FRACTIONALIZATION OF SEED STORAGE PROTEIN IN SOME SELECTED PEA AND BEAN VARIETIES USE AS FOOD IN NIGERIA BY SDS-PAGE

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Abstract
In this study, nineteen accessions of pea and beans varieties commonly grown for food in Nigeria were assessed for variability and diversity in seed storage protein by sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The electrophorogram revealed presence of protein in the rage of 11-120 kDa in the accessions. Low intra-specific heterogeneity was recorded among the accessions, however, inter-specific protein bands was remarkable. Protein fractions with molecular weight of 85 and 120kDa occurred in all the accessions and were also the most abundant. Highest protein fractions were found in accessions NGB/VU/040, NG/OA/11/08/043and NG/SA/07/0098 which are all cowpea varieties, followed by lima bean accessions NG/SA/07/133 and NGB/06/054. Protein fractions of African yam bean accessions were higher than those of pigeon pea and winged bean. At the genetic similarity index of 0.46, the accessions were separated into two main groups, with each group further partitioned into two sub-clusters based on protein band pattern. Accessions with similar protein composition clustered together irrespective of their varietal status. The study concludes that SDS-PAGE analysis is a veritable tool in the assessment of the endospermic proteins in peas and beans. Since seed storage protein is the major determinant of end-use quality of beans in Nigeria, the studied accessions had sufficient protein fractions to justify their dietary use. The genetic diversity and variability of the seed storage proteins obtained in the present study could be useful in identification of protein high yielding varieties for crop improvement and seed production.

Keywords: bean varieties; electrophoregram; protein fractions; protein variation; SDS-PAGE;

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1. INTRODUCTION

Leguminous grains are one of the most important component of global food security, a major source of dietary proteins and calories need for several millions of poor and malnourished children in Africa and Asia countries with low socio-economic index (FAO, 2004; Diouf, 2011). The bean family are dietary mainstay in the region where religious preferences discourage the consumption of animal protein. Many pea and bean species and varieties are cultivated worldwide for green pea vegetables, fresh seed grains or dried seed which are used for several purposes (Nasir et al. 2007).

In Nigeria, pea and pulse beans have the largest usable proteins content of all cultivated legumes and are arguably one of the most important plant protein. Cultivated peas are not only a sources of amino acids to huge population of Nigeria, but may also play a bioactive role and/or can be precursors of biologically active peptides with various physiological functions. In this context, plant proteins and their derivative protein hydrolysate are increasingly being used as an alternative to proteins from animal source in human nutrition (Nwosu et al. 2013). Leguminous grains contain numerous enzymes including amine oxidase (Mann, 1995) *a*-amylase **(**Eric and Stanley, 1990), protease inhibitor (Liener and Kakade, 1969), L-glutaminase (Murray and Ireland, 1980**)** and asparaginase (Sodek et al. 1980) which are of great importance in human and animal dietary

The use of biochemical markers for the assessment of genetic diversity and variability in crop plants is becoming more popular. Among the biochemical techniques, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is often used due to its easiness and effectiveness in marking genetic relationship of crop germplasm. Consequently, the technique have been effectively used to study various classes of

storage proteins in plants (Rao et al. 1992; Elfallah et al. 2008; Khoshroo et al., 2013), varietal identification, hybrids detection and determination of percentage genetic purity of F_1 progenies (Roy et al. 2010).

Traditionally the classification of legume protein is based on their solubility properties. Variation in seed protein patterns of common peas and beans species and varieties utilized as food in Nigeria have not been well studied. There is need to comparatively evaluate and determine the protein status and fidelity of such beans. This study therefore, was designed to investigate variability and genetic divergence of various peas and bean pulses consume in Nigeria using SDS-PAGE analysis. This would help to identify novel germplasm or peas variety that could serves as a source of valuable genes for protein content improve in bean varieties and species.

2. MATERIALS AND METHODS

Seed collection and preparation

Good and healthy seeds of nineteen varieties of bean and pulses collected from National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Oyo state, Nigeria were used for the study. The varietal names, accession numbers and appearances of the peas and beans are shown in Table 1 and Plate1.

Methods

Chemicals and glassware

All glassware used were of high quality from Pyrex, (USA). Chemicals used were of high analytical/molecular grade purchased from Sigma-Aldrich, Germany and Merck, Germany. The glassware were thoroughly washed with detergent solution and rinsed with warm distilled water and then autoclaved at 15 atmospheric pressure, 110 ^{o}C for 20 min. The autoclaved wares were dried in hot air oven at 80 ^{o}C before used.

