VARIATION IN THE PROTEIN LEVEL OF DIFFERENT ACCESSIONS OF AFRICAN YAM BEAN (*Sphenostylis stenocarpa)* HOCHST. EX. A. RICH. HARMS

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OCTOBER, 2015

DECLARATION

I hereby declare that this project has been clarified by me, it is a record of my own research work and to the best of my knowledge and has not being published or presented in any form. All sources of information are duly acknowledged by means of references

Daodu Oluwaseun

Sign:  .....

Date: October, 2015

CERTIFICATION

I hereby certify that this dissertation titled; **VARIATION IN THE PROTEIN LEVEL OF DIFFERENT ACCESSIONS OF AFRICAN YAM BEAN (Sphenostylis stenocarpa)** is an original effort by, **DAODU OLUWASEUN** with **Matriculation No. BTH/11/0251**, undertaken under the supervision of Dr. **J.O AGBOLADE** of the Department of Plant Science and Biotechnology, Federal University Oye-Ekiti, Ekiti state.

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ACKNOWLEGDEMENT

My heartfelt appreciation goes to my Maker for his unconditional love shown to me for the successful task of the project. All glory is credited to his holy name.

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DEDICATION

This report is dedicated to the Almighty God who by his grace protects me from the day one of my existent to this present time.

Also, I dedicate this project to those who earnestly seek to succeed in the field of Plant Science and Biotechnology.

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ABSTRACT

Nine (9) accessions of African yam bean (*Sphenostylis stenocarpa*) obtained from the germplasm unit of the National Centre for Genetic Research and Biotechnology (NACGRAB), Ibadan, Oyo state were assessed for their variation in protein level through electrophoresis of the seed proteins. Seeds (2.0g) were macerated with sterile mortar and pestle in 0.2M phosphate buffer containing 0.133M of acid (NaH2PO4) and 0.067M of base (Na2HPO4) at PH 6.5. The accessions were screened for total protein banding patterns using SDS page electrophoresis. Result shows that protein banding pattern were taxon specific. The accession with the highest number of bands was AY4 and the lowest was AY6. Variation exists not only in the number of bands but also in the intensity of the bands. The retention value (R_f) was calculated which was used to determine the molecular characterization of the proteins based on their molecular weight. Protein characterization with standard protein marker revealed that seeds of the nine accessions contain Bovine serum albumin, oval albumin, Pepsinogen, Trypsinogen and Lysozyme with molecular weight ranging 66-above, 45-65kda, 33-44kda, 24-32kda and 14-23kda respectively.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Variability is an aspect of systematic; it established that variations occur in various level of an organism or specie.

Systematic is the study of biological diversity and its origins. It focuses on understanding evolutionary relationship among organisms, species, higher taxa or other biological entities such as gene and the evolution of the properties of taxa including intrinsic traits, ecological interactions and geographic distributions. An important part of systematic is the development of methods for various aspects of phylogenetic inference and biological nomenclature/classification (society of systematic biologists, 2015).

Systematic has also been defined as the study of the units of biodiversity (Davis, 1995). Systematic differs from ecology in that ecology is concerned with the interactions of species at a particular time while systematic is concerned with the diversification of lineages through time (systematic agenda, 2000). It is the discovery of the basic units of biodiversity (species), reconstructing the patterns of relationships of species at successively higher level, building classifications based on these pattern and naming appropriate taxa and the application of this pattern. Knowledge to studying changes in organism features through time.

Systematic aims at identifying and documenting earth's biodiversity and organizing this information in a form that can be utilized by others. It also aims at forming the basis for modern classification (systematic Agenda, 2000). It is also noted from the work of Cracraft, 1995 that systematic provides a basis for biodiversity conservation priorities.

African yam bean (*Sphenostylis stenocarpa* Hochst. Ex. A. Rich.Harms) is an underutilized tropical African tuberous legume with potential to broaden man's food base. It belongs to the class Magnoliopsida, order Fabales, family Fabaceae, subfamily Papilionoideaea, and genus Sphenostylis which content other six species (Potter and Doyle, 1994). AYB is categorized under the feed legume forages. It is a perennial climbing bush; it is a dehiscent dry fruits that split open at maturity. It is a dicotyledonous plant. It forms small tuberous roots that contain more protein than sweet potatoes, potatoes or cassava roots and above ground produces good yields (2000 kg ha") of edible seeds (National Academy of Sciences, 1979). Although it is generally considered a minor crop in most areas of its cultivation, farmers in some areas of eastern Nigeria grow it as an important source of income and it is the major legume they produce (Potter, 1992, Pretty., Morison and Hine, 2003).

The family, Fabaceae is a large and economically important family of flowering plants. It includes trees, shrubs to the perennial or annual herbaceous plants, which are easily recognized by their fruit (legume). African yam bean (AYB) is a highly adaptable crop capable of producing growth even on acid and highly leached sandy soils of humid lowland tropics (Potter, 1992, Zahran, H. H, 2001), an attribute common to most under-utilized food plants known to flourish with little Inputs in areas too marginal for conventional crops, It is a crop of dual food products: pulse and tuber. The use of the exceptionally nutritious pulse (Rachie, 1973 Aminah, H, 2003) is popular in West Africa. AYB is low in fat but has protein content up to 19 and 29% in tuber and in seed grains respectively (Uguru and Madukaife, 2001). African yam bean (AYB) is underutilized specie with great genetic and economic potentials.

The pods which may sometimes be flat or raised in a ridge-like form on both margins are usually prone to shattering; they dehisce along the dorsal and ventral sutures when dry. Each pod can yield up to 20 seeds which may be round, oval, oblong, or rhomboid. There are varieties of seed color (Oshodi *et al.* 1995, GRIN. 2009) and size (Adewale *et al.* 2010) with mono-colored or mosaic types. Mono-colored seeds are white, grey, cream, light or dark

brown, purple, or black. AYB is usually grown in mixtures with yam and cassava. The crop has medicinal importance (Potter 1992, GRIN. 2009). It has also been shown to form nitrogen-fixing nodules if inoculated with slow growing Bradyrhizobium bacteria (Assefa and Kleiner, 1997). This ability to fix atmospheric nitrogen means the plant will not require large amounts of nitrogen fertilizer to meet growth demands, thus making its production affordable to the resource poor farmers living mainly in areas where it grows.

It has remarkably low susceptibility to most field and storage leguminous pests (Omitogun *et al.* 1999, D.J. Dumet. 2010). AYB's climbing habit is also utilized as it can form a living fence where it is grown on stakes around fields of cocoyam (Potter, 1992, Zahran, H. H, 2001). Various nutritional studies have revealed the potential of AYB as an alternative food supplement to most diets consumed in the third world that lack some essential nutrients resulting in severe cases of malnutrition. Oshodi et al, 1995 and GRIN, 2009 recorded comparatively higher values of amino acids (cysteine, lysine, methionine, phenylalanine and pyrone) in AYB flour than the 1985 FAOIWHO amino acid reference values recommended as the requirement for infants. In the same analysis, it was also found to be a good source of other essential amino acids. Several food products available to the world's poor are lacking in dietary nutrients and crops with such enormous nutritional potential, such as AYB, will assist the fight against malnutrition if their cultivation is developed. In contrast to this potential, some studies conducted on under-utilized legumes in Nigeria have revealed AYB to be one of the legumes with traces of anti-nutritional substances (Oboh et al., 1998, Alozie et al, 2009).

However, some food processing measures, such as dehulling, soaking and soaking/cooking, have been found to reduce significantly the contents of some of these anti-nutritional substances (Nwinuka et al., 1997, Alozie et al, 2009), thus making AYB more acceptable for human consumption. In other studies, for example, Okeola and Machuka (2001), anti-nutritional substances such as lectin in AYB have been found to possess some insecticidal properties that can be further exploited to benefit food.

The diversity spectrum of most indigenous species has gradually been reducing in parts over time. Adewale *et al.* (2010) identified that most classification for AYB had been dependent on the seed coat colours and its pattern. Practically, classification based on phenotypic description has some deficient genetic information; hence, resultant conclusions may be undue and even misleading. The available literature on the species reveals that research efforts have focused on morphological characterization, genetic diversity, and evaluation of the nutritional and chemical components. Available reports on the cytology of the crop are scanty and not precise. The aim for studies in this area becomes imperative to provide information on the systematic knowledge about the various accession of African yam bean present.

