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Full Length Research Paper

Genetic diversity assessment of *Phaseolus vulgaris* L. landraces in Nigeria's mid-altitude agroecological zone

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***Phaseolus vulgaris* L. is a valuable and highly nutritious food legume. In Nigeria, it is highly underutilized, being consumed in few parts of Plateau and Taraba States within the mid-altitude agroecological zone. In order to provide information on breeding and improvement for better acceptability, genetic diversity among eleven landraces grown within this zone, belonging to six phenotypic classes based on seed colour, was assessed using random amplified polymorphic DNA (RAPD) and soduim dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of total seed proteins. RAPD-PCR amplified products ranged from 4 to 9 bands. Number of alleles observed and mean gene diversity within the different phenotypic classes ranged from 1.0 ± 0 to 1.63 ± 0.49 and from 0.13 ± 0.19 to 0.26 ± 0.21, respectively. The highest genetic distance was between white and canberry seeded varieties (81.09%) while the least was between red and pinto accessions (9.22%). However, the highest number of polymorphic loci (40), percentage polymorphism (63.49%), Nei's gene diversity (0.26 ± 0.21) and number of alleles (1.63 ± 0.49) was found within pinto varieties. Nei's genetic similarity between landraces from the two states was 91.72% indicating a profound founder effect which could be facilitated by high germplasm exchange between these states. Jaccards similarity coefficient between the landraces based on total seed proteins bands was 1.00. However, genetic relationships and clusters drawn from RAPD and SDS-PAGE data differed, with most of the landraces belonging to separate clusters in the dendrograms obtained from both methods. High polymorphism suggests a broad genetic base among the landraces. The study of more specific seed proteins such as phaseolin as biochemical markers might better elucidate diversity present within the landraces.**

Key words: *Phaseolus vulgaris*, genetic diversity, Nigerian landraces, random amplified polymorphic DNA, seed proteins.

INTRODUCTION

Phaseolus vulgaris (syn. common bean) is an annual legume grown for its nutritional value. It is traditionally a basic food crop in many developing countries and serves as a major plant protein source for rural and urban areas (Atilla et al., 2010). The green immature pods of some varieties may be cooked and eaten as vegetable while the seeds of other varieties are harvested at a later growth stage and consumed for their high protein content. The dry seeds are also good sources of essential vitamins, minerals, soluble fiber, starch and phytochemicals (Messina, 1999) while the leaf is occasionally used as vegetable and the straw as fodder. Consumption of *P. vulgaris* has been linked to several health benefits such as reduction of cholesterol level (Rosa et al., 1998), reduction of coronary heart diseases (Anderson et al., 1998; Bazzano et al., 2001), favourable effects against cancer (Hangen and Bennick, 2002), decreasing diabetes and obesity (Geil and Anderson, 1994) and high antioxidant capacity (Heimler et al., 2005). However, the consumption of *P. vulgaris* is limited due to the presence of certain anti-nutritional components such as phytic acid, flatulence factors, saponins, lectins

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and the need for prolonged cooking (Lyimo et al., 1992).

Originally domesticated in Central and Southern America (Gepts and Debouck, 1991), *P. vulgaris* was introduced into Africa and other parts of the world by the Spanairds and Portugese and is now widely cultivated in the tropics, subtropics and temperate regions with an annual production of about 18.3 million tonnes in 2007 (FAOSTAT, 2009). Typically self-pollinating with low frequency of crossing (Wells et al., 1988), *P. vulgaris* shows wide variations in the growth habit, pigmentation, pod, seed and phenology (Leakey, 1988; Singh, 1989) reflecting the wide range of ecological and human environments under which the crop has evolved over millennia. The seeds are often smooth, plump, kidney-shape and range widely in colour (some black, brown, white, red and purple while others are mottled with two or more colours).