Protein extraction by buffer method

Protein extraction buffers were prepared using the method of Ahmed (2005). The extraction buffer comprised of 0.5M Tris-HCl (pH 8.0), 0.2% Sodium Dodecyl Sulfate (SDS), 5M urea and 1% β-marcaptoethanol balanced at pH 8.0 with con HCL and KOH. Two drops of bromophenol blue (BPB) was added. The mixture was vortexed thoroughly to homogenize and then kept in a refrigerator at 40 ^{0}C till use. Protein was extracted from the seeds by grounding seed into fine powder. 400 µl of the extraction buffer was added to approximately 0.1 g of seed flour in a 1.5 ml microtube. The solubilized samples were centrifuged at 13000 rpm for 10 minutes at room temperature and the supernatant was transferred to new 1.5 ml tube. 100 ml of the extract mixed with 20 ml of loading dye (loading dye: A few drops of bromophenol blue, SDS 10% +1.5M Tris-Hcl, pH =8.8+ β-Mercaptoethanol + Glycerol) was added and stored at 4 ^{0}C.

Preparation of gel

The gel preparation followed the method of Laemmli (1970). SDS-PAGE of total seed protein was resolved by 20% polyacrylamide slab gels in discontinuous buffer system with vertical slab gel organized in a glass sandwich. The separating gel contained 20% by weight acrylamide and 0.135% by weight N-Nmethylene-acrylamide in 0.5M Tris-HCl buffer (pH 8.8) with 0.27% SDS. The gel was polymerized chemically by adding 15 µl Tetramethylene-diamine (TEMED) and 10% Ammonium per sulphate (APS). The staking gel consisted of 30% acrylamide, 0.8% N.N-methylenebis-acrylamide in 0.25M Tris-HCl buffer (pH 6.8) of 0.2 SDS. The electrode buffer whish comprised of Tris-glycine (3.0g Tris-HCl and 14.4 g glycine per liters of buffer solution at pH 8.9) and 3.0g SDS (0.1%) was used.

Procedure for the SDS-PAGE

Ten microliters (10 µl) of sample was applied into the separation stacking gel sample wells the molecular weight marker of 11-245 kDa (BlueEye PAGE ruler) was used as the standard. A constant 200-150 mA electrical current was applied to the electrophoretic unit. The gels were stained with Coomasie Brilliant Blue R-250 containing 10% acetic acid, 45% methanol, and 45% water overnight. The gels

were then distained by washing with the solution of methanol, acetic acid and distilled water 20:5:75 respectively until the color of background disappeared and electrophoresis bands were clearly visible. The gel was photographed with a gel documentation system (Biorad, USA)

Data analysis

Electrophoregrams for each accession were scored for presence (1) or absence (0) of the polypeptide bands shown by the standard marker. All the monomorphic and polymorphic bands that were visible to the eye were scored and unclear or ambiguously bands were not used in the analyses. The data were entered into a binary data matrix and similarity index was calculated by the formula of Sneath and Sokal, (1973).

$$S=W/(A+B-W)$$

Where W is the number of bands of common mobility, A is the number of bands in type A and B is the number of bands in type B. Data analyses were conducted using NTSys-pc, version 2.2 (Exeter software, Setauket, N.Y.). Cluster analyses were conducted on similarity estimates using the unweighted pair- group method for arithmetic averages (UPGMA) and the resulting clusters were expressed as dendrogram.

3. RESULTS AND DISCUSSION

3.1. RESULTS

High and low molecular weight proteins subunits of different accessions of pea and beans were separated by SDS-PAGE electrophoresis for characterization and evaluation of protein variability and genetic diversity among the accessions. Protein banding pattern of the pea and bean varieties are presented in Figure 2. The total number of bands vary between 5 and 9 in different accessions. About 66.67% of the protein bands were polymorphic and 33.33% were monomorphic. Protein within the molecular weights of 85 – 120 kDa were common to all the samples, similarly, protein in the regions of 50-77 kDa occurred in most of the pea and bean varieties or species analyzed which protein occurrence are limited to few accessions.