1.1.1 ECONOMIC IMPORTANCE OF AFRICAN YAM BEAN

Throughout the study area, the African yam bean is grown primarily for its dry seeds, which are a nutritious pulse. In the Nkwanta District, the Konkombas mill the dry seeds into flour, which is processed into a paste with water and some condiments. This is then wrapped into plantain leaves and boiled and eaten as 'turbani'. The flour may also be mixed with cassava flour and cooked into a paste eaten with soups or sauces. The Chalas, another ethnic group in the Nkwanta District, boil the dry seeds for about three hours, replacing the water intermittently. The cooked beans are made into a sauce and eaten with 'gari', a roasted cassava product. Some of the farmers interviewed reported that the water drained after boiling the beans may be drunk by lactating mothers to increase their milk production.

In the Avatime traditional area of the Ho West District, fresh mature seeds are added to soups as a protein supplement, while dry seeds are roasted and milled into flour, which is processed into sauces or soups with additional condiments for eating with various foods. Ethnic groups around Ho roast the seeds and eat them with maize. The mature green beans are also

boiled in the pods, shelled and eaten. In Taviefe and neighbouring villages it was noted that goats and sheep feed on the dry pods. The crop also occupies a niche in the socio-cultural lives of the traditional people in this area. It is used in the preparation of special meals during the celebration of puberty rites for adolescent girls.

Although there are extensive references to the use of the tuberous roots of African yam bean as a source of carbohydrates in West Africa (Okigbo 1973; Anon 1979; Ezueh 1984; Ene-Obong 1992; Porter 1992), the roots were not used in this way in the study area. Farmers in the Nkwanta District, noted for yam production in Ghana, attach no importance to the bean tubers as their yield compares poorly to that of yam. In the Ho-West District, also noted for production of many local staples such as yam, cassava, cocoyam and plantain, farmers were not even aware of the tuber-producing ability of the crop.

Other uses to which the crop is put in the study area are implicit, as these are not obvious to the farmers. It features extensively as an intercrop in the traditional farming system throughout the study area. Unknown to the traditional farmers, it may be serving as a rich source of leaf litter for improvement of soil characteristics. The crop also modulates profusely and probably has high nitrogen-fixing ability, thereby helping to replenish soil nitrogen.

1.1.2 RESEARCH OBJECTIVES

The potential of AYB cannot be over emphasised. However, like many other crops of the third world it is still under-utilized because of inadequate information on its physiology, agronomy, lack of good planting material and improved varieties. Due to the restricted attention it receives in terms of production and research, it faces eminent danger of extinction/erosion. It is also losing out to major legume and tuber crops such as cowpea (*Vigna unguiculata*) and potato (*Solanum tuberosum*) that have been improved for better yield, quality, and disease and pest resistance. Like many other under-utilized crops, its survival as a crop has largely been sustained through tradition and knowledge of the local growers. This is evident from the Paucity of documented information on its culture in general. The specific objectives of this current study therefore are as followed:

- To establish that variation exist among the nine (9) accessions of African yam bean *Sphenostylis stenocarpa* based on the molecular weight and quantity of the seed protein through electrophoresis.

1.2 LITERATURE REVIEW

1.2.1 BACKGROUND

Adequate information is necessary in the course of proposing new research concepts. Information on most indigenous species is poor due to gross neglect over the years; such that in some cases, clues to most indigenous species have only been gotten through cultural hand-downs. Genetic resources of indigenous species are poorly conserved; therefore, most research on these species had depended on few landraces from the farmers and local markets. However, the genetic materials in farmer's hands (species irrespective) are those they (the farmers and local consumers) preferred; implying that some initially available cultivars may have been lost to selection pressure. The diversity spectrum of most indigenous species has gradually been reducing in parts over time (Adewale, B. D., Kehinde, O. B., Odu, B. O., & Dumet, D. J. (2008)). Adewale *et al.* (2010) identified that most classification for AYB had been dependent on the seed coat colours and its pattern. Practically, classification based on phenotypic description has some deficient genetic information; hence, Resultant conclusions may be undue and even misleading. There are about fifty tuberous legumes of international significance; seventeen of them are of African origin (Saxon, 1981). These tuberous legumes may be used as food/feed, insecticide, medicine and flavoring agents. A thorough review of literature suggests that among the 17 tuberous legumes from Africa, AYB is most prominent in use (Saxon, 1981). In 1979, National Research Council (NRC) published a sketchy summary of information on AYB. However, most of research conducted during the past three decades, were especially on the nutritional aspect of the crop.

1.2.2 ORIGIN OF AFRICAN YAM BEAN

The African yam bean was originated in Ethiopia. Both wild and cultivated typed now occur in tropical west Africa from Zimbabwe, throughout west African from guinea to southern Nigeria, being especially common in the latter and in Togo and the Ivory coast, and in east African from northern Ethiopia (Eritrea) to Mozambique, including Tanzania and Zanzibar. The center of diversity of African yam is only in African. Nigeria has the largest production of African yam bean (Abbey and Berezi, 1988; Potters, 1992).

1.2.3 MORPHOLOGICAL DESCRIPTION OF AFRICAN YAM BEAN (*Sphenostylis stenocarpa)*

Sphenostylis stenocarpa is a vigorously climbing herbaceous vine whose height can reach 1.5-3 metres or more depending on the height of the stakes and cultivar. The main vine/stem may or may not be pigmented. The crop produces many branches which also twine strongly on available stakes. The vegetative growing stage is noted with profound production of trifoliate leaves. The terminal leaflet length could be up to 14 cm long and 5cm broad. The pods which may sometimes be flat or rose in a ridge-like form on both margins are usually prone to shattering; they dehisce along the dorsal and ventral sutures when dry. Each pod can yield up to 20 seeds which may be round, oval, oblong, or rhomboid. There are varieties of seed color (Oshodi *et al.* 1995) and size (Adewale *et al.* 2010) with mono-colored or mosaic types. Mono-coloured seeds are white, grey, cream, light or dark brown, purple, or black. Four to ten flowers are arranged on long peduncles, which are usually on the primary and the secondary branches. Its inflorescence is raceme and exhibits acropetal mode of floral maturation (Adewale, 2011). The large and excellently attractive flowers blends pink with purple, the standard petals slightly twist backward on itself at anthesis. According to the

observation of Popoola *et al.* (2011) using 25 AYB accessions, the pollen grains had tricolporate, fenestrate and scabrate exine. They further noted that the pollen grain had three colpus which were characteristically large with window-like spaces lacking tectum. The pollen grains were single reticulate, slightly rounded without sharp corners, the spinous cover was interrupted by three protuberances (germpores) in a fixed geometrical pattern.

The flower seems to exhibit self-pollination; a peduncle can hold up to three or more pods. The usually linear and long unicarpel pods turn brown when matured. Pods may have flat or raised margin on both side. Most dried pods do dehisce along the dorsal and the ventral suture causing shattering and loss of seeds. However, Adewale (2011) observed variation in the shattering tendencies of dried pods of AYB. Each pod can yield up to twenty seeds which may be rounded, oval, oblong or truncated (Milne-Redhead and Polhill, 1971; Adewale et al., 2012). The mat or shiny seeds can be mono-coloured or mosaic. The prominent basal colours in AYB includes: white, grey, cream, light or dark brown, purple and black. The mosaic type has various modifications of speckling and varieties of colour mixtures. There appear to be a number of 'types' according to seed colour (Oshodi et al., 1995). Moreover, considerable variation existed among seed sizes.

The stem of the plant produces small underground tubers of various sizes and shapes (Adewale & Dumet, 2011). The tubers are very similar to sweet potatoes; its flesh is white and watery (Kay, 1987). There seem to be some evidence that yields of seeds and tubers are inversely related (Hutchinson & Dalziel, 1958; Milne-Redhead &Polhill, 1971; Dukes, 1981). An update research with the novel objective of understanding the assimilated partitioning pathway of the crop for biomass and agricultural yield is necessary to ascertain the above claim. Output from such study may provide primary information for breeding programme for specific varieties; such as grain, tuber or dual.

