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Genetic Variability and Interrelationships of Grain, Cooking, and Nutritional Quality Traits in Cowpea: Implications for Cowpea Improvement

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Abstract: Grain quality, cooking quality, and nutritional quality traits are some of the major attributes that enhance the uptake and utilization of improved cowpea varieties. Therefore, there is a need for a better understanding of the genetic variation and inter-relationships among these quality traits in cowpeas to integrate them into cowpea breeding programs. This study was conducted to determine genetic variability among 306 cowpea genotypes for grain quality, cooking quality, and nutritional quality traits and to understand the interrelationships among these traits for exploitation in breeding programs. The results showed highly significant differences ($p < 0.001$) among genotypes for grain quality, cooking quality, and nutritional quality traits. The mean performance for these quality traits was also very variable. These results suggest that genetic variability exists in the cowpea genotypes studied, which can be exploited in breeding programs aimed at developing high-performing varieties for the said traits. Significant ($p < 0.001$) positive correlations were detected for protein content with iron and zinc. On the other hand, nutritional quality traits did not exhibit any association with grain quality or cooking quality traits. Cooking quality traits were also shown to be significantly and positively correlated with grain quality traits. This study has identified several genotypes with desirable quality-related traits that could be used in crossing programs to generate improved varieties with consumer-preferred traits to improve the food, income, and nutritional status of many smallholder farmers that largely depend on cowpeas.

Keywords: consumer-preferred traits; cowpea; genetic variability; interrelationships; quality traits

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1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important legume crop produced and consumed in Sub-Saharan Africa by the majority of smallholder farmers, especially women. Cowpea is diploid, with 2n = 22 and a genome size of about 620 Mb [1–3]. The crop is autogamous, but outcrossing of up to 5% has been reported in the cultivated varieties, mainly due to insect activities [4]. It is a crucial food crop for meeting dietary protein needs and preventing micronutrient deficiencies [5], especially among women and children [6].

Cowpea whole grains contain 23–32% protein, 50–60% carbohydrates, and 1% fat [7], as well as considerable amounts of phytochemicals, dietary fiber, minerals (calcium, iron, and zinc), and vitamins. Iron and zinc are essential for human well-being. Their adequate supply helps prevent anemia and boost the immune system, two common problems in developing countries [8]. The composition of nutrients can vary due to several factors, including varietal differences, climatic conditions, and agronomic practices [9]. Some studies have reported mature grains to contain lower concentrations of most minerals [10] than immature green pods [11] and leaves [12].

Despite its enormous economic potential, the productivity of the crop is considerably low in Malawi, which is estimated at an average of less than 500 kg/ha among smallholder farmers [13]. This low productivity is attributed to low adoption levels of improved varieties (currently at 10%) and continued use of landraces, which tend to be low-yielding and highly susceptible to biotic and abiotic stress. Improved varieties, however, have been blamed for lacking important quality traits such as grain size, cooking time, and broth thickness. Low adoption of improved varieties and a lack of farmer and consumer-preferred varieties have been attributed to the failure of national breeding programs to involve farmers in the process of designing and developing varieties to meet their priorities and preferences [14].

Although cowpeas are nutritionally rich, the longer cooking time makes the legume unacceptable to some consumers. In a trait preference study conducted in Malawi in 2021 [15], short cooking time and broth thickness were among the priority traits for consumers to adopt and utilize a particular cowpea variety. It has also been reported that broth thickness is an important trait in the determination of cooking quality [16]. Traore et al. [17] stated that cooking qualities must be combined with other consumer preferences to encourage cowpea utilization. Other traits that influence cowpea marketing and utilization include seed coat color, seed shape, and grain size [18–22].

Considerable genetic variability has been reported for cowpea's physical, cooking, and nutritional quality traits [19,20,23–26]. In Malawi, reports on the genetic variability for grain quality (grain color, seed size, seed shape), cooking quality (cooking time, broth thickness), and nutritional quality (protein, zinc, and iron) traits on a diverse panel of cowpea genotypes are rare and non-existent. The degree of association among these quality traits has neither been fully dissected nor exploited to inform cowpea breeding programs. The present study was therefore conducted to evaluate the genetic variability of cowpea genotypes for grain, cooking, and nutritional quality traits. Further, this study was conducted to understand the degree of associations among the cowpea quality traits so that they can be exploited in a breeding program to develop cowpea varieties with consumer-preferred, market-driven, and resilient inclusive traits to enhance the food, income, and nutrition security of smallholder farmers in East and Southern Africa.

2. Materials and Methods

2.1. Study Area Description

A field experiment was conducted at Lilongwe University of Agriculture and Natural Resources (LUANAR), Bunda College of Agriculture, in the 2021/2022 growing season. The site is located at about 14°11′ S latitude and 33°46′ E longitude. The site receives a mean annual rainfall of about 930 mm (Figure 1) with an average temperature range of 17.2 °C to 19.1 °C and predominantly clay-loam soils.

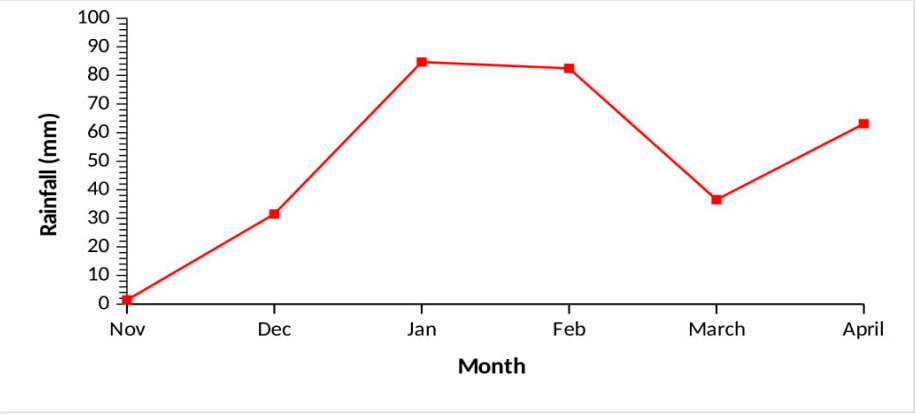

Figure 1. Monthly rainfall data at Bunda from November 2021 to April 2022.

