinction, uniformity, stability and value for cultivation and use of cowpea (Traore et al., 2022). Also, there is an information gap on the physical, dimensional, density, and imbibition properties of cowpea seeds, which could aggravate mechanized production and processing.

Although breeders have studied cowpea genetics and agronomic traits (Traore et al., 2022), there is still limited knowledge about the physical, size, gravimetric and water absorption properties. Effective design of a cowpea milling machine requires physical and mechanical seed properties. Seed sphericity in the range of 0.7 and shape (0.80) makes a machine roll faster than slide. This affects gravity-chute angles and seed uniformity. The seed's physical properties are important for the manufacture of threshing and grinding equipment, and additionally for cooking. Also, small, medium scale and commercial cowpea growers cultivate cowpea varieties without cognizance of the different grain physical, density and hydration properties for processing and value addition. The hydration kinetics of the cowpea breeding lines remain unexplored, thus limiting their industrial-level utilization and consumer acceptability. Unlike earlier works, this study defines the links between seed physical characteristics (sphericity, density, and hydration kinetics) and the practical design parameters needed to develop and optimize threshers, sorters, graders, and industrial cookers. The findings will advance understanding of seed trait variability and their biological underpinnings and aligning cowpea breeding objectives with mechanization and processing performance. The objectives of this study were to: 1) determine if considerable variability exists among cowpea breeding lines for seed physical, gravimetric, and hydration properties; 2) determine if the association between seed physical, gravimetric and hydration properties that could serve as grouping characters for selection and accounts for a higher proportion of variability in the seed weight.

Materials and Methods

Experimental location

This investigation was carried out at the Teaching and Research Farm, Federal University Oye-Ekiti, Nigeria (07°48' N, 05°29' E; 547m above sea level) between June and October 2021. The area has a tropical climate with an average annual

temperature of 24.2°C and 1313 mm annual rainfall, with September being the wettest month. The soil is sandy loam, moderately acidic (pH 5.7), and low in organic matter (0.82%).

Plant materials and experimental design

Sixteen cowpea breeding lines were obtained from the Legume Improvement Program, International Institute of Tropical Agriculture (IITA), Ibadan. The accessions varied in seed color, maturity group, seed texture, seed shape and seed size. The field evaluation was conducted at the Teaching and Research Farm, Adamawa State University, Mubi, Nigeria. The treatment was laid out in a randomized complete block design (RCBD) with three replications. Each plot was 2 rows of 5 m, with 0.75 m between rows, while a replicate comprised 16 plots, each separated by 1.5 m. At planting, two seeds of each breeding line were planted per hole at a spacing of 0.30 m plant to plant, and 0.75 m between rows. Thinning was done to have two plants per hill. A phosphorus fertilizer (P2O5) was applied, while insects were controlled with lambda-cyhalothrin sprays at different growth stages (vegetative, flowering and post-harvesting). At maturity, pods were picked and dried to 10% moisture content, and threshed. Clean seeds without deformities were selected for laboratory analysis of seed dimensions, gravimetric and hydration characteristics.

Determination of seed physical attributes

The three seed principal dimensions, viz. length (L), breadth (B), and thickness (T) of 50 grains each of 16 cowpea breeding lines were measured using digital venier calipers before and after soaking to measure functional parameters. These were further used to estimate the geometric mean diameter (GMD), arithmetic mean diameter (AMD), surface area (SA), aspect ratio (AR), and sphericity (SPH).

The formula described by Milani et al. (2007) and Mohsenin (2020) was used to estimate the GMD, AR, and SPH:

$$GMD\ (Dg) = (L \times B \times T)^{1/3} \quad (1)$$

$$AMD(Da) = \frac{(L \times B \times T)}{3} \quad (2)$$

Seed sphericity and aspect ratio are used to describe the shape of the grain. Seed sphericity was calculated as;

$$SPH\ (\varphi) = (L \times B \times T)\tfrac{1}{3}L \times 100 = \frac{Dg}{L} \times 100 \quad (3)$$

While the aspect ratio which describes the relationship between an image width and height was computed as:

$$AR = \frac{B}{L} \times 100 \quad (4)$$

The surface area (SA) of cowpea seeds was estimated as described by (McCabe et al., 1986**;** Baryeh and Mangope, 2002):

$$SA = \pi D^2 g \quad (5)$$

A thousand cowpea grains were counted in four replicates and weighed using a sensitive weighing balance having milligram accuracy. The seed bulk density (Kgm^{-3}) (Okaka and Potter, 1979), true density and porosity were also evaluated. The bulk density was determined by fluid displacement method (Yalcin and Ozaralon, 2004).

$$\text{Bulk density (BD)}(Kg/m^3) = \frac{\text{weight}}{\text{Bulk volume}} \quad (6)$$

True density was calculated using the liquid displacement method, where 50 g of the cowpea seeds was transferred into a measuring cylinder containing a known volume of 100 ml of toluene. A change in the solvent volume is called the true volume of seeds:

$$\text{True Density (TD)}(kgm^{-3}) = \frac{\text{weight}}{\text{True volume}} \quad (7)$$

Porosity was estimated based on the bulk density and true density values, using the following equation:

$$\text{Porosity (\%)(POR)} = \frac{TD - BD}{TD} \quad (8)$$

Where: TD= True density and BD=Bulk density

Cowpea seed hydration characteristics

Seed hydration was measured in terms of hydration capacity (HC) and swelling index (SI). The initial moisture content of cowpea seeds was taken using the hot air oven method. Thereafter the seeds were soaked at 30°C in proportional-integral-derivative (PID) controlled water bath (MTPH-049, Micro-technologies) with a precision of ±0.1°C until equilibrium was attained. The time (seconds) to reach equilibrium was estimated from the starting of soaking to the time after which the weight of seeds became constant (Kaur and Prasad, 2022).

