GENETIC VARIABILITY VIA PROTEIN ELECTROPHORESIS AMONG SOME NIGERIAN ACCESSIONS OF PIGEON PEA (*CAJANUS CAJAN*)

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Purpose: Lack of genetic variability is a limitation to pigeon pea improvement. Hence the need to study the genetic variability of five accessions of pigeon pea with the view of isolating those that are with high yield and early maturity.

Research Method: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE) was used in the protein extraction and genetic diversity was estimated using Paleontological Statistics (PAST).

Findings: A total of 24 protein bands were obtained ranging from 10kDa to 170 KDa. The protein band with the highest molecular weight was generated in accession NG/AO/MAY/09/021, NG/AO/11/08/108, NG/SA/07/0180 and NG/SA/07/208 while lowest molecular weight i.e 10 KDa was generated in NG/AO/MAY/09/021. Highest similarity index was recorded (45.16%) in NG/SA/07/0180 and NG/SA/07/208 and lowest (13.04%) in NG/SA/07/0180 and NG/AO/MAY/09/021. The bands showed variability based on intensity and presence or absence of any of them among the accessions. Jaccard's similarity separated the 5 accessions into two clusters at an UPGMA similarity coefficient range of 0.2 to 1.

Originality/Value: Accession NG/AO/MAY/09/021 occupies a distinct position hence could be combined with other accessions in a breeding programme.

Keywords: Genetic variability, Pigeon Pea, SDS PAGE, Similarity index, crop improvement

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INTRODUCTION

Pigeon pea (*Cajanuscajan*(L.)Huth) is an important source of vegetable protein (20–22%) (Sharma *et al*., 2011) though underutilized. It is found to be a perennial legume from the family Fabaceae. This plant is used extensively as livestock fodder and feed (Rao *et al*., 2002) and in Barbados, it is feed for pigeons (Fuller *et al.*, 2006). Pigeon pea is known by numerous names with different etymology such as eye pea, tropical green pea, gungo pea in Jamaica, red gram, and kadyos in the Philippines (Fuller *et al.*, 2006). The pigeon pea is a perennial crop that can grow into a small tree. It is a drought-tolerant legume grown in the semi-arid tropics especially in India, (Zemede *et al.*, 2009). There are various types such as the tall, dwarf, and smaller bushes varieties. Pigeon pea is of high nutritional value (Varshney *et al.*, 2012). Pigeon pea plants are also known for different medicinal benefits and other several uses. It is used as a remedy for health problems such as cough, cold, chest problems, and sore throat. It has diuretic, laxative, antidote, astringent, expectorant and sedative properties. It is also used in the manufacture of fuel, as wind breakage or shade crop for young coffee plants. It acts as a cover crop (Duke *et al.*, 1981).

Traditional pigeon pea varieties take a long period of almost ten months or more to mature. The plant is not fitted in multiple cropping systems due to its long duration to maturity (Ritesh *et. al*., 2012). To have a profitable pigeon pea, there is a need to develop a suitable type with high yield and early maturity that can be incorporated in multiple and intercropping patterns (Ramanujam and Singh, 1981). The unavailability of plant genetic variability is a limitation in a crop improvement programme (Kimani, 2000).

SDS-PAGE is a technique used in genetics, biochemistry, molecular biology, and biotechnology to separate macromolecules, such as proteins and nucleic acids, based on their electrophoretic mobility. A molecule’s mobility is determined by its length, shape, and change. Molecules are run in their natural condition, as with all types of gel electrophoresis, preserving the molecule's higher-order structure. The native page is the name given to this method. A chemical denaturant can also be added or withdrawn from the structure, causing the molecules to alter (Ninfa, 2010). It is the process of classifying molecules according to their molecular weight. The SDS molecules are negatively charged at the pH of gel electrophoresis and link to protein in a predetermined ratio, roughly one molecule of SDS for every two amino acids. As a result, the detergents ensure that all proteins have a consistent charge-to-mass ratio. By binding their primary, secondary, tertiary, and/or quaternary structure and denaturing them, they can be turned into negatively charged linear polypeptide chains, regardless of their original charge. The negatively charged polypeptide chain traversed by molecules in PAGE is inversely related to the logarithm of their molecular weight when exposed to an atmospheric electric field. By evaluating the relative ratio of the distance travelled by each protein to the