Traditionally, plant genetic diversity is assessed by measuring variations in phenotypic or morpho-agronomic traits such as yield and stress tolerance among other traits (Szilagyi et al., 2011); evidence based on biochemical markers or allozymes (Singh et al., 1991a, b); seed proteins (Gepts and Bliss, 1986) and molecular markers (Semagn et al., 2006). Morphological and biochemical markers are however influenced by the environment as well as relatively low abundant with low level of polymorphism, respectively. In contrast, seed storage proteins are highly polymorphic and these polymorphisms are genetically determined. Molecular markers also have the advantage of showing polymorphism and are less affected by the environment (Kumar et al., 2009).

Studies on assessing plant genetic diversity have been applied in conservation of genetic resources for improved acquisition, maintenance and use (Karp et al., 1997; Hodgkin et al., 1995); and in phylogenetic and taxonomic studies (Spooner et al., 2007; Labate et al., 2009; Killan et al., 2007; Kwak and Gepts, 2009). It has also been applied in ecological studies where it is used to unveil the origin, distribution and domestication of genetic resources (Szilagyi et al., 2011; Bhullar et al., 2009).

Techniques used in studying molecular diversity may be hybridization-based for example, restriction fragment length polymorphisms (RFLP) or polymerase chain reaction-based for example, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), single nucleotide polymorphism (SNP) etc. Although RAPD has been used in several genetic diversity studies, it is criticized because of its banding patterns that are not reproducible (Jones et al., 1997). This challenge could however be overcome through choice of an appropriate DNA extraction protocol that would remove any contaminants (Micheli et al., 1994); by optimizing the DNA extraction and amplification protocols used (Ellsworth et al., 1993; Skroch and Nienhius, 1995); testing several oligonucleotide primers more than once and scoring only the reproducible DNA fragments (Kresovich et al., 1992; Yang and Quiros, 1993).

While characterizing breeding lines of *P. vulgaris* using RAPD and morphological traits, Atilla et al. (2010) concluded that the main source of variation resulted from genetic factors found in the places of origin where the first selection was made. Becerra and Gepts (1994) and Gepts (1998) reported that the DNA polymorphisms between Andean and Mesoamerica genepools have been well characterized with many studies predicting less diversity among Andean cultivars than the Mesoamerican cultivars (Duarte et al., 1999; Beebe et al., 2001; Blair et al., 2006). In attempting to explain the genetic diversity in *P. vulgaris*, Gepts (1998) suggested that crop expansion from America into Europe resulted in strong founding effects, adaptation to new environments and consumer preferences. Papa et al. (2007) states that a large portion of the genome in this species appears to have been subjected to the effects of selection during domestication. Not much has been done on the diversity of *P. vulgaris* based on the seed proteins. However, Berber and Yasar (2011) generated two distinct clusters after numerical analysis of seed storage protein profiles of *P. vulgaris* using Sodium Dodecyl Sulphate PolyAcrylamide Gel Electrophoresis (SDS-PAGE) technique.

Due to the absence of studies on the diversity of *P. vulgaris* in West Africa generally and in Nigeria where it is grown and consumed in Plateau and Taraba States which belong to the mid-altitude agroecological zone, this study sought to elucidate the genetic diversity in landraces grown within the zone using RAPD and SDS-PAGE and to evaluate the level of agreement between the two methods used.

MATERIALS AND METHODS

Seeds of eleven landraces of *P. vulgaris* were collected from Mangu (9° 31′ N, 9° 06′ E), Bokkos (9° 18′ N, 9° 00′ E) and Jos North (8° 24′ N, 10° 38′ E) Local Government Areas (LGAs) of Plateau State, and Sardauna (6° 25′ N, 9° 36′ E) LGA of Taraba State all within the mid-altitude agroecological zone. The accessions were placed in six distinct phenotypic classes based on seed colour (Table 1).

For the RAPD analysis, seeds of each accession were planted in polythene bags and one-week old leaves collected for DNA extraction. The total genomic DNA was extracted using the modified Dellaporta et al. (1983) extraction protocol. Extracted DNA was dissolved in ultrapure water and stored at -4°C until use.

