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Genetic Diversity in Pigeon Pea (*Cajanuscajan*(L.) Millspaugh Germplasm Revealed by Gel Electrophoresis of the Seed Proteins

Agbolade James Oludare*, Adekoya Modinat Adejoke, David Oyinade Aderoju, Chukwuma Deborah Moradeke, Komolafe Ronke Justina, Olaiya Aderonke Eunice

Department of Plant Science and Biotechnology, Faculty of Science, Federal University Oye-Ekiti, Oye-Ekiti, Nigeria

Email address:

oludareagbolade@gmail.com (A. J. Oludare), datus_oludare@yahoo.com (A. J. Oludare)

*Corresponding author

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Abstract: Ten accessions of Pigeon pea (*Cajanuscajan*) obtained from National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Oyo state, were assessed for their genetic and phylogenetic relatedness through electrophoretic analysis of the seed proteins. 0.2g of the seeds were weighed and macerated with mortar and pestle in 0.2M phosphate buffer containing 0.133M of acid (NaH_2PO_4) and 0.067 of base (Na_2HPO_4) at pH 6.5. Protein characterization with standard marker revealed that the seeds of the ten accessions contained proteins (B.S.A, Oval Albumin, Pepsinogen, Trypsinogen and Lysozyme) with molecular weights ranging from 66kda and above, 45 – 65 kDa, 44 – 33 kda, 32-24 kDa and 23-14 kDa, respectively. The student T-test revealed that accessions PP2, PP3, PP5, PP6, PP8 and PP9 have molecular weights not significantly different from one another ($P<0.05$) while samples PP1, PP4 and PP7 showed significantly different values ($P>0.05$). All the accessions had at least two proteins and two major bands in common. The study revealed intra-specific similarities and genetic diversity in protein contents among the ten accessions of pigeon pea (*Cajanuscajan*).

Keywords: Accessions, Gel Electrophoresis, Intra-Specific, Germplasm, Genetic Diversity

1. Introduction

The name Pigeon pea was first reported from plants used in Barbados where it was used as pigeon feed. However, the seeds were given the name Pigeon pea in 1962 in the West Indies. There have been mixed opinion about the origin of this crop. Some historians believed that pigeon pea is a native of East indies, some said it was from Malaya while some authorities claimed that it originated from here in Africa. Other countries such as Egypt, China, India, Australia and Asia were also speculated as the primary and secondary centre of origin of this crop. However, due to the presence of several wild relatives of pigeon pea seed, archeological remains, and genetic diversity of this crop in India, it was concluded that 'gaundu' originated from there (Fuller *et. al.,* 2006). Archaeological findings of pigeon pea include those from two Neolithic sites in Odisha, Gopalpur and GolbaiSassan dating between 3,400 and 3,000 years ago, and sites in South India, Sanganakallu and TuljapurGarhi, also dating back to 3,400 years ago (Fuller *et al.,* 2006).

Furthermore, the early Asiatic name of the species *Cajanuscajanifolius* 'kayan', which is believed to be of Indian origin, all the more convinced historians that pigeon pea indeed originated from India. Myanmar also account for 16 species of *C. cajan*. Australia has 15 wild relatives of Pigeon pea of which 13 are endemic. *Cajanus*progenitors must have evolved along different lines in Australia and Asia because no species are common in these two continents (IGPRI, 2001). Only one wild relative of this specie is found in Africa, the *C. kerstingii. C. Volubilis*(Blanco) is found in Southeast Asia and *C. crassus* (Prain ex King), is restricted to the drier parts of the islands of Indonesia and the Philippines (Van der Maesen, 2006).

Breeding pigeon pea has been more challenging compared

to other food legumes due to various crop specific traits. Pigeon pea is an often cross pollinated crop, with an insect-aided natural out crossing range from 20 to 70% (Saxena, 1990), that has limited the use of efficient selection and mating designs possible in self-pollinating species. Pure line breeding, population breeding, mutation breeding, and wide hybridization have been used for development of new varieties in pigeon pea and have led to incremental improvements in the yield potential of this crop. To overcome this bottleneck, two genetic male-sterility (GMS) systems were discovered in pigeon pea (Saxenaand Kumar, 2003). Despite a 30% yield advantage over the non-hybrids, the GMS based hybrids could not be commercialized due to high cost of hybrid seed production. The yield-jump observed in the GMS hybrids encouraged the development of the alternative and more efficient cytoplasmic-genetic male-sterility (CGMS) system (Saxena and Kumar, 2003).