Table 1: Accession name, common name scientific name of beans, pulse and peas used for the SDS-PAGE protein analysis

S/No	Accession Number	Common Name	Scientific name
1	45 CL-T307	African yam bean	*Sphenostylis stenocarpa*
2	NG/AT/APR/09/014	African yam bean	*Sphenostylis stenocarpa*
3	93AIAR&T REP2	African yam bean	*Sphenostylis stenocarpa*
4	NG/OA/09/11/058	African yam bean	*Sphenostylis stenocarpa*
5	94A NGB	African yam bean	*Sphenostylis stenocarpa*
6	NG/OA/016	African yam bean	*Sphenostylis stenocarpa*
7	NG/OA/017	African yam bean	*Sphenostylis stenocarpa*
8	NG/SA/07/1330	Cowpea	*Vigna unguiculata*
9	NGB/VU/040	Cowpea	*Vigna unguiculata*
10	NG/OA/11/08/043	Cowpea	*Vigna unguiculata*
11	NG/SA/07/0098	Cowpea	*Vigna unguiculata*
12	NGBO1468K3	Pigeon pea	*Cajanus cajan*
13	NG/OA/11/08/012	Pigeon pea	*Cajanus cajan*
14	NGBO1456	Pigeon pea	*Cajanus cajan*
15	TB87/7030	Butter bean	*Phaseolus lunatus*
16	NG/SA/07/133	Lima bean	*Phaseolus macrocarpus*
17	NGB/06/054	Lima bean	*Phaseolus limensis*
18	NG/SA/11/0018	Winged bean	*Psophocarpus tetragonolobus*
19	NG/OA/07/0019	Winged bean	*Psophocarpus tetragonolobus*

The distinct patter and summary of protein presence in the analyzed samples based on the SDS-PAGE band formation pattern is shown in Figure 3. Seven clear protein regions viz: 11, 20, 35, 50, 77, 85, and 120 kDa were observed. Other bands were faint and not definite relatively to molecular weight marked used. The intensity of the bands indicated the abundance of the protein occurrence in that region. The result showed molecules within 85-120 kDa were the most abundant, followed by 50-77kDa among the accession, protein in the band region of 11-20kDa occurred in traces even in the accession where present (Fig 3).

The result of cluster analysis is given in the dendrogram (Figure 4) on the bases of linkage distance by the procedure of unweighted pair group method with arithmetic means (UMPGMA). Cluster analysis sorted the bean accessions into two (2) major groups (lineages) A and B at similarity index of about 0.45. Each of the major group were further slip into two clusters A1;A2 and B1;B2 respectively. The A1 cluster had 2 sub-clusters A1(i) and A1(ii). While A1(i) had a sole member (NG/OA/016), A1(ii) consisted of three African yam bean accession. The A2(i) subgroup on the other hand had six heterogeneous members of 2 closely associated cowpea, three similar pigeon pea and a distant African yam bean accession (93A IAR&T-REP2). In contrast, the sub-cluster A2(ii) had only two member which are African yam bean accessions. At similarity index of 0.58, the B major group divided into B1 and B2. The three lima bean accession converged to form the B1 sub-cluster while the B1 comprised of mixture of two winged bean and two cowpea accessions that were similar at index above 0.96. The number and names of the accessions or varieties in each group and cluster is summarized in Table 2.

3.2. DISCUSSION

The SDS-PAGE electrophorogram of the endosperm protein of different varieties and species of pea (accessions) studied revealed variation in protein composition. The variation in protein content obtained in this study was similar to the previous study on accessions of cowpea (Shuaib and Alam Zeb, 2007). Although the authors alluded that pea accessions showed low level of heterogeity and the present results aligned in this perspective but, remarkable interspecies seed protein diversity was found among different bean species. This suggest that basically, the seed protein composition varied among different beans which may be due to genetic make and differential amino acid syntheses. The pattern of protein bands in different plants can be related to their origin, evolution, and genome composition (Khoshroo et al., 2013). In addition, variation in amounts of protein in genes encoding system may account for the difference and abundance of protein bands revealed by SDS-PAGE. Protein types and their diversity varied among a variety of crop species, which may assist in detection of species at seed level and provide information on clarity of genetic resources.

Table 2. Grouping of 19 accessions of pea and beans use for food in Nigeria based on cluster analysis using SDS-PAGE analysis.