RAPD analysis

Six decamer primers (Table 2) were used to assess the molecular diversity among the accessions. PCR was done in a 25 µl volume reaction containing 2.5 µl 10X Buffer, 1.2 µl (5 mM) magnesium chloride ($MgCl_2$), 2.0 µl (2.5 mM) Deoxynucleotide Triphosphate (dNTP), 1.0 µl dimethyl sulfoxide (DMSO), 1.0 µl primer, 0.2 µl *Thermus aquaticus* (Taq) polymerase, 1.0 µl (4 ng) DNA and 16.1 µl distilled water (dH_2O). DNA amplification involved initial preheating of the DNA at 94°C for 3 min, 44 amplification cycles (each consisting of denaturing at 94°C for 20 s, annealing at 38°C for 40 s and elongation at 72°C for 1 min) and a final extension

Table 1. Description of accessions from different locations.

Accession number	Location	Phenotype
Cnb-21	Mangu, Plateau	Brown
Cnb-22	Mangu, Plateau	Black
Cnb-27	Jos, Plateau	Pinto
Cnb-28	Mangu, Plateau	White
Cnb-29	Jos North, Plateau	Red
Cnb-211	Sardauna, Taraba	Black
Cnb-212	Mangu, Plateau	Brown
Cnb-213	Bokkos, Plateau	Pinto
Cnb-214	Mangu, Plateau	Small, cranberry
Cnb-215	Sardauna, Taraba	Red
Cnb-216	Bokkos, Plateau	Pinto

Table 2. RAPD primers used for amplification.

Primer code	Primer sequence
OPD17	-TTTCCCACGG-
OPAD05	-ACCGCATGGG-
OPAE11	-AAGACCGGGA-
OPAE14	-GAGAGGCTCC-
OPAC11	-CCTGGGTCAG-
OPAE09	-TGCCACGAGG-

Primer source: Operon oligos.

phase of 72°C for 7 min.

The amplified products were run on 1.5% agarose gel in 0.5 M Tris-Borate-Ethylene (TBE) buffer at 100 V for 3 h after the addition of 0.3 µl of ethidium bromide. The gel was visualized using UV light (Figure 1). The observed bands were scored 1 where present and 0 where absent. The data generated were analysed using Numerical Taxonomic System of Statistics (NTSYS) and Popgene 1.31 software. Nei and Li (1979) formula was used to estimate the genetic similarities, distances and percentage polymorphisms within and between the phenotypic classes. The total number of alleles observed and alleles effective in detecting polymorphism were also recorded. Dendrogram showing the relationship between the landraces was constructed from Jaccard's similarity coefficients using unweighted pair group method of arithmetic means (UPGMA).

SDS-PAGE analysis

For the seed protein diversity study, seeds of each accession were ground to fine powder and 0.05 g of the powder of each accession was well mixed with extraction buffer and centrifuged at 8000 rpm for 15 min at 4°C for extraction of total seed proteins. The supernatant was collected and 8 µl of sample was added to 2 µl of each of the protein samples. The samples were denatured at 95°C for 4 min before performing PAGE. SDS-PAGE was performed using the method of Laemmli (1970) in 12% acrylamide gel.

SDS-PAGE was done with gel slabs of 1 mm thickness and 10 µl of the samples were loaded in the wells. A voltage of 120 V was applied until the bromophenol blue reached the bottom. The gel plate was fixed in a solution of 50% methanol and 10% acetic acid made up to 1000 ml with dH_2O. Afterwards, the plate was placed in staining solution (containing 0.1% Coomassie blue stain, 50% methanol and 10% acetic acid made up to 1000 ml with dH_2O) with gentle agitation on an orbital shaker. Destaining was carried out in a solution of 40% methanol and 10% acetic acid in dH_2O until protein bands were clearly visible (Figure 2).