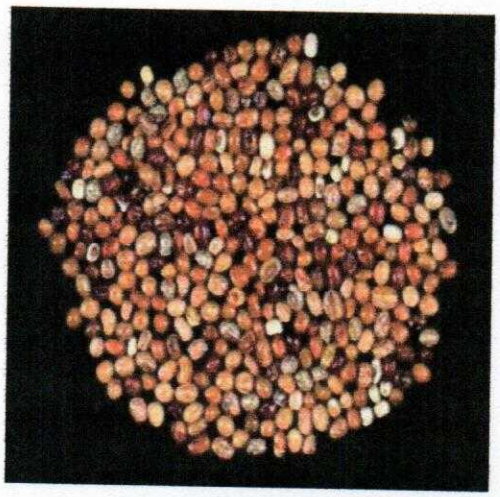


PLATE 1: Diversity in colour, colour pattern, structure, texture, brilliance, E.T.C of African yam bean seeds. Photo by D. Adewale IITA, 2011.

PLATE 2: African yam bean plant showing mature pods ready for harvest. Photo by Daniel Adewale, IITA, 2011.

1.2.3.1 QUALITATIVE DESCRIPTIONS OF AFRICAN YAM BEAN

The various forms of agronomic qualities and their level of description is showed by Adewale, 2011 who gave a concise description of African yam bean which varies from the leaf, seed, pod and tuber. The features and there level of description are given below;

1. Pod dehiscence-shattering; Non-shattering
2. Splitting of testa of seeds -absent; present
3. Seed cavity ridges on pods-absent; present
4. Seed shapes-Round; Oval; Oblong; Rhomboid
5. Testa basal colour of seeds- White; Grey; Cream; Light brown; Reddish brown; Purple, Variegation with various marbling
6. Testa texture of seeds---- Smooth; Rough; Wrinkle
7. Brilliance of seed-- Matt; Medium; Shiny
8. Eye colour pattern of seeds-- Fork-like structure below the hilum; Incision-like structure below the hilum; Vase-like structure around the hilum
9. Leaf colour-- Pale green; Green; Dark green
10. Pigmentation of plant parts-- Absent; Present
11. Intensity of pigmentation of plant part-- Slight; Moderate; Extensive
12. Tuber production-- Yes; No
13. Tuber shapes-- Round; Oval; Spindle; Irregular
14. Tuber skin colour--- Brownish-orange; Cream; Pink

Source: Adewale (2011).

1.2.3.2 QUANTITATIVE DESCRIPTIONS OF AFRICAN YAM BEAN

Quantitative description is the measured numeric values, quantity or statistical comparison derived from systematic survey, observation or analysis. The collected data of the 80 accessions of African yam beans done by Adewale, 2011 was subjected to statistical tests to see if the results are internally consistent or representative of random chance. The quantitative description of AYB done by Adewale, 2011 for 80 accessions is show below:

Agronomic characters	Minimum	Mean	SE	Maximum
Days to seedling emergence [Days]	4.00	6.17	0.05	9.00
Days to peduncle initiation [Days]	51.00	61.48	0.54	72.60
Days to 50% flowering [Days]	77.00	95.35	0.44	127.00
Terminal leave length [cm]	7.19	9.30	0.09	17.89
Terminal leave width [cm]	2.09	3.76	0.05	8.71
Peduncle length	4.66	10.04	0.20	15.40

[cm]				
Petiole length [cm]	2.80	4.13	0.05	5.13
Pods per peduncle	1.00	1.89	0.03	4.00
Internode length [cm]	3.60	7.17	0.06	10.40
100 seed weight [g]	11.46	23.62	0.21	36.00
Seed volume [cm3]	7.69	17.53	0.17	40.00
Pod length [cm]	12.70	22.81	0.15	30.64
Pod beak length [cm]	0.35	0.97	0.03	1.66
Seeds per pod	6.50	13.81	0.15	22.00
Locules per pod	8.00	16.39	0.15	26.00
Seed Weight pod [g]	1.13	3.34	0.05	7.70
Seed length [mm]	5.54	8.74	0.04	11.45
Seed width [mm]	4.91	6.46	0.03	7.59
Seed thickness [mm]	4.39	6.16	0.03	7.53
Seed	0.82	1.36	0.01	1.48

length/width ratio				
Seed width/thickness ratio	0.77	1.05	0.01	1.32
Seed length/thickness ratio	0.93	1.44	0.01	1.88
Pods per plant	14.00	28.33	0.95	101.80
Pod weight per plant [g]	21.33	158.47	5.38	597.53
Seed weight per plant [g]	19.74	101.98	4.02	466.70
Shelling percentage [%]	34.87	62.19	0.43	83.90
Grain yield per hectare [Kg]	114.84	1075.56	42.05	4875.17
Tuber yield [Kg]	8.38	95.48	8.32	336.68
Tuber weight [g]	3.44	28.45	1.86	80.04
Tubers per plant	1.20	3.20	0.10	5.00
Tuber length [cm]	8.30	12.69	0.21	16.20

Tuber width [cm]	7.20	11.63	0.27	18.00
Length/width ratio of tubers	0.81	1.12	0.02	1.45

Table 1: Sample size = 80 accessions.

Source: Adewale (2011).

1.2.4 TAXONOMY OF AFRICAN YAM BEAN (*SPHENOSTYLIS STENOCARPA)*

Sphenostylis was the botanical genus evolved by Harms (1899) to describe a group of distinctive leguminous taxon formerly grouped within the genus *Dolichos* and *Vigna*. The generic name: *Sphenostylis* arose from a Greek word *sphen*, meaning wedge shape (Allen & Allen, 1981). Therefore, the genus *Sphenostylis* comprises a group of leguminous species with dorsiventrally cuneate style with flattened stigmatic tip (Milne-Redhead and Polhill, 1971). Former grouping within the two genera was because they were found to be closely related to them; hence most species in the genus *Sphenostylis* initially bears *Dolichus* and *Vigna* synonyms (Harms, 1899, 1911). From most recent update, *Nesphostylis* is the nearest sister genus to *Sphenostylis* because both genera have dorsiventrally cuneate and flattened style (Milne-Redhead & Polhill, 1971; Potter & Doyle, 1994); however, *Nesphostylis* is distinguished from *Sphenostylis* by the presence of aril onthe seed. *Sphenostylis* is a small genus whose morphotype can be prostrate, climbing or erect. There are seven species within the genus; *Sphenostylis stenocarpa* is economically most important species (Potter, 1992). The taxonomic profile of African yam bean is presented as:

Kingdom- Plantae

Subkingdom - Tracheobionta

Super division - Spermatophyta

Division - Magnoliophyta

Class - Magnoliopsida

Subclass - Rosidae

Order - Fabales

Family - Fabaceae

Sub family - Papilionoideae

Tribe - Phaseoleae

Sub tribe - Phaseolinae

Genus - Sphenostylis E. Meyer

Species - *Sphenostylis stenocarpa* (Hochst. Ex. A. Rich.) Harms.

1.2.5 SYNONYMS OF AFRICAN YAM BEAN

The six mostly occurring botanical synonyms of AYB in the literature are: *Sphenostylis ornata* A. Chev., *Sphenostylis congensis* A. Chev., *Dolichos stenocarpus* Hochst. Ex. A. Rich., *Sphenostylis katangensis* (De Wild.) Harms, *Vigna katangensis* De Wild., *Vigna ornate* Welw. Ex. Baker. Some popular non-botanical synonyms of the crop includes: Yam pea and Tuber bean (English-Terrell et al., 1986; Rehm, 1994), Haricot igname and/or Pommedeterre du Mossi (French-Duke, 1981) and Afrikanische yam bohne and/or Knollenbohne (German - Rehm, 1994). Some indigenous lingual synonyms for AYB in Africa according to Kay (1987) are: "Diegemtenguere" (Mali), "Girigiri" (Hausa, West Africa), "Norouko" and/or "Roya" (Sudan), "Okpududu" (Igbo, Nigeria) and "Sese" (Yoruba, Nigeria). There are many dialectical synonyms for AYB. Synonyms for AYB in Igbo (Nigeria) includes: "Akidi", "Azima" (Ohafia, Abia state, Nigeria), "Uzaaku" or "Ijiriji" (Nsukka, Enugu state, Nigeria; Asoiro and Ani, 2011). In the tribal tongue of the Yorubas in Nigeria, AYB is called: "Ewe" (Ijesha, Osun state, Nigeria), "Otiili" (Ekiti, Ekiti state, Nigeria), "Ekulu" (Ipe-Akoko, Ondo state, Nigeria), "Peu" (Ijebu, Ogun state, Nigeria),"Sunmunu" (Iseyin, Oyo state, Nigeria) etc. Some other tribal names for AYB in Nigeria are: "Ihiehie" (Ishan, Edo state, Nigeria), "Iye" (Estako, Local government, Edo state, Nigeria), "Ahuma" (Tiv, Benue state, Nigeria), "Nsama" (Efik-Ibibio, Akwa Ibom and Cross River state according to Edem et al. (1990)).

