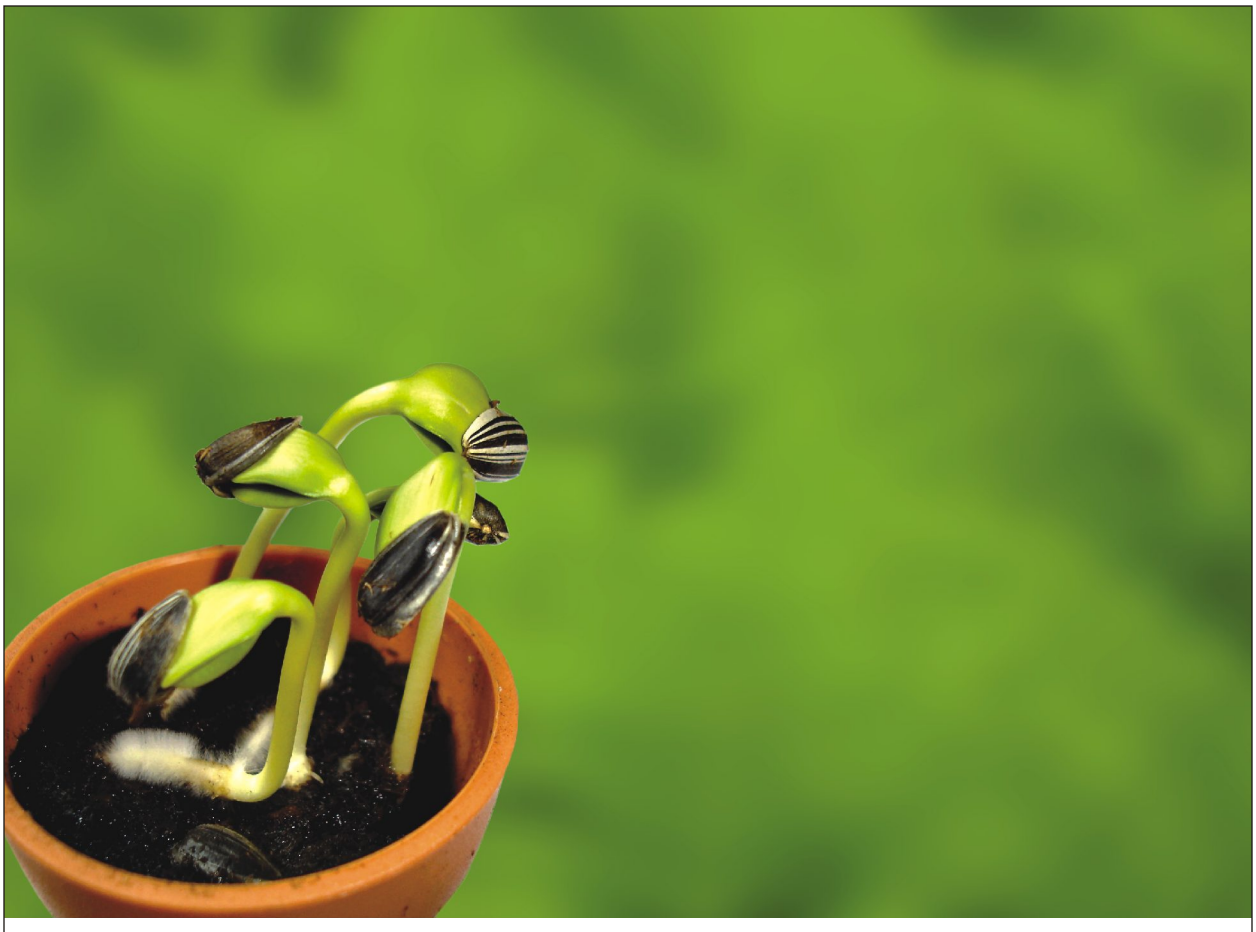

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Research Article
Protein-based Fingerprinting in Cowpea Accessions [*Vigna uniguiculata* (L.) Walp] for Deciphering Genetic Relatedness