2.2. Plant Materials

A diversity panel constituting 306 genotypes (advanced breeding lines, released varieties, and landraces) was used for this study (Supplementary Table S1). These genotypes were sourced from the International Institute of Tropical Agriculture-Nigeria genebank (IITA-44), India (61), Mozambique (24), the United States of America (22), Tanzania (16), Nigeria (13), South Africa (9), Uganda (4), Zambia (4), Hungary (2), Ghana (2), Cameroon (2), Botswana (2), Senegal (2), Russia (2), Argentina (1), Benin (1), Italy (1), Niger (1), Zimbabwe (1), Malawi (77), and 14 genotypes had unknown origins. These genotypes represent a subsample of the Centre of Innovation for Crop Improvement for East and Southern Africa (CICI-ESA) cowpea germplasm at LUANAR.

2.3. Experimental Design and Field Layout

An alpha lattice design with three replications generated using a Breeding Management System (BMS) was used for field establishment on 18 January 2022. The plot size was 2 ridges, 2 m long, each spaced 75 cm apart. Two seeds were planted per station at a depth of 3 cm, spaced 20 cm apart. Weeds were controlled by manual hoeing and uprooting to ensure weed-free conditions throughout the season. The infestation of insect pests such as aphids was controlled by the weekly application of Snowcron 500EC, a broad-spectrum emulsifiable concentrate insecticide, three weeks after germination. Data on grain quality traits were collected using the Field Book App-v5.6 [27], installed on mobile tablets. For laboratory analysis, graded and clean grains of each genotype were sampled from the three replications in the field.

2.4. Determination of Grain Quality Traits

Data for qualitative (seed shape, seed coat color) and quantitative traits (100 seed weight, seed length, seed width) of grain quality were collected following the cowpea descriptor [28]. Seed length and width were measured in millimeters on five randomly selected seeds using a ruler, and the weight of 100 seeds was determined as the weight in grams for 100 randomly selected seeds using an electronic balance.

2.5. Determination of Cooking Quality Traits

2.5.1. Cooking Time

The cooking time of each dry cowpea genotype was determined according to the method of Akinyele et al. [29]. Briefly, 20 g of dry cowpea grains were added to 450 mL of boiling water (tap water) in an aluminum cooking pot (without a top cover) on an electric hotplate. The water level was maintained by adding more boiled water intermittently. An electric kettle was used to heat the extra water, which was used for re-filling the pots during cooking. Cooking heat intensity was also maintained by using the maximum heating level of the hotplate for all cooking trials. Cooking time logging began when the water returned to the boiling point after immersing the grains in the boiling water. The cowpeas were left to cook for 30 min; thereafter, a doneness test was conducted every 5 min using the tactile method [30,31] by pressing the cooked seeds between two fingers until they were mushy, i.e., no hard material was found, just as also conducted by Munthali et al. [32] for common beans.

2.5.2. Broth Viscosity

The cooked cowpeas were removed from the pot, and then all broth and residual solids were transferred into a jar and mixed with boiling water to a total volume of 365 mL. The viscosity (cP or mPa) of 365 mL samples at 25 °C was measured using a programmable Brookfield digital viscometer (Model LV DV-I, Brookfield Engineering Laboratories, Inc., Stoughton, MA, USA) fitted with an LV spindle No. 1 as recommended in the Brookfield Labs Inc. guide (Brookfield Manual No. M14-023). A 400 mL open jar was used as a sample holder. Viscosity readings were taken after one minute at a spindle speed of 60 rpm [33]. All samples were thoroughly mixed just before taking viscosity measurements.

2.6. Determination of Nutritional Quality Traits

2.6.1. Digestion Procedure

Sample digestion was conducted according to the method proposed by Sahrawat et al. [34], with modifications. Approximately 0.2 g of finely ground cowpea seed samples were transferred to 50 mL digestion tubes, and six blanks were prepared to be used for standards. Thereafter, 2.5 mL of a sulfuric acid/selenium mixture was added to each digestion tube and blank. The sulfuric acid/selenium mixture was prepared by dissolving 3.5 g of selenium powder in 1 L of sulfuric acid and heating the mixture on a hot plate at high temperature with occasional stirring using a glass rod until the mixture turned clear and was cooled. The digestion tubes containing samples and the sulfuric acid/selenium mixture were placed in an aluminum block on a hotplate and heated to 200 °C until sample fumes were seen. The tubes were then removed from the hotplate and allowed to cool for 10 min. Then, 1 mL and 2 mL of 30% hydrogen peroxide were added to the samples and standards. The samples were replaced on the hotplate with a heavy glass vial on top of each tube and heated to 330 °C until the mixture turned clear and colorless, indicating the completion of digestion. The mixture was then allowed to cool.

N stock solution (0, 0.2, 0.4, 0.6, 0.8, and 1 mL, respectively) was added to the standards. The N stock solution was prepared by diluting 4.714 g of oven-dried ammonium sulfate in a 100 mL volumetric flask to make 10,000 ppm N. The standards are equivalent to 0, 1, 2, 3, 4, and 5% N in the plant digests.

2.6.2. Determination of Protein

The colorimetry method was used for the determination of nitrogen [35]. The N_1 reagent was prepared by dissolving 68 g sodium salicylate, 50 g sodium citrate (Tri-Sodium Citrate), and 50 g sodium tartrate in 500 mL deionized water. This solution dissolved 0.24 g of sodium nitroprusside and was diluted to 2000 mL. The N_2 reagent was prepared by dissolving 60 g sodium hydroxide in about 1500 mL of deionized water. The solution was then mixed with 28.5 mL of a 3.5% sodium hypochlorite solution and diluted to 2000 mL.