1.2.6 CYTOGENETICS OF AFRICAN YAM BEAN

The genus *Sphenostylis* exhibits a diploid chromosomal set of 2n = 22 (Lackey, 1980). The somatic chromosome set for AYB according to Baudoin and Mergeai (2001) was 2n = 18. However, the 2n = 22 counts was reported for *Sphenostylis marginata* a sister species of *Sphenostylis stenocarpa* (Peter & Davidse, 1977). Most recently, Popoola et al. (2011b) and Adesoye and Nnadi (2011) confirmed that AYB exhibits diploid somatic chromosomal status. According to Popoola et al. (2011b), majority of AYB accessions had eleven bivalent (2n =22) chromosomal status while an accession (TSs3) had the nine bivalent chromosomal status (2n = 18). The result of Adesoye and Nnadi (2011) indicated that the chromosome count ranged between 2n = 20 to 24 with 2n= 22 being the most frequent. Summarily, four bivalent chromosomal status (2n = 18, 20, 22 and 24) are identifiable in AYB. The sizes of the chromosomes of AYB were generally small in size (Popoola et al., 2011b; Adesoye & Nnadi, 2011) ranging between 0.58 to 1.84μm. While the above information seems to ascertain prevalent genetic potentialities within the species, it equally submits the cytology of the crop to further investigation.

1.2.7 GEOGRAPHICAL DISTRIBUTION AND WIDE ADAPTABILITY OF AFRICAN YAM BEAN

AYB tolerates wide geographical, climatic and edaphic ecologies. The stretch of the environment where it thrives lie within the latitudes of 15° North to 15° south and the longitudes of 15° West to 40° East of Africa (Adewale et al., 2008). There is no record of the origin of the crop in any other continent except Africa (Potter & Doyle, 1992; Potter & Doyle, 1994). Hence, the above geographical catchments could be referred to as the centre of diversity of AYB (Figure 1). This confirms the common claim that the crop is a tropical African legume.

The centre of diversity of AYB was presented by Germplasm Resources Information Network [GRIN]. GRIN (2009) presented the regional demarcation as: Northeast tropical Africa (i.e. Chad and Ethiopia), East tropical Africa (i.e. Kenya, Tanzania and Uganda), West-Central tropical Africa (i.e. Burundi, Central African Republic and Zaire), West tropical Africa (i.e. Cote d'Ivoire, Ghana, Guinea, Mali, Niger, Nigeria and Togo) and South tropical Africa (i.e. Angola, Malawi, Zambia and Zimbabwe). Nigeria is prominent for AYB production among other countries of Africa (Figure 2); the contrary opinion of Abbey and Berezi (1988) and Alozie et al. (2009) on the major growing/producing area of the crop within Nigeria notwithstanding. AYB cultivation extends from the southern states of Nigeria to the north of the country (Adewale et al., 2008). The cultivation of AYB is localized around Nkwanta and Ho-West districts of the Volta region of Ghana (Amoatey et al., 2000; Klu et al., 2001).

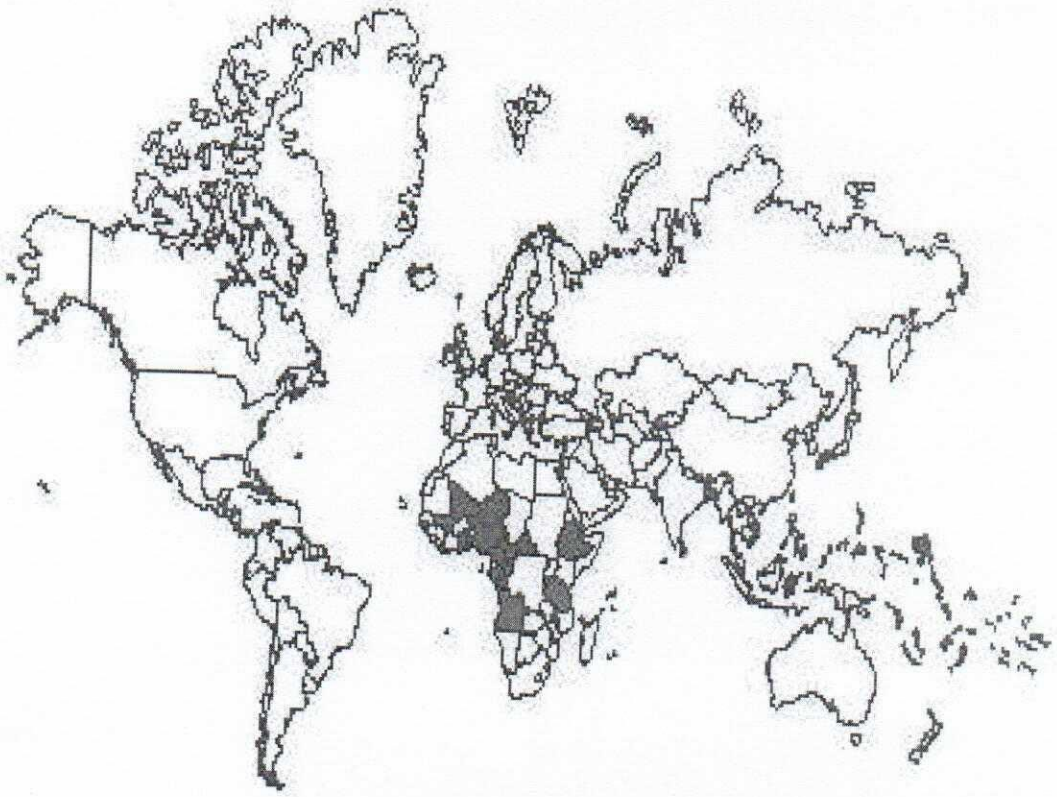


PLATE 3: The centre of diversity for African yam bean

Source: http://www.zipcodezoo.com/Plants/S/Sphenostylis%5Fstenocarpa/Default.asp (September 22, 2009).