length of the gel (RF), a deduction can be made about the relative molecular weight of the protein where the length of the gel is determined by the distance travelled by a small molecule like a tracking dye for protein samples to coat protein to impact to negative changes for every molecule to every two amino acids of the denatured protein. Purification and separation of the various subunits of the protein can be achieved by combining nature and SDS-PAGE (Song *et al.,* 2006). The molecular characterization of the Nigeria accessions of *C. cajan* is important in the improvement of the crop so that it can be fully utilized to feed an ever-expanding human population.

MATERIALS AND METHODS

COLLECTION OF ACCESSIONS

The seeds of 5 pigeon pea accessions were collected at National Centre for Genetic Resources and Biotechnology (NACGRAB) Moor plantation, Ibadan, Nigeria. Table 1 lists the pigeon pea accessions that were described in this study.

Table 1

Pigeon pea accessions

S/N	Accession number
P_1	NG/AO/MAY/09/021
P_2	NG/AO/11/08/108
P_3	NG/SA/JAN/09/149
P_4	NG/SA/07/0180
P_5	NG/SA/07/208

PREPARATION OF SAMPLES

The SDS-PAGE was performed using the Laemmli (1970) method. 5 seeds from each accession were pulverized with a mortar and pestle throughout the protein extraction process. The powder was completely homogenized in an extraction buffer comprising 0.5 M Tris-HCl (pH 6.8), 2.5 percent sodium dodecyl sulphate (SDS), 10% glycerol, and 5% 2-mercaptoethanol using a vortex. For 5 minutes, the samples were centrifuged at 10,000 rpm. The supernatants (500 l) were separated into vials. Electrophoresis of sodium dodecyl sulphate polyacrylamide gels was performed using a 4 percent stacking gel and a 12 percent resolving or separating gel. The runs were carried out in Tris-glycine (pH 8.3) buffer on a mini gel apparatus. Bromophenol blue (BPB) was added, as tracking dye, to the sample

buffer to maintain the movement of protein molecules in the gel. The gels were run at 90 V for 2 hours in an ominipac mini- vertical gel apparatus using Promega protein as a standard marker. They were gently removed and washed with 500 ml of the gel fixing solution and subsequently covered with 400 ml of Coomassie blue stain at room temperature for 3 to 4 hours and were gently agitated. The Coomassie stains were removed after staining by covering gels with 250 mls of the destaining solution. The destaining solution was changed severally until the protein bands were seen without the background staining of the gel.

DATA ANALYSIS

The 5 accessions banding patterns were analyzed and photographed. Across the lanes, the bands were scored. The molecular weights of the two proteins were compared. Each band is treated as a distinct character, with its presence or absence being coded for analysis. Using the statistical software tool PAST (Paleontological Statistics), a data matrix was created, and the coefficient of similarity tree was created by clustering the similarity data. Cluster analysis was performed by an agglomerative technique using the Unweighted Pair Group Method with arithmetic averages (UPGMA). The intensities of bands are represented by a plus symbol. The similarity index (S.I) was calculated thus:

$$\text{similarity index} = \frac{\text{Number of common bands}}{\text{Total no. of bands}} \times 100$$