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Abstract

Background and Objective: A clear understanding of the extent of genetic diversity in a germplasm is very critical for maximal exploitation and utilization of inherent resource for conservation and subsequent improvement. This paper was aimed at evaluating the diversity in cowpea accessions using seed storage protein profiling. **Materials and Methods:** The analysis of the seed storage proteins of cowpea was undertaken at the analytical bioscience laboratory of the International Institute of Tropical Agriculture (IITA) using 15 cowpea accessions obtained from the germplasm of International Institute of Tropical Agriculture (IITA), Ibadan in March, 2017. Seed storage protein was extracted and subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Distinct protein bands were scored for presence ("1") and absence ("0") and subjected to analyses using GenAlEx 6.5 and MSVP 3.21 software for genetic parameters. **Results:** SDS-PAGE analysis confirmed diversity in the seed storage proteins of cowpeas with two major clusters and sub-clusters based on population genetic distance differed according to location and this can be exploited to undertake hybridization of accessions TVu-4467 and/or TVu-8063 from Nigeria as well as TVu-12513 adopted from Zambia. Other genetic diversity parameters indicated the existence of genetic diversity in the 15 accessions. **Conclusion:** Taking the results together, the investigation confirmed it might be justifiable to carry out parental selection of cowpea accessions adopted from Nigeria, preferably TVu4467 and/or TVu8063 as well as TVu12513 adopted from Zambia for possible crossing to widen the genetic base of cowpea.

Key words: Cowpea, seed storage protein, SDS-PAGE, genetic diversity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The economic hardship orchestrated by economic recession as well as global climatic conditions have imposed serious stress on Nigerian families, especially in terms of food security and nutrition. However, the unfortunate angle to this is the fact that Nigerians are still wanting the imported food instead of exploring and exploiting the local ones, which interestingly might be better nutrition-wise. African families have varying preference to foreign cowpea varieties against local ones in the following order: Niger (52%), Burkina Faso (50%), Togo (46%), Lagos (36%) and Ghana (18-21%)[1]. This situation is unfortunate especially taking into cognizance the economic recession in Nigeria. For emphasis, most children in developing countries and the internally displaced people (IDPs) in camps are malnourished as a result of protein deficiency and as such the urgent need to utilize available but nutrient-rich food crop as cowpea.

Cowpea (*Vigna unguiculata* (L.) Walp) is a global legume and presently is the second most important legume in Africa[2-3]. Nigeria being the largest producer as well as consumer accounts for 61% of production in Africa and 58% worldwide[4]. Regrettably, this increase in cowpea production in sub-saharan Africa not withstanding, its yield has remained one of the lowest among all food legumes. The Food and Agriculture Organization (FAO) estimated average cowpea yield in West Africa to be 483 kg ha^{-1}, which was still 50% below the estimated potential production yield[5].

Plant breeding depends on the conservation and utilization of plant breeding resources for the purpose of improvement, which depends on clear understanding of the genetic variation and distinctiveness of the populations[6]. This underscores the importance attached to the analysis of genetic relationships in crop species[7]. Diversity in plant genetic resources (PGR) provides ample opportunity for plant breeders to develop new and improved cultivars with desirable characteristics[8]. Undoubtedly, various researchers have adopted different approaches in the bid to estimating genetic relatedness in a given germplasm with varying levels of informativeness and resolution power.

These includes principal component analysis, divergence analysis and utilization of morphological and agronomical data[7]. However, due to the laborious and time-consuming nature of these methods in drawing meaningful conclusions from generated data, molecular marker-based techniques have been explored and exploited by plant breeders. Molecular markers are highly heritable and polymorphic enough to enable the discrimination of closely related genotypes. Studies have affirmed that molecular genetic techniques using DNA polymorphism have been increasingly used to characterize and identify a novel germplasm for use in the crop breeding process[9]. Other markers systems such as biochemical markers that incorporates the analysis of seed storage proteins and isozymes is an inexpensive technique that measures population subdivision, genetic diversity, gene flow, genetic structure of species and comparisons among species out-crossing rates, population structure and population divergence[10].

Mature seeds of crops provide a very stable and convenient system for biochemical analysis for establishing relationship between parents and hybrids of plant species[11], resolving the taxonomy and evolutionary problems of several crop plants[12] and for selecting desirable genotypes for breeding as well as crop improvement[13]. The polymorphism of seed storage proteins has been widely applied in plant classification[14], screening of mutants for seed storage proteins[15,16], germplasm resource analysis[17,18], variety identification[19,20] as well as genetic diversity analysis[21,22].

Proteins are products of gene expression and their different sizes or biochemical characteristics are a function of the different alleles which encode different amino acid sequences[23]. Isozymes are useful in germplasm diversity analysis owing to its co-dominance nature[24], ability to reveal absence of epistatic and pleitropic effects, ease of use as well as cost effectiveness[10]. Important to note is the fact that storage proteins are direct and stable products of genes, which obviously reflect DNA diversity. This DNA diversity can thus be easily detected by various electrophoresis, especially SDS-PAGE. The evaluation of genetic variations using protein markers requires the electrophoresis of seed proteins using SDS-PAGE, which is a potent tool for application in plant breeding[11]. The electrophoretic patterns of the proteins provides insight into the polymorphisms present in the accessions and the level of relatedness.