To determine the total nitrogen in samples, 0.750 mL extracts from the digestion above were put into 25 mL glass vials, and to each of the vials containing the extracts and standards, 5 mL of N_1 solution was added. After 5 min, 5 mL of the N_2 solution was added. The vials were allowed to stand until the color developed (about 1 h), and then the absorbance of the samples and standards was read on a spectrophotometer at 655 nm. To obtain the protein percentage, the percentage of nitrogen was multiplied by 6.25.

2.6.3. Determination of Iron and Zinc

The nitrogen digestion method was used to determine iron and zinc contents. To prepare stock solutions, 0.498 g dried ferrous sulfate septahydrate ($FeSO_4 \cdot 7H_2O$) and 0.440 g dried zinc sulfate septahydrate ($ZnSO_4 \cdot 7H_2O$) were weighed for iron and zinc determination, respectively. These were then dissolved in about 200 mL dilute HCl (0.1 N) and made up to the mark with distilled water in a 1 L volumetric flask to contain 100 ppm Fe and 100 ppm Zn stock solutions, respectively. The original samples of the digestion extract were passed on the Atomic Absorption Spectrophotometer (AAS).

2.7. Data Analysis

An exploratory analysis using density plots was performed on quantitative data to check for ANOVA assumptions. Thereafter, all the analysis was performed in the R software environment [36]. All laboratory analyses were performed in duplicate. ANOVA was performed by fitting the models with the aov function of the agricolae package [37] in R, and Duncan's multiple range test was used to separate means at a 0.05 significance level. Principal component analysis (PCA) was performed using the FactoMineR package [38], and Pearson correlation was performed using the corr. function of the stats package in R. The Euclidean distance metric was used in hierarchical cluster analysis, and the associations

between groups were performed using the Ward D2 method in a cluster R package version 2.1.6 [39].

3. Results

3.1. Frequency Distribution of Quantitative Traits

The exploratory analysis showed that all the traits except cooking time, iron content, and zinc content exhibited a nearly normal distribution pattern (Figure 2).

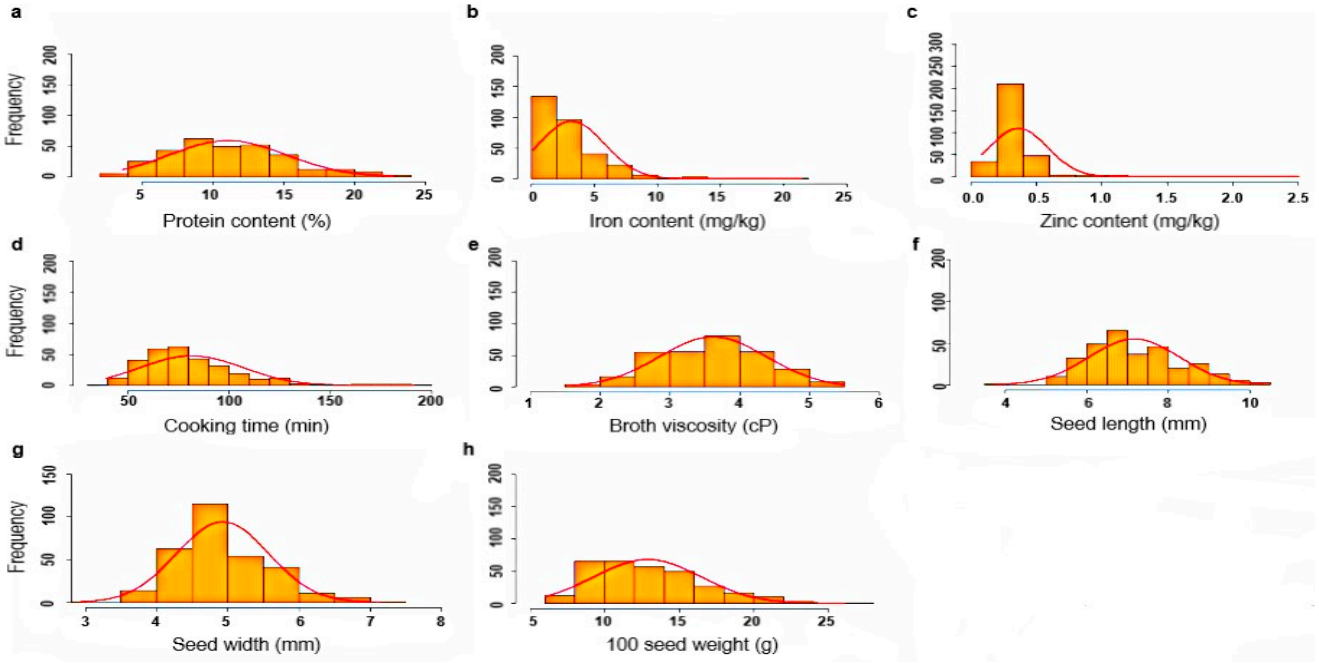

Figure 2. Frequency distribution of traits: (**a**) Protein content (%), (**b**) iron content (mg/kg), (**c**) zinc content (mg/kg), (**d**) cooking time (min), (**e**) broth viscosity (cP), (**f**) seed length (mm), (**g**) seed width (mm), (**h**) 100 seed weight (g).

3.2. Genetic Variability and Mean Performance for Grain Quality Traits

Highly significant differences ($p < 0.001$) were observed among the genotypes for all grain quality traits (Tables 1 and S1). The seed length ranged from 3.73 mm (TVu-322) to 10.33 mm (MWcp03). The highest seed width was recorded on genotype MWcp44 (7.13 mm), while genotype TVu-322 (2.93 mm) registered the lowest seed width. The 100 seed weights ranged from 6.0 g to 26.13 g. Genotype MWcp36 had the largest seed size, while genotype TVu-10169 had the smallest seed size. In terms of seed coat color, genotypes were assorted into brown (43.14%), white (19.28%), reddish-brown (13.40%), purple (11.76%), purplish-brown (4.25%), black (1.63%), and red (1.31%). Some of the genotypes (5.23%) were either mottled or had other mixed colors. For seed shape, the ovoid shape was dominant with about 60.78%, followed by the rhomboid shape (32.03%) and the kidney shape (6.54%). Globose and crowder shapes were observed in 0.66% of the genotypes (Tables 2 and S1).

