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CHARACTERIZATION AND PROFILING OF SEED STORAGE PROTEINS OF SOME UNDERUTILIZED BEANS VARIETIES USING SDS-PAGE

K. O. Soetan[1] and D. A. Animasaun[2*]

[1]Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.
[2]Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria
Correspondence: animasaun.ad@unilorin.edu.ng, kehinde.soetan@gmail.com

ABSTRACT

Seed storage proteins of seventeen underutilized beans germplasm from the International Institute of Tropical Agriculture (I.I.T.A), Ibadan, Nigeria were profiled for protein composition and abundance using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Electrophoregrams were analyzed by matching spot positions compared with standard protein marker (Page Ruler 10-200 kDa) in three independent replicates. Protein fractions between 11-140 kDa were detected of which high molecular mass and low mobility protein fractions were dominant. A total of 10 fractions were separated from the samples with the highest (8) found in Lima bean cultivars (2006-015, 2006-011, 2006-001) and Sword bean Tcg-1, while the least (4) occurred in Winged bean TPt-3. There were remarkable similarities between all the samples in their low-molecular mass fractions (11.0-33.3 kDa). Legumin and vicilin fractions abound in the samples, but protease inhibitor (11 kDa) present only in small quantity. The dendrogram of the similarity showed two main clusters which divided into sub-clusters of heterogeneous beans. The dendrogram revealed that Pigeon pea (CITA-2, A078-99), Jack bean TCe-5 and African yam bean TSs-10 were similar based on protein composition. Sword bean (TCg-1, TCg-4) and winged bean (TPt-06 and TPt-30) had the highest genetic distance (0.7 scale). The study concludes that SDS-PAGE is efficient for profiling and identifying pea proteins. The seed storage protein composition of the underutilized beans had basic protein fractions for their utilization as cheap sources of plant protein. Moreover, low content of protease inhibitor (11 kDa) signified low toxic constituent of pea protein which make the seed proteins of good quality for nutritional application.

Keywords: Pea seed proteins, protein profile, SDS-PAGE, underutilized beans, protease inhibitor.

INTRODUCTION

Legumes, in particular, members of the family Leguminosae play an important role in human and animal nutrition. They are rich sources of protein, calories, minerals and vitamins (Baloch and Zubair, 2010). Grain legumes are used as pulse in areas where they are grown due to their abundant essential amino acids, particularly lysine (Sai-Ut *et al*., 2009). In Nigeria and other sub-Saharan African countries with limited purchasing economic power, supplement of protein content of cereal diet with readily available plant protein, especially from beans and pulses to improve the nutritional status of the low-income population is imperative. Various peas and bean species are becoming an important vegetable source of proteins and a potential alternative to animal protein in most developing countries due to their manifold qualities, high nutritional value, availability, and relatively low cost (Animasaun *et al*. 2017). In addition, pea beans and their products are a rich source of biologically active components that may exert beneficial health and therapeutic effects on human and animals (Berber and Yasar, 2011). A number of these beans and pulses of different species and varieties e.g. chickpea, winged bean, pigeon pea, lentil, Lima bean, African yam bean, sword beans, broad and kidney beans etc. which are restricted to local cultivation in Nigeria are still grossly underutilized.

Utilization of beans and peas for food and feed formulation in animals as a source of protein could be the cheapest way to provide balanced amino acids requirement. Protein energy deficit, malnutrition and micro-element threat of food security in developing nations can be ameliorated using plant protein sources. However, the utility and quality of specific bean and pea for nutritional purposes depends on the type of amino acids present, absorption property and minimal mandatory rates of oxidation (Longnecker *et al*., 2002). The presence of anti-nutrients such as saponins, trypsin inhibitors, heamagglutinins, tannins, oxalates, phytates etc., could immobilize important minerals in grain legumes and consequently contribute to mineral deficits (Vasagam and Rajkumar, 2011).

