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Electrophoretic Characterization of Water Soluble Seed Proteins and the Relationship between Some Legume Species in Nigeria

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Abstract: Legume seeds are a rich source of dietary proteins consumed by humans and livestock. In this research, a total of six species of grain legumes *viz., Vigna unguiculata* sub- specie *unguiculata* (black-eyed bean), *Vigna unguiculata* (patisco), *Vigna unguiculata unguiculata* sub- specie *sesquipedalis* (yardlong bean), *Phaseolus vulgaris* (red kidney bean), *Cajanus cajan* (pigeon pea) and *Mucuna pruriens* (velvet or devil bean) consumed in eastern Nigeria were studied to assess their genetic variability and relationship. These grains were characterized by the numerical analysis of seed protein profiles obtained by using native - and SDS- PAGE techniques. The average polymorphisms of these species were 0.00% in Native-PAGE (β-mecarptoethanol absence) and SDS-PAGE (β-mecarptoethanol presence), 10.71% in SDS-PAGE (β-mecarptoethanol absence) and 57.769% in Native-PAGE (β-mecarptoethanol presence). *V. unguiculata unguiculata* sub- specie *sesquipedalis* and *P. vulgaris* showed the highest similarity index (94.12%) while *M. pruriens* and the two species *V. unguiculata unguiculata* sub- specie *unguiculata* and *V. unguiculata* showed the lowest (22.2%) in Native-PAGE. Also, *V. unguiculata unguiculata* and *V. unguiculata unguiculata* sub- specie *sesquipedalis* showed the highest similarity index (72.72%) while *P. vulgaris* and *C. cajan* showed the lowest (0.00%) in SDS-PAGE. Cluster analysis showed that water soluble proteins in *V. unguiculata unguiculata* sub- specie *unguiculata* and *C. cajan*; *V. unguiculata unguiculata* sub- specie *sesquipedalis* and *M. pruriens* are closely related to each other. The grains contained proteins with molecular weights in the range of 20-28 kDa. This study indicated that the numerical analysis of seed protein profiles offer no concrete answer to the identity of the bean species studied, however, the method provided extra banding pattern for the discrimination of these bean species consumed in Nigeria.

Key words: Legumes seeds · Grains/beans · Native-PAGE · SDS-PAGE · Genetic diversity · Seed storage proteins

INTRODUCTION

Grain legumes are important sources of food proteins. In many regions of the world, legume seeds are the unique protein supply in the diet of humans and livestock [1], thus, they are usually referred to as "poor man meat" [2]. Very often they represent a necessary supplement to other protein sources [3]. Therefore, the dietary importance of legume seeds is expected to grow in the years for the protein (and other nutrients) demand of the increasing world population and the need of reducing the risks related to consumption of animal food sources, especially in the developed countries [4, 5]. In addition, legumes replenish soil nitrogen because nodules on their root hairs contain nitrogen-fixing bacteria, which make them important in crop rotation [6]. Well known grain legumes include beans, lentils, lupins, peas and peanuts and are cultivated for their seeds, also known as pulses [7, 8]. Legume grains cultivated and consumed in Nigeria are annuals which are usually creepers or climbers are thought to have originated from Latin America. They are available all year round in Nigeria where a substantial

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quantity is cultivated in the northern part of the country by inter-planting them with crops such as maize, sorghum, sweet potatoes, coffee, cotton and yam [2]. Today, mostly domesticated populations and modern breed bean varieties are grown [2, 9].

Genetic diversity is important for improving any crop species. An important understanding of the magnitude and pattern of genetic diversity in crop plant has important implications in breeding programs and for conservation of genetic resources [10]. Genotyping of different species is necessary for characterization of different accession of crop germplasm, testing varietal purity and registration of newly developed cultivars [11]. There are numerous techniques for assessing the genetic variability and relationship, however, electrophoretic characterization of seed storage proteins remains a valid method to varietal identification and to classify plant varieties [12, 13]. Usually the electrophoretic mobility of proteins has been used to study relationships at the species and subspecies levels [14]. Storage seed proteins are suitable genetic markers because they are highly polymorphic, their polymorphism is genetically determined and the molecular sources of their polymorphism are known, they are not sensitive to environmental fluctuations, are conservative and their banding pattern is very stable which are added advantages for been used for cultivars identification purposes in crops [15]. Seed storage protein profiling based on SDS-PAGE can be employed for various purposes, such as characterization of germplasm [16, 17], varietal identification [18], biosynthetic analysis and the determination of genetic diversity and phylogenetic relationship between different species [13, 15, 19, 20]. Electrophoretic analyses are simple and inexpensive which is added advantage for use in practical plant breeding [15]. Genetic diversity of seed storage proteins via SDS-PAGE has been reported for wild and cultivated rice [21], lima bean [22], *Phaseolus vulgaris* [23] and chickpea [10, 24]. Presently, there are limited if not no information on the genetic diversity and phylogenetic relationship between the different species of legume grains cultivated in Nigeria.