Hydration capacity was measured as the gain in weight of seeds at the equilibrium stage:

$$\text{Seed weight after soaking} = \frac{\text{Initial weight}}{\text{initial weight}} \quad (9)$$

Swelling index of cowpea seeds was calculated as:

$$SI = \frac{\text{Volume after soaking}}{\text{intial volume of water}} \quad (10)$$

Statistical analysis

Data collected were subjected to F-test to determine the magnitude of variability for seed physical, gravimetric and hydration characteristics using PROC ANOVA. The association between seed physical, gravimetric and hydration characters was done using the 'ggcorplot' and 'agraph' functions in R **(**Epskamp et al., 2012). The correlation network was drawn showing variables represented at nodes connected by edges. The strength of the correlation between two variables is shown

by the thickness of the line connecting them in the network plot. This plot allows for the identification of variable clusters and their interconnections. Blue lines denote positive correlations, red lines indicate negative correlations, and faded lines represent coefficients that are close to zero. Thicker lines signify stronger correlation coefficients closer to unity. The multiple regression analysis was used to determine the contribution of the aspect ratio (X_1), bulk density (X_2), total density (X_3), FSD (X_4), seed weight after imbibition (X_5), seed dry weight after imbibition (X_6), hydration capacity after imbibition (X_7), seed width after imbibition (X_8) to seed weight (Y) among the 16 cowpea breeding lines. The second polynomial regression equation is:

$$Y = Bo + B_1X_1 + B_2X_2 + B_2X_2 + E \quad (11)$$

Y = Dependent variable (fruit weight/plot).

B_0 = Intercept constant

B_1 = Linear effect parameter

B_2 = the quadratic equation.

X_1- X_7 = Explanatory variables (aspect ratio (X_1), bulk density (X_2), total density (X_3), FSD (X_4), seed weight after imbibition (X_5), seed dry weight after imbibition (X_6), hydration capacity after imbibition (X_7), seed width after imbibition (X_8) to seed weight (Y).

£ = Residual

The strength of relation (*R*) = Coefficient of determination:

$$R = \frac{\sum i=1(xi-x)(y-y)}{\sqrt{\sum i=1(x-x)^2 \sum i=1(yi-y)^2}}$$

Results and discussion

The seed physical characteristics showed significant mean squares (P≤0.05) for seed length and width, true seed density, seed porosity, AMD and GMD, seed bulk density, seed aspect ratio, and seed sphericity at 15 per cent moisture content (Table 1). The seed hydration capacity, seed length, seed diameter, seed sphericity, and seed weight of the cowpea breeding lines after imbibition showed significant (P≤0.05) response (Table 1). The magnitude of variability among the breeding lines for seed physical properties was associated with differences in the genetic backgrounds of the cowpea breeding lines and with the influence of the environment in the expression of these physical attributes among the breeding lines. In a separate study, Correa et al. (2010) and Darfour et al. (2022) found significant responses for seed weight (g), bulk density, seed porosity, and true density of maize. A significant (P≤0.05) response for GMD and AMD before imbibition indicates non-uniform seed size Kaur et al. (2021). Additionally, the seed sizes are not clustered around a central value. As shown in Table 1, a significant difference (P≤0.05) in gravimetric properties (hydration properties and SPH) indicates that the cowpea breeding lines exhibited different mean values for geometric mean diameter and seed sphericity after imbibition. This is associated with different internal cell arrangements in the cowpea seeds. Correa et al. (2010) reported significant differences among seeds of common beans for seed metric, seed hydration and seed gravimetric traits. Furthermore, the shape characteristics of the cowpea seeds are imperative for determining the energy required for efficient milling and selecting dehydrators. The increase in seed size during hydration is associated with the enlargement of the seed structure, which shrank during drying. The increase in seed thickness and width relative to seed length might be due to differences in cell arrangement in different directions (Baryeh and Mangope, 2002). During soaking, the seed volume increases, resulting in a decrease in true density. Significant differences (P≤0.05) for seed bulk density and seed true density among the breeding lines suggest considerable internal pore spaces or voids within the seed. Several studies have reported variation in the physical properties of different pulse seeds, such as lentil (Isik and Izli, 2016), as well as pigeon pea (Sangani and Davara, 2013), beans (Ravikumar et al., 2018) and Chick pea (ÖZaktan, 2021).