Globulins and albumins are the major proteins of pea and other legumes, and these pea proteins are characterized by high levels of genetic polymorphism (Baringer *et al*., 2004). About 638 amino acid sequences of proteins and polypeptides were reportedly found in different pea varieties, including seed storage proteins

(Wu *et al*., 2006). Proteins of different leguminous sources such as soy bean (Barac *et al*., 2006; Ribotta and Rosell, 2010; Chen *et al*., 2011), pea bean (Aluko, 2008; Pownall *et al*., 2010), peanuts (Dong *et al*., 2011; Zhao *et al*., 2011) cowpea (Horax *et al*., 2004) have already been reported and could be significant in their preference and utilization. The differences in content, composition and structure of legume proteins are important for their nutritional and functional properties, and according to Zhao *et al*. (2011), the contribution of legume in to the pea protein gels was cultivar specific. Functional properties of pea protein-based products depend on several factors including protein constituents.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is an effective technique for separating tissue proteins (Konopka *et al*., 2007). The use of SDS-PAGE for seed proteins profiling and fractionalization has been reported for various crop species and cultivars (Koenig *et al*., 1990; Karihaloo *et al*., 2002; Lioli *et al*., 2005; Yüzbaşıoğlu *et al*., 2008; Animasaun *et al*., 2017). The technique is rapid and generally free from environmental influence compared with the traditional morphological and other descriptive criteria derived from field trials (Pekşen *et al*., 2005). Successful use of electrophoretic procedure for protein separation depends on the existing polymorphism of seed protein and isozymes, which are basically gene products (Tsumura *et al*., 2005). SDS-PAGE profiling of seed storage proteins in crops is a useful tool for identifying and differentiating seed materials for the purpose of seed certification and plant variety rights (Duran *et al*., 2005).

The protein banding pattern is unique for a particular genotype and is independent of seed vigor and physiological seed activity (Kamel *et al*., 2003). The use of total seed protein for varietal identification of pea using SDS-PAGE was reported by Roy *et al*. (2010). Also, Kumar *et al*., 2001 established genetic identity and determined percentage purity of sunflower F_1 hybrids using seed protein profile. Generally, there are many underutilized beans and peas varieties in Nigeria whose protein constituents have not been well reported. These underutilized beans restricted to local use, have great potential for human food and animal feed formulation as cheap and rich sources of protein to bridge the deficit, reduce malnutrition and contribute to food security. Keeping the above in view, the present study profiled the seed storage proteins of some underutilized beans and peas in Nigeria using SDS-PAGE technique, with the view of providing information that would enhance and promote utility of the species and cultivars for development of protein enriched formulation for both human and animals.

MATERIALS AND METHODS

Seed materials, chemicals and reagents: Genetically pure seeds of bean and pea varieties; Pigeon pea (2), Lima bean (3), African yam bean (3), Bambara groundnut (3), Sword bean (2), Winged bean (3) and Jack bean (1) (Table 1) which are underutilized genetic resources were obtained from the Genetic Resources Centre, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria for the study. Physical appearances of the seeds are shown in Figure 1. High purity and molecular grade chemicals and reagents were used for the study. Chemicals for the protein profiling were purchased from BDH Laboratory Supplies, Poole, England and Sigma-Aldrich (Seelze, Germany). Staining solution Roti®-Blue (Coomassie Brilliant Blue R-250) was purchased from Roth (Karlsruhe, Germany).

Methods

Reagents and buffers preparations: Total soluble proteins (Tris-HCl Soluble) in the seeds were extracted and separated by Polyacrylamide gel electrophoresis (PAGE) as described by Ahmed (2005). Extraction buffer (EB), protein sample buffer (PSB) and stock sample buffer (SSB) followed the described method. The working sample buffer consists of 4.25 ml of SSB and 75 ml β-mercapto ethanol. These components were mixed thoroughly and the volume was made up to 100 ml. To this, 10 µl of Bromophenol blue was added, to act as a tracking dye. The 30% acrylamide stock gel solution (SGS) prepared consisted of 30.08 g of acrylamide and 0.8 g of Bis-acrylamide made up to 100 ml with distilled water. The defatting solution was a mixture of chloroform, methanol and acetone in the ratio 2:1:1. The separating gel buffer (SGB), stacking gel buffer (SGLB), 10% SDS, and running gel mixture (RGM) were also prepared using the method of Berber and Yasar, (2011).