The main objective of the present study was therefore aimed at evaluating the genetic diversity and relationships in six cultivated Nigerian bean species by employing seed storage protein profiling based on electrophoresis and also to ascertain whether the electrophoresis of seed proteins is suitable for verification of taxonomic data based on the morphological ones.

MATERIALS AND METHODS

Materials: Sodium dodecyl sulfate (SDS), β-mercaptoethanol (β-ME), acrylamide, polyacrylamide, Coomassie Brilliant Blue R and molecular weight markers (14-78 kDa) used were of analytical grade and were purchased from Sigma-Aldrich Chemical Co, St Louis, MO, USA. All reagents were freshly prepared unless otherwise stated and deionized water was used throughout.

Sample Sources and Characteristics: The germplasms of six different species of mature legume grains were obtained from local markets in Anambra State, South- East region of Nigeria. The locations of collection and the seed characteristics are described in Table 1. All bean species were identified by Onyeukwu, C.J. from the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

The seeds were dehulled and ground well using a Waring commercial blender (Smart Grind, Black and Decker, Towson, MA, USA). The flour was defatted as described by [25] in three hexane extractions (10 ml hexane/g flour), each for 2 hours with slow stirring at 4°C. After the n-hexane layer was discarded, the flour was air-dried. With the aim to remove the impurities and to obtain a uniform product, the whole flour was sieved through a net with mesh size of 75μm. Flour samples were packaged in sealed low density polyethylene bags and stored in refrigerators prior to analysis.

Methods

Protein Extraction: Water soluble seed protein extracts were prepared from the six species of bean according to the method of [25] with minor modifications. A portion (30 mg) of defatted flour was mixed with 0.5 ml of deionized water in an Eppendorf tube overnight at room temperature and then centrifuged in micro-centrifuge machine (Eppendorf) at 23 000 xg for 15 min at 15 °C. The residue was re-extracted twice under the same conditions. All the extracts were combined and stored at −10°C until used. Total protein was estimated as reported elsewhere [26] using bovine serum albumin as standard protein.

Electrophoresis: Protein separation was carried out in vertical slabs using the TV50 Camlab Vertical Electrophoresis Unit. Gel electrophoresis of the extracted water soluble seed proteins were performed using 5% stacking and 12.5% separating gels according to the method reported elsewhere [27] with modifications.

Table 1: Seed characteristics of some legume grains used for electrophoretic characterizations.

Species name	Sample code	Source	Seed size
Vigna unguiculata sub-specie *unguiculata* (Black-eyed bean)	1	Ihiala (Nkwogbe market)	Bold
Vigna unguiculata (Patisco)	2	Ihiala (Nkwogbe market)	Small
Vigna unguiculata sub-specie *sesquipedalis* (Yardlong bean)	3	Ihiala (Nkwogbe market)	Small
Phaseolus vulgaris (Red kidney bean)	4	Ihiala (Nkwogbe market)	Bold
Cajanus cajan (Pigeon pea)	5	Ihiala (Nkwogbe market)	Small
Mucuna pruiens (velvet bean)	6	Uli	Bold

The polymerization mixture for native PAGE contained 16.7 ml of 30% acrylamide, 10 ml of 4x resolving gel buffer (pH 8.8),13.2 ml deionized water, 200 µl of 10% ammonium persulfate and 13.3 µl of TEMED. The stored water- soluble seed protein extracts (10 µl) were solubilised in sample buffer consisting of 4x stacking gel buffer (pH 6.8), deionized water; 10% glycerol and 0.1% bromophenol Blue and 20 µl was applied to the gel. For native PAGE under reducing condition, 5% β-ME was present in the sample buffer but was absent in non-reducing conditions.

The polymerization mixture for SDS PAGE contained 16.7 ml of 30% acrylamide, 10 ml of 4x resolving gel buffer (pH 8.8), 0.4 ml of 10% SDS, 12.8 ml of deionized water, 200 µl of 10% ammonium persulfate and 13.3 µl of TEMED. The stored water- soluble seed protein extracts (10 µl) were solubilised in sample buffer consisting of 4x stacking gel buffer (pH 6.8), 10% SDS; 10% glycerol and 0.1% bromophenol Blue. The mixture was heated in a boiling-water bath for 5 min and was placed on ice until 20 µl of the mixture was applied to the gel. SDS PAGE was also carried out under reducing and non-reducing conditions and the determination of the apparent molecular weight of each protein band was carried out using molecular weight marker proteins; ovotransferrin (78 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), actinidin (29 kDa), β-lactoglobulin (18 kDa) and lysozyme (14 kDa)) as was similarly carried out elsewhere [15, 28].

The gels were electrophoresed using a current of 15 mA and a voltage of 300 V (using Consort E844 power pack) until the bromophenol blue tracker dye reached the bottom of the gel. Gels were fixed and stained with 0.2% Coomassie Brilliant blue R-250 in methanol: acetic acid: deionized water (5:4:1, v/v/v) overnight. Afterwards, the gels were destained by using the solvent of the stain mixture; methanol: acetic acid glacial: deionized water (5:4:1, v/v/v) until protein bands became clearly visible.