Pigeon pea belongs to subtribe *Cajaninae*of tribe *Phaseoleae* under sub-family *Papilionoideae*of family *Leguminosae.C. cajan* is the only domesticated species under sub-tribe *Cajaninae*. Within *Phaseoleae*, *Cajaninae*is well distinguished by the presence of vesicular glands on leaves, calyx, and pods. Currently, 11 genera are grouped under *Cajaninae.* The members of the earlier genus Atylosia closely resembled the genus *Cajanus* in major vegetative and reproductive characters but they were relegated to two separate genera, mainly on the basis of the presence or absence of seed strophiole. Based on growth habit, leaf shape, hairiness, structure of corolla, pod size, and presence of strophiole. Van der Maesen (1980) grouped the genus into six sections. The revised genus *Cajanus* currently comprises of 18 species from Asia, 15 species from Australia, and one species from West Africa. The 18 erect species were placed under three sections: Seven species in Atylosia, nine species in section Fruticosa, and two species in section *Cajanus* that consists of the cultivated species along with its progenitor, *C. cajanifolius*. Elevenclimbing and creeping species were arranged in two sections, *Cantharospermum*(5) and *Volubilis* (6) and the remaining three trailing species were classifiedunder*Rhynchosoides*. Three *Cajanus* species have been further subdivided into botanical varieties*; C. scarabaeoides* into *Var. pedunculatus and Var. scarabaeoides; C. reticulatus into Var. grandifolius, Var. reticulatus, and Var. maritimus; and C. volubilis into Var. burmanicus and Var. volubilis* (Subbarao *et. al,* 1991).

Pigeon pea seeds contain about 20-22% of protein and essential amino acids making it an important diet for the vegetarian population and also known to contain a wide range of vitamins and minerals. The cotyledons are rich in carbohydrates (66.7%) while a major proportion (about 50%) of seed protein is located in embryo. About one-third of seed coat is made up of fiber. The quantities of important sulfur-containing amino acids such as methionine and cystine range around 1% and they are present in cotyledons and embryo; while calcium is pre-dominantly present in seed coat and embryo. The nutritional composition varies from the immature to mature seeds. Due to its high nutritive values, its seeds are cooked as food, eaten as vegetables and as side dishes (Akande *et. al,* 2014).

Pigeon pea as an important source of dietary protein is used mainly as food both for human and animal and as forage for livestock. Other part of the plant such as the woody stem is used for construction and as fuel in villages. It is also used inmedicine and industries (IGPRI, 2001).

The matured dry seeds are dehulled andthen boiled, eaten as a pulse just like any other similar edible dried beans. Dehulling greatly reduces cooking time and improves the appearance, texture, palatability, digestibility and nutritional quality of the seeds (Faris and Singh, 1990). In the Caribbean region, there is a persistent demand for vegetable pods whether canned or frozen green peas because this pea is usually combined with rice or served in a soup. In India, it may be grounded and used in a variety of meatand vegetable dishes. In Africa, it is usually served as a stew (www.gracefoods. com/site/gungopeas). Dry seeds of pigeon pea also have other uses, such as in the preparation of *tempe*, a traditional Indonesian food prepared by fermenting the legume seeds with *Rhizopus* and to produce wine (Akande *et. al,* 2014)

Since pigeon pea has strong woody stems that grow up to 4m tall and branch freely, its spindly stalks are extensively used as a cooking fuel in energy short villages of several African countries and in India, Nepal and Sri Lanka. Historically, the stalks were employed to make the charcoal used in gunpowder. Farmers in Africa grow pigeon pea for its wood instead of its grain (IGPRI, 2001)

In addition to food uses, pigeonpea has outstanding soil amelioration and conservation properties. The growth habit of this crop facilitates soil protection as the canopy continues to expand for 4 months after other crops are harvested. For more than 100 years, the legume symbiosis as shown by pigeon pea was known to be the most efficient way of transforming atmospheric nitrogen into plant nutrients (Alam and Manzoor, 2005). Leaf fall at maturity adds to the organic matter in the soil and provides additional nitrogen. The root system is reported to break plough pans, thus improving soil structure, encouraging infiltration, minimizing sedimentation and smothering weeds. (Anderson *et al.,* 2001)