The protein bands were again scored 1 where present and 0 for absence. The data generated were analysed using the NTSYS software. Dendrogram showing the relationship between the landraces based on their total seed proteins was drawn from Jaccard's similarity coefficient using UPGMA.

RESULTS

Figure 1 shows that the amplified RAPD-PCR products ranged from 4 to 9 bands. The genetic similarity between the phenotypic classes was generally high, ranging from 62.78 to 91.19% (Table 3). The highest genetic similarity of 81.55% was observed between the pinto and brown phenotypes.

Table 4 shows the polymorphic parameters of the phenotypes with the observed bands ranging from 1500 to 8000 basepairs (bp). The pinto phenotype had the highest polymorphism of 63.49% and Nei's gene diversity of 0.26 ± 0.21 among 1.46 ± 0.41 effective alleles.

The polymorphism among the black phenotype was lowest (31.75%) while the white and cranberry phenotypes were both represented by one accession each, hence the absence of polymorphic parameters for those phenotypes.

Also, the percentage genetic similarity and distance from Nei's coefficient showed very high similarity (91.72%) between the accessions from Plateau and Taraba States (Table 5).

The accessions from Plateau State had high percentage polymorphism of 93.65% compared with accessions from Taraba State which had percentage polymorphism of 58.73% (Table 6). In the accessions from Plateau State, 1.94 ± 0.25 alleles were observed out of which 1.61 ± 0.30 were effective in detecting polymorphism while in Taraba State, 1.42 ± 0.35 of the 1.59 ± 0.50 alleles were effective in polymorphism.

Dendrogram constructed from Jaccard's similarity coefficient of the results of RAPD (Figure 3) showed two distinct clusters; one containing Cnb21, Cnb27, Cnb22, Cnb211, Cnb214, Cnb29 and Cnb213 while the other contained Cnb28, Cnb215, Cnb216 and Cnb212. The highest similarity was between Cnb215 and Cnb216 while the lowest was between Cnb211 and Cnb214 with Jaccard's coefficients of 0.79 and 0.68, respectively.

The results of SDS-Page (Figure 4) showed protein bands ranging from 15 to 150 kiloDaltons. The dendrogram based on seed protein data also showed two distinct clusters. Most of the landraces, however, had very high Jaccard's similarity coefficients and showed a perfect similarity of 1.00 based on their seed protein components. Cnb211, however, stood out of its subcluster which contained Cnb27, Cnb28 and Cnb29 with Jaccard's similarity coefficient of about 0.88.

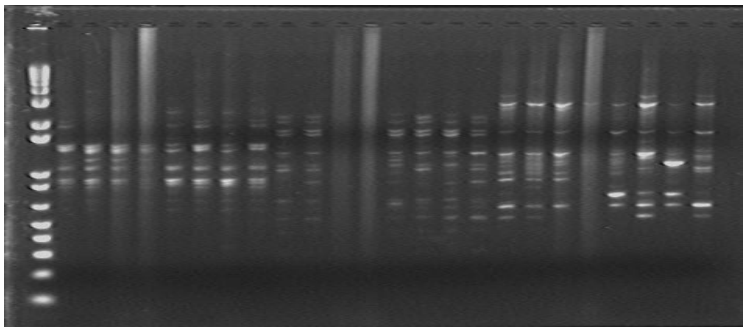

Figure 1. RAPD bands from agarose gel electrophoresis of *P. vulgaris.*

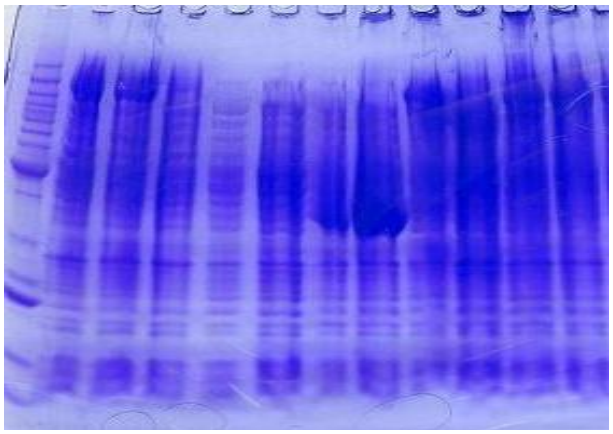

Figure 2. Total seed protein bands of *P. vulgaris* landraces from polyacrylamide gel electrophoresis.