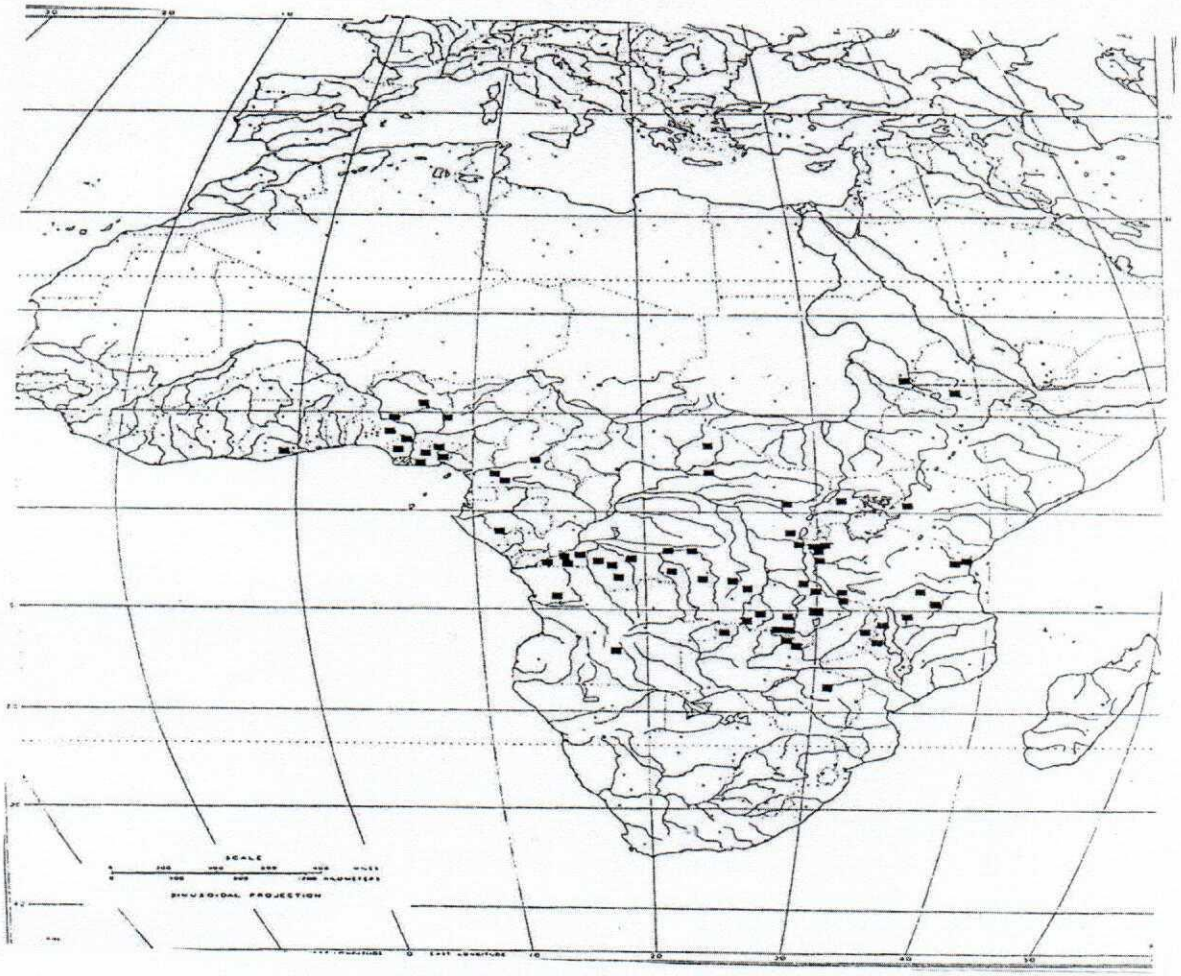


PLATE 4: Distribution of African yam bean in Africa

Source: Potter and Doyle (1992).

The suitability of AYB for the diverse ecologies (Anochili, 1984; Schippers, 2000; Betche et al., 2005) suggests that it can potentially serve as an important crop for food security since it can tolerate varied soil and climatic conditions. This quality confers on it an ecological advantage over most conventional legumes. Contrary to the remark of Klu et al. (2001) on the nearness of the crop to extinction, the ability of the crop to survive in diverse agro ecological conditions of Africa must have aided its continual existence over times. Presently in Ghana and Nigeria, there is a dwindling interest in the production of AYB among the farmers (Amoatey et al., 2000; Olisa et al., 2010). Only a very small sector of the farmers appreciates its cultivation, hence, they are the holder of the crop's genetic resources (Adewale et al., 2012).

1.2.8 INTRA-SPECIFIC VARIABILITY AND DIVERSITY WITHIN AFRICAN YAM BEAN

According to Adewale and Dumet (2009), the continual availability of the genetic resources of the crop is threatened and the cultivation of the crop may further decline due to continued neglect and underutilization. Wide exploration of AYB's genetic resources in Africa will provide assurance of its future genetic improvement (Adewale et al., 2011). Seed length, width, thickness and their ratio significantly differentiated among 80 accessions of AYB (Adewale et al., 2010). For all the genotypes tested, the seed length was longer than the width and thickness but the difference between seed width and thickness was not consistent. Reliable and predictable relationship existed between the seed length, width and thickness. The three metric measurements on AYB seeds were equally very important in the seed shape determination of the crop (Adewale et al., 2010). Kay (1987) remarked that the tuber shape of AYB was only spindle-like. The tuber shapes of AYB as observed by Adewale (2011) and Adewale and Dumet (2011) varied and included round, oval, spindle and irregular. Results from phenotypic evaluation of small ($\leq$ ten) to large ($\geq$ 30) number of accessions of AYB

revealed wide intra-specific variability for some agro-morphological variables (Potter, 1992; Ene-Obong & Okoye, 1992; Akande, 2009; Adewale et al., 2010, Popoola et al., 2011a; Adewale et al., 2012). AYB samples significantly differed in the proximate fractions of the seeds (Adeyeye et al., 1999). Significant differences were observed in the crude protein fractions, percentage ash fraction, nitrogen-free extract, crude fibre, carbohydrate content (Ameh, 2007) and fatty acid content (Adeyeye et al., 1999). Lectin extract differed significantly among two accessions of AYB (Okeola et al., 2002). The result from the study of the anti-nutritional factor content in three varieties of AYB revealed that significant ($P < 0.05$) differences exist among the genotypes for anti-nutritional factors especially for cyanogenic glycosides (Betche et al., 2005). The range of protein and starch content in AYB (Betche et al., 2005) was 22-25 g 100 g-1and 42-47 g 100 g-1, respectively.

The application of molecular tools to unravel intra-specific diversity of the crop had been notably experimentally by Moyib et al. (2008), using Random Amplified Polymorphic DNA (RAPD) technique and Adewale (2011), using Amplified Fragment Length Polymorphism (AFLP) technique. While two RAPD primers assessed diversity in 24 AYB accessions from Nigeria (Moyib et al., 2008), five AFLP primers revealed the genetic diversity among 80 AYB accessions from Nigeria and other countries (Adewale, 2011). The diversity in AYB by RAPD and AFLP techniques did not show any clear-cut eco-geographical demarcation, suggesting that environmental mutation among the accessions of AYB was low. The inherent stability of the crop across wide environment deserves further investigation and exploitation.

1.2.9 AGRONOMY OF AFRICAN YAM BEAN

Planting of AYB usually starts when the rain had stabilized; between May to July, in Ghana and Nigeria (Klu et al., 2001; Okpara & Omaliko, 1995). Two to three seeds are sown at the base of the heaps of major crops. Traditionally in Nigeria and Ghana, AYB is grown as a minor crop in mixed association with crops especially, yam and cassava (Amoatey et al., 2000; Saka et al., 2007). Sole cropping of AYB is rare; it is usually planted along with yams to share the same stake for support (Amoatey et al., 2000; Ibeawuchi et al., 2007). Scarification is not a practice to aid imbibition before germination in AYB despite the hardness of the seed (Olisa et al., 2010). AYB exhibits hypogeal germination (Adewale, 2011) which occurs between the fourth and the seventh day after planting. AYB performs better when intercropped than when grown as sole crop (Kay, 1987; Baudoin & Mergeai, 2001; Saka et al., 2007; Adeniyan et al., 2007). However, the research finding of Ibeawuchi et al. (2007) was contrary; they obtained significantly higher grain yield from AYB when planted under sole cropping system. This could justifiably be due to the lack of competition for the necessary growth resources from other crops; hence, there is a promising commercial production of the crop. Optimum plant and row to row spacing for AYB varies considerably. There is no report in literature of any recommended spacing for AYB under sole cropping. Most workers have therefore used the planting distance of most principal crops, e.g. yam, cassava etc. to which AYB is usually intercropped. The plant population of 24 200 plants/ha produced a range of 3461-3872 kg/ha grain yield from some cultivars (Kay, 1987). The multi-locational trial of 30 accessions of AYB evaluated at the spacing of 1m x 1m by Adewale (2011) gave a grain yield range of 248-4,130.46 kg/ha. Inoculation of AYB with compatible rhizobial strains could possibly prevent the use of supplementary fertilizer (Oganale, 2009). AYB like every other legume can derive adequate nitrogen through atmospheric fixation (Assefa & Kleiner, 1997). Oganale (2009)

described the crop as a profuse nodulator with some *Rhizobium,* strains. He remarked that the AYB landraces studied obtained 79.0-97.6% of their nitrogen from the atmosphere and the growth of the inoculated plants increases. Some of the quantitative traits of AYB had plastic response to fertilizer application. Fertilizer application improved the yield and yield components and some vegetative parameters of AYB. The values of most of the yield parameters increased with increasing level of NPK fertilizers up to 60 Kg/ha, however yield and other responses declined with the further increase of NPK dosage (Togun & Olatunde, 1998). Olaposi and Adarabioyo(2010) obtained the highest number of flowers with the application 60 Kg ha-1 NPK fertilizer. This suggests that 60Kg/ha NPK fertilizer treatment favoured AYB production. Phosphate application greatly enhanced grain yield and other yield parameters of AYB (Ikhajiagbe et al., 2009). The result of the factorial combination of Nitrogen, Phosphorus and staking levels by Okpara and Omaliko (1995) revealed that staking (among other factors) is a very important cultural practice for harvesting optimum grain yield of AYB. Information on the response of different cultivars to agronomic treatments for tuber yield evaluation is rare in literature. The low consideration of this second economic product of AYB in research could probably be due to its non-use in the meal of the West Africans.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 MATERIALS

Mortar and pestle, test tubes, beakers, measuring cylinder, micropipette, Ph meter, conical flask, Spatula, weighing balance, test tube rack, refrigerator, foil, tissue paper, centrifuge, electrophoresis machine and nine (9) accessions of african yam bean, Tris-base, tris-HCl, ethanol, phosphoric acid and comassive Brilliant blue G-250 were purchased from Sigma Chemical Company, St. Louis, MO, USA. Bovine Serum Albumin (BSA), Standard proteins 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) were purchased from Sigma Chemical Company, St. Louis, MO, USA.