Pigeonpea has been used successfully in coffee plantations as a cover crop to improve soil properties, reduce weed competition as well as act as a food source for predators (Venzon *et al.,* 2006). Maize yields have been increased by 32.1% in West Africa by using pigeonpea as a cover crop (Sogbedi *et al.,* 2006). Pigeon pea is used in alley cropping, and being perennial, it can be rationed successfully, that it can be harvested without cutting the root and the lower part of the plant. Thereby making the crop mature early and decreases the cost of preparing the field and planting (Sharma *et al.,* 1978)

According to Aiyeloja and Bello (2006) and Van der Maesen (2006), pigeonpea finds wide application in traditional medicine. Diarrhea, gonorrhea, measles, burns, eye infections, earache, sore throat, sore gums, toothache, anaemia, intestinal worms, dizziness and epilepsy are treated

with leaf preparations. Root preparations are taken to treat cough, stomach problems and syphilis. Stem ash is applied on wounds, and stalks and roots are chewed against toothache. Powdered seeds serve as a poultice on swellings.

In Madagascar, the leaves are used to clean teeth. In India and Java, the young leaves are applied to sores. Indochinese claim that powdered leaves help expel bladder stones. Salted leaf juice is taken for jaundice. Leaves are also used for toothache, mouthwash, sore gums, child delivery and dysentery. Scorched seed, added to coffee, are said to alleviate headache and vertigo. In Argentina the leaf decoction is prized for genital and other skin irritations, especially in females. Floral decoctions are used for bronchitis, coughs and pneumonia. Chinese shops sell dried roots as an alexiteric, anti helminthic, expectorant, sedative and vulnerary. Fresh seeds are said to help incontinence of urine in males, while immature fruits are believed to be useful in liver and kidney ailments (Aiyeloja and Bello, 2006).

Although the medicinal value of pigeonpea in Africa has not been fully exploited, leaf decoction is diuretic and is used to control nervous breakdown, pulmonary troubles, stomach troubles, naso-pharyngeal affections, smallpox, chicken-pox and measles. The roots can be used to cure venereal diseases and the seeds as sedatives. Pigeonpea leaves have been used to treat malaria in Nigeria (Aiyeloja and Bello, 2006).

Pigeon pea production has remained nearly static over the years and this can be partially attributed to unavailability of good materials and the effects of the ever changing climatic condition of our environment.(Odeny, 2007). Factors militating against the cultivation of this crop and the probable way-out of such constraints has been identified by various researchers.

Farmers' addiction to growing of traditional landraces which in turn suffers from both biotic and abiotic stress is a factor limiting the production of pigeon pea. Farmers in the developing countries should be enlightened on the importance of planting improved hybrid or varieties of this crop and to ensure that better herbicides and fungicides and fertilizers are used.

Environmental conditions such as extreme drought, poor water holding capacity of the soil et, as well as lack of roads and market infrastructures are also factors hindering better production of the crop. With the increasing water stressed areas of the World from 28-30 countries today to 50 countries with billions of people, there is a crucial need to increase both salinity and drought tolerance in legumes. For countries coping with the above stated environmental condition, for example Africa, it should be made known to them that pigeon pea cultivation is the best solution to enhancing food security in such areas (IGPRI, 2001).

Insect, pests and diseases also inhibits 'guandu' production. Pigeon pea were less infected in the past but in recent times, diseases like *Fusarium wilt,* leaf spot and powdery mildew are of economic concern. (Aiyeloja and Bello, 2006) stated that pests and diseases are more prevalent in third world countries. Fallow period to reduce vector population, plowing to bury infected plant tissues, biological and chemical control as well as chemical applications are methods of control.

Crop's long life cycle and complex genome structure makes breeding slow and particularly expensive. The establishment of the International Crops Research Institute for Semi-Arid Tropics (ICRISAT) in 1972 created a new focus and research interest leading to the recent development of Cytoplasmic male sterile (CMS) lines for commercial hybrid breeding of pigeon pea (Mallikarjuna and Saxena, 2011).