Extraction of total seed proteins for SDS-PAGE: Dry dehulled cotyledons of each seeds sample were ground to flour using electrical grinder (Binatone, Japan). About 0.2 g of each sample poured into a micro-tube was used for protein extractions by the addition of 1 ml of defatting solution with vigorous agitation, then allowed to settle for 3 hr. The supernatant was decanted and the procedure repeated trice. The samples were kept overnight for drying at room temperature. In the next day, 1 ml extraction buffer was added into each of the sample tube and incubated overnight at 10 ^{o}C. After 24 hr., the mixture was centrifuged (Sigma 3K30 Centrifuge, Harz, Germany) under cool condition at 12,000 rpm for 30 min., and the supernatant was collected. Equal volume of sample buffer was added to the supernatant; thoroughly mixed for homogenousity and then boiled in water for 3 min. The mixture was cooled to room temperature in preparation for loading.

Electrophoresis: The soluble seed proteins were subjected to SDS-PAGE as described by Laemmli (1970). The electrophoresis of the soluble protein was performed in duplicate with a discontinuous buffer system on Bio-Rad Mini Protean 3 System (Bio-Rad Laboratories, CA, USA). Ten microliters (10 µl) of sample were loaded per well. The loaded gel slabs of 1 mm thickness (3.5 cm 4% stacking and 15.5 cm 12% resolving gels) were run at constant voltage (60V) until the bromophenol blue marker had reached the bottom of the gel. Staining of gels were carried out for 6 h in 0.01% (w/v) Coomassie Brilliant Blue (CBB) and de-stained with methanol-water mixture until protein bands became clearly visible. Protein molecular weight was determined by comparison with standards (PageRuler^{TM} Protein Ladder, Fermentas, MW 10-200 kDa).

Protein profile analysis: The gels were scanned with a high resolution gel documentation system (Biorad, CA, USA). The molecular weight of each band was determined by one dimensional analysis software (Lab Image Version 2.6, Halle, Germany). The intensity, position, presence or absence of bands, were documented to discriminate the cultivars employed in the study. Data were coded as 0 (absent) and 1 (present). A hierarchical cluster analysis was performed using the average linkage method and correlation coefficient distance. The dendrogram, based on the total seed protein pattern of bean and pea varieties was constructed.

RESULTS

The electrophoretic profile of the extracted soluble protein form seventeen (17) underutilized bean and pea species/or varieties studied are presented in Figure 2. Protein isolates from the beans were separated into their component fractions with molecular weight (MW) ranging from 11 to 140 kDa. Analyses of the seed storage proteins of the beans exhibited remarkable similarities for all bean species and varieties in their low-molecular mass fractions (11.0 - 33.3 kDa). However, there was a slight fraction variation in higher molecular mass proteins above 70 kDa. The PAGE profiles showed that fractions of high molecular mass with low mobility were dominant. There were three major (100, 63 and 35 kDa) and five minor (33.3, 25, 23.3 17.0 and 11.0 kDa) regions of protein fractions in the beans, although there were other sub fractions (Figure 2).

Protein fractions which were present in each of the beans and peas are presented in Table 2. A total of 10 bands (10 fractions) were separated from the samples with the highest number of fractions (8) found in samples 8, 9, 10 and 14 while the least (4) occurred in sample 17. Fractions within the region of 245 kDa were absent in all the samples. Lima beans (*Phaseolus lunatus*) cultivars (samples 14 - 16) had protein fraction around 140 kDa which was not present in other bean samples, meanwhile, protein of molecular masses in the regions 11.0, 17.0, 23.3, 44.3, 63.0, and 135 kDa were present in all the studied beans. However, the intensity which indicates the abundance varied for the samples. Protein in the band regions of 11 - 20 kDa occurred in traces in the samples where they were present. The fractions between 33 - 68 kDa were legumin, those at the region of 90 kDa were lypoxigenase (Lox), 37 – 31 kDa were vicilin while the very low molecular mass around 11 kDa were protease inhibitors (PI).