RESULTS

Electropherogram showing protein banding pattern of the 5 accessions of Pigeon pea (*Cajanus cajan*) is given in Figure 01. A total of 24 protein bands were obtained ranging from 10kDa to 170 KDa, Table 2. The bands showed variability based on intensity and presence or absence of any of them among the accessions. Among which bands 1 and 4 were only present in accession NG/AO/MAY/09/021, bands 2 and 8 were present in accessions NG/AO/11/08/108 and NG/SA/JAN/09/149, bands 5,7,10 and 14 were present in accessions NG/SA/JAN/09/149, NG/SA/07/0180 and NG/SA/07/208, bands 6 and 9 were present in accessions NG/AO/MAY/09/021 and NG/AO/11/08/108. Bands 11, 12 and 20 were present in all accessions but at different intensities, bands 13,15,18 and 19 were absent in accessions NG/AO/MAY/09/021 band 17 is only present in accession NG/AO/11/08/108, band 16 is present in accessions NG/AO/11/08/108 and NG/SA/07/208 while bands 22 and 24 were only present in accession NG/SA/07/208 and bands 21 and 24 were not present in accession NG/SA/JAN/09/149. Bands 20 is present in all accessions and has the highest intensity. According to Abdulkareem *et al*. (2019),

the high intensities of bands are an indication of the presence of high protein content.

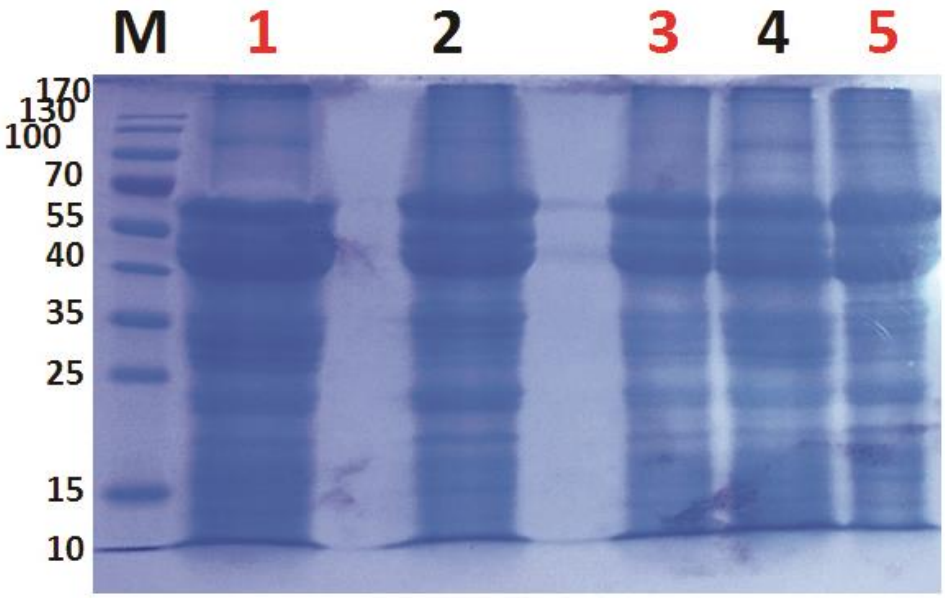

Figure 1. SDS PAGE electropherogram of seed protein for accessions 1-5 of pigeon pea. M is the standard protein marker. 1= NG/AO/MAY/09/021, 2= NG/AO/11/08/108, 3= NG/SA/JAN/09/149, 4= NG/SA/07/0180 and 5= NG/SA/07/208.

Table 2

Molecular weight and Intensities of bands present in each pigeon pea accession

Bands number	Molecular weight	NG/AO/MAY/ 09/021	NG/AO/11/08/ 108	NG/SA/JAN/ 09/149	NG/SA/07/ 0180	**NG/SA/07/ 208**
1	10	+++	0	0	0	**0**
2	11	0	+++	++	0	**0**
3	11.5	0	0	0	++	++
4	13.5	++	0	0	0	**0**
5	14.5	0	0	+	+	+
6	15	+	+	0	0	**0**
7	16.5	0	0	+	+	+
8	17.5	0	+	+	0	**0**
9	19.5	+	+	0	0	**0**
10	20	0	0	+	+	+
11	23	+++	+++	++	++	++
12	27.5	++	+	+	+	+
13	28	0	+	+	+	+