2.2 METHODS

Table 2: Sample identification and weight

Sample	Sample identification	Weight
AY92[A]	AY1	3.7
AY57	AY2	4.2
AY45C	AY3	5.0
AY45	AY4	5.7
AY94[A]	AY5	5.2
NG/AY/APR/09/014	AY6	4.7
NG/OA/09/11/058	AY7	5.6
AY93[A]	AY8	4.7

2.2.1 PREPARATION OF 0.2M PHOSPHATE BUFFER AT PH 6.5

Prepare 1 litre of 0.2M phosphate buffer with pH 6.5

$$pH = pka + \log\frac{[base]}{[acid]}$$

$$6.5 = 6.8 + \log\frac{[base]}{[acid]}$$

$$6.5 - 6.8 = \log\frac{[base]}{[acid]}$$

$$-0.3 = \log\frac{[base]}{[acid]}$$

$$\text{Antilog-o.3} = \log\frac{[base]}{[acid]}$$

$$0.501 = \frac{[base]}{[acid]}$$

$[base] = 0.501[acid]$ ---------- eqn 1

$[acid] + [base] = 0.2M$ ----------eqn 2

Substitute equation 1 in 2

[acid]+0.501[acid]=0.2M

1.501[acid]=0.2M

[acid]=$\frac{0.2M}{1.501}$ =0.133M

Substitute 0.133M in equation 2

[acid]+[base]=0.2M

0.133M+[base]=0.2M

[base]=0.2M-0.133M

=0.067M

To calculate the mass of both base and acid in 0.2M of phosphate buffer.

NaH_2Po_4 which is the aacid is added to Na_2HPo4 which is the base to produce the phosphate buffer.

The molecular weight of Na_2HPo4= 156.01g/mol

NaH_2Po_4 = 177.99g/mol

Concentration of acid in mol/dm^3 = $\frac{\text{concentration of acid in g/ } dm^3}{\text{Molar mass}}$

0.133mol/ dm^3 =$\frac{\text{mass}}{1.56.01\text{g/mol}}$

Mass = 0.133 x 156.01

=20.75g/dm^3 in 1 litre

=10.375g/dm^3 in 500ml

Concentration of base in mol/dm^3 = $\frac{\text{concentration of base in g/dm}^3}{\text{Molar mass}}$

0.067mol/ dm^3= $\frac{\text{mass}}{177.99\text{g/mol}}$

Mass = 0.067mol/dm^3 x 177.99g/mol

=11.92g/dm^3

Therefore the mass of base in 1 litre of 0.2M phosphate buffer is 11.92g/dm^3

Mass of base in 500ml = $\frac{11.92\text{g/ dm}^3}{2}$ = 5.96 g/dm^3

2.2.2 SAMPLE PREPARATION.

Two gram of each sample 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.

2.2.3 PROTEIN DETERMINATION

Protein concentration was determined by the method of Bradford (1976) using Bovine Serum Albumin (BSA) as the standard, where the protein absorbance was interpolated from the standard curve. The reaction mixture consists of 100µl of the sample solutions and 1.0ml of Bradford reagent (Bradford, 1976). The absorbance was read at 595nm.

2.2.3.1 PREPARATION OF BRADFORD REAGENT

Bradford reagent is prepared by dissolving 0.1g of commasive brilliant blue 6-250 in 5ooml 99% ethanol and 100ml of 85% (w/v) of phosphoric acid was added and it was made up to 1 litre. The reagent is then filtered to give a perfect and homogenized mixture. Filtration is done so as to remove all possible impurities in the reagent.

Table 3: Bradford method for protein determination

Reagent	**Blank**	**Test**
Crude extract(sample)	———	100ul
(Distilled)DH_2O	100ul	———
Bradford	1000ul	1000ul

2.2.3.2 CALCULATION OF PROTEIN CONCENTRATION

According to a protein standard graph, at a particular optical density (O.D) of 0.4, protein concentration equals 5.0mg/ml.

0.4=5.0mg/ml

$O.D_{new}$=x

Cross multiple

$O.D_{new}$5.0mg/ml=0.4 x

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

$X= O.D_{new} \times 12.5$

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

2.2.4 ELECTROPHORESIS ON SDS-PAGE

The molecular weight ranges of the samples were 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 min. A 10 µl aliquot was applied to a gel. The preparation of enzyme sample,

CHAPTER THREE

3.0 RESULTS

All the accessions of African yam bean (*Sphenostylis stenocarpa*) revealed considerable intra specific variation and overlap in most of their banding patterns.

Table four (4) shows the result for the determination of protein at optical density (O.D) 595nm. The result shows variation among the accessions from AY1 to AY9. The accession with the highest concentration is AY2 while AY5 has the lowest protein concentration.

Table 4: Result for determination of protein at optical density (O.D) 595nm

S/N	Protein concentration (mg/ml)
AY1	8.475
AY2	11.588
AY3	9.613
AY4	6.913
AY5	5.600
AY6	6.638
AY7	6.625
AY8	9.175
AY9	6.500

Figure one (1) is a bar chart showing the variation that existed among the various accessions of African yam bean. It shows the protein concentration per sample. AY2 has the highest protein concentration while AY5 has the lowest concentration. The bar chart is plotted in consonance with Table 4.