The relative abundance of the protein fractions exhibited by the intensity is shown in Table 2. The most abundant seed storage proteins of the underutilized beans were in the order of 63 kDa > 30 kDa > 40 kDa > 17 kDa and 135 kDa. Faint band of protein fraction of 11 kDa (protease inhibitor), which is a toxic constituent of pea protein was common to the beans but in a very little content. Low molecular mass legumin (23 kDa) was only present in six samples; Lima bean (2006-015, 2006-011 and 2006-001), sword bean TCg-1, winged bean (TPt-06, TPt-3) while protein fraction of high molecular mass legumin of the region 140 kDa was only found in sword bean TCg-1, winged bean TPt-30 and winged bean TPt-06. Protein profiles of the studied bean varieties and cultivars showed that Pigeon pea CITA-2, Pigeon pea A078-99, Sword bean TCg-4, African yam bean TSs-84 and African yam bean TSs-10 had similar fractions but differed in abundance (Table 2).

The average linkage method and correlation coefficient distance cluster analysis based on SDS-PAGE profiles of seed proteins were used to generate a dendrogram (Fig. 3). At genetic distance of 3.6 scale (i.e. above 96%), the dendrogram separated the beans into two major groups (A and B). Group A was further divided into two; A1 and A2. The A1 cluster had two different beans species (Sword bean TCg-1 and Winged bean TPt-06), while the A2 was partitioned into two sub-clusters of two members each in which the A2i comprised of Lima and winged beans of similar protein composition. The A2ii cluster was made up of two Lima bean cultivars separated at 99% similarity.

The beans and peas in the second group (B) were divided into two major clusters (B1 and B2) in which an African yam bean TSs-57 solely occupied a sub-cluster (B1i), while clusters B1ii composed of an African yam bean and three cultivars of Lima bean (Figure 3). Furthermore, the dendrogram revealed heterogeneous clustering of peas in cluster B2. Based on protein profile, African yam bean TSs 10, Jack bean TCe-5, Pigeon pea CITA-2 and Pigeon pea A078-99 were all similar with genetic distance less than 0.1.

Fig. 1,- Physical appearances of the seventeen underutilized beans and peas collected from International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

1 = Pigeon pea A078-99; 2 = Lima bean 2006-015; 3 = African yam bean TSs-57; 4 = Bambara nut TVSU-870; 5 = Sword beans TCg 4; 6 = Winged bean TPt-30; 7 = African yam bean TSs-84; 8 Jack bean TCe-5; 9 = Winged bean TPt-06; 10 = Lima bean 2006-011; 11 = Lima bean 2006-001; 12 = Bambara nut TVSU-1034; 13 = Winged bean TPt-3; 14 = Pegeon pea CITA-2; 15 = African yam bean TSs-10; 16 = Sword bean TCg-1; 17 = Bambara nut TVSU-1482