Table 1. Mean squares for seed gravimetric properties, seed physical, hydration and imbibition properties

Source of variation	Df	TD	POR	GMD	AMD	SDW	BD	AR	SPH	SLIM	SDWI	HCIM	SLIM	BDIM	SPHIM	SWTI
Varieties	15	0.009*	0.04*	125.42*	21.59*	1.29*	0.008*	3430*	14.64*	32.17*	23.25*	1329541*	13.85*	1.86*	10.57*	45.20*
Residual	32	0.0002	0.0009	18.70	0.10	0.06	0.0002	121	0.13	0.67	0.18	2678	0.33	0.45	0.26	1.84
CV (%)		1.53	11.64	13.72	4.82	2.66	2.80	16.12	8.74	8.74	7.36	2.09	3.68	6.26	5.03	5.27
R^2		0.95	0.94	0.97	0.98	0.91	3.92	0.93	0.96	0.96	0.98	0.99	0.95	0.66	0.96	0.92
Grand mean		0.84	0.22	81.45	5.45	7.42	0.55	16.12	8.74	8.74	4.71	2022.186	12.71	8.76	7.23	21.03

TD=True density, POR=Porosity, GMD=Geometric mean diameter, AMD=Arithmetic Mean Diameter, SDW=Seed Width, BD=Bulk Density, AR=Aspect Ratio, SPH-Seed Sphericity, SLIM=Seed Length after Imbibition, SDWT-Seed width after imbibition, HCIM=Hydration Capacity after Imbibition, SLIM=Seed Length after Imbibition, BDIM=Bulk Density after Imbibition, SPIM=Seed Sphericity after Imbibition, SWTIM=Seed Weight after Imbibition.

The coefficient of variability (CV %) ranges from 1.59% in true density to 11.64% in seed porosity. Seed porosity is a function of seed moisture content, which is the excess of grain density over the bulk density per unit of the grain density. The mean values for seed porosity range from 0.07 (CP18) to 0.45 (CP17) (Table 2). Seeds with low values for seed porosity are characterized by few air spaces, reduced water absorption, and high desirability to clump together, influencing processing and handling. The AMD is the average of all seed dimensions in cowpea, while the GMD determine the shape and aperture (screen-hole sizes) in the engineering design for separating or screening equipment for cowpea grains. The top three cowpea breeding lines for geometric mean diameter were CP22 (155.4), CP17 (160.33) and CP1 (177.82), while CP16 (0.31), CP18 (0.43) and CP14 (0.31) had the least values (Table 2).

Table 2. Least square mean for seed gravimetric properties, seed physical, imbibition and hydration attributes of Twenty-six (26) varieties of cowpea

Variety	TD	POR	GMD	AMD	SDW	BD	AR	SPH	SLIM	SDWI	HCIM	BDIM	SPIM	SWTI
CP22	1.00a	0.43a	155.225ab	7.90b	7.77bc	0.59ab	62.25def	6.45a	10.49bc	5.75d	2814.35b	10.58a	8.45ab	26.90b
CP19	0.89b	0.26c	69.54e	6.06e	7.80bc	0.58bc	45.15fgh	4.89bcd	9.14cd	4.11e	1749h	8.48def	6.07fg	20.23ef
CP1	0.87bc	0.24cd	116.30cd	7.33cd	7.80bc	0.59ab	68.42d	4.78cd	10.21bc	6.99bc	2103fy	8.75b-f	7.52d-g	21.03def
CP23	0.87bc	0.18ef	109.67d	7.18d	8.33a	0.58bc	69.65cd	4.93bcd	9.80cd	6.81bc	1633.0i	8.14ef	7.67c-f	17.26gh
CP26	0.87bc	0.35ab	136.77bc	7.56bcd	6.43f	0.43g	65.87d	0.09g	10.34bc	6.84bc	2220.1e	9.01b-e	7.73b-e	22.76cd
CP7	0.85cd	0.20def	0.56f	1.59g	7.50cde	0.54ef	8.99i	1.15ef	3.48gh	0.33h	2018.0g	8.77b-f	7.69c-f	20.23ef
CP14	0.84d	0.14f	0.31f	1.48g	7.50cde	0.55def	3.15i	0.65fg	2.83h	1.21g	136.0k	7.93ef	7.69c-f	17.05gh
CP21	0.84d	0.29c	116.30cd	7.06d	7.10e	0.53ef	46.69e-h	6.25a	9.98c	4.37e	2502.0c	8.45ef	8.40a-c	24.23c
CP24	0.83de	0.18ef	177.81a	8.57a	7.20de	0.52f	46.69e-h	5.33bc	12.43a	8.17a	2357.0d	9.59a-d	8.61a	23.13cd
CP5	0.83de	0.14f	129.09cd	7.71bc	0.57b-e	0.57b-e	64.08de	5.13bcd	11.38ab	6.63c	2201.0e	8.42ef	8.11a-d	22.13cde
CP6	0.83de	0.07g	66.33e	5.86e	8.16ab	0.62a	58.63d-g	5.38b	5.50ef	6.71c	2163.0f	7.74f	6.90g	20.33ef
CP8	0.83de	0.16ef	2.55f	2.27f	7.80de	0.55c-f	125.04a	1.13ef	4.69fg	4.11e	2141.0g	9.82ab	8.18a-d	20.13ef
CP2	o.82de	0.15ef	62.01e	5.77e	6.43f	0.54ef	106.32b	4.72d	6.16e	6.43cd	3200.0a	8.31ef	6.90g	30.07a
CP16	0.81ef	0.18ef	0.31f	2.27f	7.46cde	0.55ef	42.06gh	0.09g	5.05ef	2.08f	2020g	8.65c-f	7.0efg	20.17ef
CP18	0.78f	0.50a	0.43f	1.18g	7.80bc	0.58b-e	9.34i	1.13ef	2.53h	0.14h	1745h	7.93ef	7.33efg	19.27fg
CP17	0.73g	0.45a	160.04a	7.42bcd	5.61g	0.43g	87.29c	5.39b	8.50d	7.42b	1447g	9.67a-c	6.51h	14.83i