Table 3. Percentage genetic similarity and distance between the phenotypic classes of *P. vulgaris.*

Phenotype	Brown	Black	Pinto	White	Red	Cranberry
Brown		66.44	81.55	70.51	81.53	57.39
Black	40.89		71.12	62.81	62.78	73.53
Pinto	20.39	34.07		77.70	91.19	55.87
White	34.94	46.44	25.24		71.11	44.44
Red	20.42	46.56	9.22	34.09		49.99
Cranberry	55.53	30.75	58.21	81.09	69.34	

Values above the diagonal = percentage genetic similarity; Values below the diagonal = percentage genetic distance.

Table 4. Descriptive statistics showing polymorphic parameters of *P. vulgaris* phenotypes.

Phenotype	ONA (Mean ± SD)	ENA (Mean ± SD)	NGD (Mean ± SD)	NPL	PP (%)
Brown	1.54 ± 0.50	1.38 ± 0.36	0.22 ± 0.21	34	53.97
Black	1.32 ± 0.47	1.22 ± 0.33	0.13 ± 0.19	20	31.75
Pinto	1.63 ± 0.49	1.46 ± 0.41	0.26 ± 0.21	40	63.49
White	1.00 ± 0.0	1.00 ± 0.0	0.00	0	0
Red	1.57 ± 0.50	1.40 ± 0.35	0.24 ± 0.21	36	57.14
Cranberry	1.00 ± 0.0	1.00 ± 0.0	0.00	0	0

ONA: Observed number of alleles; ENA: effective number of alleles; NGD: Nei's gene diversity; NPL: number of polymorphic loci; PP: percentage polymorphism.

Table 5. Percentage genetic similarity and distance between the locations of *P. vulgaris.*

Location	Plateau State	Taraba State
Plateau State		91.72
Taraba State	8.64	

Values above the diagonal = percentage genetic similarity; Values below the diagonal = percentage genetic distance.

Table 6. Descriptive statistics showing polymorphic parameters of *P. vulgaris* phenotypes from two locations.

Location (State)	ONA (Mean ± SD)	ENA (Mean ± SD)	NGD (Mean ± SD)	NPL	PP (%)
Plateau	1.94 ± 0.25	1.61 ± 0.30	0.35 ± 0.14	59	93.65
Taraba	1.59 ± 0.50	1.42 ± 0.35	0.24 ± 0.21	37	58.73