Protein Profile Analysis: Gel photographing and documentation were carried out with the obtained results. Data were coded as 0 (absent) and 1 (present). The dendrogram, based on the total seed protein patterns of bean cultivars, was constructed with the program PyElph version 1.4 using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Average polymorphism was calculated as a ratio of total number of polymorphic bands (TNPB) to total number of bands (TNB) (TNPB: TNB) multiplied by 100. Jaccard's similarity index was calculated as ratio of similar bands to total bands between two species multiplied by 100 [10].

RESULTS AND DISCUSSION

The water soluble seed protein profiles of six Nigerian grains species, obtained by one-dimensional Native- and SDS-PAGE along with molecular weight marker proteins (in the case of SDS-PAGE) in absence and presence of β-ME are presented in Fig. 1 and 2 respectively. The protein patterns of the species were inspected visually and compared with each other. Analyses of cotyledon proteins exhibited high similarities between all bean genotypes in their slow-mobility range for Native-PAGE and at their fast-mobility range for SDS-PAGE. Also, the six bean species contained proteins mostly in the range of 20-28 kDa (Fig. 2).

Protein Profiling: The Native- and SDS-PAGE of seed proteins of six different bean species were carried out in the presence and absence of β-ME to investigate the genetic diversity at the molecular level. Seed storage protein profiling showed distinct polymorphism in electrophoretic banding patterns that led to the detection of 54 and 52 polypeptide bands respectively under Native-PAGE in the absence and presence of β-ME and 56 and 42 bands respectively under SDS-PAGE in the absence and presence of β-ME (Table 2).

Out of 52 bands detected under Native-PAGE in the presence of β-ME only 30 were polymorphic while 6 out of the 56 bands were polymorphic under SDS-PAGE in the absence of β-ME. The rest were monomorphic. Also, no polymorphic band was detected under Native-PAGE in the absence of β-ME and SDS-PAGE in the presence of β-ME. The average polymorphisms were 0.00% for Native-PAGE in the absence of β-ME and SDS-PAGE in the presence of β-ME, 57.769% for Native-PAGE in presence of β-ME and 10.71%for SDS-PAGE in the absence of β-ME (Table 3).

Table 2: Data matrix of water soluble seed proteins of six bean species based on electrophoresis in the absence and presence of β-ME

Bean species/electrophoretic condition																							
Native PAGE in absence of β-ME						Native PAGE in presence of β-ME						SDS-PAGE in absence of β-ME						SDS-PAGE in presence of β-ME					
1	2	3	4	5	6	1'	2'	3'	4'	5'	6'	1	2	3	4	5	6	1'	2'	3'	4'	5'	6'
1	1	1	0	1	1	0	0	1	1	1	0	1	1	1	0	0	1	1	1	1	0	1	1
1	1	1	1	0	0	0	0	0	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1
0	0	0	0	1	1	1	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	1	1
0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1
0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0
1	1	1	1	1	0	1	1	1	1	1	1	0	0	0	0	0	1	0	1	0	0	0	0
0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	1	0	1	0
1	1	1	0	0	1	1	1	1	1	1	1	0	0	0	0	0	1	0	1	0	0	0	0
0	0	1	0	0	0	1	1	1	1	1	1	1	1	1	1	0	1	0	0	0	0	1	0
1	1	0	1	0	0	0	1	0	0	0	1	0	1	1	0	0	1	0	1	0	0	0	0
0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0
0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1
0	1	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	1	0	1	0	0	0	0
1	1	1	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0
0	0	0	1	1	1	1	1	1	1	0	0	1	0	1	0	0	0	1	0	1	1	0	1
0	0	0	1	0	1	1	1	1	1	0	0	1	0	1	0	0	1	1	0	0	0	0	0
0	0	0	0	0	1							0	1	0	1	0	0	1	0	0	0	0	0
0	0	0	0	1	1							1	1	0	0	0	0	0	0	1	0	0	0
1	0	1	1	1	0							1	0	1	1	0	1	0	0	1	0	0	0
0	1	0	1	0	0							1	0	0	0	0	1	1	1	0	0	0	0
0	1	1	1	1	1							0	1	0	1	1	0	0	0	1	1	0	1
												1	0	1	0	0	0	0	0	1	0	0	1

1) *V. unguiculata* sub-specie *unguiculata* (Black-eyed bean), 2) *V. unguiculata* (Patisco), 3) *V. unguiculata* sub-specie *sesquipedalis* (Yardlong beans), 4) *P. vulgaris* (Red kidney bean), 5) *C. cajan* (Pigeon pea) and 6) *M. pruriens* (Velvet bean). 1-6 and 1? - 6? denote species in the absence and presence of β-ME respectively

Table 3: Average polymorphism of six bean species in Native- and SDS-PAGE experiments

Experimental condition	TNB	TNPB	AP (%)
Native-PAGE in absence of β-ME	54	0	0.0000
Native-PAGE in presence of β-ME	52	30	57.69
SDS-PAGE in absence of β-ME	56	6	10.71
SDS-PAGE in presence of β-ME	42	0	0.0000