There are over 15,000 accessions and 2000 wild relatives of cowpea held at the International Institute of Tropical Agriculture, (IITA) Genebank[25]. Regrettably, non-utilization of majority of the germplasm collections due to genetic redundancy, duplicated lines, little knowledge of their genetic characterization is hampering the efforts of conservation and improvement[26], especially with the incapacitation of most developing countries to sustainably feed its citizens. The implication is that these accessions need to be properly characterized, conserved and utilized for integration in breeding programmes. In this study, therefore, an attempt was made using protein-based profiling to generate information on 15 accessions of cowpeas from IITA germplasm with the following objectives:

- Screen the cowpea accessions collected from IITA for genetic diversity
- Identify/distinguish duplicated accessions from IITA
- Identify desirable accessions for crop improvement

MATERIALS AND METHODS

Experimental materials: This work was carried out in March-April, 2017. Fifteen cowpea accessions were used for this study which were obtained from the germplasm collection of the International Institute of Tropical Agriculture, IITA, Ibadan, Nigeria. Details of the experimental materials were presented in Table 1.

Protein extraction: Cowpea seeds coats were removed and seed storage proteins were extracted as described by Jha and Ohri[27]. After powdering, it was homogenized using 0.5 M Tris-HCl buffer in the ratio of 1:10 (w/v). The paste was put into Eppendorf tubes and centrifuged at 13,000×g for 10 min. The supernatant was collected as protein fractions and stored at -20°C for analysis.

Protein profiling using SDS-PAGE: During analysis, the extracted protein was mixed with sample loading buffer (0.5 M Tries [pH 6.8] 50% glycerol, 10% SDS, 2-β mercaptoethanol and bromophenol blue [*In vitro* gen™ pre-stained marker) in the ratio of 1:5, denatured at 95°C, cooled on ice and loaded on SDS-PAGE for separation[28].

Constant current was applied while varying the voltage initially 100 V and later increased to 150 V. The resolving gel (30:0.8% acrylamide/Bis, 1.5 M Tries buffer (pH 8.8), 10% SDS, APS and TEMED concentrations was 12 and 15% while the stacking gel concentration was 5%. The staining gel was performed in 0.0025% Coomassie brilliant blue R250 containing 40% methanol and 20% acetic glacial acid (v/v) in distilled water at room temperature for about 4 h and gently agitated. Destaining was done in the same solution without dyes repeatedly until the protein bands were clearly seen. Electrophoresis was performed at room temperature until the blue marker reached the bottom of the gels.

Gel documentation and analysis: The banding patterns were visualized and photographed with VMR Trans-illuminator ECN 730-1386. Distinct bands were scored for presence or absence of bands and subjected to analysis. UPGMA-based dendrogram was constructed using Multivariate Statistical Package[29] (MVSP) version 3.1 as well as Jaccard's coefficient of similarity. Genetic diversity, genetic distance, Shannon's information index, molecular variation and principal coordinate analysis (PCoA) were performed[30] using GenAlEx 6.5.

RESULTS

Number of bands in the accessions: The electropherogram from SDS-PAGE revealed a total of 17 polypeptide bands from which 9 bands were monomorphic and 6 were polymorphic. The band sizes ranged from 15.44-251.39 kDa. The accessions with the highest number of bands were accessions TVu595, TVu5590, TVu12277, TVu13076, TVu14046, TVu14085, TVu14114 and TVu15323 each with 14 bands; accessions TVu10710, TVu4467, TVu8063 and TVu15695 followed with 13 bands each. Accessions TVu11707 and TVu11883 had 12 bands while accession TVu12513 had 11 bands.

UPGMA-based dendrogram of cowpea accessions investigated: Four major clusters, I-IV were detected based on Jaccard's similarity coefficient of protein bands. To a large

Table 1: Germplasm description of the 15 accessions of cowpea [*V. unguiculata* (L.) Walp] used

Accessions	Cultivar name	Biological status of accessions	Origin	Region	Source
Tvu596	FarinMoriki	Traditional cultivar/Landrace	Nigeria	West Africa	IITA
TVu10710	Ex Kagera	Traditional cultivar/Landrace	Nigeria	West Africa	IITA
TVu4467	Ex Enugu	Traditional cultivar/Landrace	Nigeria	West Africa	IITA
TVu5590	KR 617	Traditional cultivar/Landrace	Niger	West Africa	IITA
TVu8063	Ex Yamoussoukro Market	Traditional cultivar/Landrace	Cote d'Ivoire	West Africa	IITA
TVu11707	Kampandila Mwaba, Lundazi	Traditional cultivar/Landrace	Zambia	East Africa	IITA
TVu11883	Ex Thyolo	Traditional cultivar/Landrace	Malawi	East Africa	IITA
TVu12277	TN 80-42	Traditional cultivar/Landrace	Niger	West Africa	IITA
TVu12513	ZM 3137-2	Traditional cultivar/Landrace	Zambia	East Africa	IITA
TVu13076	VITA 5-LS-T-2	Breeding/Research Material	Nigeria	West Africa	IITA
TVu14046	G-78	Traditional cultivar/Landrace	Central African Republic	East Africa	IITA
TVu14085	G-264	Traditional cultivar/Landrace	Central African Republic	East Africa	IITA
TVu14114	G-367	Traditional cultivar/Landrace	Central African Republic	East Africa	IITA
TVu15323	PS 87CH-527	Traditional cultivar/Landrace	Chad	West Africa	IITA
TVu15695	IT 87S-1390	Breeding/Research Material	Nigeria	West Africa	IITA