Table 1. Mean square values for seed length, seed width, and one hundred seed weights.

Source of Variation	DF	Seed Length	Seed Width	HSDWT
Rep	2	124.68 ***	9.49 ***	543.50 ***
Block:Rep	99	4.67 ***	2.14 ***	10.90 **
Genotype	305	18.55 ***	6.31 ***	38.30 ***
Residuals	4183	1.18	0.54	7.00

DF = Degrees of freedom, HSDWT = 100 seed weight, *** highly significant at $p < 0.001$, ** highly significant at $p < 0.01$.

Table 2. Mean performance of the top ten and bottom ten genotypes was ranked based on 100 seed weights.

Genotype	Seed Length (mm)	Seed Width (mm)	HSDWT (g)	Seed Coat Color	Seed Shape
Top 10 genotypes					
MWcp36	9.33 ± 1.29	6.53 ± 1.06	26.13 ± 0.67	Brown	Rhomboid
MWcp03	10.33 ± 0.98	6.67 ± 1.05	23.70 ± 1.56	Purple	Ovoid
MWcp67	7.80 ± 0.94	5.53 ± 0.52	22.57 ± 3.32	Reddish-brown	Rhomboid
MWcp60	8.13 ± 1.13	6.13 ± 0.74	22.23 ± 1.53	Reddish-brown	Rhomboid
UAM14-126-19-2	8.60 ± 1.24	5.80 ± 0.68	21.70 ± 7.01	White	Rhomboid
MWcp45	8.67 ± 1.35	6.00 ± 1.07	21.30 ± 0.70	Reddish-brown	Rhomboid
MWcp24	8.87 ± 0.74	6.20 ± 0.86	21.03 ± 1.24	Reddish-brown	Rhomboid
MWcp53	8.73 ± 1.28	5.93 ± 0.59	21.00 ± 4.75	Reddish-brown	Ovoid
MWcp04	8.00 ± 1.77	5.27 ± 0.70	20.97 ± 2.78	Reddish-brown	Rhomboid
TVu-14004	9.80 ± 0.77	5.73 ± 0.70	20.93 ± 4.16	White	Kidney
Bottom 10 genotypes					
TVu-3526	5.40 ± 0.74	4.07 ± 0.70	7.87 ± 0.50	Purple	Ovoid
TVu-1177	5.67 ± 0.62	3.73 ± 0.46	7.77 ± 0.40	Brown	Ovoid
TVu-3063	6.87 ± 1.06	4.33 ± 0.90	7.30 ± 0.79	White	Ovoid
TVu-81	5.4 ± 0.63	3.73 ± 0.46	7.23 ± 0.95	White	Rhomboid
TVu-3228	6.27 ± 1.10	4.73 ± 0.70	7.00 ± 0.40	Purplish-brown	Ovoid
TVu-3229	5.20 ± 0.68	4.07 ± 0.46	7.00 ± 1.11	Purple	Ovoid
TVu-972	5.80 ± 0.94	3.87 ± 0.64	6.97 ± 0.32	Brown	Ovoid
TVu-17060	5.07 ± 0.80	3.87 ± 0.64	6.53 ± 0.65	Purple	Ovoid
TVu-3217	6.13 ± 0.83	4.27 ± 0.59	6.13 ± 0.70	Brown	Rhomboid
TVu-10169	6.27 ± 1.28	3.8 ± 0.56	6.00 ± 1.42	Black	Rhomboid
Grand mean	7.17	4.93	12.86		
Pr (>F)	0.00	0.00	0.00		
CV (%)	15.14	14.97	20.51		

CV = Coefficient of variation, Values with the same within the column.

3.3. Genetic Variability and Mean Performance for Cooking Quality Traits

Highly significant differences ($p < 0.001$) were observed among genotypes for cooking quality traits, cooking time, and broth viscosity (Table 3). Genotype TVu-15631 recorded the shortest cooking time (39 min), while genotype MWcp07 reported the longest cooking time (191 min) (Tables 4 and S1). The minimum broth viscosity at 25 °C was recorded at 1.6 cP for IT10K-834-3, whereas the maximum was observed for TVu-3252 (5.5 cP).

Table 3. Mean square values for cooking quality traits in the study.

Source of Variation	DF	Broth Viscosity	Cooking Time
Genotype	303	1.17 ***	1274.80 ***
Residuals	304	0.45	10.70

DF = Degrees of freedom, *** highly significant at $p < 0.001$.

3.4. Genetic Variability and Mean Performance for Nutritional Quality Traits

The genotypes exhibited highly significant differences ($p < 0.001$) for both protein (%) and mineral (iron and zinc) contents (mg/kg) (Table 5). The highest average protein content was recorded on genotype TVu-3243 (22.92%), while the lowest protein content was reported on genotype TVu-3263 (3.6%) (Tables 6 and S1). The highest iron content (21.44 mg/kg) was recorded on genotype TVu-3533, whereas the lowest level (0.12 mg/kg) was reported on genotype MWcp26. Genotype MWcp37 had the highest zinc level (2.63 mg/kg), while Raha 1 and TVu-1472 had the lowest zinc level (0.08 mg/kg).

Table 4. Mean values for cooking quality traits (top ten and bottom ten genotypes were ranked based on cooking time).