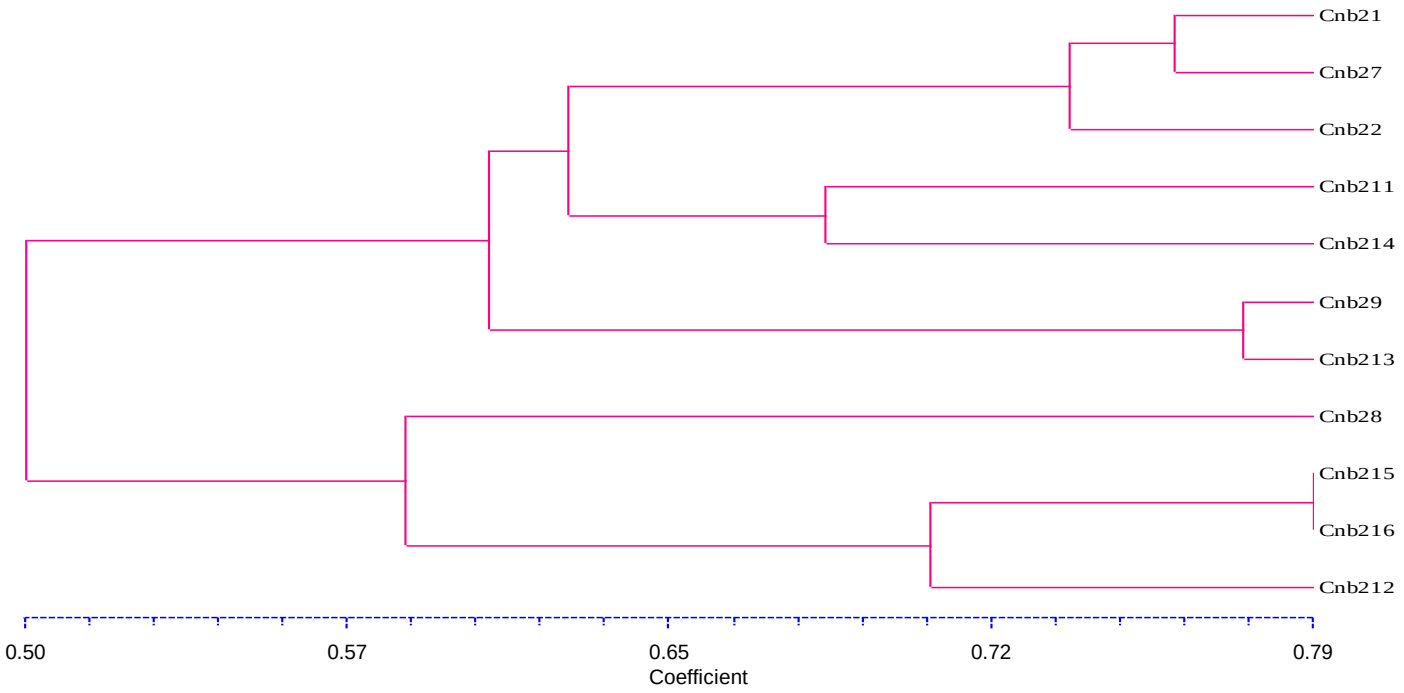

Figure 3. Dendrogram from RAPD analysis showing the relationship between *P. vulgaris* landraces.

DISCUSSION

The Nei's genetic similarity ranging from 62.78 to 91.19% and percentage polymorphism which ranged from 31.75 to 63.49% reveals a wide and diverse genetic base in *P. vulgaris* landraces in the mid-altitude agroecological zone of Nigeria. The similarity supports their grouping as same species; however, the high polymorphism despite a wide range of genetic similarity implies variations in genes that code for the same character in the different phenotypes studied, hence the landraces are thought to have a broad genetic base. This agrees with Franklin et al. (2009) and Biswas et al. (2010), who reported inter-varietal similarity ranging from 50 to 95% and 58.58 to 98.51% in *P. vulgaris* landraces in the Nilgiris biosphere reserve and from three eco-geographical regions of Bangladesh, respectively. Szilagyi et al. (2011) in their evaluation of genetic diversity in *P. vulgaris* observed low genetic similarity between genotypes of different group clusters and high genetic similarity between genotypes within the same cluster, suggesting a wide variation of DNA, larger between varieties from different groups and smaller