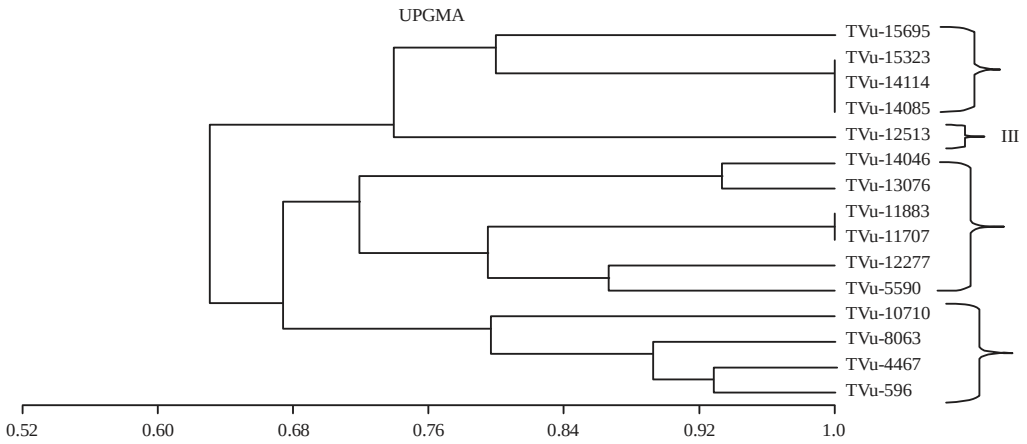

Fig. 1: Relationship among 15 accessions of *V. unguiculata* L. Walp

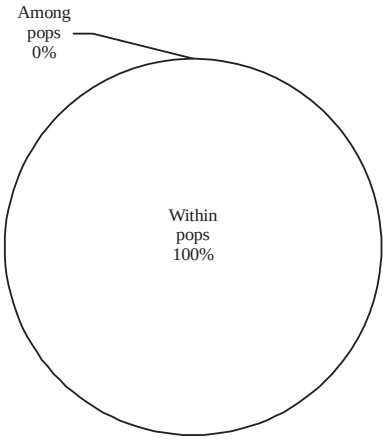

Fig. 2: Percentages molecular variance in two cowpea populations (West and East Africa)

extent, clustering pattern was based on population except two accessions, TVu15323 (Chad) and TVu15695 (Nigeria) that were clustered together with accessions in cluster IV. Dendrogram clustered cowpea accessions based on countries of collection/adoption into IITA germplasm. TVu596, TVu10710, TVu4467 and TVu8063 from Nigeria were clustered together while TVu5590 and TVu12277 adopted from Niger were also clustered together with infiltration of TVu11707 from Zambia (Fig. 1).

UPGMA-based Jaccard's coefficient of similarity: Jaccard's coefficient of similarity showed that the highest coefficient of similarity 1.00, which was between TVu14114 (Central African Republic [CAR]) and TVu14085 (CAR); TVu15323 (Chad), TVu14085 (CAR) and TVu14114 (CAR).TVu4467 (Nigeria) share 0.929 coefficient of similarity with TVu596 as well as between TVu8063 and TVu596. However, the lowest coefficient of similarity was 0.412, which was between TVu12513 (Zambia), TVu4467 and TVu8063 (Nigeria). Additionally, TVu12513 (Zambia) share coefficient similarity of 0.471 with TVu596 (Nigeria) as well as between TVu15695 (Nigeria), TVu11707 (Zambia) and TVu11883 (Malawi) (Table 2).

Genetic distance of cowpea accessions: The result from genetic distance revealed that the widest distance (3.61) were observed between TVu13076 (Nigeria) and TVu10710; TVu14046 (CAR) and TVu11707 (Zambia) while narrowest (1.73) was observed between TVu15695 (Nigeria) and TVu11707 (Zambia) and between TVu14114 (CAR) and TVu12513 (Zambia) (Table 3).