TNB = total number of bands, TNPB = total number of polymorphic bands and AP = average polymorphism

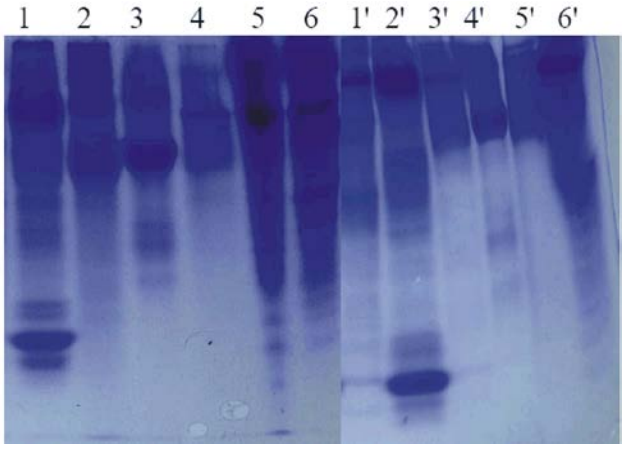

Fig. 1: Electrophoregram of water soluble seed storage proteins using Native-PAGE in the absence of β-ME (lane 1-6) and presence of β-ME (lane 1'-6'). 1) *V. unguiculata* sub-specie *unguiculata* (Black-eyed bean), 2) *V. unguiculata* (Patisco), 3)*V. unguiculata* sub-specie *sesquipedalis* (Yardlong beans), 4) *P. vulgaris* (Red kidney bean), 5) *C. cajan* (Pigeon pea) and 6) *M. pruriens* (Velvet bean)

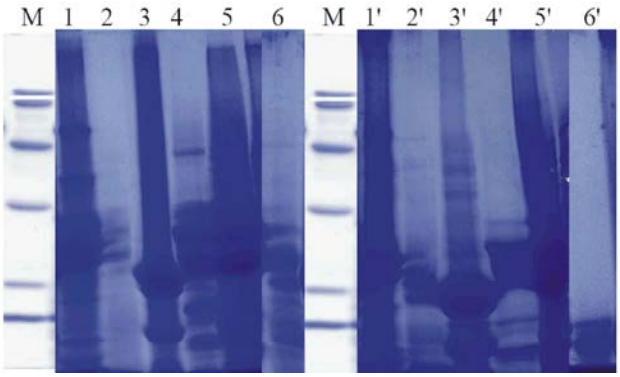

Fig. 2: Electrophoregram of water soluble seed storage proteins using SDS-PAGE in the absence of β-ME (lane 1-6) and presence of β-ME (lane 1□-6□). 1) *V. unguiculata* sub-specie *unguiculata* (Black-eyed bean), 2) *V. unguiculata* (Patisco), 3) *V. unguiculata* sub-specie *sesquipedalis* (Yardlong beans), 4) *P. vulgaris* (Red kidney bean), 5) *C. cajan* (Pigeon pea), 6) *M. pruriens* (Velvet bean) and M) molecular weight marker proteins

Table 4: Similarity index of six bean species using Native-PAGE in the presence and absence of β-ME.

Jaccard's Similarity index (%)													
Native-PAGE in absence of β-ME							Native-PAGE in presence of β-ME						
Sp	1	2	3	4	5	6	Sp	1'	2'	3'	4'	5'	6'
1	100						1'	100					
2	75.00	100					2'	72.73	100				
3	80.00	70.59	100				3'	70.00	77.78	100			
4	50.00	55.56	47.06	100			4'	66.67	73.68	94.12	100		
5	35.29	31.58	44.44	52.63	100		5'	52.63	58.82	80.00	87.50	100	
6	22.22	22.22	31.58	30.00	57.14	100	6'	55.56	75.00	71.43	66.67	76.92	100

Sp) species, 1) *V. unguiculata* sub-specie *unguiculata* (Black-eyed bean), 2) *V. unguiculata* (Patisco), 3) *V. unguiculata* sub-specie *sesquipedalis* (Yardlong beans), 4) *P. vulgaris* (Red kidney bean), 5) *C. cajan* (Pigeon pea), 6) *M. pruriens* (Velvet bean).1-6 and 1' - 6' denote species in the absence and presence of β-ME respectively

The average polymorphism of 0.00%, 10.71% and 57.69% of water soluble proteins in the bean species suggests presence of polymeric proteins which most probably must have evolved from monomerically distinct proteins. It is possible that the polymeric proteins upon treatment with SDS and β-ME dissociated and/or aggregated to form proteins of distinct molecular masses, hence 0.00% average polymorphism in SDS-PAGE experiment in presence of β-ME.