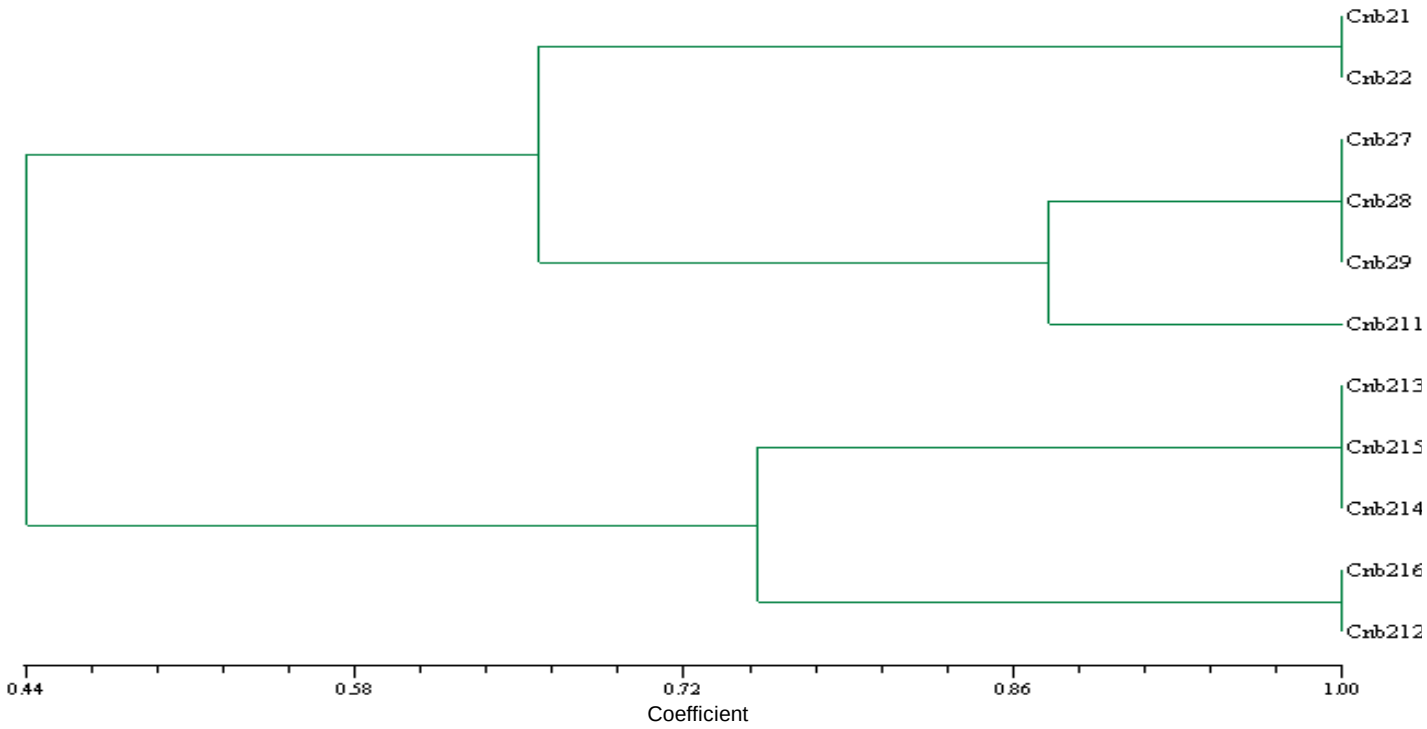

Figure 4. Dendrogram of the total seed protein bands from SDS-PAGE showing the relationship between *P. vulgaris* landraces.

within groups. The high polymorphism observed is supported by Gepts (1998), Menezes et al. (2004), Palomino et al. (2005), Atilla et al. (2010) and Biswas et al. (2010) who all reported that great molecular diversity exists in collections of *P. vulgaris* around the world. Sicard et al. (2005) confirmed this genetic diversity using SSR markers but reported low heterozygosity within the accessions studied. The low heterozygosity observed was believed to be due to the inbreeding nature of the crop.

Between the locations studied, *P. vulgaris* landraces also showed very high similarity as well as high percentage polymorphism within each state. The very high genetic similarity between the accessions from Plateau and Taraba States is thought to be a result of founder effect as Gomez et al. (2004) suggested founder effect for the absence of detectable differentiation in molecular markers of *P. vulgaris* cultivars between agroecological zones. The self fertilizing mating system in *P. vulgaris* may also contribute to this similarity with the limitation of gene flow between cultivars. Moreover, Sicard et al. (2005) opined that selection by farmers could be responsible for the observed diversity in *P. vulgaris* but not in *Phaseolus coccineus* landraces in Italy. Such selection in addition to the possibility of an initial introduction of higher seed diversity into Plateau State may be responsible for the higher polymorphism observed when compared to Taraba State.