Analysis of molecular variation of cowpea accessions (AMOVA): Molecular variation of cowpea accessions showed that among population variance was zero percent while within population variation was 100%. However, attempt was made to resolve molecular variation of the accessions based on loci. Result revealed that for locus 1 among population was 7% while within population was 93%. Percentage molecular variance in locus 6 was observed to be 30% for among population variation and 70% for within population variance. For locus 14, among population variance was 23% while within population variance 77% (Fig. 2-5).

Pooled genetic parameters over loci and population: For pooled population, genetic diversity was 0.369±0.018, unbiased genetic diversity was 0.396±0.019 while Shannon's information index was 0.550±0.020. However, based on population, genetic diversity was 0.377±0.019, unbiased genetic diversity was 0.399±0.020 while Shannon's information index was 0.561±0.021 for West African population. For East African population, the result revealed

Table 2: UPGMA Jaccard's coefficient similarity matrix

Coefficients	Tvu 596	Tvu 10710	Tvu 4467	Tvu 5590	Tvu 8063	Tvu 11707	Tvu 11883	Tvu 12277	Tvu 12513	Tvu 13076	Tvu 14046	Tvu 14085	Tvu 14114	Tvu 15323	Tvu 15695
TVu-596	1.000														
TVu10710	0.800	1.000													
TVu4467	0.929	0.857	1.000												
TVu5590	0.867	0.688	0.800	1.000											
TVu8063	0.929	0.733	0.857	0.800	1.000										
TVu11707	0.625	0.563	0.667	0.733	0.563	1.000									
TVu11883	0.625	0.563	0.667	0.733	0.563	1.000	1.000								
TVu12277	0.750	0.688	0.800	0.867	0.688	0.857	0.857	1.000							
TVu12513	0.471	0.500	0.412	0.653	0.412	0.533	0.533	0.563	1.000						
TVu13076	0.706	0.647	0.647	0.706	0.750	0.688	0.688	0.706	0.625	1.000					
TVu14046	0.647	0.588	0.588	0.750	0.688	0.733	0.733	0.750	0.667	0.933	1.000				
TVu14085	0.647	0.688	0.588	0.750	0.588	0.625	0.625	0.750	0.786	0.706	0.750	1.000			
TVu14114	0.647	0.688	0.588	0.750	0.588	0.625	0.625	0.750	0.786	0.706	0.750	1.000	1.000		
TVu15323	0.647	0.688	0.588	0.750	0.588	0.625	0.625	0.750	0.786	0.706	0.750	1.000	1.000	1.000	
TVu15695	0.688	0.733	0.625	0.688	0.625	0.471	0.471	0.588	0.600	0.647	0.588	0.800	0.800	0.800	1.000

Table 3: Genetic distance across the accessions

Coefficients	TVu-595	TVu-10710	TVu-4467	TVu-5590	TVu-8063	TVu-11707	TVu-11883	TVu-12277	TVu-12513	TVu-13076	TVu-14046	TVu-14085	TVu-14114	TVu-15323	TVu-15695
TVu-595	0.00														
TVu-10710	2.45	0.00													
TVu-4467	3.32	3.32	0.00												
TVu-5590	2.45	2.83	3.32	0.00											
TVu-8063	2.83	3.16	3.00	3.16	0.00										
TVu-11707	3.16	2.45	3.32	2.00	3.46	0.00									
TVu-11883	3.16	2.45	3.00	3.16	3.16	2.45	0.00								
TVu-12277	3.32	3.00	2.00	2.65	3.32	2.65	2.65	0.00							
TVu-12513	2.83	3.16	2.65	3.16	3.46	3.16	2.83	3.00	0.00						
TVu-13076	2.65	3.61	2.45	2.65	2.65	3.32	3.32	3.16	2.65	0.00					
TVu-14046	2.24	2.65	2.83	3.00	2.65	3.61	3.32	2.83	3.00	2.83	0.00				
TVu-14085	3.00	3.00	3.46	2.65	2.65	2.65	3.00	3.16	3.00	3.16	2.83	0.00			
TVu-14114	2.65	3.32	2.45	3.00	3.32	3.32	3.00	2.83	1.73	2.00	2.45	3.16	0.00		
TVu-15323	2.45	2.00	3.00	2.45	2.83	2.83	2.83	2.65	3.16	3.00	2.24	3.00	2.65	0.00	
TVu-15695	3.32	2.65	3.16	2.24	3.32	1.73	2.65	2.45	3.00	3.46	3.46	2.45	3.46	3.00	0.00

Table 4: Pooled genetic parameters over loci per population

Populations	Mean±SE	N	Na	Ne	I	He	uHe
Population 1	Mean	9.000	2.000	1.630	0.561	0.377	0.399
	SE	0.000	0.000	0.053	0.021	0.019	0.020
Population 2	Mean	6.000	2.000	1.619	0.539	0.361	0.394
	SE	0.000	0.000	0.072	0.035	0.030	0.033
Grand mean and SE over loci and populations							
Total	Mean	7.500	2.000	1.625	0.550	0.369	0.396
	SE	0.261	0.000	0.044	0.020	0.018	0.019