Table 2 (continued)

Bands number	Molecular weight	NG/AO/MAY/ 09/021	NG/AO/11/08/ 108	NG/SA/JAN/ 09/149	NG/SA/07/ 0180	**NG/SA/07/ 208**
14	33.5	0	0	+	+	+
15	35	0	++	+	+	+
16	36.5	0	++	0	0	+
17	38	0	+	0	0	**0**
18	40	0	++++	++	++	++
19	50	0	+++	+	++	++
20	65	+	++++	++++	++++	++++
21	100	+	+	0	+	+
22	129	0	0	0	0	+
23	148	0	0	0	0	+
24	170	+	+	0	+	+

+, bands present; 0, bands absent, ++, +++, ++++ band intensity

The separation of the 5 accessions into two clusters showed genetic variations. The result of the clusters analysis is given in a dendrogram (Figure 2) based on the similarity coefficient. The UPGMA clustering method based on Jaccard's similarity separated the 5 accessions into two clusters at a UPGMA similarity coefficient range of 0.2 to 1 (Figure 2).

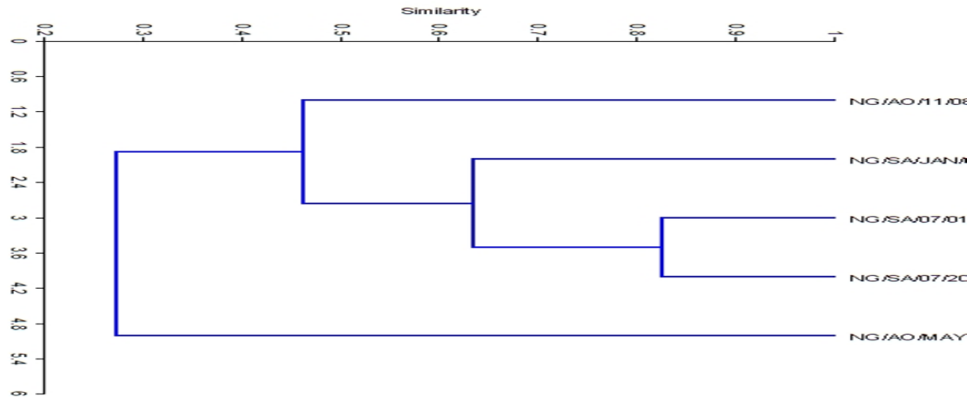

Figure 2. Dendrogram showing the relationship among the 5 accessions of pigeon pea.

Accession NG/SA/07/0108 and NG/SA/07/208 are clustered as same and were found at a similarity coefficient of 0.83, accession NG/SA/07/JAN/149 form a node at a similarity coefficient of 0.62 that is the three accessions are similar at this similarity coefficient. Accession NG/AO/11/08/108 which is in group 1 with the other three accessions (NG/SA/07/0108, NG/SA/07/208 and NG/SA/07/JAN/149) form a node at similarity coefficient 0.47. The only accession NG/AO/MAY/ 09/021

which is group 2 forms cluster to group 1 at a similarity coefficient of 0.27 and this accession is shown as being distantly related to others, it shows accession NG/SA/07/208 and NG/SA/07/0108 are closely related and accession NG/SA/07/0108 and NG/AO/11/08/108 shows a little distant from each other. It can therefore be deduced from Figure 2 that accession NG/SA/07/0108 and NG/AO/MAY/ 09/021 had the least similarity and maximum distance.

