VARIATION IN THE PROTEIN LEVEL OF DIFFERENT ACCESSION OF BAMBARA GROUNDNUT

(Vigna subterranea) (L Verdc.)

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(BTH/11/0250)

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CERTIFICATION

I certify that this project work was written by Bamidele Georgina Oluwayemisi, of matriculation number BTH/11/0250 in the department of Plant Science and Biotechnology, of Federal University Oye Ekiti, Ekiti State.

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DEDICATION

This project work is fully dedicated to God Almighty, the Lord of the universe who has been my strength and shield and also to my parent.

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My sincere gratitude goes to the unchangeable God who made everything possible for me since I began my educational career till this present moment.

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ABSRACT

Bambara groundnut (Vigna subterranean L.) is a protein rich legumes, with food security potential. The nine (9) accessions of the Bambara Groundnut were obtained from National Centre for Genetic Resources and Biotechnology (NACGRAB) Ibadan, Oyo State Nigeria. These were accessed for their genetic and phylogenic relatedness through electrophoresis of the seed's proteins. Protein characterization with standard gel marker revealed that the 9 accessions contained proteins (Bovine Serum Albumin, Oval Albumin, Pepsinogen, Trypsin and Lysozyme) with molecular weight ranging from 66kda and above, 45-65kda,33-44kda,24-33kda, 14-23kda respectively. All the accessions as BSA, Oval Albumin and Pepsinogen in common. The study revealed intra- specific similarities genetic diversity in protein contents among the 9 accessions.

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CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Variability in the protein level is an aspect of systematics. Systematics is the study of biological diversity and its origins. It focuses on understanding evolutionary relationships among organisms, species, higher taxa, or other biological entities, such as genes, 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.*(SSB 2015)*

Because of the large unknown nature of biodiversity, systematic studies remain a fountainhead of discoveries and new ideas in biology. Biosystematics deserves more cultivation and the attention of our brightest minds. It is in a position to yield increasing returns to scale, with a variety of benefits for both science and society.*(journals on systematic biology, volume 64 may,2015)*

Systematic uses taxonomy as a primary tool in understanding, as nothing about organism's relationships with other living thing can be understood without it first being properly studied and described in a sufficient detail to identify and classify it correctly.

Today's systematic gemerally make extensive use of molecular biology and of computer programs to study organisms *(schuh , Randall T.2009)*

Study on Bambara beans originated from North Africa and through migration of indigenous peoples moved as far south as KwaZulu-Natal. It is confined to the Northern Province, Swaziland and KwaZulu-Natal. The name of this African groundnut originates from

Bambara, a district on the Upper Niger near Timbuctoo. Because it is widely cultivated throughout tropical Africa, the Bambara district has no pre-eminent claim to the plant *(Waziri et al 2013).*Diversity of opinion exists among the different tribes as to who first brought bambara to the southern part of Africa. The Bolebedu of Letaba claim they came with it from the north. They arrived south of the Limpopo before the Venda, who in turn asserts that they brought bambara from Central Africa to the Transvaal. The latter contention is substantiated by two factors: The Venda name iNduhu-mvendaî, meaning the groundnut of Vendaland, which is still frequently used, shows that there is some truth in the contention. Bambara groundnut *[Vigna subterranean (L.) Verdc]* is an indigenous grain legume grown mainly by female subsistence farmers in drier parts of sub-Saharan Africa. *(Advance in agricultural biotechnology 1 2011)*

Bambara groundnut *[Vigna subterranean]* is one of the most important food crops after groundnut and cowpea. It is widely cultivated in the West and Central Africa. *Vigna subterranean* also known by its common names Bambara groundnut, Bambara-bean, Congo goober, earth pea, ground-bean, or hog-peanut is a member of the family Fabaceae. *Vigna subterranean* ripens its pods underground; much like the peanut also they can be eaten fresh or boiled after drying [*Doku 1997.*] Bambara groundnut has the ability to adapt to divers and marginal agro-climatic conditions, as it is the case of the northern Cameroon. Its seeds are highly nutritious containing 65% of carbohydrates and 18% of proteins. Chemical analyses showed that they contain 32.72% of total essential amino acids and 66.10% of total non-essential amino acids Lysine is the major essential amino acid and represents 10.3% of the total essential amino acid *[Heller and Begemann 1995].* Bambara groundnut is known as being of high nutritional value, as an atmospheric nitrogen fixer and to possess high levels of drought, pest and disease tolerance.