In Native-PAGE and absence of β-mecarptoethanol, *V. unguiculata unguiculata* sub- specie *unguiculata* and *V. unguiculata unguiculata* sub- specie *sesquipedalis* showed highest similarity index (80%) while lowest similarity index (22.2%) was found between *V. pruriens* and two other species (*V. unguiculata unguiculata* sub- specie *unguiculata* and *V. unguiculata* (Table 4). Also, in Native-PAGE and presence of β-ME, *V. unguiculata sesquipedalis* and *P. vulgaris* showed highest similarity index (94.12%) while lowest similarity index was found between *V. unguiculata unguiculata* sub- specie *unguiculata* and *C. cajan* (52.6%) (Table 4). Similar but not identical results were obtained for seed storage protein of cultivars of *Sesamum indicum* L [29] and cultivars of Cicer (chickpea) [10].

For SDS-PAGE in the absence of β-ME, *V. unguiculata unguiculata* sub- specie *unguiculata* and *V. unguiculata unguiculata* sub- specie *sesquipedalis* showed highest similarity index (72.72%) while *C. cajan* and *M. pruriens* showed lowest similarity index (14.29%). Also, SDS-PAGE in the presence of β-ME, showed highest similarity index (62.50%) between *V. unguiculata unguiculata* sub- specie *sesquipedalis* and *M. pruriens* while *P. vulgaris* and *C. cajan* showed lowest similarity index (0.00%) (Table 5). Similar but not identical results were obtained for seed storage protein of cultivars of *Sesamum indicum* L [29] and cultivars of Cicer (chickpea) [10].

Table 5: Similarity index of six bean species using SDS-PAGE in the presence and absence of β-ME

Jaccard's Similarity index													
SDS-PAGE in absence of β-ME							SDS-PAGE in presence of β-ME						
Sp	1	2	3	4	5	6	Sp	1'	2'	3'	4'	5'	6'
1	100						1'	100					
2	41.67	100					2'	44.44	100				
3	72.72	50.00	100				3'	50.00	22.22	100			
4	36.36	60.00	33.33	100			4'	18.18	15.38	36.36	100		
5	23.53	26.67	15.38	61.54	100		5'	46.15	28.57	46.15	0.00	100	
6	60.87	38.11	63.16	31.58	14.29	100	6'	37.50	22.22	62.50	36.36	46.15	100

Sp) species, 1) *V. unguiculata* sub-specie *unguiculata* (Black-eyed bean), 2) *V. unguiculata* (Patisco), 3) *V. unguiculata* sub-specie *sesquipedalis* (Yardlong beans), 4) *P. vulgaris* (Red kidney bean), 5) *C. cajan* (Pigeon pea), 6) *M. pruriens* (Velvet bean).1-6 and 1?-6? denote species in the absence and presence of β-ME respectively

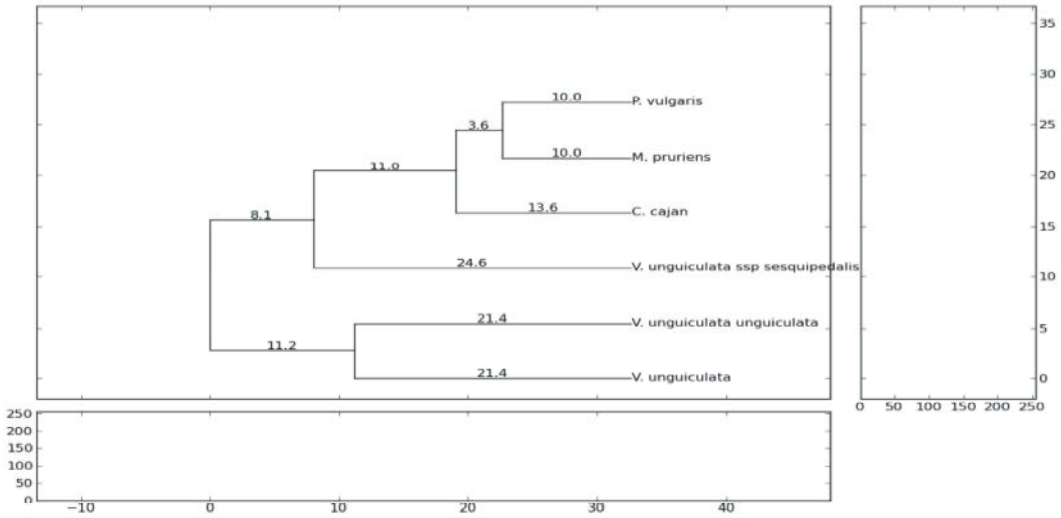

Fig. 3: UPGMA dendrogram depicting phylogenetic relationships among the six bean species based on their water soluble seed protein profiles obtained by Native-PAGE in the absence of β-ME