Population 1 (West Africa): TVu-595, TVu-10710, TVu-4467, TVu-5590, TVu-8063, TVu-12277, TVu-13076, TVu-15323, Tvu-15695. Population 2 (East Africa): TVu-11701, TVu-11883, TVu-12513, TVu-14046, TVu-14085, Tvu-14114. N: Number in population, Na: Number of alleles, Ne: Number of effective alleles, I: Shannon's information index, He: Genetic diversity, uHe: Unbiased diversity

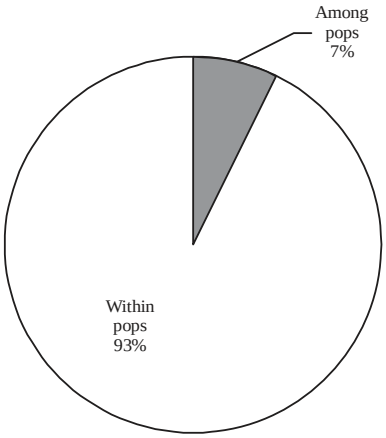

Fig. 3: Percentages molecular variance in locus 1 across the cowpea accessions investigated

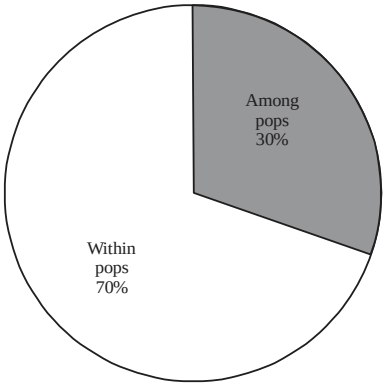

Fig. 4: Percentages molecular variance in locus 6 across the cowpea accessions investigated

0.361±0.030, 0.394±0.033 and 0.539±0.035 for genetic diversity, unbiased genetic diversity and Shannon's information index, respectively (Table 4).

Principal coordinate analysis (PCoA) of cowpea accessions: Principal coordinate analysis results revealed two major clusters with four sub-clusters. Sub-cluster 1 had TVu8063,

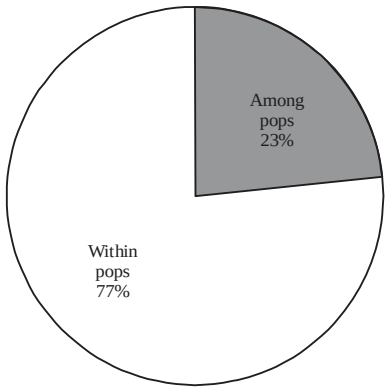

Fig. 5: Percentage molecular variance at locus 14 across the cowpea accessions investigated

TVu596 and TVu11883 while sub-cluster 2 had TVu4467, TVu15695, TVu14046 and TVu14085. Importantly is that the clustering was not based on region where the accessions were adopted from with some exceptions. From the three axes, cumulative percentage contribution to variation in the cowpea accessions were 16.39, 29.05 and 39.27% for principal coordinate 1, 2 and 3. Total contribution to variability was 84.71% (Fig. 6).

DISCUSSION

Protein profile analysis of cowpea accessions using SDS-PAGE revealed 17 bands with a range of molecular weights from 8.30-251.39 kDa and six polymorphic bands detected. These results are corroborated by the findings of two findings[31,32]. The polymorphic bands were observed in TVu596, TVu10710, TVu4467, TVu5590, TVu8063 and TVu11883 with low molecular weights ranging from 15.44-14.85 kDa. A study reported that seven cowpea landraces were accessed for genetic variability in their seed proteins[33]. Using SDS-PAGE, the pattern observed from the electrophoresis of seed proteins revealed molecular heterogeneity in the total proteins. The common bands