Bambara groundnut is a predominantly self-pollinated crop and is grown as locally adapted landraces.*[FAO 2001]*

Food legumes have a major role to play in the fight against malnutrition. It is therefore necessary that their levels of consumption, which are already too low in a number of developing countries, should be increased *(Borget, 1992)*. Legumes serve as a source of protein to a large proportion of the population in the poor countries of the world by being the least expensive and easily stored and transported non-processed protein source for rural and urban dwellers *(Rachie and Silvester, 1997)*

Little research has been done to date to improve bambara groundnut. The work done on the crop has been limited to mass selection of a few local varieties, followed by a purification phase for the main agronomic characteristics. International Institute of Tropical Agriculture (IITA) has recently evaluated a large collection of bambara groundnut comprising more than 1000 introductions collected from all over Africa (Baudoin and Mergeai, 2001).

1.1.1 ECONOMIC IMPORTANCE OF BAMBARA GROUNDNUT

It can fix atmospheric nitrogen through symbiosis with rhizobia. As a leguminous crop, Bambara Groundnut is useful in crop rotations because it may improve the nitrogen status of soil. Mukurumbira (1985) found that Bambara Groundnut has a higher residual nitrogen effect than groundnut, maize or fallow. The crop has also been reported to be drought tolerance and able to produce some yield where other crops such as groundnut *(Arachis hypogaea)* fail (Linnemann and Azam-Ali, 1993).

1.1.2 RESEARCH OBJECTIVES

To establish that variation occurs in the protein level of the nine (9) accessions of Bambara groundnut based on the molecular weight and quantity of the seed's protein through electrophoresis.

1.2 LITERATURE REVIEW

1.2.1 ORIGIN AND DISTRIBUTION

Bambara groundnut, (*Vigna subterranean (L) Verdc.)* is of North African origin and has been widely cultivated in tropical Africa for centuries. Bambara groundnut is an indigenous African leguminous crop and one of the most important pulses grown on the continent *(Doku and Karikari, 1969).* The crop has been widely cultivated in tropical regions since the seventeenth century. In addition to sub- Saharan Africa, it is now found in many parts of South America, Asia and Oceania *(Baudoin and Mergeai, 2001).*

Bambara groundnut was domesticated in the semi-arid zone of West Africa, probably around the headwaters of the Niger River, from where it spread in ancient times to Central Africa, and more recently to the Malagasy Republic, Asia and South America *(Tweneboah, 2000).*

The crop is indigenous to West Africa where it has a long history of cultivation although there is now limited production in parts of Asia and South and Central America *(Gibbon and Pain, 1985).* Bambara groundnut is a hardy plant particularly well suited to the growing conditions found in the savanna regions with a Sudanese and Sudano–Guinean climate *(Baudoin and Mergaei, 2001).*

The crop is found wild in West Africa. It has been cultivated throughout tropical Africa for many centuries. It was taken at an early date to Madagascar, probably by Arabs. It has reached Brazil and Surinam early in the seventeenth century and was later taken to the Philippines and Indonesia *(Purseglove, 1992).*

1.3 THE PLANT

Bambara groundnut (*vigna subterranean)* is a herbaceous, intermediate, annual plant, with creeping stems at ground level differences in the length of internodes result in bunched, intermediate (semi-bunched) and spreading types. The general appearance of the plant is bunched leaves arising from branched stems which form a crown on the soil surface. Stem branching begins very early, about 1 week after germination, and as many as 20 branches may be produced. Each branch is made up of internodes, and those near the base are shorter than the more distant ones. The plant has a well developed tap root with profuse geotropic lateral roots. The roots form nodules for nitrogen fixation, in association with appropriate rhizobia. Leaf and flower buds arise alternately at each node. Leaves are pinnately trifoliate, glabrous with erect petiole, thickened at the base. Two stipels subtend the terminal leaflet, while only one is assigned to each of the two lateral leaflets. The oval leaflets are attached to the rachis with marked pulvini. The terminal leaflet is slightly larger than the lateral leaflets, with an average length of 6 cm and an average width of 3 cm. *(Bamishalye et al 2011)* The flowers are borne on hairy peduncles, which arise from the nodes of the stems. Usually, two flowers are attached to the peduncle by pedicels. A good knowledge of the flower structure is essential for breeding the crop. Important observations have been reported by *(Doku and Karikari (1971a).* The flowers are typically papilionaceous .The peduncles reach their maximum lengths at the initiation of pod