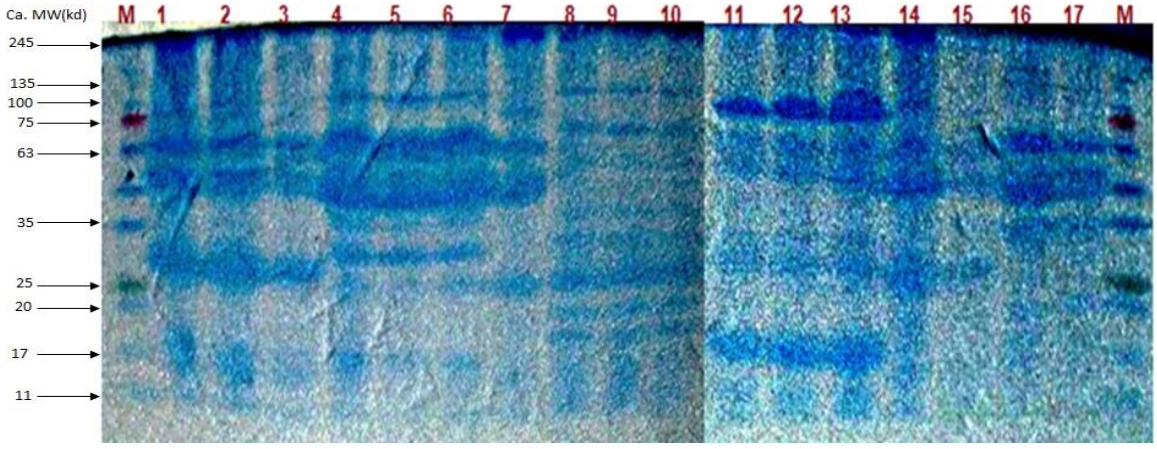

Fig. 2. Electrophoregram of protein profiles for seventeen underutilized bean and pea germplasm from International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Sample ID: 1: African yam bean TSs-84; 2: African yam bean TSs-10; 3: African yam bean TSs-57; 4: Bambara TVSU-870; 5: Bambara TVSU-1034; 6: Bambara TVSU-1482; 7: Jack bean TCe-5; 8: Lima bean 2006-015; 9: Lima bean 2006-011; 10: Lima bean 2006-001; 11: Pigeon pea CITA-2; 12: Pigeon pea A078-99; 13: Sword bean TCg-4; 14: Sword bean TCg-1; 15:Winged bean TPt-30; 16: Winged bean TPt-06; 17: Winged beanTPt-3.

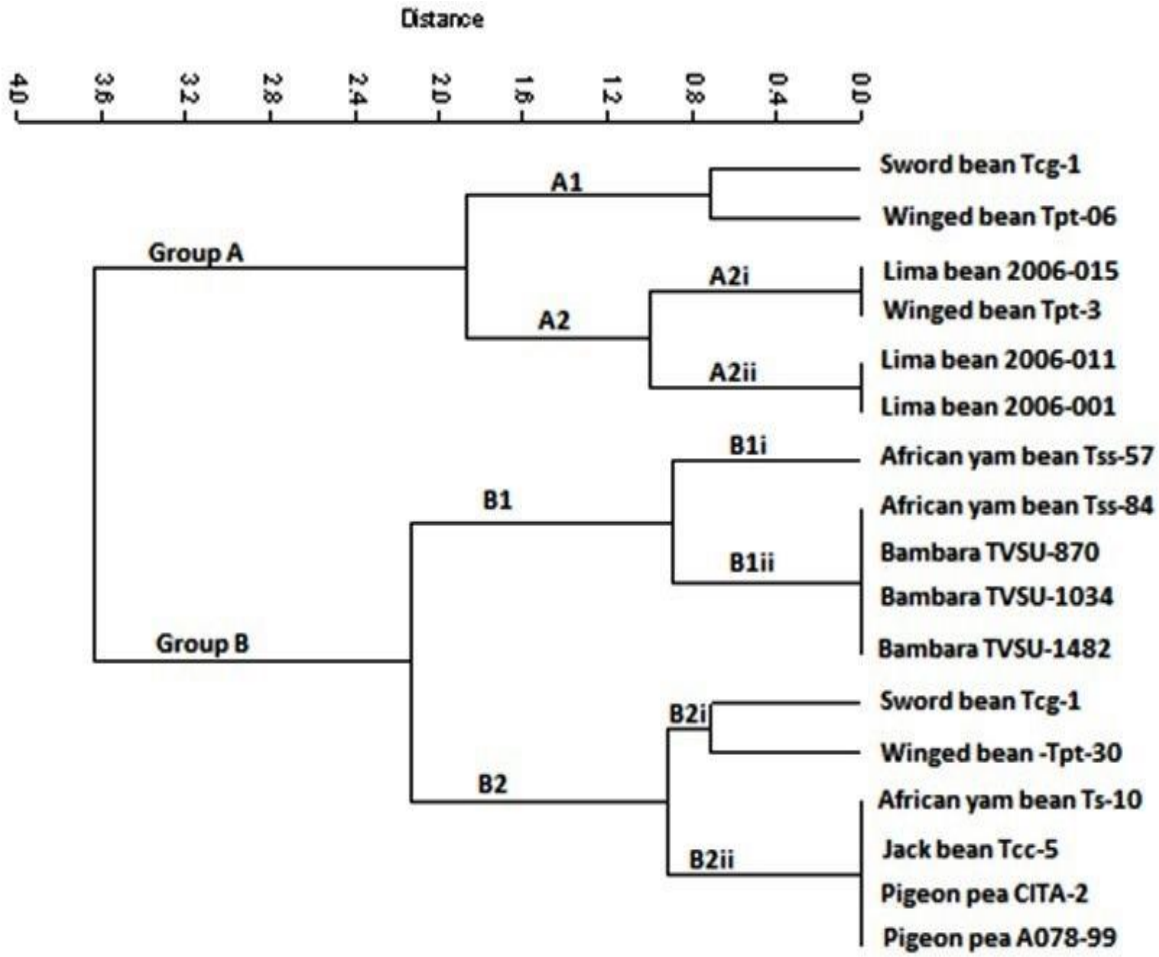

Fig. 3. Dendrogram of seventeen underutilized bean and pea varieties based on protein profile similarity.