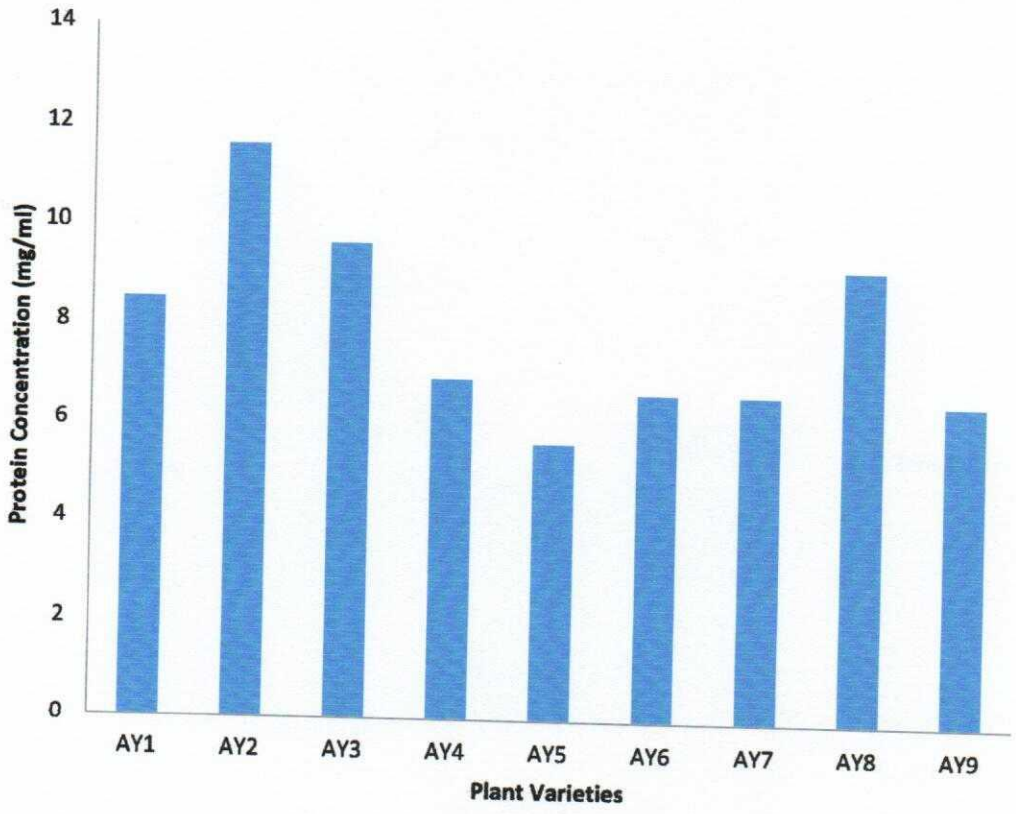
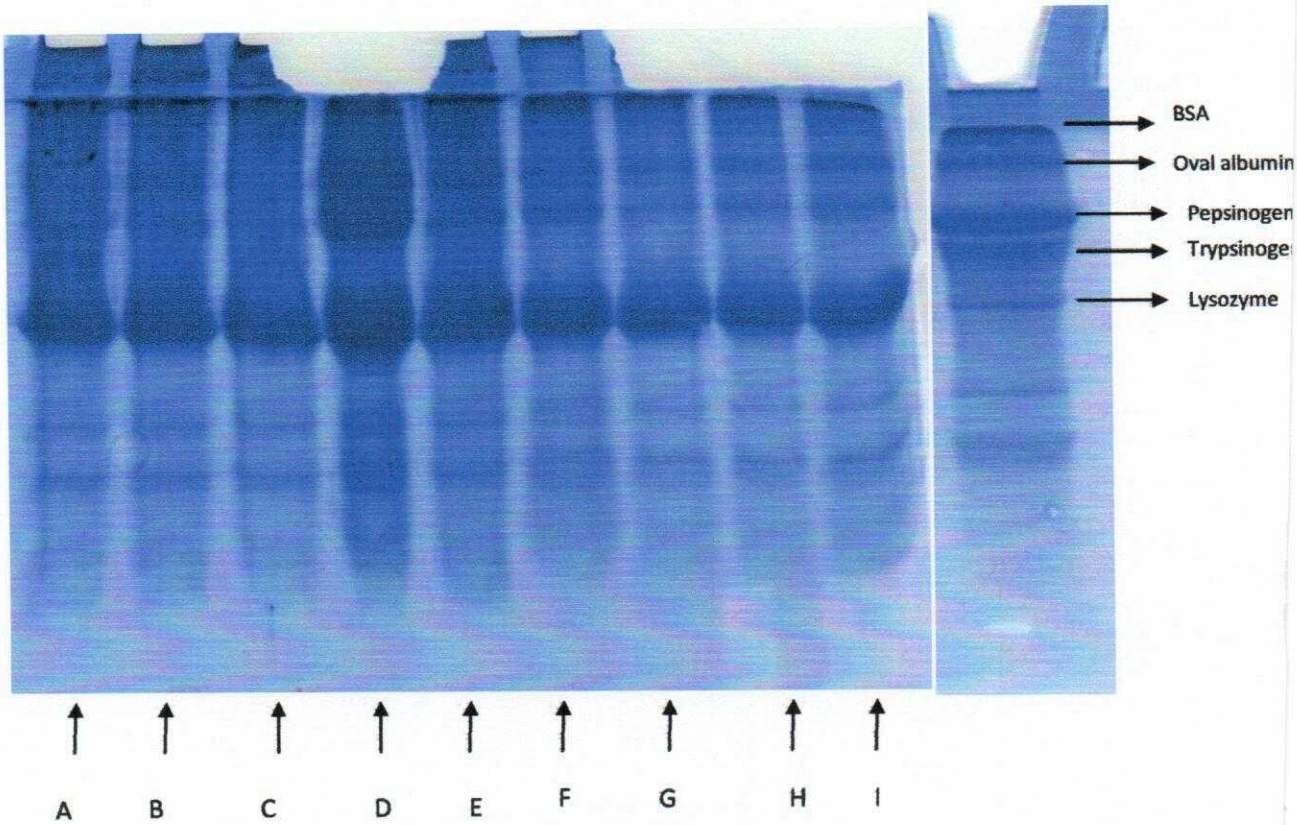


FIGURE 1: BAR CHART SHOWING PROTEIN CONCENTRATION PER SAMPLE

Plate 5 shows the result of SDS-PAGE and the standard gel. The number of bands observed varies from five (5) in AY6 accession to six (6) in AY1, AY2, AY3, AY5, AY7, AY8, AY9 to seven in AY4 accession. All the accessions have at least one major band revealed in the third (3) bands of various accessions. The standard gel is used for the estimation of the typed of protein present in the samples. The proteins present in the standard gel are used to determine the molecular weight of the samples. Bovine serum albumin has molecular weight of 66 and above in kilodalton, Oval albumin ranges between 45-65kda, Pepsinogen ranges between 33-44kda, Trypsinogen ranges between 24-32kda and Lysozyme 14-23kda.



A=AY1; B=AY2; C=AY3; D=AY4; E=AY5; F=AY6; G=AY7; H=AY8; I=AY9.

PLATE 5: STANDARD GEL AND RESULT OF SDS PAGE

Figure two (2) is the standard protein graph for Bovine Serum Albumin protein estimation.

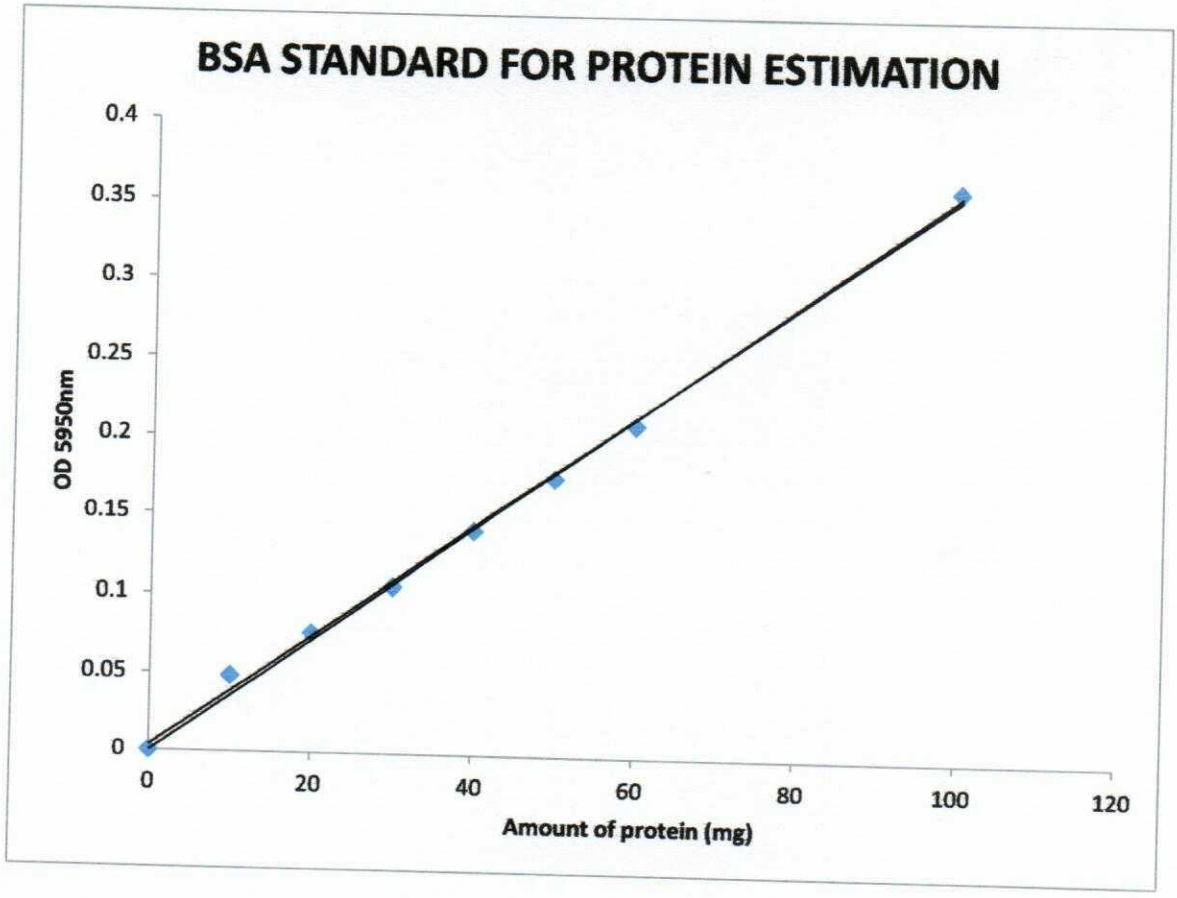


FIGURE 2: STANDARD PROTEIN GRAPH

Weber and Osborn (1976)

Figure 3 is graph showing log M_w versus R_f for the estimation of molecular weight of proteins. The retention factor (Rf) was calculated by measuring the length of the gels and the length of bands in the each gels. The Rf was plotted against the logarithm of the molecular weight (logWm). The molecular weights of the proteins were determined by calculating the anti-log of the value gotten from the graph.