Various breeding methods should be incorporated by breeders to produce more wild relatives of pigeon pea which have been found to posses traits like resistance to pests and diseases (Sharma*et al.*, 2003), salinity tolerance (Subbarao *et al.,* 1991), and high protein content (Saxena *et al.,* 2006).Cultivation of pigeon pea in the same area using the same technology will not enhance its production. Pigeon pea production can be improved by using modern method of breeding with better market prices (Agbolade *et. al, 2013*).

The presence of variability in the species of any organism is an assurance of evolutionary survival and the possibilities of improving these species for characters of significance. Selection, a tool for effective classification is being guided and preceded by variation; hence vivid knowledge of the within species variation is a fundamental track to effective classification and improvement (Ramanujan, 1975; Chedda and Fatokun, 1982). Hence, this study has been designed toreveal genetic diversity among ten accessions of *Cajanuscajan*(L.)millspaugh through gel electrophoresis analysis of the seed proteins, towards improvement for sustainable development.

2. Materials and Methods

2.1. Seed Acquisition

Seeds of ten accessions of the Pigeon Pea (*Cajanuscajan(L,)* millspaugh were obtained from the Genetic Resources Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo-State, Nigeria. Sample number and identification of the studied accessions are presented in the table below.

Table 1. *Sample Number, Identification and Weight.*

Sample Number	Sample Identification	Weight(g)
NG/AO/MAY/09/021	PP_1	2.4
NG/SA/JAN/09/149	PP_2	2.8
NG/AO/MAY/09/O21	PP_3	2.6
NG/SA/07/208	PP_4	2.4
NG/AO/MAY/10/021	PP_5	2.2
NG/SA/07/134	PP_6	3.0
NG/SA/07/191	PP_7	2.0
NG/SA/01/210	PP_8	2.4
NGBO1456 ^{REG}	PP_9	2.4
NG/SA/07/190	PP_{10}	2.2

2.2. Sample Preparation

Two gram of each sample was macerated using a sterile mortar and pestle; it was soaked in 10 ml of 0.2M phosphate buffer, Ph 6.5 and left in the refrigerator for 24 hours. The samples were then centrifuged to obtain a supernatant and the protein concentrations were also determined using Bradford method. The supernatants obtained were used for the SDS-PAGE.

2.3. Protein Determination

Protein concentration was determined by the method of Bradford, as contained in Weber and Osborn (1976), using Bovine Serum Albumin (BSA) as the standard, where the protein absorbance was interpolated from the standard curve. The reaction mixture consists of 10 µl of the sample solutions and 1.0ml of Bradford reagent (Weber and Osborn, 1976). The absorbance was read at 595nm.

Mathematically, according to a standard protein graph, at every optical density (O.D) of 0.4, the protein concentration is 5.0mg/ml.

Therefore, at 0.4 = 5.0mg/ml

$O.D_{new} = X$

$5.0mg/ml \times O.D_{new} = 0.4X$

X=O.Dnew/*5.0mg/ml/0.4

X=0.4Dnew*12.5

Meaning that every new O.D generated will be multiplied by 12.5 to get the protein concentration.

2.4. Preparation of Bradford Reagent

0.1g of coomassie brilliant blue 6-250 was weighed and dissolved in 50ml of 99% ethanol and made up to 1000ml using 100ml of 85% (w/v) of phosphoric acid. The mixture was then filtered to give a homogenize mixture with minimal or no impurities.

2.5. Electrophoresis on SDS-Page

The molecular weight range of the samples was determined by SDS polyacrylamide gel electrophoresis as described by Weber and Osborn (1976). Standard proteins were as contained in Sigma Molecular Weight Markers Calibration Kit for SDS polyacrylamide gel electrophoresis (Daltons Mark VII-L, Molecular Weight Marker Range 14,000-70,000). The vial was reconstituted in 1 ml of sample buffer, mixed properly and placed in a boiling water bath for 2 minutes and 10 µl aliquot was applied to a gel. The preparation of enzyme sample, running conditions, staining and destaining were as described earlier on Weber and Osborn (1976).