formation, but their pedicels reach theirs at the time of anthesis. The interval between the openings of successive flowers in a raceme varies from 24 to 48 hours; that of flowers on the same peduncle does not exceed 24 hours, but rarely do they open at the same time. When flowers open during the early hours of the morning, they are yellowish-white, but towards the evening, the colour changes through various shades of yellow to brown. Flowers that are produced towards the end of the plant's life are usually light brown (Advance agricultural biotechnology 60-72) . The flower has a pair of hairy epicalyces. The calyx consists of five hairy sepals (four on the upper side and one on the lower side). The four upper sepals are almost completely joined, while the lower sepal is largely free. The epicalyx and calyx completely enclose the corolla in the early budding stage. The epicalyx drops off during the course of entry of the fertilized flower into the soil, but the calyx persists on the developing pod. The standard encloses the wing and keel until the flower opens. When the standard petal opens, it is bent over to about half its length. There is a hollow at the tip of the keel, through which ants occasionally enter both the unopened and open flowers (*Doku and Karikari 1971b; Baudion and mergael, 2001)*. The stamens are diadelphous, nine with partly fused filaments, and one isolated vexillary stamen. Upon pollination and fertilization, the peduncle elongates to bring the ovaries to or just below ground level. Apparently, reproductive development is not completely inhibited by light. The pod grows first, and reaches its mature size about 30 days after fertilization. The seed develops in the following 10 days. Mean temperature during the seasons influences the time taken to achieve physiological maturity. Bunch types tend to mature earlier than spreading types. Fruit development has been reported to be influenced by photoperiod (*Linnemann and Azam-Ali1993)*.

PLATE 1: Images of Bambara groundnut plant Source: Wikipedia/http. Bambara image.

Long photoperiods delay or even prevent fruit set in some cultivars. Flowering is considered day-neutral, but continuous light was shown to delay flowering by 6-11 days in a few genotypes *Nishitani et al.* 1988). The pods usually develop underground, and may reach up to 3.7 cm, depending on the number of seeds they contain. Most varieties have single-seeded pods, but pods with three seeds were frequently found in ecotypes collected in *Congo Goli and Ng 1988.* Mature pods are indehiscent, often wrinkled, ranging from a yellowish to a reddish dark brown colour. Seed colour also varies, from white to creamy, yellow, brown, purple, red or black. Various testa patterns are found, including mottled, blotched or striped, in addition to the predominantly uniformly coloured seeds.

1.4 MORPHOLOGY OF BAMBARA GROUNDNUT

1.4.1 DESCRIPTION OF THE SEED

Vigna is an Annual crop plant in the family *fabaceae* and a staple food grown mainly in Africa. The seed possesses a hard coat. They are either green or purple when mature. The seeds are formed 40 days after fertilization. At maturity, the seeds vary considerably in colour and size and are smooth and extremely hard when dry. Seed colour varies from cream white, brown, yellowish brown, red, spotted, purple and black *(Stephens, 2003).* Bambara groundnut growth

habit is either spreading or the bunched type. The spreading types are cross-pollinating while bunched types are self-pollinating, and the latter usually matures earlier *(Goli, 1997).* Morphological structure of the crop matches that of the groundnut (*Arachis hypogea*), in that it bears its fruit below the ground.

1.5 TAXONOMY AND BOTANICAL DESCRIPTION

The species *Vigna subterranea* belongs to the genus *Vigna*, and subtribe *Phaseolinae,* the tribe *Phaseoleae* and the family *Papilionaceae (Baudoin and Mergeai, 2001).* Bambara groundnut which since 1980, has been renamed *Vigna subterranea* after having been known as *Voandzeia subterranea* for more than a century (Borget, 1992). In 1763, Linnaeus described it in *Species Plantarum,* and named it *Glycine subterranea*, in accordance with his system of nomenclature. Du Petit–Thouars (1806) found the crop in Madagascar and proposed the name *Voandzeia subterranea (L). Thouars*, which was widely used by subsequent researchers for over a century. Recently, detailed botanical studies were undertaken by *Maréchal et al. (1978);Lowa 2014),* who found great similarities between bambara groundnut and plant species of the genus *Vigna.* This confirmed studies done by Verdcourt, who seized the opportunity in 1980 to propose the current name *Vigna subterranean (L) Verdc.* (Goli, 1995).