Results from cluster analysis placed accessions of different phenotypes in the same cluster. For instance, Cnb215 and Cnb216 are morphologically distinct and belong to the red and pinto phenotypes, respectively; however, dendrogram from RAPD results placed them in the same sub cluster with Jaccard's similarity coefficient of 0.79. This further highlights the high genetic similarity between the phenotypes. In addition, the dendrogram from SDS-PAGE showed more similarity between the accessions than RAPD with Jaccard's similarity coefficients of 1 implying a greater similarity in the total seed proteins of the landraces compared to RAPD. Although morphologically distinct, the landraces studied were highly similar in terms of their DNA and total seed protein content. The diversity inferred earlier at the level of individual genes coding for the same character is a likely explanation for the observation of this distinction in phenotypes despite the similarity in DNA and total seed proteins. This however requires further investigation of the genes responsible for the different phenotypic types observed in *P. vulgaris*.

While some investigators proposed that seed protein profiles may be useful as an indicator of taxonomic relationships within some species (Cooke, 1984; Lioli et al., 2005; Duran et al., 2005), others say the method is insufficient for the discrimination at the cultivar level (Panella et al., 1993; Yüzbaşıoğlu et al., 2008). The lack of agreement in the group clusters resulting from the total seed protein content and the DNA shows that seed proteins are not good estimators of genetic diversity.

In the same vein, Sultana and Ghafoor (2008) reported high inter-accession diversity from RAPD and isozymes;

while, it was lowest in the case of seed proteins for lentil hence concluded SDS-PAGE of the total seed protein could not be used for investigating inter- or intra-accession diversity. Similarly, Berber and Yasar (2011) reported that SDS-PAGE of total seed proteins was not sufficient in distinguishing between *P. vulgaris* cultivars. Ghafoor and Ahmad (2005) while studying genetic diversity in *Vigna mungo* also reported that SDS-PAGE cannot be used for identification of various genotypes on the basis of intra-specific variation because accessions that differed on the basis of characterization and evaluation of morpho-agronomic data exhibited similar banding patterns in their seed proteins. Thanh et al. (2006) therefore recommended the use of protein components (albumin, prolamin, globulin and glutelin) to expose the minor variations between the cultivars of specific crops. Similarly, in the case of *P. vulgaris*, diversity in the storage protein phaseolin besides the other protein types may further distinguish cultivars. In addition, Celis and Bravo (1984) and Beckstrom-Sternberg (1989) suggested that the genotypes with similar protein banding patterns be studied for more detailed agronomic and biochemical analyses including two-dimensional electrophoresis and DNA markers.

The diverse genetic base observed has important implications in the improvement of the crop which presently requires serious breeding attention so as to breed for lower anti-nutritional factors and a shortened cooking time both of which currently limits it use. At present, breeding programmes for *P. vulgaris* explore only a low proportion of the available genetic diversity (Perseguini et al., 2011). This results in a narrow genetic base for elite cultivars (Cooper et al., 1997). Since the success of any breeding programme is intimately related to appropriate choice of divergent parents, information on the genetic diversity in the available germplasm would be important; allowing for the exploitation of heterosis to improve crop plants. Such information is also necessary for gene bank management for purposes of improved acquisition of germplasm for inclusion in gene banks especially in Nigeria where *P. vulgaris* is highly underutilized and relatively unknown.

Further studies using more specific molecular markers (such as simple sequence repeats and single nucleotide polymorphisms) are recommended to elucidate more information on the overall genetic diversity and particular genes responsible for specific characters of agronomic interest. The knowledge of such genes will enhance the breeding for improvement of the crop through marker assisted selection. An understanding of the centres of origin of *P. vulgaris* landraces in Nigeria from molecular analysis of the chloroplast DNA and more specific studies of the seed proteins are other areas requiring research.

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