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Selection of new recombinant inbred lines and dual-purpose cowpea genotypes based on total protein and its fractions, amino acids, and nutritional quality

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ABSTRACT

Nutritional quality is a lever for the adoption of new varieties. This study aimed to assess the protein quality of 29 cowpea genotypes for breeding purposes. The collection includes 4 local and donor parental varieties, 10 and 11 progenies of F7-30 and F8-38 families, and 4 dual-purpose varieties. Crude protein and protein-soluble fractions of cowpea grains were determined using the methods of Kjeldahl, Osborne, and Campbell, respectively. Amino acid content was quantified using high-performance liquid chromatography. The descriptive analysis shows high intra and inter-familial trait variability among genotypes. Protein contents varied from 25.5% to 35.86%. The main protein fractions were albumin (24.36–73.34 g/100 g protein) and glutelin, followed by globulin and prolamin. Glutamine/glutamic acid, asparagine/aspartic acid, and phenylalanine + tyrosine were prevalent. Methionine + cysteine was the most limiting amino acid. However, apart from CWS-F7-30-9a, CWS-F7-30-7a, and CWS-F8-38-50, all investigated genotypes meet the requirements of all essential amino acids (EAAs) as recommended by FAO/WHO/UNU adults and 2–5 year olds. The genotypes had a mean predicted protein efficiency ratio of 3.68, making them excellent protein sources. This study identifies genotypes with high protein, good EAA profile, and high protein quality for breeding programs and other specific usages.

1. INTRODUCTION

The world's population is expected to increase by nearly 2 billion persons in the next 30 years, from the current 8 billion to 9.7 billion in 2050, posing significant challenges to the global food sector in providing secure and safe food supplies [1]. Legumes and grains play an important role in human nutrition and are one of the food sources to achieve the sustainable objective of providing a good amount of essential nutrients, particularly low-cost protein [2,3]. Cowpea (*Vigna unguiculata*) is one such rustic, versatile, and hardy legume crop that is resistant to heat and adaptable in areas with water scarcity and low-fertile soils [4,5]. Its high protein, low carbon footprint, less growth period, and high productivity in marginal areas will allow the fulfillment of three sustainable development goals, i.e., SDGs 2, 3, and 13 [3]. Cowpea has gained more attention recently from consumers and researchers worldwide due to its exerted health-beneficial properties including anti-diabetic, anti-cancer, anti-hyperlipidemic, anti-inflammatory, and anti-hypertensive properties [6].

Malnutrition is a major health concern for children in poor and developing nations, where most of their diet consists of cereal-based foods. The lack of protein, iron, and zinc in their diets is particularly worrying [7]. Cowpeas are vital to millions of smallholder farmers as they provide both income and essential nutrients [8]. Cowpea protein is a good source of essential amino acids (EAAs) such as lysine, leucine,

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and isoleucine. However, it lacks cysteine and methionine [9,10]. Apart from protein, cowpeas also contain complex carbohydrates, dietary fiber, and resistant starch, which make them an excellent source of nutrition [3]. The quality of protein and nutrient value of foods depends on their amino acid content and how efficiently they are utilized by the body after digestion, absorption, and utilization [11].

There can be significant variations in the chemical composition and nutritional properties of cowpea varieties due to factors such as plant nutrition conditions, cultural practices, and genetic modification [12]. Thus, concerns about the harmful effects of genetic modification on human health should not only be directed toward foods produced by rDNA technology but also toward those produced through conventional breeding methods, as they can also introduce unintended compositional changes that may have negative effects on human health. [13]. Despite being the second largest producer of cowpea [13], Niger is still facing serious malnutrition issues. While most breeding efforts have focused on selecting high-yielding varieties that can withstand biotic and abiotic stress [14], little attention has been given to selecting genotypes based on their nutritional quality. It is important to evaluate the nutritional value of high-yielding varieties developed by plant breeders to ensure their significance in addressing malnutrition [15]. The analysis of the nutritional composition of various cowpea genotypes is crucial in selecting genetically distinct lines that can be potential parental genotypes in developing cowpea cultivars with enhanced nutritional value. This is particularly important in Sub-Saharan Africa, where there is a high prevalence of nutritional deficiencies. Therefore, this study aimed to evaluate the genetic variability among new inbred cowpea lines, and local and dual-purpose varieties to identify unique and complementary high protein, good EAA profile, and superior protein quality parental genotypes for a breeding program.

2. MATERIAL AND METHODS

2.1. Study Area

The nutritional analyses were carried out in the Department of Food and Technology laboratories of the Research Institute of Applied Sciences and Technologies (IRSAT/DTA) and the LABESTER laboratory in the Faculty of Life Science Joseph Kizerbo University of Ouagadougou, Burkina Faso.

2.2. Plant Materials

A total of 29 varieties from the collection of the CowpeaSquare project were selected. The collection includes 4 local and donor parental varieties, 10 inbred lines of the F7-30 family resulting from the cross between CS036 (local receiving parent from Niger) and CS117 (donor parent from INERA, Burkina Faso), eleven progenies of the F8-38 family from the cross between CS133 (local receiving parent from Niger) and CS098 (donor parent from IITA of Ibadan, Nigeria), and 4 dual-purpose varieties.

2.3. Experimental Design for Seed Multiplication

The varieties were multiplied under the same conditions in pure culture during the 2020–2021 rainy season on the experimental site of Dan Dicko Danckoulodo University of Maradi (Niger). The experimental plots consist of one block per family. One row of six pockets per variety with 1 m spacing. The spacings within rows and between blocks were 1.5 and 2 m. Two seeds were sown per hole. Standard cowpea agronomic management practices were followed. No pesticide was sprayed, and weeding was done manually.

2.4. Sample Preparation

The pods were gathered from the accessions grown until they reached the physiological maturity stage, after which they were sun-dried. The seeds were sorted manually and cleaned thoroughly to remove unhealthy, insect-infested seeds, soil, dust, and husks. Once cleaned, the seeds were ground into a fine powder using a laboratory seed grinder and stored in sterile polystyrene pots.

2.5. Determination of Protein Content

The protein content of cowpea varieties was determined according to the Kjeldahl method AOAC 979.09 [16].

2.6. Determination of Soluble Protein Content

The extraction (fractionation of soluble proteins) was carried out according to the method developed by Osborne and Campbell [17] (Fig. 1). Protein fraction concentrations were determined according to Bradford's method [18]. The absorbances were read using a microplate spectrophotometer (Epoch, Biotek, USA). Bovine serum albumin was used as the standard protein to draw the standard curve.