The relative mobility was calculated using the following expression:

$$R_f = \frac{\text{Distance of protein migration}}{\text{Length of gel after staining}} \times \frac{\text{Length of gel before staining}}{\text{Distance of dye migration}}$$

R_f values of the standards were then plotted against the logarithms of their molecular weight. The molecular weight of the enzyme preparations was then interpolated from the curve.

2.6. Data Analysis

The molecular weight values obtained were subjected to student t-test to evaluate the significance level of differences among the various accessions studied.

3. Results

All the ten accessions of Pigeon Pea (*Cajanuscajan(L.Millspaugh.)* revealed considerable intra-specific variation and overlap in most of their banding patterns.

Table 2 shows the result of protein determination at optical density 595nm. All theseaccessions contain proteins. However, sample 5 (NG/AO/MAY/10/021) shows the highest degree of protein concentration while sample 3 (NG/AO/MAY/09/021) contains the lowest amount of protein.

Table 2. *Protein determination at optical density (o.d) 595nm.*

Samples	Protein Concentration (mg/ml)
PP_1	7.213
PP_2	9.250
PP_3	5.713
PP_4	6.900
PP_5	9.800
PP_6	9.050
sPP_7	6.850
PP_8	8.963
PP_9	9.113
PP_{10}	9.038

Figure 1 is a bar chart showing the protein concentration per accessions. The protein concentration varies from PP1 to PP10. Sample E (PP5) has the highest protein concentration while sample C (PP3) contains the lowest concentration of protein. The chart was plotted in consonance with Table 2.

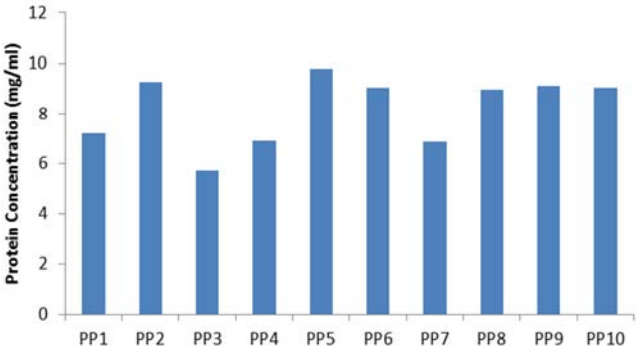
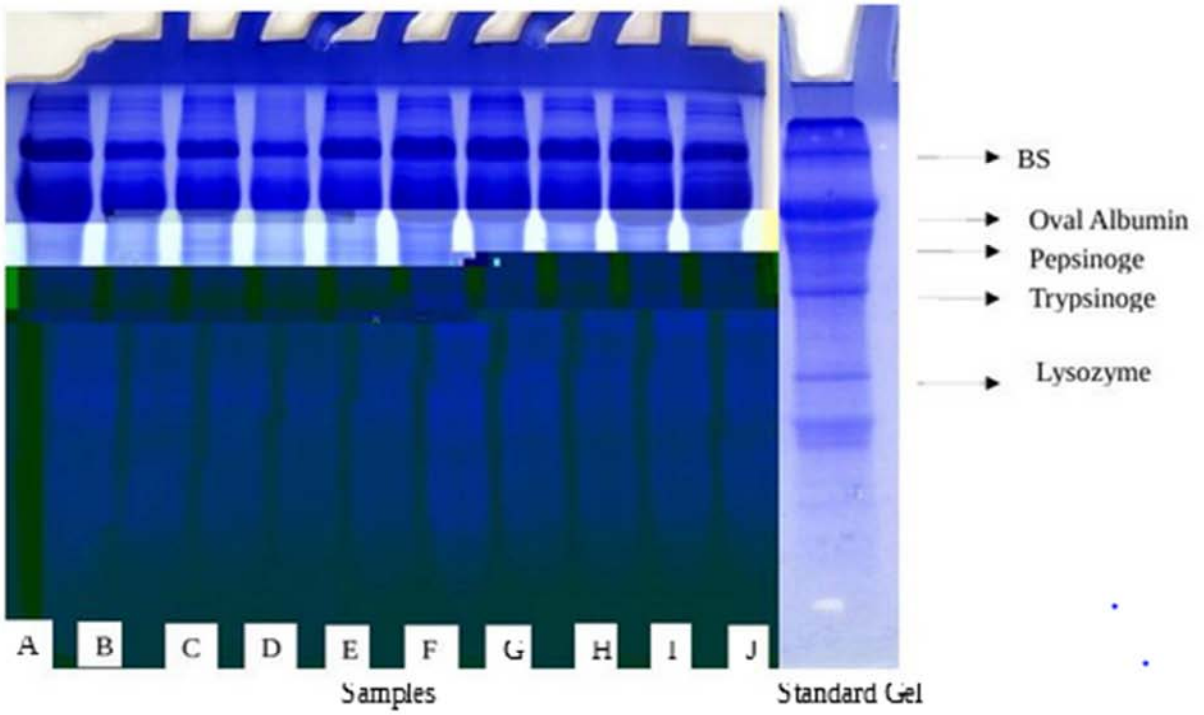