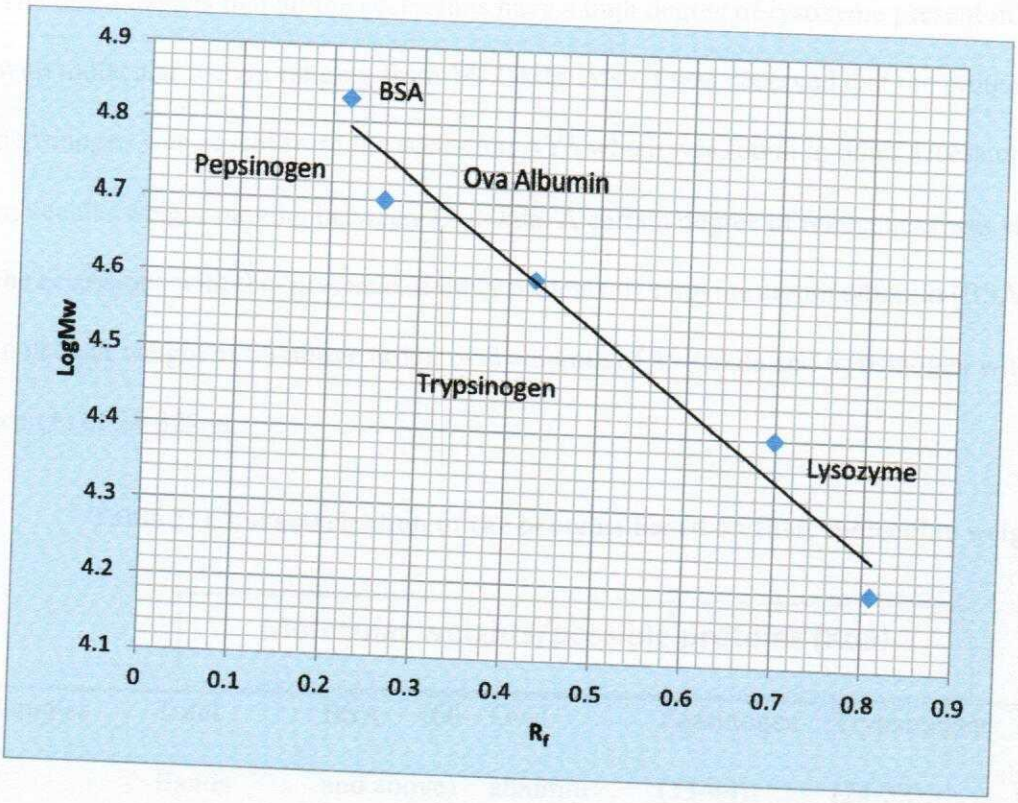


FIGURE 3: GRAPH SHOWING LOG M_W VERSUS R_F FOR THE ESTIMATION OF MOLECULAR WEIGHT OF PROTEINS

Table 5 shows the characterisation of the proteins based on their molecular weights. The result depicts that all the accessions have a high degree of lysozyme present in their bands with molecular weight ranging from 14-23kda. Also it was observed that the protein pepsinogen was absent from the accession AY5 which was found in other accessions with molecular weight ranging between 33-44kda. A certain degree of relatedness was show by all the accessions with the presence of other proteins like bovine serum albumin (BSA) with molecular weight of 66-above (kda), oval albumin of 45-65kda and trypsinogen with molecular weight of 24-32kda.

Table 5: Characterization of the proteins based on their molecular weights

		Molecular weights/possible proteins (Kda)				
Samples	Total Bands	BSA (66 and above)	Oval albumin (45-65)	Pepsinogen (33-44)	Trypsinogen (24-32)	Lysozyme (14-23)
AY1	6	1	1	1	1	2
AY2	6	1	1	1	1	2
AY3	6	1	1	1	1	2
AY4	7	2	1	1	1	2
AY5	6	1	2	0	1	2
AY6	5	1	1	1	1	1
AY7	6	1	1	1	1	2
AY8	6	1	1	1	1	2
AY9	6	1	1	1	1	2

CHAPTER FOUR

4.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1 DISCUSSION

The relationship of a group of accessions can be determined directly through electrophoresis which deals with protein which is the primary product of gene actions. Hence, any similarities and differences observed in the banding patterns of protein extracts of organisms is an indication of genetic similarities and differences among them.

Plate five (5) shows the result of electrophoresis of crude protein from the nine accessions of African yam bean studied. Table 4 also shows the list of the accession and the protein concentration determined for each accession. The determination of the protein concentrations were done at a particular optical density of 595nm. Table 5 depicts characterization of the proteins based on their molecular weight. It was shown that the proteins contain varying molecular weight which are categorized within the range of 66 and above in Bovine serum albumin, 45-65kda in Oval albumin, 33-44kda in pepsinogen, 24-32kda in trypsinogen and 14-23kda in Lysozyme. The result of electrophoresis shows that some of accessions are quite dissimilar both in terms of number and intensity of the bands while some of other one shows a certain degree of relatedness. This is similar to observation made by Agbolade et al, 2013. The number of bands observed varies from five (5) in AY6 accession to six (6) in AY1, AY2, AY3, AY5, AY7, AY8, AY9 to seven in AY4 accession. All the accessions have at least one major band.

Despite the similarities observed in the banding patterns, a close examination revealed that differences still abound among the African yam bean accessions. These similarities are expected and the differences noticed are understandable because both 'nature and nurture' determines the phenotype of an organism. The differences observed might be due to the impact

of environment (nurture). It follows quite logically that different accessions belonging to the same species are expected to be more phylogenetically related.

Table 4 shows the result for determination of protein at optical density (O.D) 595nm which gives the concentration of protein in each accession. AY2 has the highest protein concentration while AY5 has the lowest. Similarities and differences in protein composition of the accessions are represented in Table 5 which characterized the proteins based on their molecular weight. According to a standard protein graph (Weber and Osborn, 1976), the result shows that all the accessions have a high degree of lysozyme with molecular weight ranging from 14-23kda. Unlike AY5 which has no pepsinogen in it bands, other accessions have a minimum degree of pepsinogen in them with the molecular weight of 33-44kda. All the accessions shows a certain degree of relatedness with the presence of other proteins like bovine serum albumin (BSA) with molecular weight of 66-above (kda), oval albumin of 45-65kda and trypsinogen with molecular weight of 24-32kda.

The protein characterized in the samples are of great value to health and shows economical important to human. Bovine serum albumin is used as a nutrient in cell and microbial culture. It is also used to determine the quality of other protein by comparing an unknown quality of protein into known amount of BSA. Oval albumin maybe administered in case where poisoning by heavy metals (such as iron) is suspected (wiki/ovalbumin, 2014). Pepsinogen is a product of pepsin in an inactive precursor form by the chief cell. It helps in digestion of protein (wiki/pepsinogen, 2014). Trypsinogen which has it activated form called trypsin. It helps break down food protein (wiki/trypsinogen, 2014). Lysozyme protect against ever-present danger of bacterial infection. It is a small enzyme that attacks the protective cell walls of bacteria. (David Goodsell, 2000).

It is worthwhile to emphasize that logically, differences in electrophoretic 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 protein having identical electrophoretic mobility is known (Gottlieb, 1971), yet the assumption is made that bands derived from two different accessions that migrate the same distance in polyacrylamide gel are considered to be produced by a gene(s) common to both accessions,

The result of the electrophoresis banding patterns of the studied accessions of African yam bean reveals some diagnostic characteristics that could be used for taxonomic decision. Similarities and differences observed in this work agreed with the studies of Agbolade et al, 2013, Jesse Machuka, 2001 and Olaniran Temitope et al, 2012, who employed comparative electrophoresis protein banding pattern of different species and accessions in establishing relation among various taxa.

4.2 CONCLUSION

The differences observed in the protein profiles of the accessions of African yam bean studied are indicative of genetic variation in protein content and thus may be useful in the taxonomic delimitation of the difference accession belonging to the same species of *Sphenostylis stenocarpa*. Thus the seed protein electrophoresis appear to demonstrate close relationship and distinctiveness of the difference accession studied and could therefore be important in their infra generic delimitation. Also the present of the various protein found in the bands of the accessions has shown a varying importance which are essential to human and the plant wellbeing.

4.3 RECOMMENDATION

Presence of all these proteins in majority of the accessions requires more investigation in other to exploit the nutritional quality and usefulness of this crop African yam bean. Also further studies should be carried out on the systematic studies including the physiological and the genotypic classification of the accessions of African Yam Bean (*Sphenostylis stenocarpa*).

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