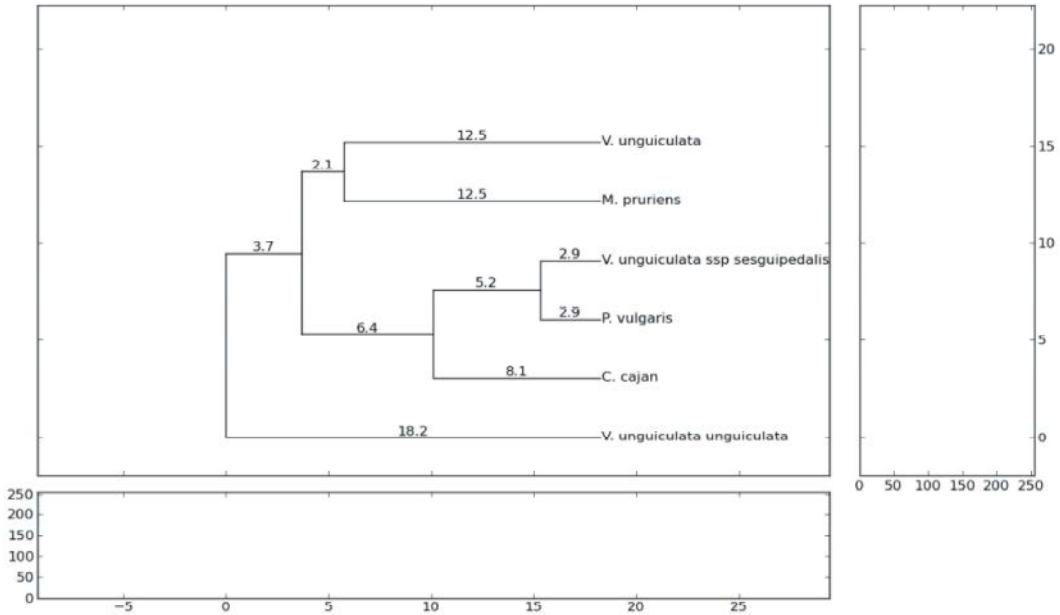

Fig. 4: UPGMA dendrogram depicting phylogenetic relationships among the six bean species based on their water soluble seed protein profiles obtained by Native-PAGE in the presence of β-ME.

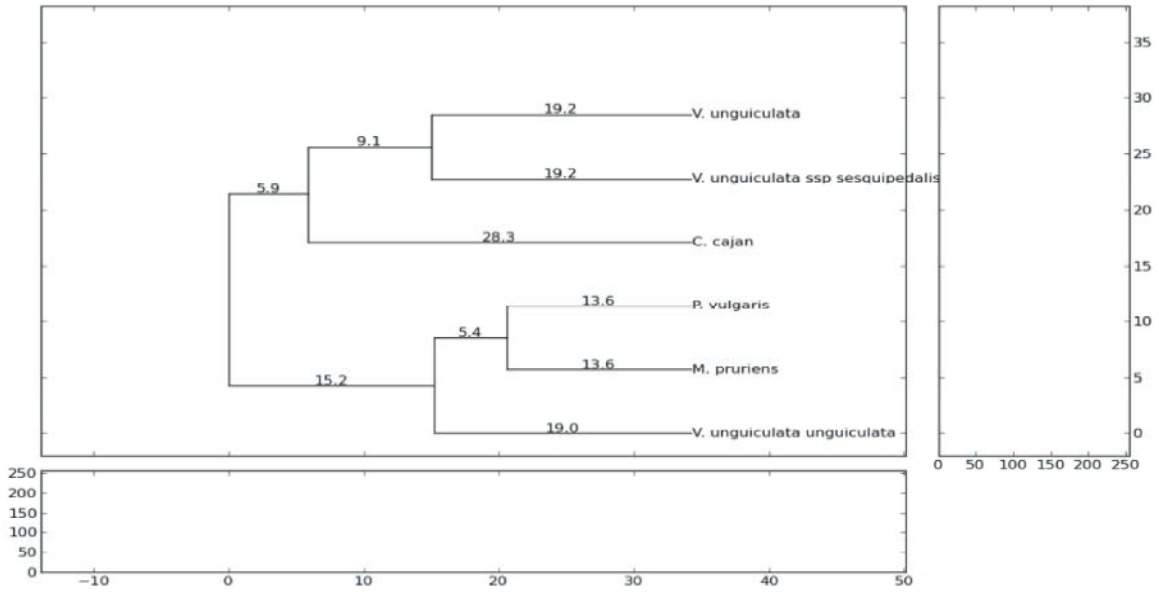

Fig. 5: UPGMA dendrogram depicting phylogenetic relationships among the six bean species based on their water soluble seed protein profiles obtained by SDS-PAGE in the absence of β-ME