Entry	Cooking Time (min)	Broth Viscosity (cP)	SDCCOL	SDSHP
Top 10 genotypes				
TVu-15631	39.00 ± 1.41	4.60 ± 0.28	Red	Kidney
TVu-328	45.50 ± 2.12	4.20 ± 0.14	Mottled	Ovoid
IT98K-131-2	46.50 ± 4.95	3.75 ± 1.34	Brown	Rhomboid
TVu-22	47.50 ± 3.54	3.90 ± 0.14	Mottled	Kidney
TVu-3550	48.50 ± 0.71	5.00 ± 0.00	Reddish-brown	Kidney
IT00k-126-3	58.50 ± 2.12	4.60 ± 0.28	Brown	Ovoid
TVu-14004	49.50 ± 2.12	3.60 ± 1.41	White	Kidney
MWcp40	49.50 ± 2.12	3.85 ± 1.34	Purple	Ovoid
TVu-13265	49.50 ± 2.12	3.85 ± 1.34	White	Kidney
TVu-2661	49.50 ± 3.54	2.75 ± 0.64	Brown	Ovoid
Bottom 10 genotypes				
TVu-11674	137.50 ± 0.71	2.95 ± 0.64	Brown	Rhomboid
MWcp31	145.00 ± 2.83	3.20 ± 0.28	Brown	Ovoid
MWcp17	159.50 ± 0.71	4.20 ± 0.14	White	Rhomboid
MWcp61	160.50 ± 3.54	4.60 ± 0.28	Mottled	Ovoid
MWcp64	167.50 ± 0.71	3.30 ± 0.28	Brown	Ovoid
MWcp54	171.00 ± 5.66	4.10 ± 0.14	Mottled	Rhomboid
MWcp29	171.50 ± 6.36	4.00 ± 0.14	Purple	Ovoid
MWcp46	180.50 ± 0.71	2.40 ± 0.0	Mottled	Rhomboid
MWcp43	181.00 ± 10.41	3.65 ± 0.21	White	Rhomboid
MWcp07	191.00 ± 7.07	2.65 ± 0.64	Mottled	Ovoid
Grand mean	81.52	3.62		
Pr (>F)	0.00	0.00		
CV (%)	4.01	18.48		

CV = Coefficient of variation, SDCCOL = Seed coatcolor, SDSHP = Seed shape.

Table 5. Mean square values for nutritional quality traits in the study.

Source of Variation	DF	Protein	Iron	Zinc
Genotype	305	34.42 ***	13.58 ***	0.10 ***
Residuals	306	1.05	0.05	0.00

DF = Degrees of freedom, *** highly significant at $p < 0.001$.

Table 6. Mean values for nutritional quality traits (top ten and bottom ten genotypes were ranked based on protein content).

Entry	Protein (%)	Iron (mg/kg)	Zinc (mg/kg)	SDCCOL	SDSHP
Top 10 genotypes					
TVu-3243	22.92 ± 0.25	8.39 ± 0.00	0.45 ± 0.00	Brown	Ovoid
MWcp305	22.21 ± 0.85	6.56 ± 0.00	0.34 ± 0.00	Brown	Ovoid
TVu-2706	21.32 ± 0.10	3.20 ± 0.00	0.39 ± 0.00	Reddish-brown	Kidney
TVu-3094	21.29 ± 0.15	1.29 ± 0.00	0.36 ± 0.00	White	Kidney
MWcp69	21.21 ± 0.75	8.67 ± 0.00	0.39 ± 0.00	Purple	Ovoid
MZcp 024	20.97 ± 0.60	5.00 ± 0.00	0.34 ± 0.00	Brown	Ovoid
TVu-3524	20.40 ± 0.10	1.19 ± 0.00	0.29 ± 0.00	Brown	Rhomboid
IT 99K-529-1	20.29 ± 0.15	7.15 ± 0.00	0.45 ± 0.00	Brown	Ovoid
TVu-10169	20.18 ± 0.70	1.93 ± 0.00	0.33 ± 0.00	Black	Rhomboid
TVu-1483	20.04 ± 0.20	1.64 ± 0.00	0.43 ± 0.00	Purplish- brown	Ovoid
Bottom 10 genotypes					
TVu-1015	4.29 ± 0.10	1.58 ± 0.00	0.27 ± 0.00	Brown	Ovoid
MWcp601	4.19 ± 0.03	1.89 ± 0.00	0.22 ± 0.00	Brown	Ovoid
MWcp48	4.17 ± 0.15	1.90 ± 0.00	1.77 ± 0.02	Purple	Ovoid
MWcp46	4.09 ± 0.03	2.00 ± 0.00	0.23 ± 0.00	Mottled	Rhomboid

Table 6. *Cont.*

Entry	Protein (%)	Iron (mg/kg)	Zinc (mg/kg)	SDCCOL	SDSHP
IT90K-76	4.06 ± 0.10	1.57 ± 0.01	0.31 ± 0.00	Brown	Ovoid
TVx-3236	4.00 ± 0.03	2.67 ± 0.00	0.27 ± 0.00	White	Rhomboid
TZcp67	3.78 ± 0.20	1.94 ± 0.00	0.18 ± 0.00	Brown	Ovoid
MWcp50	3.64 ± 0.10	1.67 ± 0.00	0.24 ± 0.00	Purple	Ovoid
MZcp 004	3.63 ± 0.07	1.98 ± 0.00	0.17 ± 0.00	Brown	Ovoid
TVu-3263	3.60 ± 0.15	1.24 ± 0.00	0.28 ± 0.00	Purple	Rhomboid
Grand mean	11.05	3.21	0.36		
Pr (>F)	0.00	0.00	0.00		
CV%	9.27	7.10	1.23		

CV = Coefficient of variation, SDCCOL = Seed coat color, SDSHP = Seed shape.