Table 1. Accession name, common name, scientific name of the beans and peas used for the SDS-PAGE protein fragmentation.

SN	Accession name	Common name	Scientific name
1	A078-99	Pigeon pea	*Cajanus cajan*
2	CITA-2	Pigeon pea	*Cajanus cajan*
3	TSs-57	African yam bean	*Sphenostylis stenocarpa*
4	TSs-84	African yam bean	*Sphenostylis stenocarpa*
5	TSs-10	African yam bean	*Sphenostylis stenocarpa*
6	TVSU-870	Bambara nut	*Vigna subterranean*
7	TVSU-1034	Bambara nut	*Vigna subterranean*
8	TVSU-1482	Bambara nut	*Vigna subterranean*
9	TCg 4	Sword bean	*Canavalia gladiate*
10	TCg-1	Sword bean	*Canavalia gladiate*
11	TPt-30	Winged bean	*Psophocarpus tetragonolobus*
12	TPt-06	Winged bean	*Psophocarpus tetragonolobus*
13	TPt-3	Winged bean	*Psophocarpus tetragonolobus*
14	2006-015	Lima bean	*Phaseolus lunatus*
15	2006-011	Lima bean	*Phaseolus lunatus*
16	2006-001	Lima bean	*Phaseolus lunatus*
17	TCe-5	Jack bean	*Canavalia ensiformis*

Table 2. Presence and relative abundance of protein fractions in seventeen underutilized beans and peas analyzed by SDS-PAGE.

S/N	Protein region (kDa)	Bean and pea samples																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	245	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	140	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
3	135	+	+	-	++	++	+	+	+	+	+	+++	+++	+++	++	+	+	+
4	92	+	+	-	-	-	-	+	-	-	-	+++	+++	+++	++	+	-	-
5	63	++	+	+++	++	+++	+++	++	+	+	++	+++	+++	++	++	++	++	++
6	40	++	+++	+++	++	+++	+++	++	+	-	-	++	+++	+++	++	++	++	++
7	30	++	+++	+++	++	+++	++	+	++	++	+	+	++	+++	++	++	++	++
8	23	-	-	-	-	-	-	-	++	++	+	-	-	-	++	-	++	++
9	17	++	++	++	+	+	+	+	+	++	+	+++	+++	+++	++	+	+	+
10	11	+	+	+	+	+	+	+	+	+	+	++	+	++	+	+	+	+

Sample ID: 1: African yam bean TSs-84; 2: African yam bean TSs-10; 3: African yam bean TSs-57; 4: Bambara TVSU-870; 5: Bambara TVSU-1034; 6: Bambara TVSU-1482; 7: Jack bean TCe-5; 8: Lima bean 2006-015; 9: Lima bean 2006-011; 10: Lima bean 2006-001; 11: Pigeon pea CITA-2; 12: Pigeon pea A078-99; 13: Sword bean TCg-4; 14: Sword bean TCg-1; 15:Winged bean TPt-30; 16: Winged bean TPt-06; 17: Winged beanTPt-03.

DISCUSSION

Seed storage proteins of peas and beans varieties are heterogeneous, comprising of several fractions of biologically active proteins. The SDS-PAGE separated the total seed storage proteins of the studied varieties into components with molecular weight (MW) range from 11.0 kDa to 140.0 kDa. There were variations in protein fractions of the studied beans. Although bean and pea cultivars are reportedly similar in their protein composition (Shuaib and Alam, 2007), the quantity of the polypeptide may vary among the cultivars, and fraction variation may also exist among the varieties or closely related species as observed in the present study. Protein fractions of 11, 17, 30 63 and 135 kDa were common to all the studied beans, but differed in abundance. This suggests that these regions are conserved for the beans. The observed variation in seed storage protein could be genetically controlled by the encoding system and the difference in the protein coding and profile can be of evolutionary importance (Khoshroo *et al*., 2013). For instance, the occurrence of lypoxigenase (Lox) (92.7 - 93 kDa) in abundance in just four samples (Pigeon pea CITA-2, Pigeon pea A078-99, Sword bean TCg-4 and Sword bean TCg-1) showed that they are similar in term of the protein fraction. Thus, the existing variation in conserved seed storage protein may be of great significance in delimiting, conservation and utilization of the genetic resources.