Figure 1. *Bar chart showing protein concentration per accession.*

Figure 1 is the result of SDS-PAGE of the ten accessions as compared with thestandard gel. The number and thickness of band depict the quantity and the molecular weight of proteins present per sample. Each sample has at least 3 clear bands. Samples A to I contain 7 bands while sample J has 8 bands.



Samples: A is PP_1, B is PP_2, C is PP_3, D is PP_4, E is PP_5, F is PP_6, G is PP_7, H is PP_8, I is PP_9 and J is PP_{10}

Figure 2. *Result of SDS-PAGE and the Standard Gel.*

Figure 2 is the BSA standard for protein estimation. It is used for the estimation of the concentration of proteins in the sample by comparing an unknown concentration of protein to a known concentration of Bovine serum albumin (BSA).

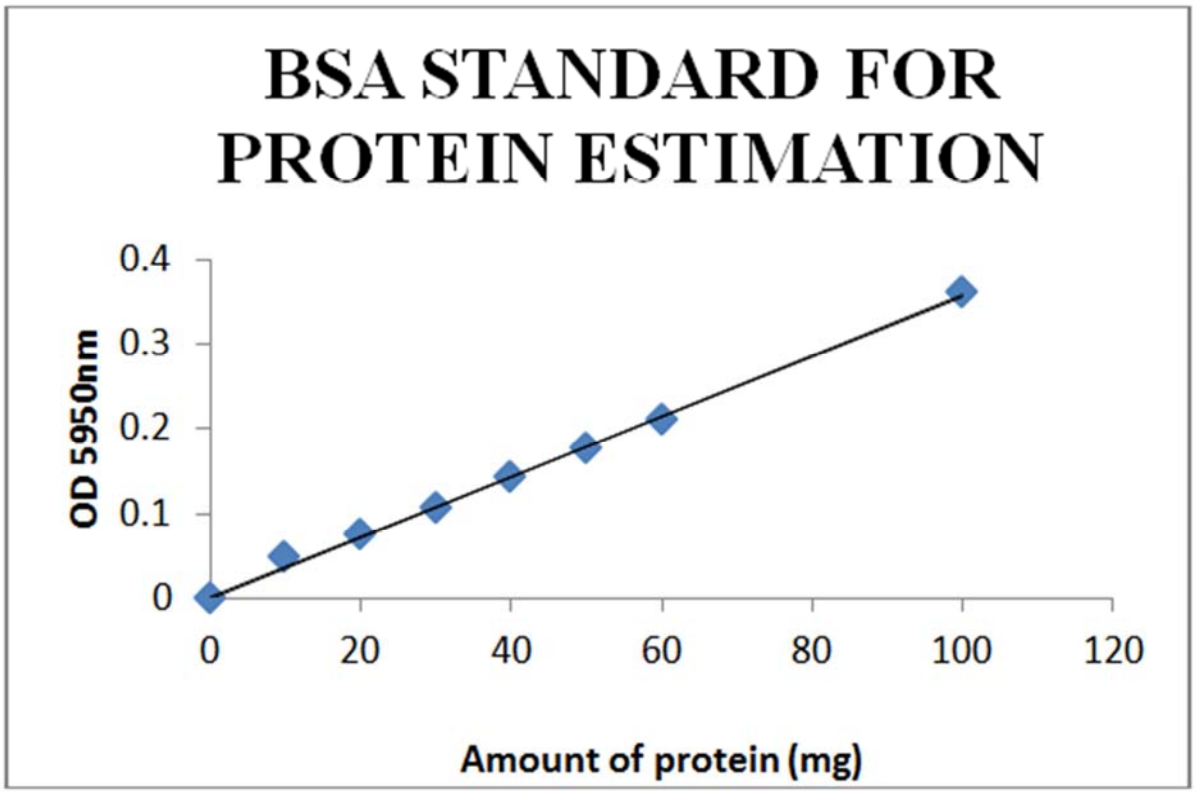


Figure 3. *Standard protein graph; SOURCE: Weber and Osborn (1976).*

Table 3 below shows the characterization of the seed proteins. The proteins were characterized based on the molecular weight of the bands in comparison to the standard gel.

Table 3. *Characterization of Protein based on their Molecular Weight.*

Samples	Total Bands	BSA(66 and Above)	Oval albumin(45-65)	Pepsinogen(33-44)	Trypsinogen(24-32)	Lysozyme(14-23)
PP_1	7	1	3	2	1	0
PP_2	7	1	2	2	1	1
PP_3	7	1	2	2	2	0
PP_4	7	2	1	2	2	0
PP_5	7	1	2	2	1	1
PP_6	7	1	2	2	1	1
PP_7	7	2	1	3	1	0
PP_8	7	2	1	1	2	1
PP_9	7	1	2	2	2	0
PP_{10}	8	1	3	2	2	0

4. Discussion

The relationship of a group of specie or accession can be determined through electrophoresis which deals with proteins, the primary product of genes. Therefore any similarities or differences observed in the banding pattern of proteins extracted from an organism are an indicative of genetic similarities and differences among them. (Agbolade *et. al, 2013*)

Result of the electrophoresis of the crude protein as revealed in Plate 1 shows that some samples are quite dissimilar both in terms of number and intensity of the bands while others show a certain degree of relatedness. Accessions 1 to 9 have seven bands each while the last accession has 8 bands. Samples A, B, C, and E, have about 5 clear bands and 2 faint bands while samples F, G, H, and I have 6 clear bands and 1 faint band, sample D has just 4 clear bands and 