3.5. Correlation Coefficients among Grain, Cooking, and Nutritional Quality Traits of Cowpea Genotypes

The relationships among the grain quality, cooking quality, and nutritional quality traits are presented in Table 7. Protein content was significantly positively correlated with iron content (r = 0.28, $p < 0.001$) and zinc content (r = 0.21, $p < 0.001$). Iron content was positively correlated with zinc content (r = 0.24, $p < 0.001$). Cooking time was significantly and positively correlated with seed length (r = 0.21, $p < 0.001$), seed width (r = 0.26, $p < 0.001$), and 100 seed weight (r = 0.19, $p < 0.001$). Highly significant positive correlations were also observed for seed length and seed width with 100 seed weights (r = 0.71 and r = 0.75 at $p < 0.001$, respectively).

Table 7. The correlation matrix among grain, cooking, and nutritional quality traits of cowpea genotypes.

Trait 1	Trait 2	r	95% CI	t	df	*p*
Protein	Iron	0.28	[0.17, 0.38]	5.05	304	<0.001 ***
Protein	Zinc	0.21	[0.10, 0.31]	3.72	304	0.005 **
Protein	Broth	−0.07	[−0.18, 0.04]	−1.23	302	>0.999
Protein	CT	−0.03	[−0.14, 0.08]	−0.53	302	>0.999
Protein	Seed.L	−0.06	[−0.17, 0.05]	−1.09	304	>0.999
Protein	Seed.W	−0.03	[−0.15, 0.08]	−0.59	304	>0.999
Protein	HSDWT	−0.06	[−0.17, 0.05]	−1.08	304	>0.999
Iron	Zinc	0.24	[0.13, 0.34]	4.34	304	<0.001 ***
Iron	Broth	−0.04	[−0.15, 0.08]	−0.61	302	>0.999
Iron	CT	−0.08	[−0.19, 0.03]	−1.45	302	>0.999
Iron	Seed.L	-7.24×10^{-03}	[−0.12, 0.10]	−0.13	304	>0.999
Iron	Seed.W	0.03	[−0.09, 0.14]	0.47	304	>0.999
Iron	HSDWT	−0.06	[−0.17, 0.05]	−1.08	304	>0.999
Zinc	Broth	0.03	[−0.08, 0.14]	0.5	302	>0.999
Zinc	CT	−0.04	[−0.15, 0.07]	−0.75	302	>0.999
Zinc	Seed.L	0.07	[−0.04, 0.18]	1.29	304	>0.999
Zinc	Seed.W	0.06	[−0.05, 0.17]	1.06	304	>0.999
Zinc	HSDWT	0.03	[−0.09, 0.14]	0.48	304	>0.999
Broth	CT	−0.04	[−0.16, 0.07]	−0.76	302	>0.999
Broth	Seed.L	6.74×10^{-03}	[−0.11, 0.12]	0.12	302	>0.999
Broth	Seed.W	−0.03	[−0.14, 0.08]	−0.54	302	>0.999
Broth	HSDWT	−0.09	[−0.20, 0.02]	−1.61	302	>0.999
CT	Seed.L	0.21	[0.10, 0.31]	3.72	302	0.005 **
CT	Seed.W	0.26	[0.15, 0.36]	4.67	302	<0.001 ***
CT	HSDWT	0.19	[0.08, 0.30]	3.44	302	0.013 *
Seed.L	Seed.W	0.89	[0.86, 0.91]	33.49	304	<0.001 ***
Seed.L	HSDWT	0.71	[0.65, 0.76]	17.57	304	<0.001 ***
Seed.W	HSDWT	0.75	[0.70, 0.80]	19.88	304	<0.001 ***

r = Correlation coefficient, CI = Confidence interval, t = t-value, df = Degrees of freedom, *p* = *p*-value, CT = Cooking time, Seed.L = Seed length, Seed.W = Seed width, HSDWT = 100 seed weight, Broth = Broth viscosity. *, **, ***=Significant at 5%, 1% and 0.1%, respectively. (*p*-value adjustment method: Holm (1979)).

3.6. Genetic Diversity among the Cowpea Genotypes

3.6.1. Principal Component Analysis for Grain Quality, Cooking Quality, and Nutritional Quality Traits

Table 8 shows principal component analyses (PCA) for the quantitative quality traits among the genotypes. The genotypes were plotted on two dimensions based on the PCA results (Figure 3). All the traits were grouped into three principal components (PC) (eigenvalues ≥ 1), which accounted for 65.17% of the variability. The first PC explained about 33.36% of the total variability present and was mainly associated with seed width, seed length, and 100 seed weight. The second PC accounted for 18.81% of the total variability, which was correlated with iron, zinc, and protein contents. The third PC explained nearly 12.98% of the total variation and was mainly associated with broth viscosity.

Table 8. Trait contributions, eigenvalues, and cumulative percentage of the components.

Traits	Principal Components			Trait Contributions (%)		
	PC1	PC2	PC3	PC1	PC2	PC3
Seed length	0.57	0.04	0.11	32.13	0.16	1.30
Seed width	0.58	0.06	0.05	33.59	0.34	0.26
100 seed weight	0.53	−0.00	−0.02	28.51	0.00	0.05
Cooking time	0.22	−0.13	−0.29	5.08	1.62	8.65
Broth viscosity	−0.04	−0.08	0.89	0.16	0.65	79.84
Protein	−0.06	0.56	−0.20	0.31	31.92	3.84
Iron	−0.03	0.60	0.01	0.08	36.04	0.01
Zinc	0.04	0.54	0.25	0.14	36.04	6.05
Eigenvalue	2.67	1.51	1.04			
% of variance	33.36	18.81	12.98			
Cumulative % of variance	33.37	52.19	65.17			