The electrophoregram revealed that proteins with higher molecular weight (63 - 135 kDa) were more prominent and more abundant as characterized by higher intensity. However, the degree of abundance as signified by the band intensity varied. In an earlier study on functional properties of pea proteins, Barac *et al.* (2011) demonstrated that the proteins are in three basic groups consisting of a subunit of vicilin, as well as two subunits of convicilin (77.9 kDa, 72.4 kDa). The authors noted that legumin (40.89, 22.3 and 23.1 kDa) were the most abundant in the peas. The present study is in congruence with the earlier report as proteins in the regions of 40 - 92 kDa were in abundance. Also, Animasaun *et al*. (2017) elucidated similar pattern of protein profiles among common beans used as food in Nigeria. Furthermore, Kimura *et al*. (2008), reported similar protein profile in pea, fava bean, cowpea and French bean. In another study, Tavano *et al*. (2008) demonstrated that chickpea seed storage protein analyzed by SDS-PAGE showed the presence of polypeptide globulin fractions of 12.4 - 67 kDa while Paredes-Lopez *et al*. (2006) documented protein of molecular weight distribution between 16.6 and 66.4 kDa in chickpea flour.

SDS-PAGE analyses of cotyledon proteins exhibited remarkable similarities among the bean varieties, but nevertheless, there are inter-specific variation in term of abundance and even polypeptide fractions (Dziuba *et al*., 2014). Seed storage protein and polypeptide types could define the utility of the leguminous protein sources and consequently, information on availability and abundance of protein fractions in beans and peas is imperative for preferential selection for nutrition purposes. The SDS electrophoregram revealed that proteins of lower molecular weight (11 - 20 kDa) were less in abundance, with regions basically consisting of lysozymes and protease inhibitor polypeptides (Barac *et al*., 2011). The presence of these proteins in lesser amount is an indicator that the bean varieties and species could be utilized as sources of cheap proteins. This is apparent as presence of low molecular weight protein in particular; protease

inhibitor (11 kDa) in small quantity would reduce the toxic constituent of the bean (Liener and Kakade, 1969). The obtained protein fragments in this study signify that all the bean species and varieties employed are potential good sources of plant based proteins for man and animals.

The dendrogram generated by average linkage method and correlation coefficient distance cluster analysis based on SDS-PAGE protein profile revealed the beans are related based on protein content. Polypeptides with molecular weights 17, 30 and 63 kDa were the most conserved for delimiting African yam bean, jack bean, pigeon pea and Bambara groundnuts. This indicates that there might be a very close genetic relationship among the species. The abundance of leguminin and vicilin in the beans inform their utility as cheap and common source of protein and could be used alternatively. Other protein fractions varied remarkably and may be employed to classify or separate the beans into different classes (Yüzbaşıoğlu *et al*., 2008). It has been proposed that seed protein profiles are a veritable tool for studying relationships among and within some species (Duran *et al*., 2005; Lioli *et al*., 2005). In contrast, some were of the opinion that protein profile information may be insufficient for the discrimination at the cultivar level (Panella *et al*., 1993; Yüzbaşıoğlu *et al*., 2008; Berber and Yasar, 2011). Meanwhile, this study allured that SDS-PAGE of seed proteins showed banding patterns that is sufficient for discrimination of the bean species, and our findings in this study corroborate the report of Lioli *et al*. (2005) that protein profile data could be utilized in species and varietal characterization.

Conclusion: In conclusion, this study demonstrated that SDS-PAGE protein profiles could provide enough information as typing tool for the differentiation of underutilized bean and pea genotypes in Nigeria. In addition, this study showed that there are inter-varietal and inter-specific variations in protein composition of the underutilized beans. Meanwhile, from nutritional point of view, the studied beans are good alternative sources of protein as they contain little amount of protease inhibitor which make them safe for nutrition formulation as human food and animal feeds.

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