TD=True density, POR=Porosity, GMD=Geometric mean diameter, AMD=Arithmetic Mean Diameter, SDW=Seed Width, BD=Bulk Density, AR=Aspect Ratio, SPH-Seed Sphericity, SLIM=Seed Length after Imbibition, SDWT-Seed width after imbibition, HCIM=Hydration Capacity after Imbibition, SLIM=Seed Length after Imbibition, BDIM=Bulk Density after Imbibition, SPIM=Seed Sphericity after Imbibition, SWTI=Seed Weight after Imbibition.

Genotypes CP22 (8.57), CP24 (9.0), and CP5 (7.71) had high mean AMD values. The dry seeds of CP23 (8.33) and CP6 (8.17) are wider than those of other entries. Genotype CP6 performed best for seed bulk density (0.62) and aspect ratio (125.04). Higher values for GMD compared with AMD for some cowpea breeding lines imply that there are likely many more small-seeded entries than large-seeded ones among the entries. Breeding lines with larger AMD than GMD had larger seed size. Seed sphericity is relevant in seed processing due to changes in shape and moisture content, which directly affect the efficiency and outcome of mechanized operations such as sorting, grading and drying. Seed sphericity and aspect ratio are associated with GMD and AMD, which are important for effective seed processing. The top breeding line for true density showed uniform performance for bulk density. A large numerical difference between the seed bulk density and true density indicates high porosity, suggesting that the seeds have many more intercellular air spaces or air pockets. This is important in the design of equipment for sorting, grading and processing seeds. True density differs among the genotypes. Both bulk density and true density enable agricultural engineers to construct size hoppers, storage facilities, and cowpea planters. During seed processing, the ability of a grain to roll depends on its sphericity, which ranges from 0.09 (CP16) to 6.44 (CP22) (Table 2).

Table 3. Multivariate analysis for seed gravimetric properties, seed physical, hydration and imbibition properties

Variables	Dim 1	Dim 2	Dim 3
True density	0.23	0.72	0.42
Porosity	0.66	-0.33	0.54
GMD	0.95	0.03	0.0006
AMD	0.93	0.16	-0.15
SDW	-0.39	0.78	-0.32
BD	-0.35	0.73	-0.31
AR	0.69	0.05	-0.57
SPH	0.90	0.20	-0.19
SLIM	0.87	0.23	0.04
SDWI	0.90	0.06	-0.34
HCIM	0.51	0.48	0.03
SDLIM	-0.29	0.85	0.26
SDIM	0.48	-0.05	0.74
SPIM	-0.23	0.87	0.20
SWTI	0.36	0.57	0.14
Eigenvalue	6.22	3.95	1.89
Percentage contribution (%)	41.46	26.39	12.26
Cumulative contribution (%)	41.46	77.85	80.11

TD=True density, POR=Porosity, GMD=Geometric mean diameter, AMD=Arithmetic Mean Diameter, SDW=Seed Width, BD=Bulk Density, AR=Aspect Ratio, SPH-Seed Sphericity, SLIM=Seed Length after Imbibition, SDWT-Seed width after imbibition, HCIM=Hydration Capacity after Imbibition, SLIM=Seed Length after Imbibition, BDIM=Bulk Density after Imbibition, SPIM=Seed Sphericity after Imbibition, SWTI=Seed Weight after Imbibition.

Cowpea seed metric and hydration attributes (seed length and width, seed diameter and weight, seed swelling index and hydration capacity) differed significantly among cowpea breeding lines measured after imbibition. The seed hydration capacity was the highest (3200) in CP2, while CP14 had the lowest (136). After imbibition, the breeding line CP24 performed best for seed length and seed width. The breeding lines showed a different range of sphericity after imbibition. A high sphericity for CP 24 (8.61) and 22 (8.45) implies its ability to roll easily on any of its axes. The mean values recorded for seed sphericity after imbibition were the highest compared to values recorded for seed sphericity before imbibition. This difference reflects variations in the seed structure, maturity and moisture content. The gravimetric properties differed among the cowpea breeding lines before and after imbibition.

