UMYU Scientifica, Vol. 2 NO. 2, June 2023, Pp 007 – 015
https://doi.org/10.56919/usci.2223.002
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Phenotypic and Protein Variations among Selected Cowpea (*Vigna unguiculata L. Walp.*) Varieties.

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ARTICLE HISTORY

Received March 7, 2023
Accepted May 28, 2023
Published June 22, 2023

KEYWORDS

cowpea, diversity, morphology, variation, *Vigna*

ABSTRACT

The study seeks to evaluate the level of genetic diversity among selected cowpea varieties using phenotypic traits and seed storage proteins. Twenty cowpea varieties were used in the study. They were planted and phenotypically characterised. Seed proteins were evaluated at maturity. A high phenotypic variation was observed among the selected cowpea with the evaluated phenotypic traits, while a low level of variation was observed with protein evaluation. The variation captured by the phenotype (>90%) was higher as compared to the protein (< 30%) analysis in the study. Regardless of whether the phenotype or protein analysis was used, three major clusters were generated, with most germplasms in cluster III. While the variation observed within the phenotypic traits might have been due to environmental influences, the low variation exhibited using seed storage proteins implies a high level of similarity among cowpea samples. Hence, a more stable marker type should be explored to identify the true level of genetic diversity within cowpea germplasm.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is a staple food and forage legume in many underdeveloped nations. It offers food and forage for animals and improves soil fertility by fixing nitrogen in the soil via its root nodules. It performs well in harsh conditions, making it a popular crop among rural farmers for cash generation (Amusa *et al.*, 2022). Although the effects of global climate change have hampered the progressiveness of cowpea production, this is mostly due to the small gene base commonly found among cowpea varieties due to their inbreeding character (Ajetobi and Abiodun, 2010; Boukar *et al.*, 2020).

Cowpea cultivars have relatively limited genetic diversity owing to their inbreeding reproductive tendency. As a result, adding new accessions to crop breeding programmes is essential for increasing genetic diversity (Nogueira *et al.*, 2021). Cowpea genetic progress can be hastened when extensive genetic variety and information on these genetic resources are available. Priority should be given to collecting these genetic resources and studying genetic diversity within and across landraces for varietal development (Sadia *et al.*, 2009).

The importance of genetic diversity's role in any breeding program's success cannot be overemphasised. It promotes the efficient use of available variations incorporation in breeding programmes through diverse selection processes depending on the goal of the crop improvement (Mafakheri *et al.*, 2017). Therefore, the genetic variation within the germplasm needs to be periodically checked, especially for a crop like cowpea with a narrow genetic base. Finding outstanding genotypes among the available germplasm is a fantastic method to launch a breeding programme and raise the productivity and output of this crop. For an improvement endeavour to be successful, genetic diversity is crucial. Variability is influenced by genetic factors, edaphic and climatic factors, and environmental influences. The variability of a quantitative property that is handed on to the following generation is known as heritability. It gives a meaningful biometrical estimate and has been used to test the effectiveness of selection since it helps separate heritable and environmental components from the total variation (Gupta *et al.*, 2019).

In estimating genetic diversity, phenotypic (both quantitative and qualitative) markers are useful (Rajab *et al.*, 2021; Sadia *et al.*, 2009). Although phenotypic markers are susceptible to phenotypic plasticity due to environmental influences (Rajab *et al.*, 2021), they allow for the evaluation of variety in the presence of environmental variables that cannot be accounted for

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How to cite: Amusa O. D. and Igbari A. D.. (2023). Phenotypic and Protein Variations among Selected Cowpea (*Vigna unguiculata L. Walp.*) Varieties. UMYU Scientifica, 2(2), 007 – 015. https://doi.org/10.56919/usci.2223.002

when genotypic variation is included. As a result, it is sometimes augmented with biochemical markers (seed storage proteins) in assessing the amount of diversity across examined crop species such as cowpea (Luo *et al.*, 2004; Rajab *et al.*, 2021).

Protein characterisation and selecting suitable lines/genotypes are critical for breeders. Seed storage protein electrophoresis is a technique used to examine genetic diversity and categorise plant types. Because seed protein is not affected by environmental variations and banding patterns, it is relatively stable and may be used to identify cultivars (Choudhary *et al.*, 2015). Hence, this study aimed to examine the genetic variability among selected cowpea accessions using phenotypic and protein variants.