Accession group	Cluster	Sub-cluster	No of accession	Accession name
A	A1	A1(i)	1	NG/OA/016
		A1(ii)	3	NG/OA/09/11/058; 94ANGB; NG/OA/017
	A2	A2(i)	6	NGB/VU/040; NGB/11/08/043; NGB01488K3; NGB01456; NG/OA/11/08/012
		A2(ii)	2	45 CL-T307; N/AT/APR/09/014
B	B1	-	3	TBB7/7030; NGB/06/054; NG/SA/07/133
	B2	-	4	NG/SA/11/0018; NG/SA/11/0019; NG/SA/07/0098; NG/SA/07/1330

Protein with molecular weight in the regions of 85-120 kDa and were common and in abundance in all the accessions, most had protein within 50-77 kDa while the rest marked protein varied for the varieties and species studied. The presence and intensity of these protein fractions specifies that the genes coding for them are conserved. Leguminous gluten proteins are either high (50 -120 kDa) or low (11- 40 kDa) molecular weight proteins (Lawrence and Sheperd, 1980). SDS-PAGE analysis of seed protein of selected peas and bean used as food in Nigeria revealed four high molecular weight proteins (50kDa, 77kDa, 85kDa and 120kDa) and three low molecular proteins (11kDa, 20kDa and 35kDa) which conformed to Shuaib and Alam Zeb, (2007). The presence of protase inhibitor (11kDa), Lysozyme (20kDa), Legumin (23kDa), β-Lactoglobulin (25kDa), Vicillin (35kDa), Ovalbumin (50kDa), Convicillin (77kDa), Bovine serum albumin (85kDa) and β-Galactosidase (120kDa) in most bean varieties utilized as food in Nigeria accounts for their nutritional functions. Faint band of protein fraction 11 kDa show low of protase inhibitor which was termed toxic constituent of foodstuff (Liener and Kakate, 1969). Since seed storage protein is the major determinant of end-use quality of peas and beans, information on types and abundance of proteins is important for preferential selection for breeding and nutrition purposes.

Fig 1: Physical appearances of the pea and pulse bean varieties and species analyzed for protein variability using SDS-PAGE

A= African yam bean, 45CL-T307; B = African yam bean, NG/AT/APR/09/014; C = African yam bean, 93/AIAR&T-REP2; D = African yam bean, NG/OA/09/11/058; E = African yam bean, 94ANGB; F = African yam bean, NG/OA/016; G = African yam bean, NG/OA/017; H = Cow pea, NG/SA07/1330; I = Cow pea, NGB/VU/040; J = Cow pea, NG/OA/11/08/043; K = Cow pea, NG/SA/07/0098; L = Pigeon pea, NGBO1468K3; M = Pigeon pea,

NG/OA/11/08//012; N= Pigeon pea, NGBO1456; O = Butter bean, TB87/7030; P = Lima bean, NG/SA/07/133; Q = Lima bean, NGB/06/054; R = Winged bean, NG/SA/11/0018; S = Winged bean, NG/OA/07/0019

Cluster analysis showed low intra-varietal variations among the beans studied, as varieties of the same species coexist in the same cluster at similarity index above 0.96. Low variation in seed storage proteins have been reported in genotypes of sesame germplasm (Fazal et al. 2012) and chickpea accessions (Ghafoor et al. 2003). In term of intra-varietal seed protein constituent, the present result was in agreement with these earlier studies and also corroborated the findings of Mehrani (2002) on a low level of intra-specific variation in seed protein among cowpea varieties. In contrast, Nisar et al. (2007), elucidated there was remarkable intra-specific variation in seed proteins among local and exotic chickpea germplasm. Meanwhile, in the present study, remarkable inter-varietal dissimilarity was obtained in the seed storage protein of peas and beans accessions as varieties and accessions from different species clustered together which indicated they are genetically linked based on protein composition. Varieties of the same species which fell into different clusters are likely to be gradually undergoing genetic isolation as seed proteinare stable and not readily controlled by environmental influences.