Multivariate analysis

Principal component analysis is an important data reduction method that efficiently reduces dimensionality and eliminates components that contribute little to variation or lack meaningful correlations with the trait of interest. The PCA conducted on 15 traits reveals eigenvalues and cumulative variance. The first three principal component axes have eigenvalues of 6.22, 3.59, and 1.84, respectively, accounting for a cumulative 80.12% of the total variability (Figure 1).

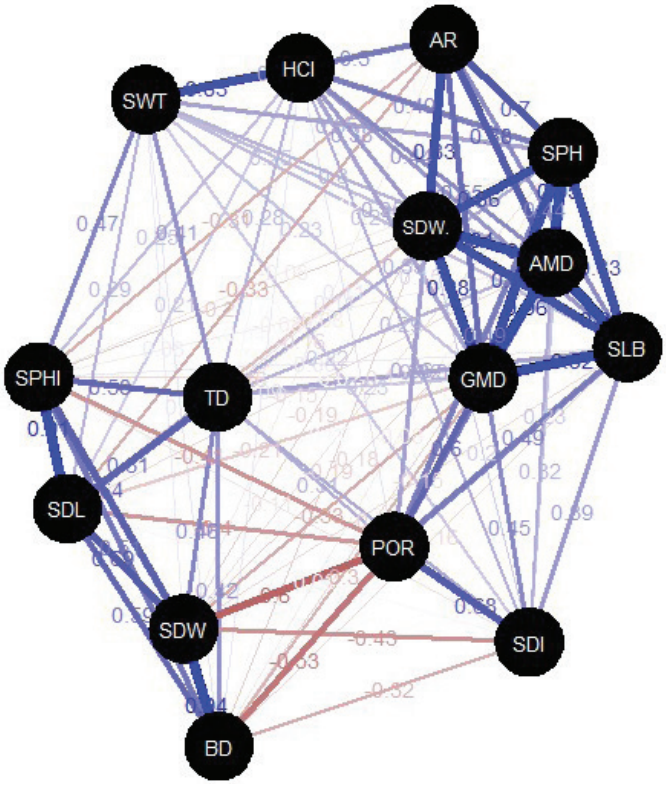


Figure 1. Correlation coefficients and correlation network of gravimetric properties (BD, AR, TD, POR) physical properties (SDL, SDW, STHK, GMD, AMD, SA, SPH) and hydration properties (SWI and HC) in 26 cowpea breeding lines

Specifically, PC 1, PC 2, and PC 3 contribute 41.4%, 26.39%, and 12.26%, respectively. Parameter loading stresses the relationship between variables, while the core plot explains the distribution of cowpea breeding lines. Loadings on PC 1 either align positively or negatively, as indicated by the correlation coefficients. Components with eigenvalues surpassing 3.59 are deemed significant (Gözen, 2008). Variation in seed structural and physical properties underscores their key role in effective milling and selecting dehydrators for cowpea grains. Seed physical properties, such as seed diameter, along with geometrical properties such as GMD, AMD and SPH, show strong positive coefficients, showing considerable discriminatory ability, while negatively associating with SDW and BD. The second PC axis clearly discriminates between SPH and SDL, with each showing high positive coefficients on PC 2 and low negative coefficients on PC 1, indicating high discriminatory power for seed physical properties. The PC biplot is essential for a multi-dimensional selection criterion focusing on target traits (Figure 2).