DISCUSSION

From the study, it was observed that the protein profiling of the five accessions of pigeon pea through SDS PAGE helped to find that the amount of protein present in them. Al –wadi and Lashin (2007) observed that the differences in protein migration correspond to the amino acids composition. The protein band for the highest molecular weight i.e 170 KDa was generated in accession NG/AO/MAY/09/021, NG/AO/11/08/108, NG/SA/07/0180 and NG/SA/07/208 while that of lowest molecular weight i.e 10 KDa was generated in NG/AO/MAY/09/021 only. The bands observed in different accessions are nine in NG/AO/MAY/09/021, fifteen in NG/AO/11/08/108, thirteen in NG/SA/JAN/09/149, fourteen in NG/SA/07/0180 and seventeen in NG/SA/07/208 respectively.

A total of 24 bands were scored out of which 21 bands were polymorphic with the percentage polymorphism of 87.5% and 3 were monomorphic with the percentage monomorphism of 12.5%. This is similar to the report of Ghafoor and Arshad (2008) where 25 bands were recorded among which 20 were polymorphic with a total of 80% polymorphism percentage and 5 bands were monomorphic with a monomorphism percentage of 20%. Shah *et al.* (2015) got a band of 33 out of which 30 were polymorphic with a percentage polymorphism of 90.09% and 3 bands were monomorphic with a percentage monomorphism of 9.01%. The similarity index (S.I) calculated after the protein profiling of the five accessions of *C. cajan* was maximum between NG/SA/07/0180 and NG/SA/07/208 (45.16%), the S. I value between NG/SA/JAN/09/149 and NG/SA/07/0180 was 39.29%, 35.71% similarity index was obtained between NG/AO/11/08/108 and NG/SA/JAN/09/149, NG/AO/MAY/09/021 and NG/SA/JAN/09/149 was 13.64%, however, the lowest S.I value was observed between accessions NG/AO/MAY/09/021 and NG/SA/07/0180 with 13.04%. Table 3.

Shrivastava and Gupta (2010), observed that similar accessions cannot be grown or planted because their results will yield the same product which was supported by Kakaei and Kahrizi (2011). Yi *et al*. (2008) reported that relative closeness maybe because there is no cross-boundary check among divisions and farmers exchange seeds that may disperse plant from one location to the other. Accession NG/AO/MAY/09/021 was observed to be an isolated group and shows

that it is not closely related to other accessions and can be used for crop improvement. As observed by Shah *et al*. (2015) in their research, stronger range variation can be established with another group when crossed because they are not similar genetically. Species-specific bands identified may be exploited for the hybrid identification inbreeding method (Maity *et al.*, 2009). It is also noted by Maity *et al*. (2009) that contrasting parents may be identified based on a distance between accessions of different cluster and can be used in the crossing programme for generating wider variability for choosing an improved crop.

Table 3

Similarity index of different accession of Pigeon Pea

	NG/AO/MAY/09/021	NG/AO/11/08/108	NG/SA/JAN/09/149	NG/SA/07/0180	NG/SA/07/208
NG/AO/MAY/09/021	100%				
NG/AO/11/08/108	29.12%	100%			
NG/SA/JAN/09/149	13.64%	35.71%	100%		
NG/SA/07/0180	13.04%	27.59%	39.29%	100%	
NG/SA/07/208	15.38%	31.25%	36.67%	45.16%	100%

CONCLUSION

In conclusion, the presence of genetic variability is important for improving any crop species. Understanding the quantity and patterns of genetic diversity in crop plants has significant implications for breeding programs and genetic resource conservation. A large number of cultivars that make use of limited genetic resources are being developed and released.Plant breeders frequently focus their efforts on a small number of adapted lines for genetic improvement, which were more likely to yield short-term economic advantages but may have increased vulnerability to insect pests and other biotic stresses in the long run. Therefore, it is concluded that SDS PAGE (Sodium Dodecyl Polyacrylamide Gel Electrophoresis) is a good marker for detecting the protein profile of *Cajanus cajan* by showing that accession NG/AO/MAY/09/021 can be used in a breeding programme because the accession showshigh genetic variability and low similarity index, while accession NG/SA/07/0180 and NG/SA/07/208 may not be used together in a breeding programme since they appear to be closely related or same.

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