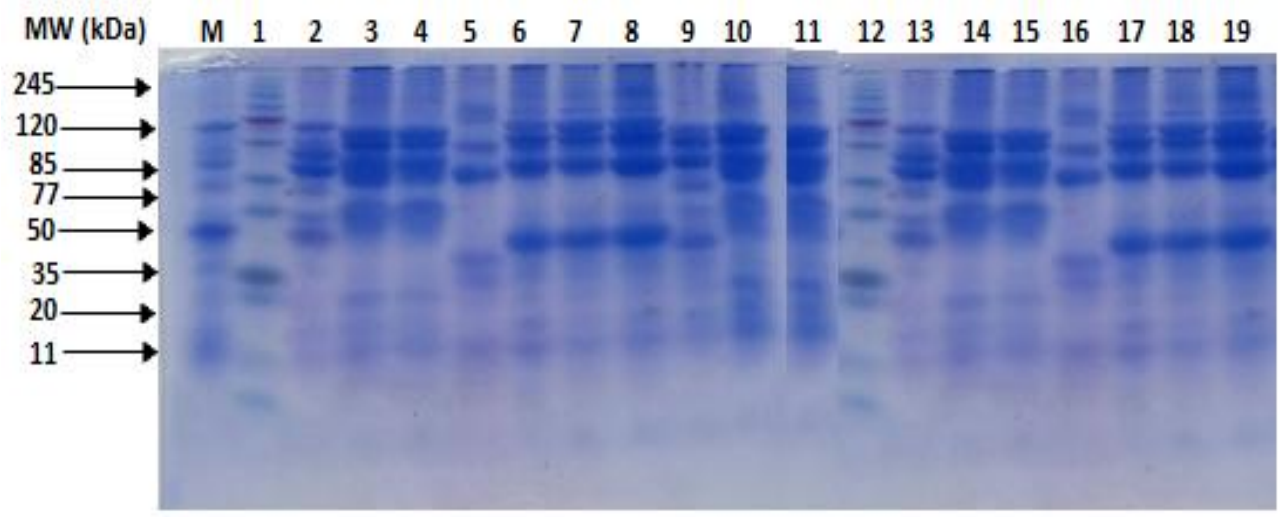

Fig. 2: Electrophoregram of seed storage protein pattern of some selected pea and bean varieties use as food in Nigeria

1= Africa yam bean 45 CL-T307; 2= Cowpea NG/SA/07/1330; 3= Cowpea NGB/VU/040; 4=Cowpea NG/OA/11/08/043; 5=Cowpea NG/SA/07/0098; 6=Pigeon pea NGBO1468K3; 7= Pigeon pea NG/OA/11/08/012, 8= Pigeon pea NGBO1456; 9= Butter bean TB87/7030, 10= Lima bean NG/SA/07/133; 11= Lima bean NGB/06/054; 12= Africa yam bean NG/AT/APR/09/014; 13= Africa yam bean 93AIAR&T REP2, 14= Africa yam bean NG/OA/09/11/058; 15= Africa yam bean 94ANGB; 16= African yam bean NG/OA/016; 17= African yam bean NG/OA/017, 18= Winged bean NG/SA/11/0018; 19= Winged bean NG/OA/07/0019.

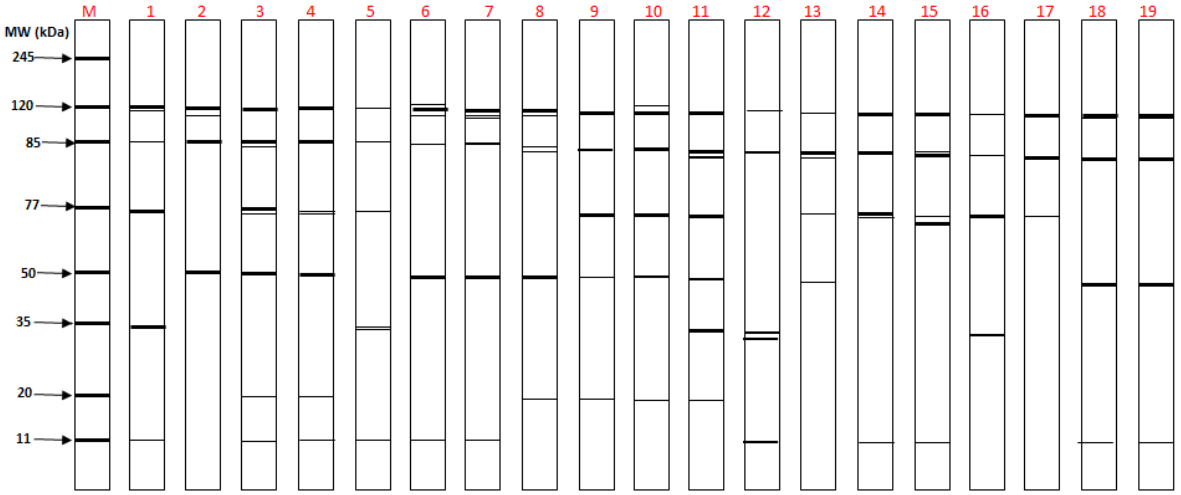

Fig 3: Zymogram of total soluble seed proteins of some pea and bean varieties/accessions use for food in Nigeria obtained through SDS-PAGE