2.7. Determination of Amino Acid Content

The amino acid contents were determined according to the Waters PICO•TAG high-performance liquid chromatography method described by Bidlingmeyer *et al.* [19].

2.8. Evaluation of Protein Quality

The chemical scores (CS) of EAAs of the accessions were calculated by expressing the limiting EAA of seed proteins as a percentage of the same EAA in the standard protein (hen egg protein) Block and Mitchell [20]. The amino acid showing the lowest percentage was called the "limiting amino acid" representing the CS. The predicted protein efficiency ratio (P-PER) was estimated according to the regression equation described by Alsmeyer *et al.* [21].

P-PER = −0.468 + 0.454 (Leucine) – 0.105(Tyrosine)

Net protein value (NPV) = (lowest amino acid score × percent of protein)/100 [11].

2.9. Statistical Analysis

The data set was analyzed using R software version 4.2.2 [22]. Descriptive statistics, including mean, standard deviation (SD), and third upper quartile, were used to analyze the data. To identify significant differences among genotypes, an analysis of variance was conducted. Pearson's correlation and principal component analysis (PCA) were performed to study the relationship between parameters and identify similarities and differences among accessions based on nutritional traits. Additionally, hierarchical cluster analysis (HCA) was conducted using Ward's clustering method and squared Euclidean distance. Finally, the dendrogram was generated based on the neighbor-joining algorithm using the hclust package.

3. RESULTS AND DISCUSSION

3.1. Genetic Variation of the Biochemical Traits

Table 1 summarizes the results of the estimation of the studied biochemical traits. The descriptive statistics of the data show high variability for each trait. Proteins ranged from 25.5% to 35.86%. The higher variabilities among genotypes were found in prolamin

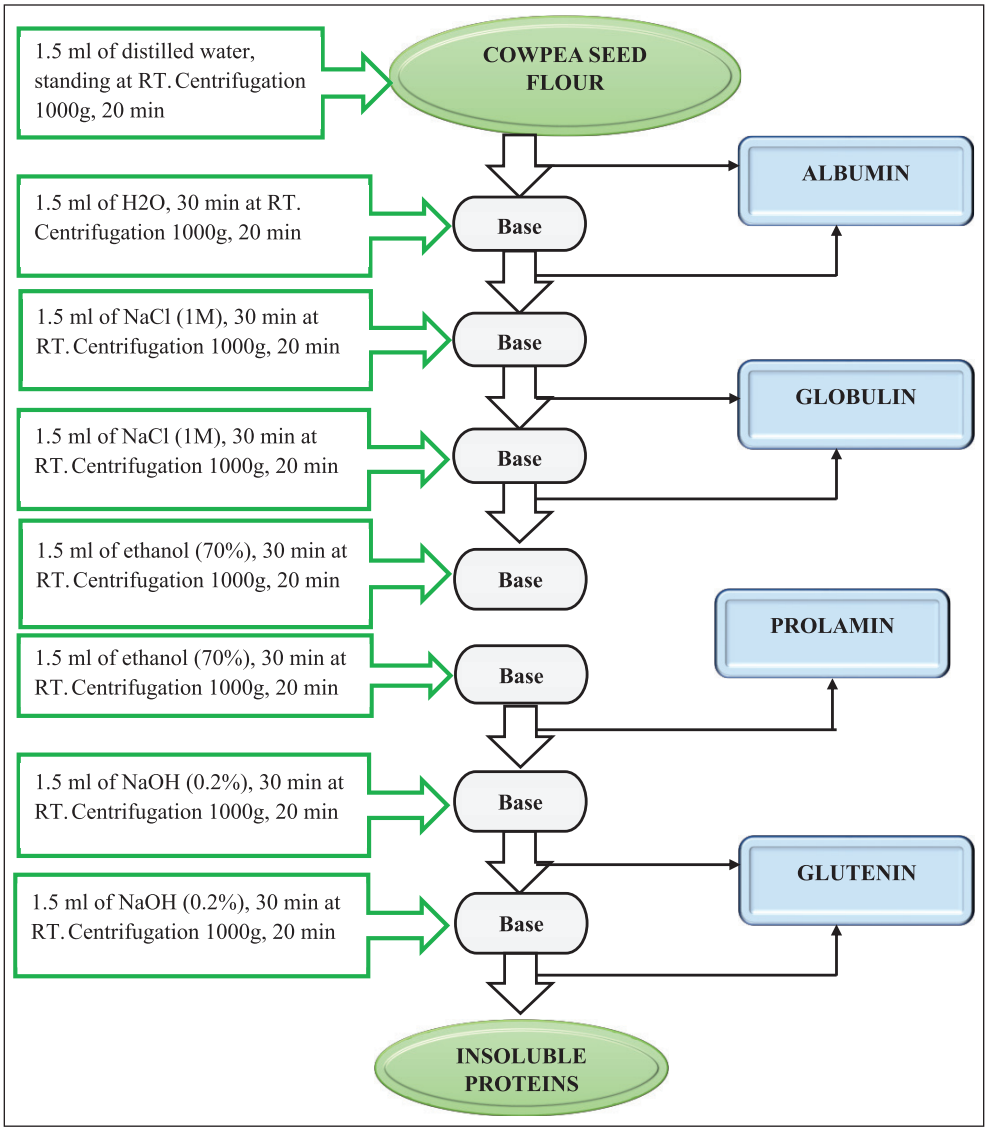


Figure 1. Fractionation steps of cowpea seed proteins by Osborne and Campbell [17].

(0–3.84 g/100 g of protein, with a coefficient of variation (CV) of 1.74, cysteine (0.01–0.12 g/100 g, CV = 0.58), and NPV with CV of 0.51. The mean, SD, and third upper quartile of nutritional parameters (crude protein, soluble proteins, amino acid, and protein quality) of the cowpea accessions investigated are shown in Tables 1–3.

3.2. Seed Crude Protein and Protein Fractions of Cowpea Genotypes

3.2.1. Total protein content

Cowpea is a significant food grain legume grown and consumed by smallholder farming communities in Sub-Saharan Africa. This crop plays a vital role in meeting the dietary protein requirements and addressing micronutrient deficiencies [23]. The study investigated the protein content of different cowpea genotypes and found highly significant ($p < 0.001$) inter and intra-familial differences (Table 2). The protein content ranged from 26.38% to 32.35% for F7-38 genotypes, 28.92% to 35.86% among F8-38 genotypes, and 25.5% to 28.72% for dual-purpose cultivars. The genetic variability observed could contribute to achieving food security, improved nutrition, and conservation of the biodiversity of cowpeas [24].