MATERIALS AND METHODS

Collection and Planting of Sampled varieties

Twenty cowpea samples used in the study (Table 1) were planted in a screen house at the Botanical Garden (Latitude 6° 30′ 52′′ N, Longitude 3° 25′ 56′′ E), University of Lagos, Nigeria. Accessions were planted with three seeds per hole (in a 2 kg pot filled with soil) and three replicates per accession, laid in a complete randomised design setup. Normal agronomic practices, which include watering and weeding when necessary, were performed throughout the experiment.

Phenotypic evaluation of sampled genotypes

Cowpea Descriptors of the International Board for Plant Genetic Resource (IBPGR, 1983) were used to assess the study's phenotypic traits. Eight quantitative and six qualitative traits, which include Plant habit (PHb), Plant height (PHt), Terminal leaflet shape (TLS), Terminal leaflet length (TLL), Terminal leaflet width (TLW), Terminal leaflet length/width ratio (TLLWR), Leaf petiole length (LPL), Terminal leaflet petiole length (TLPL), Leaf rachis length, Terminal leaflet base shape (TLBS), Terminal leaflet top shape (TLTs), Pigmentation (PIG), Number of branches per plant (NBP), Hairiness of Plant (HPt), were evaluated among sampled accessions.

Extraction of seed proteins and Polyacrylamide gel electrophoresis profiling of seed proteins

Seed protein profiling was done with modifications (Sonker et al., 2018). Ten seeds for each accession evaluated were first dehulled and then grounded into a fine powder in a mortar and pestle. This was then mixed with 500 µL of extraction buffer (0.5 M Tris HCl pH 8.0, containing 0.2% SDS, 5 M Urea and 1% β-mercaptoethanol) by vortexing for 10 secs and centrifuge at 15,000 rpm for 5 mins. The supernatants of the samples were transferred into new Eppendorf tubes.

A total of 20 µL of the supernatant was mixed with 5 µL bromophenol blue dye (0.05% w/v) and placed in a polyacrylamide gel electrophoretic setup comprising 12.5% resolving gel (6.0 mL 40% Acrylamide, 3.7 mL 1.5 M Tris pH 8.3, 200 µL 10% Ammonium persulphate (APS), 10 µL tetramethylene diamine (TEMED), 5.3 mL ddH_2O) and 4% stacking gel (0.9 mL 40% Acrylamide, 1.3 mL 0.5 M Tris pH 6.3, 80 µL 10% APS, 5 µL TEMED, 3.0 ddH_2O). The setup was run at 150 V for 2 h. The resulting gel was then stained in silver nitrate before visualisation. Protein bands were scored presence (1) and absence (0) of bands.

Table 1: List of sampled cowpea varieties used in the study

SN	ID	Source
1	BBR	IAR7T
2	Ife-brown	IAR&T
3	Drum	Market
4	Oloyin	Market
5	White beans	Market
6	TVu-11883	IITA
7	TVu-11952	IITA
8	TVu-11953	IITA
9	TVu-145	IITA
10	TVu-2027	IITA
11	TVu-5333	IITA
12	TVu-845	IITA
13	TVu-13076	IITA
14	TVu-14085	IITA
15	TVu-12277	IITA
16	TVu-125113	IITA
17	TVu-13868	IITA
18	TVu-100	IITA
19	TVu-293	IITA
20	TVu-3629	IITA

IAR&T: Institute of Agricultural Research and Training, Ibadan, Nigeria; IITA: International Institute for Tropical Agriculture, Ibadan, Nigeria; Market: Bodija market, Ibadan, Nigeria

Analysis of data

Descriptive statistics were conducted on the phenotypic data generated using RStudio software (2022). The mean of sample replicates and matrixes generated for protein data was used to develop a dendrogram. Genetic similarity among accessions was estimated based on Euclidian distance coefficients. Principal components and Cluster analysis were performed using the

unweighted pair group method based on arithmetic averages (UPGMA) using RStudio software (2022).

RESULTS

Phenotypic variations among sampled genotypes

Phenotypic analysis of sample accessions revealed varied levels of variability among traits used (Table 2). Plant habits gave the highest variation (CoV = 46.72%), and the Terminal leaflet length/width ratio gave the least variation (CoV = 16.84%) among traits used in the study. Variations were observed in qualitative traits except for pigmentation and Terminal leaflet shape among sample traits. Both traits, pigmentation and Terminal leaflet shape, were monotonous.