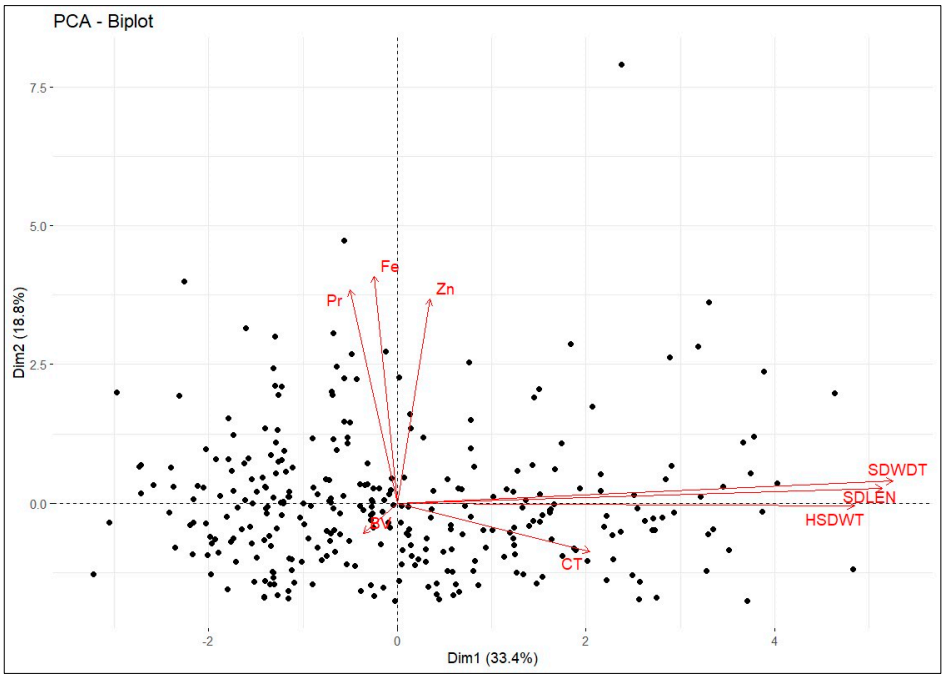

Figure 3. Genotype by trait biplot. A total of 306 cowpea genotypes measured for eight quality traits: Pr = Protein content, Fe = Iron content, Zn = Zinc content, CT = Cooking time, BV = Broth Viscosity, SDLEN = Seed length, SDWDT = Seed width, HSDWT = 100 seed weight, black dots represent the different genotypes evaluated.

3.6.2. Hierarchical Clustering of Genotypes Based on Grain Quality, Cooking Quality and Nutritional Quality Traits

As shown in the dendrogram (Supplementary Figure S1), cluster analysis grouped the genotypes into two major clusters based on the quality traits. Cluster I comprised 234 genotypes (gold color), while Cluster II comprised 72 genotypes (blue color). The genotypes in Cluster I were relatively small-seeded with shorter cooking times, while those in Cluster II were generally large-seeded with longer cooking times and higher protein and iron content (Table 9).

Table 9. Descriptive statistics of the clusters.

Cluster	N	Statistic	Pr (%)	Fe (mg/kg)	Zn (mg/kg)	CT (min)	BV (cP)	Seed.L (mm)	Seed.W (mm)	HSDWT (g)
I	234	M	10.95	3.26	0.35	76.98	3.68	6.74	4.66	11.43
		Min	3.60	0.32	0.08	39.00	1.60	3.73	2.93	6.00
		Max	22.92	21.44	1.23	145.00	5.50	8.93	5.73	18.80
II	72	M	11.39	3.06	0.40	96.03	3.43	8.57	5.79	17.58
		Min	3.64	0.12	0.09	49.50	2.20	5.67	4.53	12.80
		Max	21.21	14.64	2.63	191.00	4.90	10.33	7.13	26.13

Pr = Protein, Fe = Iron, Zn = Zinc, CT = Cooking time, BV = Broth Viscosity, Seed.L = Seed length, Seed.W = Seed width, HSDWT = 100 seed weight.

4. Discussion

Most resource-constrained smallholder farmers in sub-Saharan Africa, especially women, rely on cowpeas as their major source of protein and other mineral elements such as iron and zinc, thereby contributing to their improved nutritional status and health well-being. In dryland areas, cowpea is a major source of income to support their daily livelihoods. At the same time, cowpea is one of the crops used to adapt to climate-related changes due to its resilience in these areas. Current cowpea breeding strategies in the region must, therefore, take into consideration its grain, nutritional value, and cooking quality, which influence farmers/consumers' choices and consumption.

This study observed significant variations in the genotypes for grain quality, cooking quality, and nutritional quality traits (Tables 1, 3 and 5). This suggests that genetic variability exists in the current set of cowpea germplasm in Malawi, which could be exploited in breeding programs aimed at developing high-performing varieties for the said traits. Assessment of genetic variability for different traits is an important pre-breeding step as it provides an opportunity for plant breeders to develop new and improved varieties with desirable characteristics that are both farmer- and consumer-preferred. Earlier studies [6,40,41] reported wide genetic variation in grain quality, crude protein, and micronutrient contents in cowpeas, suggesting that the nutritional value of varieties could be improved.

In the study, seed coat color among the genotypes was quite variable, predominated by brown color (43.14%), followed by white (16.39%), and reddish-brown (13.40%). In cowpeas, seed coat color is an important aspect of consumer preference, which directly influences the marketability of the grain. This implies that variety development programs need to respond to specific and unique market preferences for improved uptake of the varieties. Trait preference studies in different regions and countries reported these unique preferences [19,21,22,42,43]. For instance, in Malawi, Mozambique, and Tanzania, through a trait preference survey [15] among farmers, consumers, traders, and processors, seed coat color was among the most preferred characteristics.

The genotypes in the study also varied for seed shape, with over 51.67% being ovoid and 32.03% rhomboid. This finding is similar to Kim et al. [44], who reported that out of the 245 cowpea genotypes in the study, 66.9% were egg-shaped (ovoid) and 24.9% were rectangular (rhomboid). This suggests that the ovoid shape is a dominant seed shape in cowpeas.