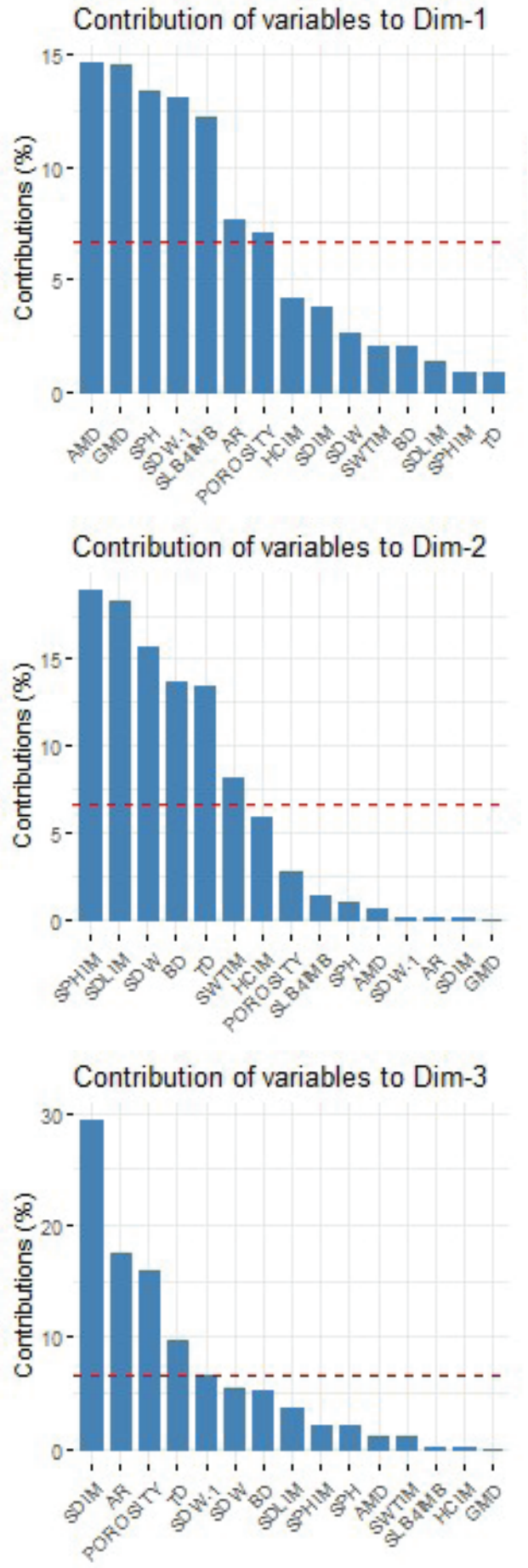


Figure 2. Contribution of seed physical, gravimetric and hydration properties dimension 1, 2 and 3

The vector length from the biplot origin illustrates how well a trait is positioned in the biplot. Conversely, a short vector suggests a weak or non-existent correlation with other traits (Juan et al., 2021), possibly due to a poor fit of the biplot on PCs 1 and 2. Seven breeding lines are distributed in the first quadrant (Q1), three in the second quadrant (Q2), four in the third quadrant (Q3), and two breeding lines in the fourth quadrant (Q4), CP22 exhibits high variability, positioned prominently in the top-right corner of Q1 (Figure 2).

As shown in Figure 3, the grouping of the sixteen cowpea breeding lines into two cluster promising for specific traits. The cluster comprising SP 8, 7,16, 14 and 18 to a cluster promising for POR, GMD, SPH and SDW. While CP 22, 26, 24, 25, 1, 19, 5, 2 and 23 to another cluster promising for SPH, TD, POR, GMD and AMD. Overlap of seed gravimetric, physical, and hydration properties showed overlap for traits. Findings from the biplot analysis are similar to the correlation network for overlap of tested traits.