3 faint ones and sample J has the highest number of bands with 7 clear bands and 1 faint band. Sample F has the highest quantity of proteins due to the thickness of its bands while sample D has the faintest of all bands making it the less protein concentrated sample. All the samples have at least 2 major bands. Sample A has the thickest of all bands making it most concentrated with protein at this region. The thickness of the bands indicates the molecular weight of the protein, hence, the thicker the band the heavier the protein present at that region. The number of bands per sample establishes the amount of protein present in that sample. Since sample A to I have a total number of seven bands each, they contain equal number of protein, while sample J has a total of eight bands making its protein content higher than the rest by just 1 band (Table 3).

Despite the similarities observed in the banding pattern, a close examination revealed that differences abound among the pigeon pea (*Cajanuscajan)* accessions. However, these similarities and differences observed are understandable because both “nature” (gene) and “nurture” (environment) determine the phenotype of an organism. These differences might be due to the influence of “nurture” (environment). It follows quite logically that different accessions belonging to the same species are expected to be more phylogenetically related.

Furthermore, still according to Table 3 above, the protein concentration per sample varies. Sample 5 (NG/AO/MAY/10/021) has the highest number of protein concentration while sample 3 (NG/AO/MAY/09/O21) has the lowest concentration. The characterization of the samples protein based on their respective molecular weight done in this table establishes that Lysozyme according to the standard protein graph is least common in the samples, each sample has one or two Bovine Serum Albumin protein, almost equal amounts of Oval Albumin and Pepsinogen and some Trypsinogen. Bands with molecular weight lesser than Lysozyme were regarded as invalid. This is a similarity found among this specie. Despite the fact that these samples belong to the same species and have the same physiology, there exist a certain degree of similarities and differences at the molecular level.