The protein content of the dual-purpose accessions in F7-30 was lower compared to new lines and F8-38 parents. Although there was no significant difference between parents and offspring in the F8-38 family, some offspring had a higher protein content. In the F8-38 family, although the difference in the mean between parents and offspring was not statistically significant, some of the offspring, such as CWS_F8_38_48, exhibited protein content exceeding parental level, and all other assessed genotypes. The extreme values may indicate a transgressive segregation. This result is consistent with Purnamasari and Syukur's [25] findings that transgressive segregation can occur for protein content in cowpeas.

More than half (16 out of 29) of the genotypes recorded protein content greater than 30%, which can be beneficial for food additives and protein-rich food formulations, such as infant formula, playing a crucial role in fulfilling SDG 2, which aims at achieving zero hunger

Table 1. Descriptive statistics of 29 cowpea genotypes for 19 biochemical traits.

	N	Missing	Minimum	Maximum	Median	Mean	St. Dev	Coef. var
Protein	29	0	25.5	35.86	30.08	30.08	2.01	0.07
Albumin	29	0	24.36	73.34	53.22	51.78	10.75	0.21
Glutelin	29	0	15.3	35.36	25.1	24.69	5.23	0.21
Globulin	29	0	3.45	11.47	6.08	6.34	1.93	0.30
Prolamin	29	0	0	3.84	0	0.55	0.95	1.74
Threonine	29	0	0.16	0.39	0.3	0.29	0.05	0.17
Valine	29	0	0.16	0.36	0.23	0.23	0.04	0.18
Isoleucine	29	0	0.12	0.32	0.18	0.19	0.04	0.19
Leucine	29	0	0.23	0.46	0.34	0.35	0.05	0.14
Lysine	29	0	0.45	0.97	0.72	0.72	0.11	0.15
Histidine	29	0	0.11	0.34	0.16	0.17	0.04	0.25
Arginine	29	0	0.26	0.76	0.57	0.54	0.12	0.21
Tyrosine	29	0	0.13	0.41	0.18	0.19	0.05	0.26
Methionine	29	0	0.04	0.25	0.09	0.09	0.04	0.40
Cysteine	29	0	0.01	0.12	0.03	0.04	0.02	0.58
Phenylalanine	29	0	0.18	0.42	0.25	0.26	0.05	0.18
NPV	29	0	9.46	68.78	19.75	20.97	10.68	0.51
EAA/NEAA	29	0	0.71	0.91	0.80	0.80	0.04	0.05
P.PER	29	0	2.29	5.49	3.61	3.68	0.73	0.20

NPV = Net protein value, EAA/NEAA = Essential amino acid/nonessential amino acid, P.PER = Predicted protein efficiency ratio.

by tackling malnutrition in poor children. These genotypes exhibited considerably higher protein content compared to other studies, with a mean value of 30% compared to 24% found in a collection of 120 varieties studied by Padhi *et al.* [3], 20.28% to 27.32% reported by Sombié *et al*. [26] in Burkina Faso, and 25.27% by Wibowo *et al*. [27]. This variability could result from differences in genetic composition [28] and study areas. According to Padhi *et al.* [3], the protein content may differ based on the type of cultivar or soil conditions. Gerrano *et al.* [24] reported that total protein was highly influenced by location and affected the vegetative and physiological growth of the crop.

3.2.2. Seed protein fractions

Cowpea contains a complex and unique protein profile with an array of seed proteins including globulins, albumins, glutelin, and prolamin [29,30]. Upon protein fractionation, the dominant protein constituent among accessions was the water-soluble fraction, albumin, which varied significantly among accessions. The fraction ranged from 24.36 ± 1.65 to 73.34 ± 1.03 g/100 g of protein (Table 2). This fraction is categorized as enzymatic and metabolic proteins [31,32]. In the F7-30 family, 90% of offspring have greater albumin levels than their parents. Among F8-38 genotypes, CWS_F8_38_9 and CWS_F8_38_17 exhibited higher albumin contents but in the same range as their local parent CS113. The albumin levels recorded in this study were higher than 19.6%–22.5% reported by Teka *et al.* [33] but close to that reported by Freitas *et al.* [34]. The discrepancies of levels in protein fraction may result from the extracting method employed or genetic and environmental variability. Even though values were in range with other studies, glutelin was the second most abundant fraction contrary to other findings where glutenin, behind globulin and albumin, was the third most prevalent fraction in cowpeas [33,35,36]. However, this result is consistent with the findings of Gupta *et al.* [32], who found glutelin as the second most dominant fraction among high-protein cowpea genotypes. The F7_30 offspring CWS_F7_30_11a, CWS_F7_30_11b, and CWS_F7_30_2 with, respectively, 32.74 ± 0, 31.75 ± 0.72, and 31.63 ± 0.51% had higher glutelin levels than their parents and other offspring within the same family. The glutenin-rich offspring among F8-38 genotypes are CWS_F8_38_45, CWS_F8_38_6, and CWS_F8_38_7.

The salt-soluble fraction (globulin) ranging from 3.45% to 11.47% was the third most dominant protein fraction. Except for the offspring CWS_F7_30_2, the two parents show higher globulin than their offspring in the F7-30 family. The mean of the F8-38 descendants was higher than the parental mean. Ten out of eleven performed better than the best single parent.

Prolamin is the least abundant fraction. The significantly lower prolamin level observed in this study is typical of *Vigna* species [37]. The progenies of F7-30, CWS_F7_30_9b, CWS_F7_30_11a, and CWS_F7_30_12 exhibited higher prolamin content than the local parent CS036. Their other counterparts and the donor parent CS117 recorded a total absence of prolamin. Three F8-38 genotypes resemble the local parent, while the others, including the donor parent, lack prolamin. The CS099 (0.86 ± 0.18) was the only genotype containing prolamin among the dual-purpose varieties.