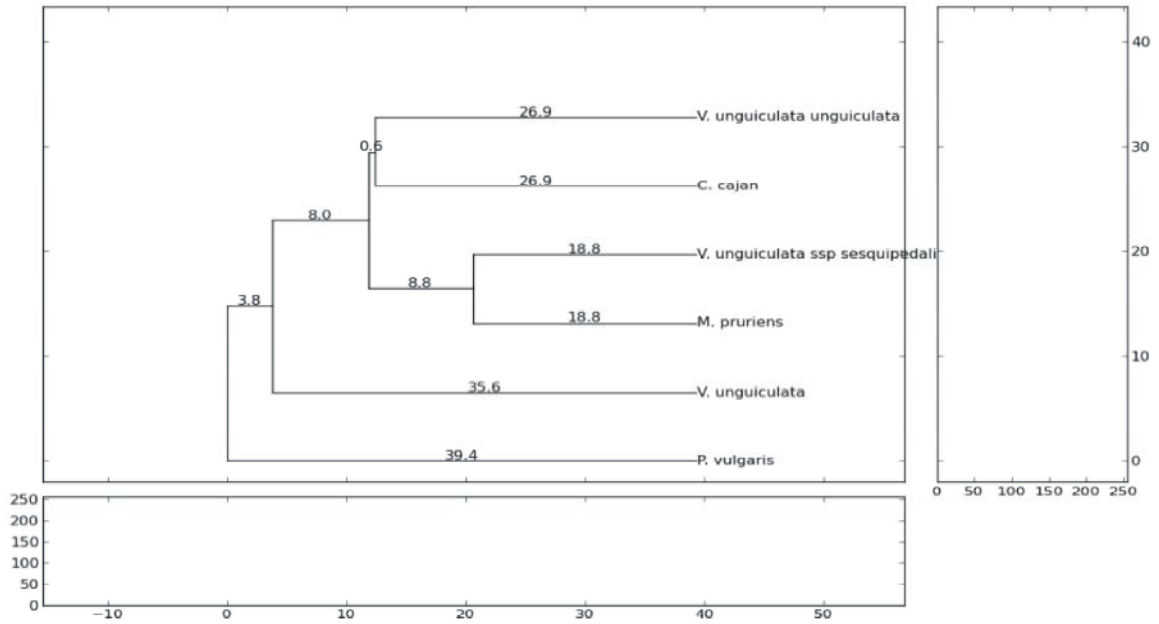

Fig. 6: UPGMA dendrogram depicting phylogenetic relationships among the six bean species based on their water soluble seed protein profiles obtained by SDS-PAGE in the presence of β-ME.

The data obtained from Native- and SDS-PAGE analysis were used for construction of dendrograms using unweighted pair group mean and arithmetic average (UPGMA). The dendogram of the six bean species obtained by Native-PAGE in the absence of β-ME showed two clusters. The cluster analysis revealed that some bean species (*V. unguiculata* and *V. unguiculata unguiculata* sub- specie *unguiculata*; *P. vulgaris* and *M. pruriens*) are very closely related to each other with respect to their water soluble proteins (Fig. 3). The cluster analysis in the presence of β-ME revealed that *V. unguiculata* and, *M. pruriens*; *P. vulgaris* and *V. unguiculata sesquipedalis* are very closely related in terms of their water soluble proteins while *V. unguiculata* sub-specie *unguiculata* occupied distinct place (Fig. 4).

Also, the dendogram obtained by SDS-PAGE in the absence of β-ME showed two clusters which when analyzed showed that *V. unguiculata*, *V. unguiculata* sub-specie *sesquipedalis* and *C. cajan*; *P. vulgaris*, *M. pruriens* and *V. unguiculata* sub-specie *unguiculata* are closely related (Fig. 5) while others *V. unguiculata* sub-specie *unguiculata* and *C. cajan*; *V. unguiculata* sub-specie *sesquipedalis* and *M. pruriens*) are very close to each other (Fig. 6) in the presence of β-ME. Also, similar but not identical results were obtained by for seed storage protein of cultivars of *Sesamum indicum* L [29] and cultivars of Cicer (chickpea) [10].

CONCLUSION

Our findings indicated that electrophoresis of seed proteins supplied additional banding patterns for the discrimination of the six investigated bean species. The average polymorphism of 0.00%, 10.71% and 57.69% of water soluble proteins in the bean species suggests presence of polymeric proteins which most probably must have evolved from monomerically distinct proteins. In Native-PAGE and SDS-PAGE and in presence and absence of β-ME, the different bean species show different similarity index. According to the results gathered from this study under SDS-PAGE in the absence of β-ME, it can be suggested that *V. unguiculata* sub-specie *unguiculata*, *C. cajan*, *V. unguiculata* sub-specie *sesquipedalis* and *M. pruriens* grown in Nigeria come from a narrow gene pool. Finally, this study indicated that the numerical analysis of seed protein profiles were relatively sufficient as a typing tool for the differentiation of bean species and thus provide useful information in order to distinguish Nigerian bean lines, improvement of already existing genetic resources, assessment of genetic diversity and improve the efficiency of breeding processes.