Table 2: Phenotypic variations observed within traits evaluated

Traits	Min	Max	Mean	SEM	CoV (%)
Quantitative					
PHt (cm)	10.00	147.00	45.54	2.22	46.72
TLL (cm)	4.86	11.40	8.93	0.17	17.97
TLW (cm)	2.40	7.80	5.04	0.11	20.22
TLLWR	1.02	2.75	1.86	0.03	16.84
LPL	0.20	0.52	0.36	0.01	22.37
TLPL (cm)	2.90	11.50	8.63	0.22	24.10
LRL (cm)	3.30	11.80	9.02	0.22	23.15
NBP	2	11	4.94	0.19	37.20
Qualitative					
PHb	Erect (n = 14)	Sub-erect 2 (n = 6)			
TLS	Deltoid (n = 20)				
TLBS	Truncate (n = 16)	Aequilateral (n = 4)			
TLTS	Aristate (n = 11)	Acuminate (n = 9)			
PIG	None (n = 20)				
HPt	Not hairy (n = 19)	Hairy (n = 1)			

Stdev: standard deviation; PHt: Plant height, TLL: Terminal leaflet length, TLW: Terminal leaflet width, TLLWR: Terminal leaflet length/width ratio, LPL: Leaf petiole length, TLPL: Terminal leaflet petiole length, LRL: Leaf rachis length, NBP: Number of branches per plant, PHb: Plant habit, TLS: Terminal leaflet shape, TLBS: Terminal leaflet base shape, TLTS: Terminal leaflet top shape, PIG: Pigmentation, HPt: Hairiness of Plant

Clustering of accessions based on phenotypic traits

Clustering analysis using phenotypic traits (Figure 1) of sampled cowpea accessions revealed a dendrogram with three clusters and an outgroup (IT84S-2264-4). The first cluster had two accessions (TVu-100 and TVu-145). While Cluster II had eight accessions subclustered into two groups. The subcluster IIa contained only two accessions, TVu-11883 and TVu-293, while the second subcluster, IIb, contained six accessions (TVu-13868, Oloyin, white, TVu-5333, Ife-brown, and TVu-3629). Similarly, the third cluster contained the remaining nine accessions, subclustered into two groups. Subcluster IIIa comprises four accessions while the second subcluster IIIb is composed of five accessions accordingly.

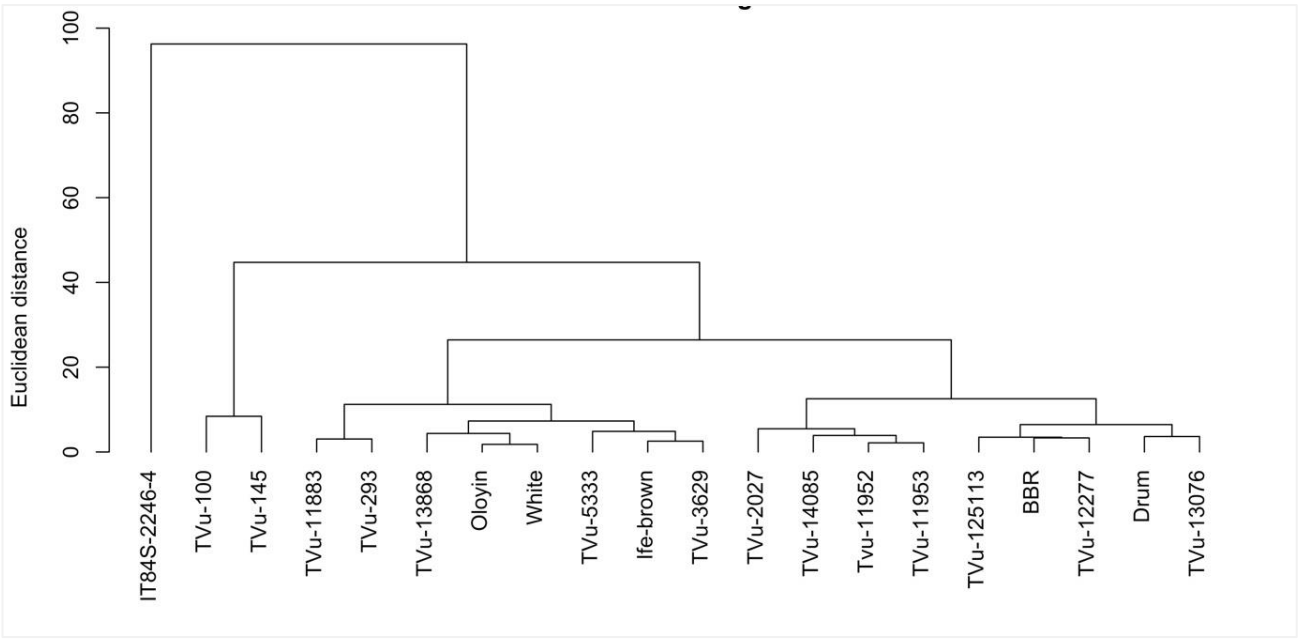

Figure 1: Dendrogram of selected accessions using phenotypic traits

The principal component analysis revealed a PC1 and PC2 with 98.15% total variability observed among accessions evaluated (Figure 2). The first principal component accounted for 96.01% of the variability observed among the evaluated accessions, while the second axis explained 2.14%. Similar to the dendrogram, IT84S-2246-4 stood out as an outgroup and two major clusters were observed.