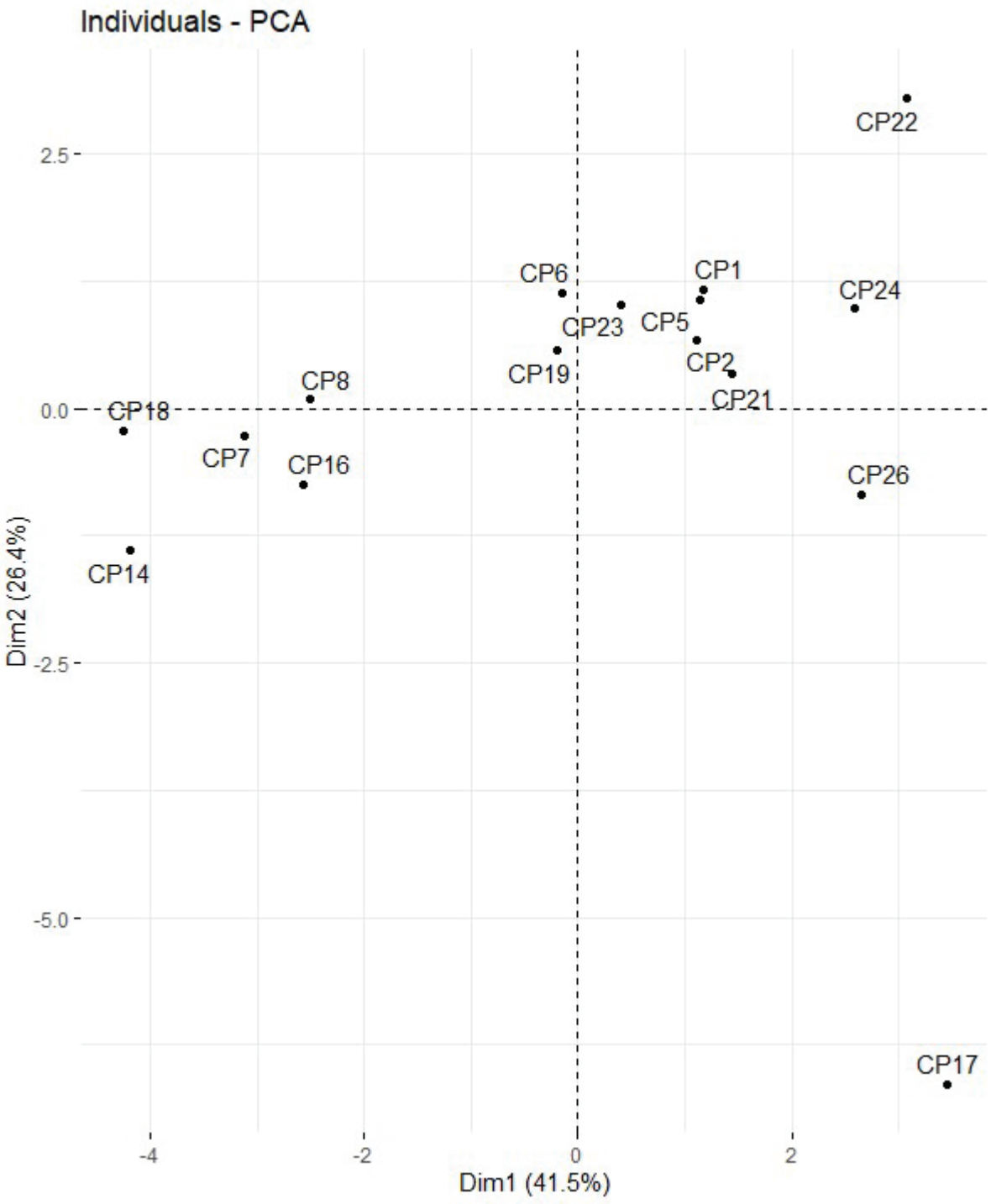


Figure 3. Dispersion of sixteen cowpea breeding lines based on 15 physical, gravimetric, hydration properties in the plot of PC1 by PC2

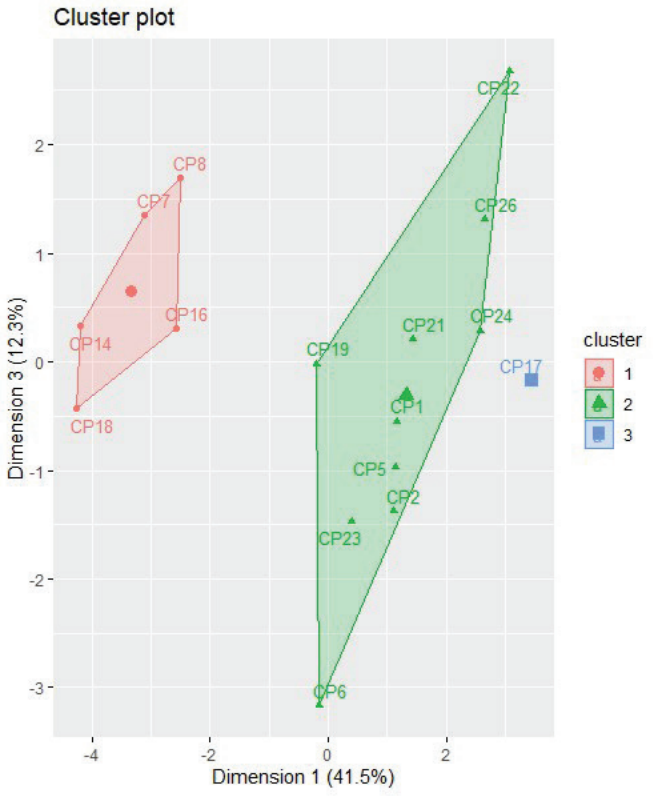
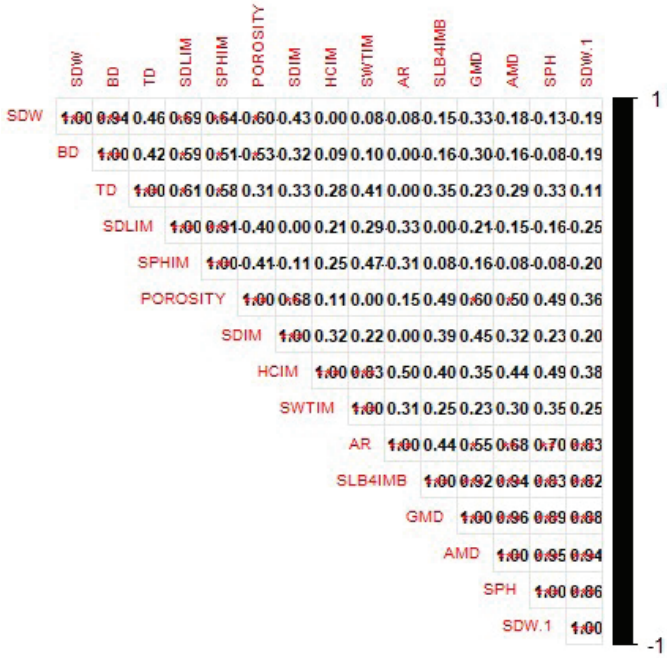


Figure 4. Cluster plot of sixteen cowpea breeding lines based on 15 physical, gravimetric, hydration properties in the plot of PC1 by PC3