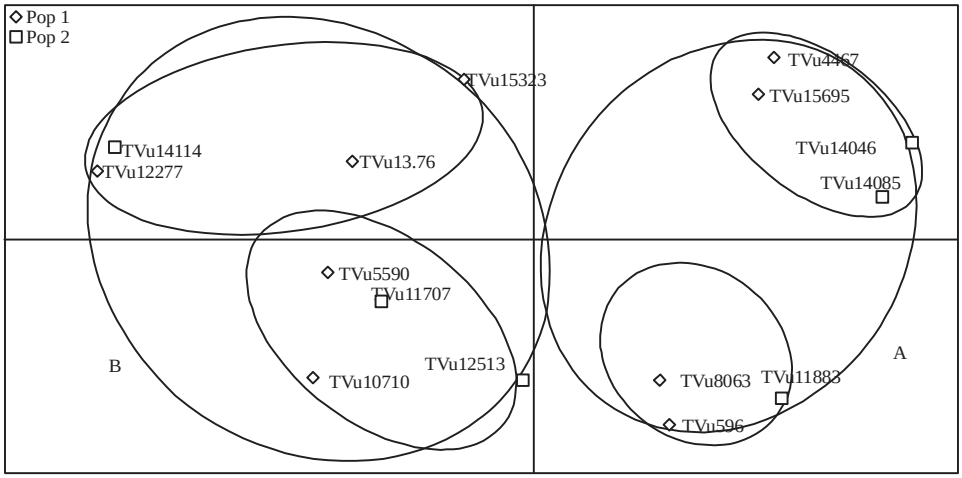

Fig. 6: Principal coordinate analysis (PCoA) based on SDS-PAGE data illustrating the genetic relationship among 15 accessions of cowpea and populations (West and East Africa) from GenALex

discovered in the 7 landraces studied indicated proximal relationship in the varieties while distinct bands were equally seen which indicated varietal differences. The nature of protein profiles as a tool for biochemical fingerprinting in cowpea has been established[31]. However, a low degree of polymorphism was observed in the cowpea cultivars studied. On the strength of the outcome of the results by Singh *et al.*[34], there is the presence of enough genetic divergence on the basis of seed storage in the pea cultivars studied. This outcome prompted the recommendation of KPMR-906 with wide genetic diversity for utilization in crop improvement programmes.

It had been reported that cowpea is among the 5th top food legumes that are grown worldwide and has a presence on every continent except Oceania and Australia[31]. The west African sub-region contributes about 95% of global cowpea production, with Nigeria being the largest producer in the world[32]. This notwithstanding, cowpea landraces are dramatically disappearing and unfortunately being replaced by improved, higher yielding modern cultivars from modern breeding programmes[35,36] and this has resulted to erosion of crop genetic diversity[31].

Successful conservation of germplasm largely depends on the understanding of the extent of genetic diversity within and among crop species[37]. There is therefore an urgency to ensure that the diversity in landraces is characterized and subsequently conserved. Germplasm with wider genetic base provides enough buffer and resilience against climatic and environmental challenges[31]. Seed storage proteins play important role in the defence against insect and pathogens as well as act as nitrogen and energy sources[38]. The reliability of protein data is obviously hinged on the fact that seed storage proteins are highly independent of environmental factors, which are comparable to those of other molecular markers. Electrophoretic techniques in plant classifications utilize gel medium supports, which contributes to the reliability of data generated therein[39-41].

The UPGMA-based dendrogram generated two major clusters with sub-clusters, which was based generally on the region the accessions were adopted. Cluster I included accessions which were highly polymorphic while other clusters were monomorphic with the exception of TVs11883 in cluster III which was polymorphic. The correlation of low molecular weight and protein polymorphism can be further explored. A positive association between high molecular weight and monomorphism was expressed by Atoyebi *et al.*[42]. This was sharply contradicted by the report of Eid[32].

Interestingly also is the fact that the result on principal coordinate analysis (PCoA) agreed with that of the dendrogram. The clustering together of TVu15695 (Nigeria) in cluster II and TVu11883 (Malawi) and TVu11707 (Zambia) in cluster I could be explained from the standpoint that there might have been introgressions before adoption. For instance, TVu15695 is a breeding/research material, which obviously should be as a result of hybridization. Clustering together of cowpea accessions is an indication that they might share common genetic characteristics. It should be noted that the existence of common bands among accessions is an indication that species bearing them share close genetic affinity as well as common ancestry. Protein bands are coded for by genes. The implication of the above is that genes have been fixed in species bearing them over evolutionary time.

Undoubtedly, the higher the similarity coefficient, the more phylogenetically related the species are and vice versa. Jaccard's coefficient of similarity revealed that TVu12513 (Zambia) was not phylogenetically related to TVu4467, TVu596 and TVu8063 (Nigeria) on the basis of coefficient of similarity. This result corroborated with the result on the dendrogram. Similarly, TVu15695 (Nigeria) was genetically distinct from TVu11707 (Zambia) and TVu11883 (Malawi) thus the grouping into different clusters. Surprisingly, TVu14046 (Central African Republic) share 0.933 (93.3%) similarity with TVu13067 (Nigeria). This might be explained from the premise that TVu13076 is a breeding/research material, which might be a product from a cross related to TVu14046. This similarity could also be explained as duplication[43].