1= Africa yam bean 45 CL-T307; 2= Cowpea NG/SA/07/1330; 3= Cowpea NGB/VU/040; 4=Cowpea NG/OA/11/08/043; 5=Cowpea NG/SA/07/0098; 6=Pigeon pea NGBO1468K3; 7= Pigeon pea NG/OA/11/08/012, 8= Pigeon pea NGBO1456; 9= Butter bean TB87/7030, 10= Lima bean NG/SA/07/133; 11= Lima bean NGB/06/054; 12= Africa yam bean NG/AT/APR/09/014; 13= Africa yam bean 93AIAR&T REP2, 14= Africa yam bean NG/OA/09/11/058; 15= Africa yam bean 94ANGB; 16= African yam bean NG/OA/016; 17= African yam bean NG/OA/017, 18= Winged bean NG/SA/11/0018; 19= Winged bean NG/OA/07/0019.

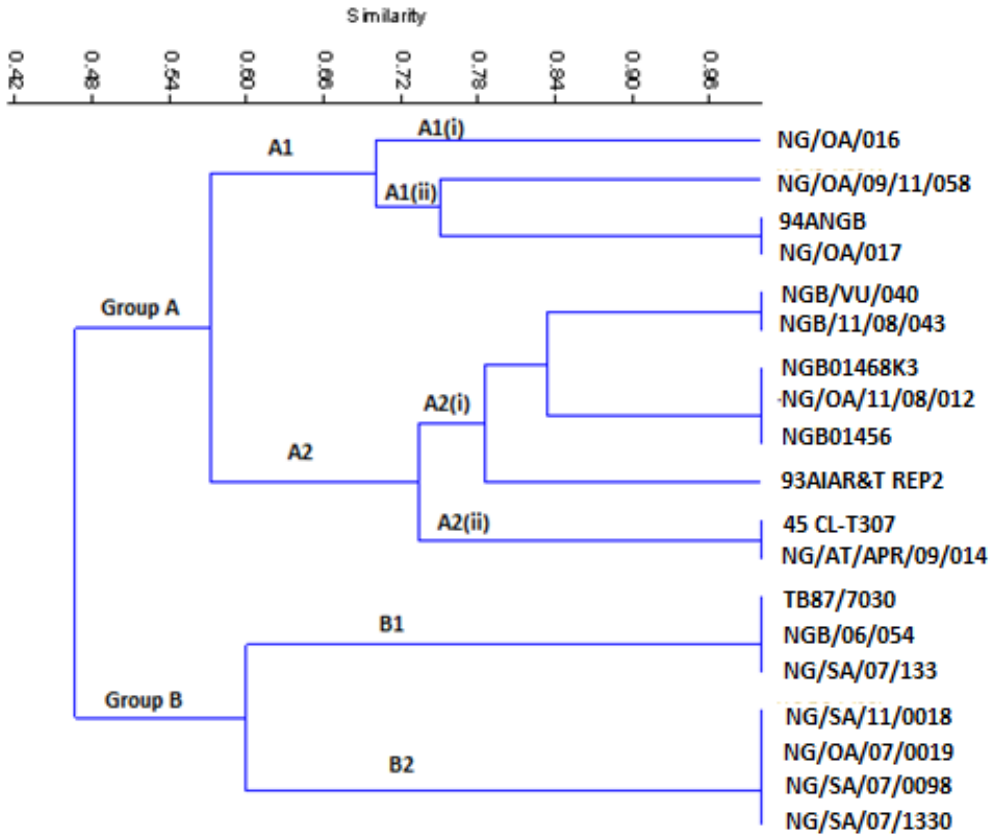

Fig. 4: Dendrogram of nineteen accessions of pea and bean varieties use for food in Nigeria based on SDS-PAGE bands

5. CONCLUSION

The study showed that SDS-PAGE is an effective method for fractionating seed storage proteins in leguminous grains. The low intra-specific diversity obtained in this result showed that accessions of same variety were similar in protein content. However, those of different species varied in some protein fractions in quality and quantity. The results showed the selected peas and beans had sufficient protein fractions which could be used to predict their fidelity in term of utilization for dietary purposes. Since seed storage protein is the major determinant of end-use quality (a highly selected trait) of peas and beans, the study of genetic diversity and variability in seed storage proteins of the leguminous grains would enhance usability and preference of the identified protein high yielding varieties for crop improvement and seed production in Nigeria.

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