1.5.1 SCIENTIFIC CLASSIFICATION

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida

Phylum:	Angiosperms
Order:	Poales
Family:	Fabacaea
Sub-family:	Papilonoideae (Baudoin and Mergeai, 2001).
Genus:	Vigna
Species:	Subterranean

(Lowa, 2014)

Bambara groundnut is a small herb that grows to about 0.30–0.35 m in height, and like the groundnut has compound leaves of three leaflets. Both prostrate and erect forms occur. The much–branched stems root at the nodes to form a bunched herbaceous annual with a thick taproot which forms a profusion of lateral roots towards its tip *(Tweneboah, 2000)*. The general appearance of the plant is bunched 6 leaves arising from branched stems which form a crown on the soil surface. Stem branching begins very early, about one week after germination, and as many as twenty branches may be produced *(Goli, 1995)*. The plant has a bushy habit. It consists of about ten running stems with very short internodes. Roots grow from the nodes at each stem. The leaves with erect petioles are alternate and trifoliate. The peduncles are auxiliary, elongating from the stem nodes, each peduncle bearing one to three flowers (usually two). The plant is considered to be autogamous *(Baudoin and Mergaei, 2001)*. Pale yellow flowers are borne on the freely branching stems and after fertilization the stem of the flower grows down towards the soil, taking the developing seed with it. The pod (1.25–2.5 cm in diameter) is drawn into the soil and ends up lying about 1 cm beneath the surface. The pods usually contain only a single seed but

sometimes there are two *(Ocran et al 2004)*. Mean temperature during the seasons influences the time taken to achieve physiological maturity *(Linnemann and Azam–Ali, 1993)*.

1.6 VARIETIES OF BAMBARA GROUNDNUT

The crop is indigenous to sub-Sahara Africa and there has been limited research into developing new varieties so all varieties are considered to be traditional. They appear in colours of black, white, cream, brown, red and mottled. Other varieties from Burkina Faso are also grown *(Ocran et al., 1998; Akintayo 2009)*. Several varieties are recognized in Africa differing in the shape of the leaves and the size, hardness and the colour of the seeds. The greatest variation is found in Togo and Zambia *(Purseglove, 1992)*. No cultivars of bambara groundnut have been named, but genotypes are distinguished on the basis of seed attributes (colour, size, hardness) and plant form (bushy or spreading). Sometimes names are based on the location where the seed was collected *(Brink et al., 2006)*.

PLATE 2: Varieties of *Vigna subterranea* Source: Wikipedia/http .bambara images

1.7 PROPAGATION AND PLANTING

The crop is always grown from seed and is sown in either mixed cultivation with cereals (pearl millet, root crops or other legumes) or in pure stands *(Gibbon and Pain, 1985; Ocran et al 2004)* reported that the crop may be grown either as a single stand or intercropped with groundnut, millet or sorghum. In rotations, it may be planted as an opening crop perhaps followed by cassava, or in the second year it may be intercropped with cereals, vegetables, groundnuts or other pulses. *(Bamishalye et al 2011). Doku (1995)* stated that there is also a trend towards mixed cropping with yams, the bambara groundnut being planted on yam mounds protect the mounds from erosion, conserves moisture and creates fewer temperature fluctuations in the mound. The crop performs best on deeply ploughed field with a fine seedbed, eventually allowing the plant to bury its developing fruits. Ridging is advisable if the soil is shallow or prone to water logging *(Brink et al., 2006). Baudoin and Mergeai (2001)* reported that proper loosening of the soil helps pod penetration during fructification and improves the yield. *Tweneboah (2000)* also mentioned that a well prepared friable seed bed is required to enable the plants bury their pods after fertilization. *Tindall (1997)* indicated that seeds, normally shelled are sown on beds or ridges in rows 40-50 cm apart, 20-30 cm between plants. According to *Ocran et al. (1998),* the recommended row spacing is usually 10-45 cm with an intra row spacing of 15-17 cm. One seed is sown per hole 3-5 cm deep. Seed rate varies in several location, that is 35 kg/ha in Tanzania; 25-45 kg/ha in Kenya; higher rate of 60-75 kg/ha in South Africa when rat damage is expected *(FAO, 1961). Gibbon and Pain (1985)* observed that the normal seed rate is 30-60 kg/ha of shelled nut giving 150,000 plants/ha. Sowing dates vary considerably within locations. In Zambia and Botswana, for example, sowing takes place from November to February. Sometimes phased planting occurs, examples, in Skumaland, Tanzania *(Brink et al.,*