Result obtained from the genetic distance also proved that TVu13076 (Nigeria) is genetically distinct from accessions TVu10710, TVu14046 (CAR) and TVu11707 (Zambia) as their genetic distances were 3.61, which was the widest. What it does suggest is that the wider the genetic distance, the more distinct the species are. Given the extent of genetically un-relatedness of some cowpea species, especially between Nigeria accessions and Zambia accessions, one wonders the underlying factor surrounding the analysis of molecular variation result obtained where within population variation was 100%. The understanding was that by clustering accessions into different clusters should mean that they should show molecular variations. However, the probable explanation is that variation was not significant enough to cause visible variability.

This work also attempted to resolve the molecular variation of the different accessions based on loci the population notwithstanding. Comparing locus 1, 6 and 14, molecular variation among population were 7, 30 and 23% while within population molecular variation were 93, 70 and 77%, respectively. Worthy to note is that genes are located on different loci encoding proteins. The difference observed in molecular variations among the cowpea accessions based on the three loci may not have made significant impact on the molecular variations comparing the populations. Going by other genetic parameters such as genetic diversity, unbiased diversity and Shannon's information index derived from protein banding pattern, revealed that west African cowpea accessions are more diverse than east African population. This might be attributed to the two accessions, TVu13076 and TVu15695 that are used as breeding/research materials. What this might suggest is that the two accessions could encode genes whose proteins produce outstanding qualities that adds to the total variability.

From principal coordinate analysis (PCoA) percentage cumulative variability contribution from the 3 axes are 16.39, 29.05 and 39.27% giving total contribution of 84.71% to the variability observed, especially within population. The efficiency and informativeness of any technique adopted to essay genetic diversity might be enhanced exponentially if many of such techniques are compared and more importantly are assessed in combination[44,45]. Though RAPD markers were used to evaluate diversity in cowpea accessions obtained from IITA, Ibadan, Nigeria[46], the outcomeshowed that Asian cowpea accessions were genetically distinct from African accessions. This notwithstanding, it was wondered how cowpea accessions adopted from Nigeria and Benin both from West Africa were genetically distinct. However, this was explained by attributing it to plant breeders introducing species from diverse geographical backgrounds in the bid to widen the genetic base among existing stock[47]. This present study using protein profiling showed higher genetic parameters such as genetic diversity, unbiased diversity and Shannon's information index in West African cowpea accessions when compared with the east African accessions. There is in existence a coherent relationship between the extent of genetic diversity within landraces and geographical distribution of the landraces[6]. This work reported a geographical or region-based clustering which corroborates with the result on genetic distance. The implication of this within population diversity reported in this present study is that parental selection should be made among accessions that are genetically distinct. From this result it might be justifiable enough to suggest selection, especially between cowpea accessions from Nigeria, probably TVu4467 and/or TVu8063 and TVu12513 adopted from Zambia. Due to the higher genetic parameters, the possibility is that there was lower intra-population similarity and higher proportion of polymorphic loci resulting in increased heterozygosity[48]. This might be the underlying reason for the differentials that exist between the two populations.

Higher genetic diversity might provide genetic barrier against different biotic and abiotic stresses[49,50]. The knowledge of genetic diversity that exists within and among genotypes of any crop is fundamental to estimating the potential of genetic gain in breeding programmes and for effective conservation and sustainable utilization of available genetic resources.

Accessions with similar banding patterns are recommended for further studies to understand their agronomic and biochemical traits to provide information for their better management and utilization.

CONCLUSION

Seed storage protein profiles bear genetic imprints and as such play useful roles in plant identification and characterization. The outcome of this study revealed genetic variation in 15 accessions of cowpea as revealed by its protein profiles. The utilization of SDS-PAGE as a cost effective technique was able to detect inherent variation and thus offering useful information for breeders.

At the moment, a plethora of advanced tools are available for plant diversity studies. The current thinking favours the usage of DNA marker systems. However, the limitation imposed by the cost of these DNA marker systems makes biochemical markers the method of choice at least as a baseline. Considering the size of the global cowpea germplasm, more work is advocated to completely characterize them, identify distinct accessions and recommend genome-wide crosses to generate more variation that can be adopted to engender food security. It is suggested though implicitly from the results taken together that parental selection of cowpea accessions be done in accession(s) adopted from Nigeria, preferably TVu4467 and/or TVu8063 as well as TVu12513 adopted from Zambia for possible crossing. Looking to the future, DNA marker systems can be incorporated for the characterization of crop plants.

SIGNIFICANCE STATEMENTS

This study has revealed variation in the accessions under consideration as it relates to cowpea production in Nigeria which is directly beneficial in quelling malnutrition as it is considered as the poor man's meat. This research will provide other scientists with requisite information to undertake breeding programmes andhence, broadening the genetic base which is both important for crop improvement and conservation of germplasm.

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