The wide variation and extreme values observed for the protein fraction among progeny compared to their respective parental genotypes may express transgressive segregation. This may result from the heterosis effect in hybrid F1 due to the bi-parental nature of the progenies and the recombination power of the heterozygous local parent used for the crosses. The higher soluble protein contents exhibited by F7 and F8 lines with means exceeding that of the parents indicate that progenies with higher content could be selected without losing potential even at further generations. Padi [38] reported that a progeny that exhibited distinguished performance at F2 also sustained their performance at advanced homozygous generation. According to Tchiagam *et al.* [29],

Table 2. Crude protein and protein fractions of 29 inbred lines and multipurpose cowpea cultivars.

Family	Variety	Protein (%)	Prolamin (g/100 g protein)	Albumin (g/100 g protein)	Glutenin (g/100 g protein)	Globulin (g/100 g protein)
F7-30	CS036†	28.95 ± 0.32	0.72 ± 0.1	40.67 ± 2.49	27.77 ± 0	**9.98 ± 0.18**
	CS117	29.11 ± 0.01	0 ± 0	24.36 ± 1.65	22.34 ± 0.55	**7.34 ± 0.55**
	CWS_F7_30_1	29.37 ± 0	0 ± 0	48.21 ± 0.27	26.49 ± 0.18	6.76 ± 0
	CWS_F7_30_11[a]	26.38 ± 0.32	**1.79 ± 0.6**	52.54 ± 0.61	**32.74** ± 0	6.44 ± 0.3
	CWS_F7_30_11[b]	29.54 ± 0.33	0 ± 0.00	46.86 ± 5.41	**31.75 ± 0.72**	4.96 ± 0.3
	CWS_F7_30_12	**32.35 ± 0.01**	**2.04 ± 0.98**	49.36 ± 0.25	26.61 ± 0.16	6.83 ± 0.16
	CWS_F7_30_2	**31.48 ± 0.32**	0 ± 0	35.95 ± 2.79	**31.63 ± 0.5**1	**9.11 ± 0.59**
	CWS_F7_30_3	**32.14 ± 0.66**	0 ± 0	**67.03 ± 1.1**2	24.79 ± 1	6.53 ± 0.49
	CWS_F7_30_7[a]	29.56 ± 0.32	0 ± 0	**58.85 ± 0.27**	25.1 ± 0.36	4.79 ± 0
	CWS_F7_30_8	30.34 ± 0.33	0 ± 0	55.87 ± 3.17	26.17 ± 0.88	4.12 ± 0.09
	CWS_F7_30_9[a]	30.05 ± 0.32	0 ± 0	53.22 ± 4.78	22.95 ± 0	5.63 ± 0.47
	CWS_F7_30_9[b]	30.57 ± 0.34	**2.22 ± 1.13**	55.69 ± 0	28.62 ± 0.17	5.62 ± 0.17
	3rd Quartile	30.92	0.99	55.92	29.37	7.27
	p. value	3.198e-08	2e-16	1.38e-07	8.91e-10	3.89e-09
F8-38	CS098†	31.25 ± 0.64	0 ± 0	51.67 ± 3.07	21.89 ± 0	4.24 ± 0.09
	CS133	30.72 ± 0.01	**1.05 ± 0.35**	**69.46 ± 0.17**	24.11 ± 0.87	3.79 ± 0.3
	CWS_F8_38_17	29.33 ± 0.33	**3.84 ± 0.81**	**61.07 ± 0.54**	15.3 ± 0.36	6.11 ± 0.73
	CWS_F8_38_36	29.45 ± 0	0.84 ± 0	56.94 ± 1.36	22.84 ± 1.27	3.45 ± 0.12
	CWS_F8_38_37	31.34 ± 0.32	0 ± 0	44.32 ± 0.51	19.06 ± 0.51	5.92 ± 0.43
	CWS_F8_38_45	30.82 ± 0.31	0 ± 0	57.54 ± 0.52	**25.13 ± 1.39**	6.08 ± 0.17
	CWS_F8_38_46	30.08 ± 0.01	0 ± 0	55.26 ± 0.27	16.81 ± 0.18	6.58 ± 0.36
	CWS_F8_38_48	**35.86 ± 0.32**	**1.89 ± 0.07**	29.99 ± 1.56	22.94 ± 0.15	**11.47 ± 0.07**
	CWS_F8_38_50	**31.69 ± 0.32**	0 ± 0.0	54.71 ± 1.77	21.59 ± 0	5.37 ± 0.08
	CWS_F8_38_56	30.36 ± 0.01	0 ± 0.00	54.42 ± 0.79	15.42 ± 0.18	5.29 ± 0
	CWS_F8_38_6	**32.31 ± 0.62**	0.59 ± 0.25	50.82 ± 0.25	**26.76 ± 0.33**	6.49 ± 0
	CWS_F8_38_7	28.92 ± 0	0 ± 0.0	57.14 ± 0.83	**35.36 ± 1.11**	5.81 ± 0.18
	CWS_F8_38_9	30.89 ± 0.01	0 ± 0	**73.34 ± 1.03**	16.48 ± 0.34	6.47 ± 0.09
	3rd Quartile	31.54	0.84	57.91	24.04	6.86
	p. value	1.97e-09	2e-16	8.018e-12	1.73e-11	3.42e-11
Dual purpose (Gold)	CS001	26.6 ± 0.33	0 ± 0	52.86 ± 0.3	25.59 ± 0.2	7.33 ± 0.2
	CS052	28.66 ± 0.01	0 ± 0	42.78 ± 2.79	29.96 ± 0.56	6.05 ± 0.28
	CS099	**28.72 ± 0**	**0.86 ± 0.1**8	41.66 ± 0	**30.14 ± 0.3**7	**10.67 ± 0.89**
	CS127	25.5 ± 0.02	0 ± 0	**59.08 ± 4.1**4	19.72 ± 0.42	4.53 ± 0.21
	3rd Quartile	28.68	0.84	57.91	24.0450	6.865
	p. value	9.78e-05	2e-16	8.018e-12	1.73e-11	3.42e-11

†= local parent; = donor parent from research, Values represent means ± standard deviation in triplicate, Rows Colored in green = parental varieties, values in bold = top three values upper to the third quartile.

there is evidence of positive control by dominant genes for albumins, globulins, and prolamins.