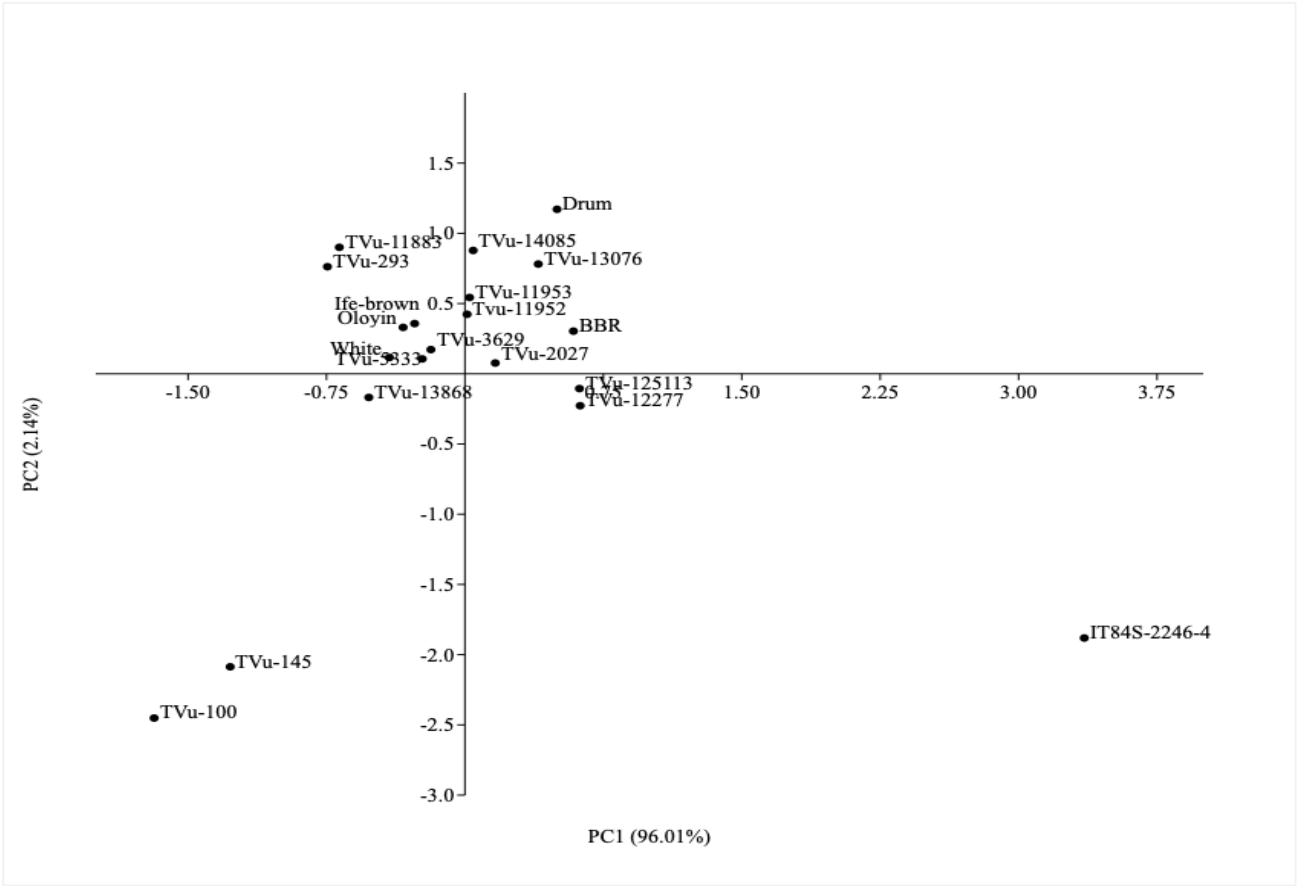

Figure 2: Principal component analysis of selected accessions using phenotypic traits

Clustering of accessions based on protein fractions

The present study detected 64 bands ranging from 21 – 169 kDa among the twenty varieties evacuated. Clustering analysis using seed storage protein fractions (Figure 3) showed four major clusters and TVu-12277 as an outgroup. The first cluster had three accessions, TVu-11883, white and BBR. The second cluster had seven accessions, including TVu-11883, TVu-2027, TVu-125113, TVu-3639, IT84S-2246-4, and TVu-13388. The third accession had Drum, TVu-100, TVu-11952, TVu-13078, TVu-145, Ife brown and TVu-14085. The fourth cluster contained only two accessions, oloyin and TVu-145.