The other important trait in cowpea breeding is grain size. Seed size is directly correlated with grain yield, and as such, it is considered an essential market trait [18]. Seed size is measured as grams per 100 seeds, and Moses and Zibokere [24] grouped seed size into small (10–15 g), medium (15.1–20 g), large (20.1–25), and very large (greater than 25 g). In the present study, genotypes exhibited wide genetic variability for seed size, which ranged from 6 to 26.13 g. This range is within most of the reported seed sizes [23,26]. It was noted that most of the landraces were larger-seeded than the improved varieties. As noted before, most farmers prefer large-seeded varieties, and this might explain the reasons behind the low adoption levels of improved varieties in Malawi since all the improved varieties have small grain sizes.

With cowpeas being an important source of protein and other elements for many smallholder farmers, breeding programs must continue to develop and release more nutritious varieties. The genetic variability for nutritional quality properties such as protein, zinc, and iron exhibited in the study is a sure way to set the trajectory towards sustainable, improved variety development that meets the nutritional needs of farmers, especially women and children. Genetic variability for nutritional quality in cowpeas is a common phenomenon [6,12,20,40,41], which means that selection for these traits in cowpeas can increase genetic gain.

Cooking time is usually associated with energy sources. In sub-Saharan Africa, where the predominant energy source is fuel wood, most households struggle to prepare meals that require more energy. Shorter cooking varieties are usually a strategy for energy savings. The findings of the study established some considerable genetic variation among genotypes, with some taking a minimum of 39 min to cook. Some studies have reported a minimum of 35 min [45] to cook. Contrastingly, some genotypes in the study took 191 min, which suggests that they are less economical in terms of energy consumption. Related to cooking quality is broth viscosity, which very few studies have looked at in cowpeas as a genetically controlled trait. This study suggests that the variability recorded is genetically controlled, as considerable variation was observed in the genotypes, which ranged from 1.6 to 5.5 cP. In Malawi, it has been reported that broth thickness is one of the key determinants of the cooking quality of the legume [16], and if a variety lacks this trait, it can rarely be taken up by farmers/consumers.

Significant positive correlations were detected among the 306 genotypes for protein content with iron and zinc (Table 7). This is in agreement with the positive correlation between crude protein and Fe contents in 11 genotypes reported by Moura et al. [25] and Boukar et al. [6,40]. Since the presence of genetic variability has already been elucidated for these traits, it is, therefore, possible to improve protein, iron, and zinc contents without adverse interactions, as also espoused by Nielsen et al. [46] and Jean Baptiste et al. [47], who indicated some heritability for crude protein content in cowpea.

On the other hand, nutritional quality traits did not exhibit any association with grain quality or cooking quality traits. The non-significant associations between grain quality and nutritional quality traits suggest that desirable traits can be incorporated through trait introgression techniques. This is in agreement with several reported [25,41,48] results where cooking time was not associated with protein content in their study. However, cooking quality traits were shown to be significantly and positively correlated with grain quality traits. For example, cooking time is correlated with seed length, seed width, and 100 seed weight (cooking time increases with seed size). The implication is that as breeding programs strive to develop varieties that are large-seeded with short cooking times to meet market demands, there will be a trade-off between these two traits. There is a need to devise breeding strategies that maximize both of these traits, as well as a careful selection of parental materials. In addition to this, there is a need to dissect the genetic mechanisms of these traits and determine whether any of them can be amenable to trait integration techniques.

A genotype by trait biplot was produced using the first two PCs, which explained 52.19% of the variability (Figure 3). This was used to identify the best-performing and

genetically dissimilar genotypes that could be further explored for breeding. The random scattering of the cowpea genotypes across the quadrant, as seen in the biplot, suggests that the genotypes were genetically different. The genotypes clustered based on their performance for the traits; thus, those genotypes located close together had relatively similar values for specific traits. The biplot shows that many genotypes clustered near the origin, so they are likely derived from the same parents. Genotypes located further from the biplot origin had more extreme values for a specific trait than genotypes closer to the origin. For example, genotypes MWcp03 and MWcp36 showed extreme values for seed length, width, and 100 seed weight, whereas TVu-3533 showed higher values for protein, iron, and zinc concentrations. On the other hand, TVu-322 had the highest broth viscosity, and MWcp46 had the longest cooking time. Furthermore, MWcp37 was distant from the rest of the genotypes, indicating that the genotype is genetically dissimilar from most of the genotypes. Concentrations of protein, iron, and zinc were independent of broth viscosity, cooking time, seed length, width, and 100 seed weight. This suggests that protein, iron, and zinc can be improved without altering the rest of the traits. Noticeably, broth viscosity was not associated with cooking time or the quantitative grain quality traits. However, cooking time was positively related to the quantitative grain quality traits. This result further suggests that increasing the seed size in cowpeas may increase the cooking time but not the broth viscosity. As already elucidated, the landraces from Malawi were generally large-seeded with longer cooking times, but these tend to be common among smallholder farmers. This was further confirmed through cluster analysis, which clustered the majority of the landraces from Malawi into Cluster II, which had large-seeded genotypes with relatively higher protein and iron content. Nevertheless, genotypes in Cluster I were small-seeded, and within the cluster were most of the improved varieties and advanced lines from the IITA, which is an indication that they were selected for similar traits, mostly small seed size, which is contrary to farmer preferences.

5. Conclusions

This study has identified genetic variability in grain quality traits, cooking quality traits, and nutritional quality traits among the cowpea genotypes that constitute breeding lines and landraces. This study has further identified relationships among the quality traits. Significant and positive correlations among protein, iron, and zinc contents suggest the possibility of improving the concentrations of these nutrients simultaneously. Non-significant associations between grain quality and nutritional quality traits suggest that desirable traits can be incorporated through trait introgression techniques. This study has also identified several potential genotypes with desirable quality-related traits that could be used in crossing programs to generate improved varieties with consumer-preferred traits to improve the food, income, and nutritional status of many smallholder farmers that largely depend on cowpeas.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14040633/s1, Table S1: List of genotypes used in the study, Analysis of variance, summary statistics and Genotype mean values for grain, cooking, and nutritional quality traits; Figure S1. Hierarchical clustering dendrogram analysis.

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