3.3. Amino Acid Profile of Cowpea Genotypes

The whole-seed amino acid compositions of the studied cowpea cultivars are displayed in Table 3 and Supplementary Table 1. The investigated genotypes exhibited variations in amino acid composition compared to other genotypes from other studies. However, like other studies [11,35,39], glutamine/glutamic acid, asparagine/aspartic acid, and phenylalanine + tyrosine were dominant with a secondary prevalence of arginine, leucine, and lysine. The high glutamine and asparagine are beneficial as they constitute cardinal reservoirs of amino for the body. Glutamine also serves as a primary fuel source for the intestinal tract and plays a crucial role in controlling glycogen synthesis and protein degradation [37]. The uniqueness and quality of protein in food mainly depend on its amino acid composition and the physiological utilization after digestion and absorption [11]. As for the progeny CWS_F8_38_48, CWS-F7-30-3 exhibited the highest levels of total amino acids (TAAs) (7.21 g/100 g DW), sulfur amino acids (methionine and cysteine with 0.25 and 0.12 g/100 DW, respectively), as well Phenylalanine and tyrosine.

Compared to the donor parent (CS117), the inbred lines of the F7-30 family are higher in all EAAs except the sulfur amino acid

Table 3. Amino acid composition in grams per 100 g of protein for the whole seeds of new recombinant inbred lines (RILs) from two families, dual-purpose cowpea genotypes, and hen egg protein.

Family	Varieties	Cyst	His	Thr	Val	Met	Ile	leu	Phe	Lys	met+cyst	phe+tyr
	CS117	**1.6**	4.03	7.94	6.00	2.70	4.56	9.29	6.72	18.97	**4.30**	11.32
	CWS-F7-30-1	0.79	4.92	9.07	6.84	3.02	5.45	10.41	7.75	22.82	3.81	13.11
	CWS-F7-30-11[a]	0.76	5.51	**9.96**	7.57	3.23	5.86	11.21	8.35	23.61	3.99	14.03
	CWS-F7-30-11[b]	0.57	4.13	7.51	5.47	1.98	4.32	8.73	6.50	18.74	2.56	10.68
	CWS-F7-30-12	0.61	**6.52**	**11.12**	**8.97**	**3.73**	**6.89**	**13.04**	**9.62**	**26.82**	**4.34**	**16.84**
F7-30	CWS-F7-30-2	0.79	**5.54**	9.90	7.37	2.74	**6.05**	11.54	8.73	**25.23**	3.53	**14.58**
	CWS-F7-30-3	**1.10**	**6.69**	**12.64**	**9.86**	**3.32**	**7.64**	**13.87**	**10.62**	**31.22**	**4.42**	**18.17**
	CWS-F7-30-7[a]	0.68	4.23	9.03	6.65	1.44	5.67	10.03	7.66	22.49	2.13	12.46
	CWS-F7-30-8	0.77	5.45	9.94	**7.82**	**3.32**	5.77	**11.58**	8.31	24.17	4.09	14.26
	CWS-F7-30-9[a]	0.63	3.96	8.22	5.83	1.16	4.80	9.19	6.73	20.53	1.79	11.60
	CWS-F7-30-9[b]	0.71	4.97	9.24	6.92	2.73	5.58	10.51	7.72	23.04	3.45	12.79
	CS098[▯]	1.48	5.18	9.35	7.41	2.97	5.74	10.64	7.87	22.54	4.44	13.56
	CS133[†]	1.14	5	**9.41**	7.4	**3.65**	5.66	10.64	7.91	22.22	**4.79**	13.72
	CWS-F8-38-17	1.63	4.07	5.8	6.63	2.92	5.45	10.06	7.67	17.51	4.55	12.9
	CWS-F8-38-36	0.51	4.67	8.83	6.39	2.05	5.15	9.75	7.28	22.05	2.56	12.62
	CWS-F8-38-37	0.62	4.63	9.01	6.92	1.87	5.17	10.6	7.75	21.96	2.49	13.17
	CWS-F8-38-45	0.73	**5.45**	**10.81**	**7.97**	2.13	**6.07**	**12.07**	**9.14**	**25.66**	2.86	**15.01**
F8_38	CWS-F8-38-46	**1.73**	4.95	9.34	7.09	**3.71**	5.18	10.26	7.36	20.08	**5.44**	12.87
	CWS-F8-38-48	**4.33**	**12.34**	**12.43**	**12.75**	**8.91**	**11.33**	**16.49**	**14.97**	**33.83**	**13.24**	**29.5**
	CWS-F8-38-50	0.41	3.55	7.17	5.05	1.63	3.91	7.91	5.69	17.53	2.04	9.78
	CWS-F8-38-56	1.04	4.71	6.52	7.04	2.72	5.68	11.03	8.22	20.06	3.75	13.53
	CWS-F8-38-6	**1.93**	**6.05**	7.71	**8.67**	2.47	**7**	**13.15**	**9.9**	**24.5**	4.4	**16.66**
	CWS-F8-38-7	0.97	4.48	8.96	6.73	2.78	5.16	9.64	7.01	19.3	3.74	11.96
	CWS-F8-38-9	1.55	4.12	4.83	5.01	2.36	4.45	7.17	5.82	13.78	3.91	10.57
	CS001	0.90	3.92	7.44	5.65	1.97	4.26	8.26	5.86	17.57	2.87	10.52
Dual purpose (Gold)	CS052	1.01	**4.55**	**8.86**	**6.99**	**3.03**	**5.34**	**9.59**	**7.09**	**21.11**	**4.04**	**12.76**
	CS099	**1.09**	4.34	8.39	6.43	2.83	4.81	9.32	6.69	19.26	3.92	11.90
	CS127	0.56	3.41	6.86	5.01	2.15	4.08	7.58	5.56	15.56	2.71	9.45
Ref. Protein	**Hen egg**	-	2.2	4.7	6.6	-	5.4	8.6	-	7.0	5.7	9.3

[†]= local Parent; [▯] = donor parent from research. Values are means of analyses in triplicate, Rows Colored in green represent **parental varieties**, and **values in bold**= top three values upper to the third quartile**. Cys**= cysteine, **his**= histidine, **thr**= threonine, **Val**=valine, **met**=methionine, **Ile**=isoleucine, **Leu**=leucine**, Phe**= phenylalanine, lys = lysine, **met+cyst** = methionine + cysteine, **phe+tyr** = phenylalanine + tyrosine, **Ref. Protein**= reference protein.

(methionine + cysteine) (Table 3). In the F8-38 family, the progenies occupied the first top three of the upper quartiles for all EAAs except for threonine, methionine, and methionine + cysteine in which they were in the same range with donor parent (CS133). The progenies of the two families exhibit extreme levels (high or low) in both directions and some similarities of EAAs compared to their parental levels. This may indicate that the later generations (F7 and F8) persistently exhibit transgressive segregation to some extent over their parents and inheritance.