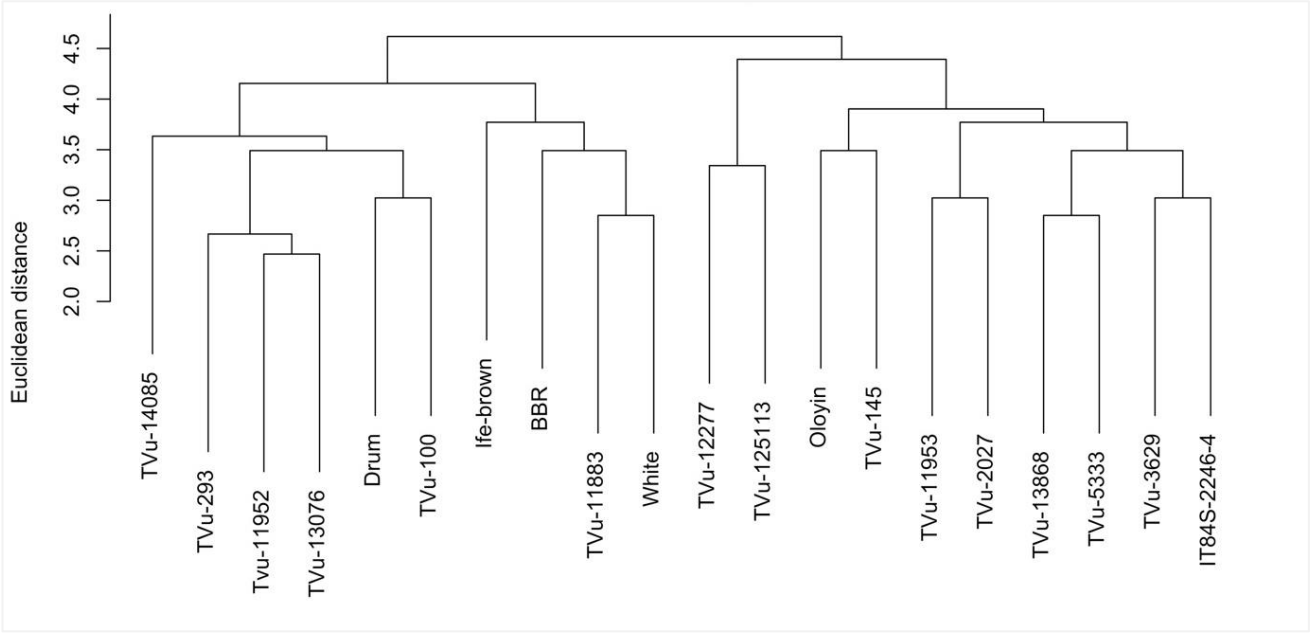

Figure 3: Dendrogram of protein analysis among the genotypes

The principal component analysis only captured 25.56% with the first two principal components (Figure 4). While the first principal component explained only 15.10% of the variability, the second principal component explained 10.46% of the variability observed among the sampled accessions using the seed storage protein fractions.