REFERENCES

1. Pedalino, M., M. Paino D'urzo, A. Costa, S. Grillo and R. Rao, 1990. Biochemical characterization of Cowpea seed proteins. Ng, N.Q. and L.M. Monti (ed). Cowpea Genetic Resources: Contributions in Cowpea Exploration, Evaluation and Research from Italy and the international institute of Tropical Agriculture; Meeting, Ibadan, Nigeria, February 6-7. Vii 200p. International Institute of Tropical Agriculture: Ibadan, Nigeria. Illus. Maps. Paper, pp: 81-89
2. Olusanya, J.O., 2008. Legume nuts. Essential of Food and Nutrition. 1st ed Apex Books Limited, Lagos, pp: 95-96.
3. Duranti, M., 2006. Grain legume proteins and nutraceutical properties. Fitoterapia; 77: 67-82.
4. FAO 1999. FAO Yearbook Production, pp: 53.
5. Mandal, S. and R.K. Mandal, 2000. Seed storage proteins and approaches for improvement of their nutitional quality by genetic engineering. Current Sci., 79(5): 576-589.
6. Franche, C., K. Lindström and C. Elmerich, 2009. Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil., 321: 35-59.
7. Graham, T.A. and B.E.S. Gunning, 2003. Localization of legumin and vicilin in bean cotyledon cells using fluorescent antibodies. Nature, 228: 81-82.
8. Jayasena, V. and K. Quail, 2004. Lupin: A Legume with a Future. Food and Beverage Asia., 12: 16-22.
9. Apata, D.F. and A.D. Ologboho, 1994. Biochemical evaluation of some Nigerian legume seeds. Food Chem., 49: 333-338.
10. Jahangir, A.D., A.K.R. Shahzada, M.K. Srivastava and A.W. Aijaz, 2014. Biochemical and cytological analysis of five cultivars of *Cicer* (chickpea). Afri. J. of Biotech., 13(11): 1281-1286.
11. Chowdhury, M.A., B. Vandenberg and T. Warkentin, 2002. Cultivar identification and genetic relationship among selected breeding lines and cultivars in chickpea (*Cicer arietinum* L.) Euphytica, 127: 317-325.
12. Manella, G., S.V. Onofaro, A. Tonini and V. Mangifico, 1999. Seed storage protein characterization of *Solanum* species and of cultivars and androgenic lines of *S. melongena* L. by SDS-PAGE. Seed Sci. Technol., 27: 23-35.
13. Isemura, T., N. Shiyo and M. Shigeyuki, 2001. Genetic variation and geographical distribution of Azuki bean (*Vigna ungularis*) landraces based on the electrophorogram of seed storage proteins. Breed. Sci., 51: 225-230.
14. Scandalios, J.G. and T.R.F. Wright, 1991. *Advances in Genetics* Academic Press, pp: 123.
15. Sadia, M., S.A. Malik, M.A. Rabbani and S.R. Peaece, 2009. Electrophoretic characterization and the relationship between some *Brassica* species. Electron. J. Biol., 5: 1-4.
16. Javid, I., A. Ghafoor and R. Anwar, 2004. Seed storage protein electrophoresis in groundnut for the evaluating genetic diversity. Pak. J. Bot., 36: 25-29.

17. Iqbal, S.H., A. Ghafoor and N. Ayub, 2005. Relationship between SDS-PAGE markers and *Ascochyte blight* in chickpea. Pak. J. Bot., 37: 87-96.
18. Sheidai, M., A. Hamta, A. Jaffari and M.R. Noori-Daloli, 2000. Morphometric and seed protein studies of *Trifolium* species and cultivars in Iran. Pl. Genet. Resour. Newsl., 120: 52-54.
19. Sammour, R.H., 1991. Using electrophoretic techniques in varietal identification, biosystematic analysis, phylogenetic relations and genetic resources management. J. Islam. Acad. Sci., 4: 221-226.
20. Ghafoor, A., Z. Ahmad, A.S. Qureshi and M. Bashir, 2002.Genetic relationship in *Vigna mungo* (L.) Hepper and *V. radiate* (L.) Wilczek based on morphological traits and SDS-PAGE. Euphytica., 123: 367-378.
21. Thanh, V.O.C. and Y. Hirata, 2002. Seed storage protein diversity of three rice species in the Mekong Delta. Biosph. Conservation, 4: 59-67.
22. Lioi, L., F. Spaovoli and R. Bollini, 1999.Variation and genomic polymorphism of lectin related protein in lima bean (*Phaseoluslanatus* L.) seed. Genet. Resour. Crop Evol., 46: 157-182.
23. Ferreira, J.J., E.A. Lvarrez, M.A. Roca and R. Giraldez, 2000. Determination of out crossing rate of *Phaseolus vulgaris* L. using seed protein markers. Euphytica, 113: 259-263.
24. Ghafoor, A. and M. Arshad, 2008. Seed protein profiling of *Pisum sativum* (L.) germplasm using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for investigation of biodiversity. Pak. J. Bot., 40(6): 2315-2321.
25. Sammour, R. H., M.N. EI-Shourbagy, A.M. Abo-Shady and A.M. Abasary, 1995. Characterization of Sunflower Seed Proteins by Electrophoretic Techniques. Arab Gulf. J. Scient. Res., 13(3): 591-604.
26. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin Phenol reagent. J. Biol Chem., 193(1): 265-275.
27. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4.Nature, 227: 680-685.
28. Sanjeev, R., M. Rajesh, B. Madhuri and P.V.R. Narendra Babu, 2012. Comparative evaluation of protein extraction methods from few leguminous seeds. Inter. J. of Adva. Biotech. and Res., 3(2): 558-563.
29. Aejaz, A.D., R.C. Abhikshit and A. Neelakantan, 2014. A study on seed protein profile of Indian cultivars of *Sesamum indicum* L., Int. J. Curr. Biotechnol., 2(6): 10-17.