Association analysis

The association study shows a consistent relationship between seed gravimetric properties and physical attributes, with many correlation coefficients being positive and moderate to high. The correlation network plot indicates that seed weight is significantly associated with other key characteristics, denoting an inter-character association that improves processing effectiveness. Specifically, seed weight shows positive and significant correlation coefficients with bulk density (r=0.94**), seed porosity (r=0.60*), seed length (r=0.69*), seed sphericity (r=0.64*), and seed diameter (r=0.43*) (Table 4). The seed bulk density, before imbibition, has a moderately positive correlation with seed length (r=0.59*), seed sphericity (r=0.51*), and porosity (r=0.63**). Seed porosity is positively correlated with GMD (r=0.60*), AMD (r=0.49*), seed diameter after imbibition (r= 0.68*), and seed sphericity (r=0.49*). Hydration capacity shows a significantly positive correlation coefficient with seed width after imbibition (r=0.83**), a moderate correlation with aspect ratio (r=0.50*), AMD (r=0.49**), and seed sphericity (r=0.59*). The aspect ratio and geometric mean diameter showed a positive, significant correlation, consistent with anticipated physical properties, supporting more accurate machinery calibration for handling, sorting, grading, and planting. Additionally, the aspect ratio and AMD with positive and significant correlation coefficient (r=0.68**) suggests consistent seed geometry within a seed lot. The aspect ratio also correlates positively with seed sphericity (r=0.70**) and swelling index (r=0.83**). The GMD and AMD are positively associated (r=0.98*), showing their similar diameter measurement. The GMD is with high positive correlation coefficient with seed sphericity (r=0.89**) and the swelling index (r=0.83**). Seed sphericity is positively associated with AMD (r=0.95**), GMD (r=0.89**), seed length before imbibition (r=0.94**), aspect ratio (r=0.70**), and seed porosity (r=0.50*). The positive association between seed length and width demonstrates uniformity in seed shape and size. This uniformity enhances mechanical handling, sorting, and planting processes, yielding more consistent results. A significant positive association between seed sphericity and seed weight is expected to improve mechanical equipment, improving seed quality and uniformity. Moreover, the strong positive association between seed sphericity and AMD indicates that larger seeds tend to be more spherical, simplifying processing by allowing engineers to use the same machinery (seed and air separators) effectively for sorting and handling processed seeds. Earlier studies have primarily reported physical or hydration properties in isolation (Doe-Addo et al., 2024; Traore et al., 2022). Current findings provide an integrated analysis of cowpea seed metric, gravimetric, and hydration traits. The identification of strong associations among geometric mean diameter, sphericity, and bulk density addresses long-standing gaps noted by Baryeh and Mangope (2002) and Horn et al. (2015) regarding the mechanization of cowpea postharvest operations.

Table 4. Correlation coefficient between seed gravimetric properties, seed physical, hydration and imbibition properties



The network analysis shows an intricate association structure among seed physical and gravimetric properties. The SPH, SLB, AR, SDI, and BD represent the primary hub due to their broad connectivity (Figure 1). SDI, SLB and AR are principal controlling traits in the association between characters. The correlation network shows the grouping of seed gravimetric, physical, and hydration properties into two major clusters, with character overlap within and between clusters. The swelling index, porosity, seed density, and bulk density formed a cluster, with overlap between the gravimetric and physical

characters within the group. This implies an interdependence between the seed's gravimetric and physical attributes. The gravimetric attributes related to mass and density, and the physical attributes (mechanical strength, moisture content, and geometric dimensions) are interdependent and can be used to forecast and understand the behaviour of the materials used in construction and processing. Similarly, seed sphericity and true density formed another cluster, indicating an interrelationship among the seed gravimetric and physical attributes of cowpea breeding lines. The second cluster shows a network of seed gravimetric (geometric mean diameter, arithmetic mean diameter, aspect ratio), physical (seed length before imbibition, seed dry weight, seed sphericity), and hydration characters (hydration capacity after imbibition and seed weight), along with their interconnectivity.

Regression analysis

The multiple regression analysis shows the dependent variable, seed weight (Y), is modeled as a function of several variables: aspect ratio (X_1), bulk density (X_2), total density (X_3), FSD (X_4), seed weight after imbibition (X_5), seed dry weight after imbibition (X_6), hydration capacity after imbibition (X_7), seed width after imbibition (X_8). The prediction equation representing the contributions of these variables to seed weight is as follows:

$Y= 7.32 + 0.01 (X_1) + 12.25 (X_2) + 0.02 (X_3) + 0.00 (X_4) + -1.02 (X_5) + 0.01 (X_6) + 0.05 (X_7) + 1.06 (X_8)$.

Although the model explains 98% of the total variation (R^2=0.99), retaining all eight predictors may introduce multi-collinearity and increase the risk of over-fitting. To ensure model parsimony, a sensitivity test was conducted, revealing that some predictors contributed only marginally. Therefore, implementing a variable selection process, such as stepwise regression or an alternative selection method, is advised to refine the model and improve its stability and relevance. This model shows a statistically significant relationship, with an R^2 value indicating that 98% of the total variation is explained. A positive 'b' value (0.01), a positive intercept value (a=7.32), and a significantly moderate positive linear regression coefficient (R^2=0.99) and adjusted R^2 of 0.99 at a 0.05 level of probability. This model displayed an R^2 value of 0.99 and an adjusted R^2 of 0.99, along with a positive intercept of 7.32.

Conclusion

A wide diversity was recorded in seed metrics, physical properties, and hydration properties. The study selected surrogates such as seed diameter, GMD, AMD, and SPH as useful for scoring large germplasm. Seed physical parameters indicate consumers' preferences throughout diverse farming systems and determine cooking time and the quality of cooked seeds. Phenotyping of the diversity panel of seed metric, physical and hydration properties of cowpea to determine variation and select traits effective for the determination of a relationship that could be used to devise a breeding program intended for enhancing seed metric and cooking quality.

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