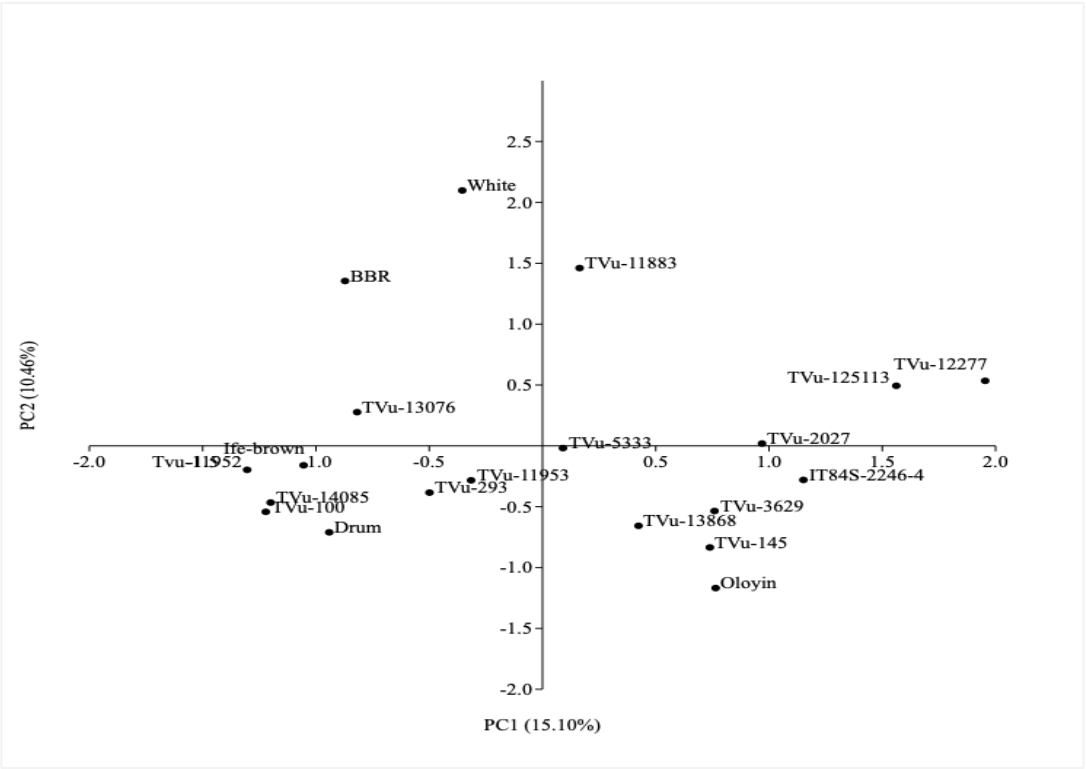

Figure 4: Principal component analysis of selected accessions using protein fractions

Clustering of accessions based on both phenotypic traits and protein fractions

When both phenotypic and protein data were combined, three major clusters and an outgroup (IT84S-2246-4) were observed (Figure 5). The first cluster contained only two accessions, TVu-100 and TVu-145. Both clusters II and III had two subclusters each. Subcluster IIa contained five accessions (White, Ife-brown, Oloyin, TVu-5333), while IIb contained three accessions (TVu-13868, TVu-11383 and TVu-293). Subcluster IIIa consist of four accessions (TVu-2027, TVu-14085, TVu-11952, TVu-11953) while IIIb contained four accessions (Drum, TVu-13078, BBR, TVu-12277 and TVu-125113).

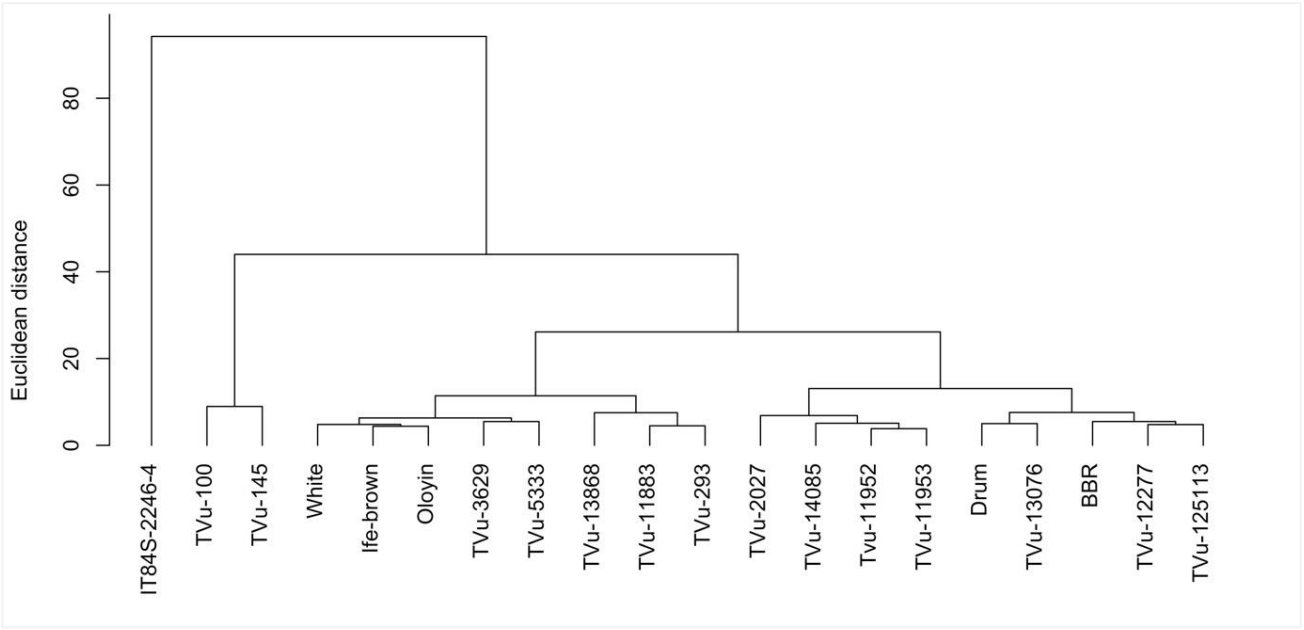

Figure 5: Dendrogram analysis of combined traits (i.e. phenotypic traits and protein fractions)