2006). In the derived savanna zone of Ghana, two crops are possible, the first crop sown in May - June and the second crop in October *(Bamishalye et al 2011).* In the north the main planting period is between August–September *(Tweneboah, 2000).* In the Guinea savanna zone, the crop is usually grown during the minor season (September-November) when the rainfall is reliable. In the Sudan Savanna zone, it is usually cultivated towards the end of the single long rainy season *(Doku, 1995).* Cultivation of *vigna subterranean* on a large scale and in pure stand is not very common. The crop is mostly grown by women, intercropped with major commodities such as maize, millet, sorghum, cassava, yam, peanut and cowpea. Grown in rotation, bambara groundnut improves the nitrogen status of the soil *(Mukurumbira 1985).* Bambara groundnut thrives better in deep, well-drained soils with a light, friable seedbed . Many farmers grow the crop on a flat seedbed, but the use of ridges or mounds is also common in a few countries. Planting density is usually low in farmers' fields, especially when crops are not in rows. In experimental plots, recommended plant density ranged from 6 to29 plants/m2 *(Rassel 1960).* Farmers do not normally apply chemical fertilizers to bambara groundnut fields. The nitrogen requirement is met by natural N2 fixation, as indicated by several nodulation studies *(Somasegaran et al. 1990).* Yield increase as a result of phosphate or potassium application has not always been confirmed *(Nnadi et al. 1981).* Bambara groundnut has a reputation for resisting pests, and compares favourably with other legumes such as groundnut or cowpea in this regard. In humid environments, however, fungal diseases such as Cercospora leafspot, Fusarium wilt and Sclerotium rot are common *(Masindeni 2006; Begemann,1988a).* In such circumstances, spraying with the fungicide benlate (1 kg/ha) has proved beneficial

Harvested pods are air-dried for several days before threshing. The raw product is sold at markets, as pods or seeds. In dry areas, materials for planting the following season are usually kept by farmers as pods. This reduces or eliminates attacks by insects.(K.P.2005)

1.8 GROWTH AND DEVELOPMENT

Bambara beans take 7 to 15 days to germinate. Seed stored for about 12 months germinate well, but longer storage results in loss of viability. Flowering starts 30 to 35 days after sowing and may continue until the end of the plants life (Brink *et al.,* 2006). It is a typical short-day plant and the following variations were observed:

- Day neutral but fruit forming is delayed by long days.
- Day neutral but fruit setting is prevented by long days.
- Delayed flowering and no fruit set under long-day conditions.
- No flowering under long day conditions.

Vegetative growth takes place in spring and early summer and pods form only in late summer and autumn.

- Pod and seed development take place approximately 30 to 40 days after fertilization.
- The fruit of bambara groundnut develops on or below the soil surface.
- The pod develops first. This takes up to 30 days after fertilization.
- The seed develops during a further 10 days (*Linneman and Azam–Ali, 1993).*
- Seeds are mature when the parenchymatous layer surrounding the embryo has disappeared and the pods become light brown between 100-180 days *(Baudoin and Mergeai, 2001)*

1.9 MANAGEMENT

Weeding of Bambara groundnut takes place 1-3 times, often with a hoe. Earthing up to cover the young pods is common, and may be done by hand, with a hoe or with ox-drawn equipment. Earthing up improves yield, but is labour intensive; it is often combined with weeding *(Brink et al., 2006). Purseglove (1992)* also reported that the rows are usually earthed up and in some areas are lightly covered with soil to promote fruit production. *Tweneboah, (2000)* mentioned that the plants are hand weeded when 10 cm high and mounded or earthed up at flowering time to encourage development of the pods underground.