The student T-test revealed that accessions PP2, PP3, PP5, PP6, PP8 and PP9 have molecular weights not significantly different from one another($P<0.05$) while samples PP1, PP4 and PP7 showedsignificantly different values ($P>0.05$). (Appendix 1 and 2)

It is worthwhile to emphasize that ultimately and logically, the differences in electrophoresis mobility of protein fractions obtain from two sources are of greater import for taxonomic purpose than the similarities of mobility. The possibility of two dissimilar proteins having identical electrophoretic mobility is known (Hayward *et. al.,* 1970), yet the assumption is made that bands derive from two different accessions that migrate the same distance in polyacrylamide gel are considered to be produced by gene(s) common to both accessions.

The proteins observed in the samples are of great value to the health. Lysozyme is a small, stable enzyme, making ideal for research into protein structure and function. It attacks the protective cell wall of bacteria, thereby protecting us from the ever-present bacterial infection. In addition to this, lysozyme also benefits bladder health, inflammation management and wound repair. However, Trypsinogen and Pepsinogen functions as storage of an inactive form of trypsin and pepsin and kept in the pancreas to be release in significant amount when required for protein digestion (Wikipedia), while Oval albumin is presumed to be a storage protein, Bovine serum albumin (BSA)is used to stabilize some restriction enzymes during digestion of DNA to prevent adhesion of the enzyme to reaction tubes, pipet tips and other

vessels. It is also commonly used to determine the quantity of other proteins by comparing an unknown protein to known amounts of BSA.

The result of the electrophoretic banding patterns of the studied accessions of pigeon pea (*Cajanuscajan)* reveals some diagnostic characteristics that could be used for taxonomic decision. Similarities and differences observed in this work agreed with the studies of Odeny (2007,) Flower and Ludlow (1987), and Agboladee*t al.,*(2013), who employed comparative electrophoretic protein banding pattern of different species and accession in establishing relation among various taxa.

5. Conclusion and Recommendation

Pigeon pea remains underutilized despite its wide range of uses and nutritive value. This study has contributed additional valuable information to the nutritional composition and genetic variabilities among its germplasms. It is recommended that this work be used as basis and refer to for future research and molecular studies on pigeon pea. The study however did not isolate the best of the samples but compared all; it can therefore be referred to for comparative study associated with the samples used in this text. It can also be used as knowledge based for farmers who seek ways of improving their crop as one of the major constraints to pigeon pea cultivars is pest and diseases. Information on the utilization, production and medicinal properties of pigeon pea is also available in this study. Enough information on the protein content of this crop is available in this context for better recommendation to the vegetarian population. Presence of all these proteins in majority of the accessions requires more investigation in other to exploit the nutritional quality and usefulness of pigeon pea (*Cajanuscajan).*

Appendix 1

Table A1. *Mean, Standard Deviation and Standard Error Values of the Variables.*

One-Sample Statistics				
	N	**Mean**	**Std. Deviation**	**Std. Error Mean**
VAR00001	3	.8647	.20561	.11871
VAR00002	3	1.0300	.12767	.07371
VAR00003	3	.9167	.21127	.12197
VAR00004	3	.8367	.28042	.16190
VAR00005	3	1.0700	.03606	.02082
VAR00006	3	1.0133	.01155	.00667
VAR00007	3	.8367	.05033	.02906
VAR00008	3	1.0067	.02309	.01333
VAR00009	3	1.0200	.02646	.01528
VAR00010	3	1.0100	.00000[a]	.00000

a. t cannot be computed because the standard deviation is 0.

Appendix 2

Table A2. *Result of the T-Test Analysis of Variables.*

One-Sample Test						
	Test Value = 0.9000					
	t	**df**	**Sig. (2-tailed)**	**Mean Difference**	**95% Confidence Interval of the Difference**	
					Lower	**Upper**
VAR00001	-.298	2	.794	-.03533	-.5461	.4754
VAR00002	1.764	2	.220	.13000	-.1872	.4472
VAR00003	.137	2	.904	.01667	-.5081	.5415
VAR00004	-.391	2	.733	-.06333	-.7599	.6333
VAR00005	8.167	2	.015	.17000	.0804	.2596
VAR00006	17.000	2	.003	.11333	.0846	.1420
VAR00007	-2.179	2	.161	-.06333	-.1884	.0617
VAR00008	8.000	2	.015	.10667	.0493	.1640
VAR00009	7.856	2	.016	.12000	.0543	.1857

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