Methionine and cysteine were the least abundant amino acids. The low levels of methionine and cysteine in combination with high levels of lysine can be complemented by including them in cereal preparations. This can help to obtain complete protein with all EAAs, as cereals are deficient in lysine but have excess methionine [39–42]. Except for methionine + cysteine, valine, and isoleucine, all investigated genotypes exhibit higher EAA content than hen egg reference protein [43].

3.4. Evaluation of Protein and Nutritional Quality of Cowpea Genotypes Based on Their Amino Acid Composition

The results of some parameters of protein quality and nutritional value of the studied genotypes are shown in Table 4.

The nutritional value of protein depends primarily on its capacity to satisfy the needs for nitrogen and EAAs [35]. The body cannot produce EAAs, so they must be obtained through the diet [39]. One of the methods of assessing protein quality is the CS. It is derived by comparing the EAA content of the test protein to that of a reference quality standard protein, such as a hen egg with a biological value of 100 [43]. Analysis of this result revealed that the first limiting amino acids were methionine + cysteine, followed by isoleucine and valine (Table 4). The new lines CWS-F7-38-48 and CWS-F8-38-56 did not present limitations in any of the EAAs. Even though there is a general limitation in sulfur amino acids and a secondary limitation of isoleucine and valine for some of the genotypes, apart from CWS-F7-30-9a, CWS-F7-30-7a, and

Table 4. Estimation of protein quality of the whole seeds from new RILs of two families and dual-purpose (Gold) cowpea genotypes.

Family	Varieties	EAA (g/100 g protein)	NEAA (g/100 g de protein)	EAA /NEAA	P-PER	NPV	Limiting AAS		
							1st	2nd	3rd
F7-30	CS117	60.21	76.04	0.79	3.26	21.98	met+cyst	Ile	Val
	CWS-F7-30-1	70.28	85.91	0.82	3.69	19.65	met+cyst		
	CWS-F7-30-11[a]	75.30	92.85	0.81	4.03	18.45	met+cyst		
	CWS-F7-30-11[b]	57.37	71.54	0.80	3.05	13.25	met+cyst	Ile	Val
	CWS-F7-30-12	**86.71**	**108.54**	0.80	**4.69**	**24.63**	met+cyst		
	CWS-F7-30-2	**77.10**	93.80	**0.82**	4.16	**19.49**	met+cyst		
	CWS-F7-30-3	**95.86**	**115.24**	**0.83**	**5.04**	**24.94**	met+cyst		
	CWS-F7-30-7[a]	67.21	82.15	0.82	3.58	11.03	met+cyst		
	CWS-F7-30-8	76.36	**97.40**	0.78	**4.17**	21.74	met+cyst		
	CWS-F7-30-9[a]	60.42	76.59	0.79	3.19	9.46	met+cyst	Ile	Val
	CWS-F7-30-9[b]	70.72	87.02	0.81	3.77	18.50	met+cyst		
F8-38	CS098	71.70	93.33	0.77	3.77	24.36	met+cyst		
	CS133[†]	71.89	89.34	0.80	3.75	**25.80**	met+cyst		
	CWS-F8-38-17	60.11	70.69	**0.85**	3.55	23.39	met+cyst		
	CWS-F8-38-36	66.15	82.60	0.80	3.39	13.20	met+cyst	Ile	Val
	CWS-F8-38-37	67.91	95.09	0.71	3.77	13.67	met+cyst	Ile	
	CWS-F8-38-45	**79.31**	**108.64**	0.73	**4.39**	15.44	met+cyst		
	CWS-F8-38-46	67.97	90.08	0.75	3.61	**28.70**	met+cyst	Ile	
	CWS-F8-38-48	**123.06**	**135.50**	**0.91**	**5.49**	**68.78**			
	CWS-F8-38-50	52.45	66.32	0.79	2.69	11.33	met+cyst	Ile	Val
	CWS-F8-38-56	65.98	84.56	0.78	3.98	20.00			
	CWS-F8-38-6	**79.45**	**102.71**	0.77	**4.79**	24.93	met+cyst		
	CWS-F8-38-7	64.06	82.27	0.78	3.39	18.99	met+cyst	Ile	
	CWS-F8-38-9	47.53	60.50	0.79	2.29	21.17	met+cyst	Ile	Val
Dual purpose (Gold)	CS001	54.93	71.40	0.77	2.79	13.40	met+cyst	Ile	Val
	CS052	**66.56**	**81.92**	**0.81**	**3.29**	**20.32**	met+cyst	Ile	Val
	CS099	62.07	76.30	**0.81**	3.22	19.75	met+cyst	Ile	Val
	CS127	50.20	63.67	0.79	2.56	12.13	met+cyst	Ile	-

[†]= local Parent; = donor parent from research, Rows colored in green represent parental varieties, values in bold= **top three values upper to the third quartile**. **Ile** =isoleucine, **met+cyst** = methionine + cysteine, **val** = valine, **NPV** = net protein value, **P-PER** = Protein efficiency ratio, **EAA**= Total essential amino acid, **NEAA**= Total nonessential amino acids.

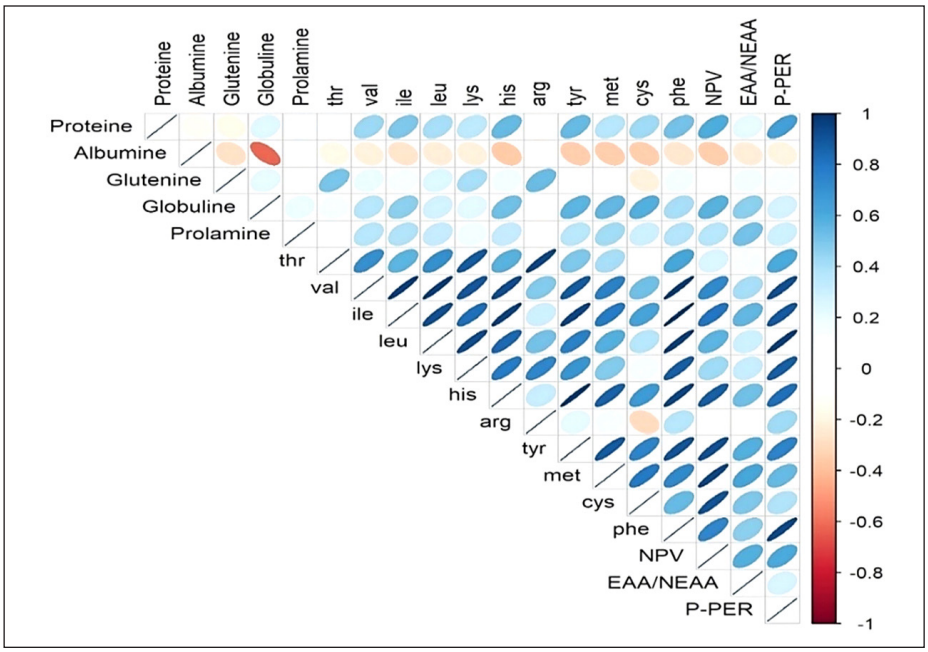
CWS-F8-38-50, all of the investigated genotypes in this study meet the FAO/WHO/UNU [43] adult and 2–5-year-old children's requirements for all EAAs.