.The principal component analysis revealed a PC1 and PC2 with a 97.20% total variability observed among accessions evaluated (Figure 6). The first principal component accounted for 94.35% of the variability observed among the evaluated accessions, while the second axis explained 2.85% of the variability. Similar to the dendrogram, IT84S-2246-4 stood out as an outgroup and two major clusters were observed.

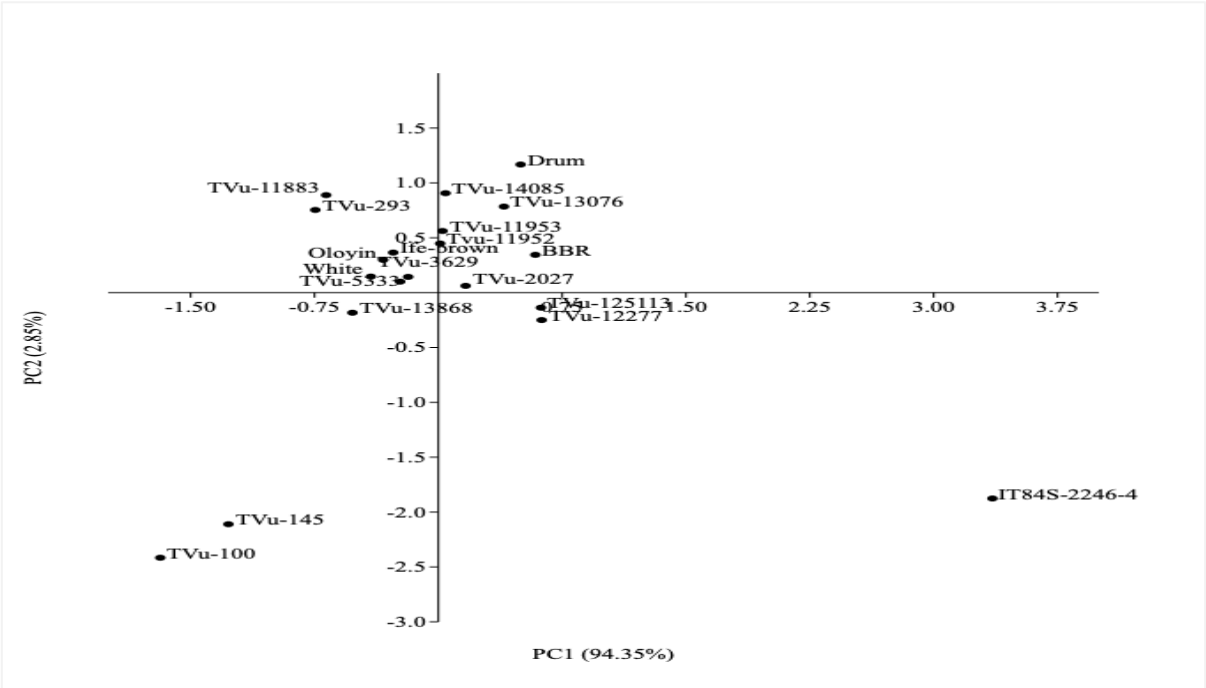

Figure 6: Principal component analysis of selected accessions using phenotypic traits and protein fractions.

DISCUSSION

Genetic diversity is crucial when it comes to improving the genetics of an inbreeder crop like cowpeas. The degree of genetic diversity can reflect the level of genetic progress for future breeding. Hence, the knowledge of available genetic diversity in germplasms can be used in supporting the appropriate selection of cross-combinations among large sets of parental genotypes will promote the effective utilisation of genetic variants in breeding programmes (Rajab *et al*., 2021).