The progeny resulting from the cross between F7-30 and F8-38 displayed enhanced biological values and protein quality across all parameters tested. They exhibited high EAAs and a high ratio of EAAs to non EAAs, resulting in an increased protein efficiency ratio and NPV. However, the offspring in the F8-38 family performed similarly to the donor parent (CS133) for NPV. The new line CWS-F7-38-48 had the highest NPV (68.78) and P-PER (5.49). The mean NPV of the F7-30 family (18.46) and dual-purpose genotypes (16.4) were close to the 17.62 reported byElhardallou *et al*. [11]. The mean P-PER value of nine out of twenty-nine genotypes investigated was higher than 3.9 for the hen egg. About 93% and all of the genotypes had P-PER higher than that of milk and soy protein with, respectively, 2.5 and 2.2. Any PER value that exceeds 2.7 is considered to be an excellent protein source [44]. Henceforth, these genotypes with a mean P-PER of 3.68 are excellent protein sources.

3.5. Association of Biochemical Traits

The relationships between the nutritional parameters were assessed using Pearson's correlation test and presented in Figure 1. It can be inferred that a highly significant positive correlation was found between protein and NPV ($r = 0.6$, $p < 0.001$) as well as P-PER ($r = 0.65$, $p < 0.001$). This result indicates that cultivars with high protein content tend to have higher NPV and P-PER. The total protein was positively and significatively associated with all EAAs except for threonine and arginine. There was a negative correlation between albumin and globulin content ($r = -0.62$, $p < 0.001$). This result indicates that cultivars with high albumin content usually have lower levels of globulin content.

This may explain the higher albumin levels and lower globulin content exhibited by these genotypes compared to those reported by other studies [34,36]. The negative relationship may result from competition on the absorption site. Robson and Pitman [45] reported that these traits share similar chemical properties and, therefore, compete for absorption, transport, and function in plant tissues. There are positive correlations between globulin and methionine, cysteine,



Cys= cysteine, **his**= histidine, **thr**= threonine, **Val**=valine, **met**=methionine, **Ile**=isoleucine, **Leu**=leucine, **Phe**= phenylalanine, lys = lysine, **met+cyst** = methionine + cysteine, **phe+tyr** = phenylalanine + tyrosine, r > 0 represented by blue color = positive correlation, r < 0 colored in red = negative correlation. The intensity of the color indicates the strength of the correlation at p < 0.05, **NPV** = net protein value, **P-PER** = Protein Efficiency ratio, **EAA**= Total essential amino acid, **NEAA**= Total non-essential amino acids

Figure 2. Pair-wise correlation for biochemical traits of 29 cowpeas inbreed lines donor and receiving local parents and dual-purpose genotypes.

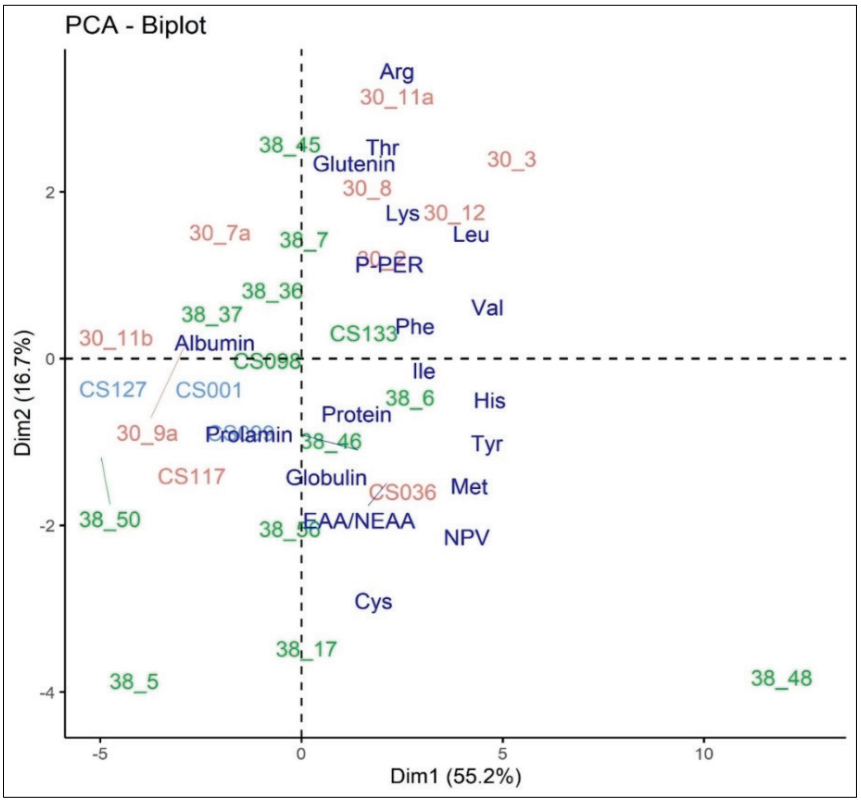
tyrosine, histidine, NPV, and EA/NEA. Glutelin shows moderate positive correlation with threonine (r = 0.51, p < 0.01) and arginine (r = 0.53, p < 0.001). Valine shows a strong positive correlation with P-PER (r = 0.91, p < 0.001). Jankowski *et al.* [46] suggest that correlated traits can be simultaneously selected in breeding programs. Breeding for high crude protein content will hence result in increased concentrations of histidine, tyrosine, phenylalanine, NPV, cysteine, and PER. The result also suggested a possible simultaneous selection of globulin with high-sulfur amino acids.

3.6. Principal Component Analysis

The PCA analysis shows that the first two PCs alone explained 71.9% of the variation among the studied cowpea genotypes for the investigated nutritional traits (Fig. 2). The first four vectors had eigenvalues higher than one with a cumulative variance of 86.56%, indicating significant variability among the cowpea genotypes evaluated for protein, protein fractions, amino acids, and protein quality. The PC 1, explaining 55.2% of the variation among genotypes, is mainly contrasted by the effect of high positive loading of valine, leucine, isoleucine, lysine, and histidine. Glutenin, threonine, and arginine made significant contributions with high positive loadings in the PC2 (16.17). Cysteine and NPV contributed to negative loading. The genotypes at the top right quadrant are characterized by high arginine, threonine, and glutenin levels. This quadrant mainly comprises offspring of the F7_30 Family (Fig. 3).

3.7. Hierarchical Clustering Analysis

HCA employed to evaluate the multivariate association between biochemical characteristics grouped the 29 genotypes into three distinct and divergent clusters as illustrated by Figure 4. Cluster I contains 21 (72.4%) accessions characterized by a high level of arginine (0.60 g/100 dw) (Fig. 4 and Supplementary Table 2) reported to boost the immune system and antioxidant activity [47], plays a crucial role in cardiovascular diseases and together with lysine have moderating effect on hypercholesterolemia [48]. The genotypes of this cluster are also reached in glutelin (26.83 g per 100 g of protein) with health benefits through slowing starch digestibility in food. This reduces the glycemic index [49] involved in the occurrence or severity of chronic diseases such as diabetes [50]. The higher glutenin content exhibited by the cultivars of this cluster makes them suitable for bakery products due to their elastic property in dough formation [51] and can be used for bread and other bakery product fortification. Cluster II consisted of 7 (22%) genotypes characterized by low P-PER (3.11), valine (0.20), methionine (0.07), and glutelin (21.02 g/100 g protein). The third cluster consists of 1 (3.4%) genotype characterized by the highest concentrations of methionine (0.25 g/100 g), protein (35.86), and globulin (11.47 g/ 100g protein) with high P-PER (5. 49) and NPV (68.78) but low albumin. This high protein content makes them suitable for protein supplements, making protein isolates, and beneficial for combating high protein-energy malnutrition. It could also be used as a source for designing high-protein and palatable food [3]. Consuming 100 g of this progeny could cover 71.72% of the WHO daily recommendation (0.83 g/kg) in protein for a 60 kg adult. However, it is necessary to point out that 5.0%–37.0% of the total protein in cowpeas (mainly globulins) has been reported to be nutritionally unavailable [7]. The high percentage ratio (47.59%) of an EAA to the TAAs was much above the 39% considered to be adequate for ideal protein food for infants, 26% for children and 11% for adults FAO/WHO/UNU [43]. This percentage ratio was very close to that of eggs (50%). The genotype may also be a potential parental candidate for the breeding program due to its



Cys= cysteine, **his**= histidine, **thr**= threonine, **Val**=valine, **met**=methionine, **Ile**=isoleucine, **Leu**=leucine**, Phe**= phenylalanine, **lys** = lysine, **Arg**= arginine, **Tyr**=tyrosine, **NPV** = net protein value, **P-PER** = Protein Efficiency ratio, **EAA**= Total essential amino acid, **NEAA**= Total non-essential amino acids

Figure 3. PCA biplot indicating the distribution of nutritional traits in 29 samples based on their protein, protein fractions, EAAs content, and protein quality.

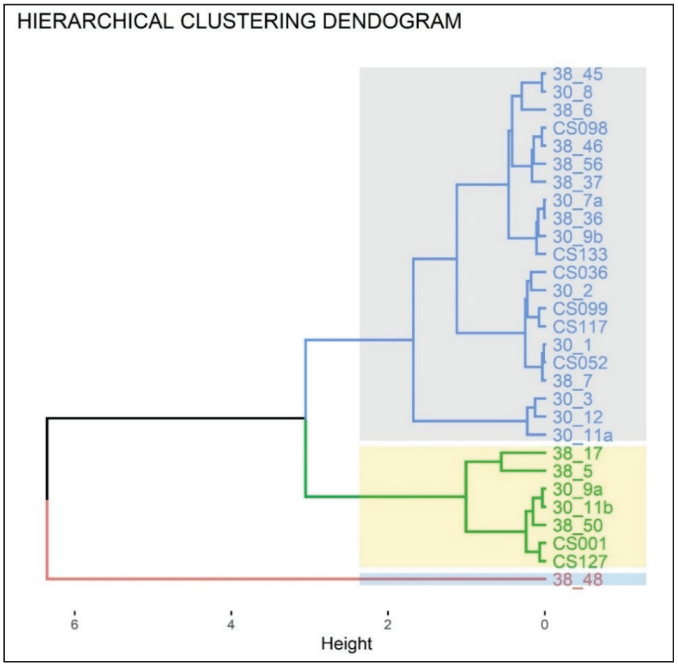


Figure 4. Dendrogram based on Ward's method depicting the genetic relationship among 29 cowpea genotypes using seed EAAs, protein, protein fractions contents, and protein quality.

high sulfur amino acid content enhancing methionine and cysteine levels and at the same time mineral content since it was reported that cysteine has positive effects on mineral absorption in staple foods [52]. The offspring CWS-F8-38-46 and CWS-F7-30-2 were very similar to their donor parent (CS098) and local receiving parents (CS036) contrary to the offspring CWS-F8-38-48, CWS-F8-38-50, and CWS-F7-30-11b which exhibited dissimilarities with their parents.

4. CONCLUSION

The investigated genotypes had a mean P-PER of 3.68, making them excellent protein sources. The offspring CWS-F7-30-12, CWS-F7-30-2, CWS-F7-30-3, CWS-F8-38-45, CWS-F8-38-46, CWS-F8-38-48, and CWS-F8-38-6 were identified as nutri-dense genotypes with high protein (>30%), high EAA and EAA to NEAA ratio contents, high P-PER, and NPV. These are promising traits to provide practical support in developing high-value cowpea populations using effective breeding strategies with a higher economic and social value.

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6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

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12. SUPPLEMENTARY MATERIAL:

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