In the present study, phenotypic traits were assessed among selected cowpea genotypes. A high variation was observed within these genotypes ranging from 16.84 – 46.72, signifying a high genetic variation harboured within these germplasms. This was also supported by the first two principal components accounting for up to 98.01% of the total variability observed among the samples used. This is similar to reports from Mafakheri *et al*., (2017), Nogueira *et al*., (2021) and Rajab *et al*., (2021). The variation observed was attributable to environmental influence since most phenotypic traits are susceptible to environmental change. Hence, they are more subjective than other measurements (protein) used in the study. However, using phenotypic traits to assess genetic variation is a standard way for many species because it is direct, inexpensive and easy (Odireleng, 2012). Phenotypic evaluation has been used for phenotypic variation studies in several crops, which includes wild cowpea (*Vigna unguiculata*) (Ogunkanmi *et al*., 2019), common bean (*Phaseolus vulgaris*) (Nogueira *et al*., 2021), urdbean (*Vigna mungo*) (Barik *et al*., 2021) to mention a few.

The effective utilisation of genetic variety within germplasm collections requires a thorough understanding of their properties. Plant breeders are keenly interested in phenotypic and agronomic features, typically used to characterise accessions. The study's findings supported the existence of significant phenotypic variations among the cowpea genotypes examined, providing an intriguing starting point for efforts to generate new and hybrid varieties of plants. However, Phenotypic traits alone cannot reveal the presence of genetic variation in crop populations due to phenotypic pleiotropism. There have been growing numbers of plant diversity studies using more stable markers to identify genetic variation in crop populations (Mafakheri *et al*., 2017). SDS-PAGE is one of the most used methods for separating and characterising proteins and determining the degree of genetic diversity because of its validity and simplicity in identifying the genetic structure of agricultural germplasm. Also, storage proteins are largely immune to environmental factors, so it is considered a trustworthy approach (Ullah *et al.* 2009). However, a low variation was observed using seed proteins, yielding the first two principal components accounting for 25.56% of the total variability observed, similar to Ullah *et al*., (2009) and Ghafoor & Arshad (2008). However, this present result does not corroborate the high diversity reported by Mafakheri *et al*., (2017) and Rajab *et al*., (2021). These authors reported a high variation among the cowpea genotypes evaluated. Although these studies utilised cowpea samples with diverse germplasm from different countries of origin as opposed to the Nigerian accessions used in this study, the inbreeding nature of the crop may also account for the low level of diversity among the samples used. Inbreeding increases homozygosity which invariably reduces genetic diversity (Gumede *et al*., 2022).

SDS-PAGE has been used for genotypic variation studies in several crops species which includes pea (*Pisum sativum*) (Shah et al., 2021), common bean (*Phaseolus vulgaris*) (Ayelign *et al*., 2020), soybean (*Glycine max*) (Malik *et al*., 2009), pumpkin (*Cucurbita maxima*) (Ikram *et al*., 2021) to mention a few. While the combination of both phenotypic and protein variations has also been used to assess genetic diversity among species which include common bean (*Phaseolus vulgaris*) (Nogueira *et al*., 2021) and cowpea (*V. unguiculata*) (Rajab *et al*., 2021).

Clustering genotypes based on similarity or dissimilarity is advantageous for cowpea breeders because it allows for selecting the most significant genotypes in the population for hybridisation in cowpea improvement programmes. Because of the strong contextual influence on the varied expression of the measured phenotypic traits, there is a discrepancy between the phenotypic and genotypic clusters, partially explained by this. The interaction between genotype, environment and genotype-by-environment effects determine the phenotypic performance (Rajab *et al*., 2021). The similarity of protein bands among genotypes revealed their close genetic connections. These findings corroborate Gupta *et al.* (2010), who claimed that SDS-PAGE was frequently employed to separate proteins directly connected to the genetic makeup of wild, cultivars, or recently descended cereal plants. Additionally, Bertozo and Valls (2001) found significant variation in seed storage proteins among various species; as a result, it is advised to employ a variety of species to boost the genetic diversity of the germplasm.

CONCLUSSION

The study revealed that the level of diversity within the selected cowpea varieties was high using their phenotypic traits but low using seed storage proteins. While the variation observed within the phenotypic traits might have been due to environmental influences, the low variation exhibited using seed storage proteins implies a high level of similarity among cowpea samples. Hence, a more stable marker type should be explored to identify the true level of genetic diversity within cowpea germplasm.

ACKNOWLEDGEMENT

The authors wish to thank Emmanuella Adaeze Emereuwa, Oreoluwa Ideraoluwa Olayiwola, Omotola

Victoria Osunkojo, Azeezat Adeola Adetola, and Fechi Daniella Ugbo from the Department of Cell Biology and Genetics who assisted with both the phenotyping and protein profiling of cowpea varieties used in the study. And Prof. L.A. Ogunkanmi, who made his screen house available for use during the study.

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