1.10 NUTRITIONAL REQUIREMENT

Farmers do not normally apply chemical fertilizer to bambara groundnut fields. The nitrogen requirement is met by natural nitrogen fixation as indicated by several nodulation studies *(Doku, 1996; Ncube et al 2007).* The nodules on their roots can fix atmospheric nitrogen and therefore ensure their nitrogen nutrient supply without recourse to nitrogen in the soil. However, there are some cases where, for various reasons, assimilation is poor and the application of nitrogen fertilizer has a positive effect particularly in the early period of growth, when root development is rapid. Later application may suppress nodulation. The dose normally ranges from 30 to 50 kg of nitrogen per hectare *(Borget, 1992).* According to *Baudoin and Mergeai (2001),* as with almost all legumes, bambara groundnut is capable of symbiosis with nitrogen–fixing bacterial belonging to the genus Rhizobium. The maximum quantity of nitrogen, which can be obtained by symbiotic fixation, is 100 kg/ha. The crop is able to meet its nitrogen requirements but it is known to respond favourably to application of about 250 kg/ha of single super phosphate applied before planting *(Tweneboah, 2000).* The addition of nitrogen at planting time or later at the rate

of 60 kg/ha of sulphate of ammonia, approximately three weeks after sowing appears to be economic and in Malagasy, seeds are placed in holes containing cow dung.

1.11 HARVESTING

Harvesting begins about four months after sowing when the pods are mature and the plant leaves are beginning to yellow. The plants are simply pulled out of the ground, with the attached nuts *(Akintayo, 2009).* In a dry environment, harvesting takes place when the entire foliage dries up. In humid ecosystems, however, podrotting or early seed germination (in the pod) may take place while the leaves are still partially green. Harvesting is then recommended before full foliage drying *(Goli, 1995).* According to *Karikari (1998),* in Botswana, immature pods are usually harvested about two months before the pods dry completely. Although a farmer may harvest a crop as immature for immediate use, bambara groundnut of commerce are available only as mature dry seeds.

Harvesting of bambara groundnut is done by pulling or lifting the plant. For the bunched-habit type, most pods remain attached to the root crown. Detached pods left in the ground are collected manually. In a dry environment, harvesting takes place when the entire foliage dries up. In humid ecosystems, however, pod-rotting or early seed germination (in the pod) may take place while the leaves are still partially green.

1.12 CLIMATE AND SOIL REQUIREMENTS

The crop does better than most other bean crops in poor soils and grows best with moderate rainfall and sunshine *(Williams et al., 1980; Padi 2003).* Under less favourable growing conditions, such as limited water supply and infertile soil, it yields better than other legumes, for example, groundnut. The crop will grow on soils in hot, dry regions that are marginal for groundnuts and other pulses, as for example the savanna ochrosols of Africa *(Azam-Ali et al 2001).*It was also reported that the crop is the least demanding for mineral elements and thrives in soils which are considered too marginal for groundnut. Bambara groundnut can be grown on a range of soils, especially light loams and sandy loams but may be successfully grown on heavier soils than groundnuts. Generally it performs better on poor soils than groundnuts. Light soils make harvesting easier, soils rich in nitrogen may produce excessive vegetative growth which is undesirable for seed production *(Tweneboah, 2000).* Also reported that the crop is the most drought resistant pulse, producing a crop under conditions of high temperature and low rainfall, where other pulses fail to thrive. According to (*AMADOU et al.,2001),* the fertility coefficient (the pod: flower ratio) was higher during the dry than in the wet season. He therefore suggested that the dry season would be more favourable for the cultivation of the crop.

Bambara groundnut tolerance of drought and ability to yield in soils too poor to support the growth of more favoured legumes are all factors which contribute to its continued popularity with poor farmers *(Azam–Ali, 1992).* The crop is very drought–resistant but for good yields requires moderate rainfall of 750–1000 mm during the rainy season and a dry period for harvesting *(Tweneboah, 2000).* Production can occur under rainfall of 600–700 mm per annum but optimum growth occurs with 900–1200 mm per annum *(Gibbon and Pain, 1985 EDJE et al., 2003).* The crop is adapted to a wide range of soils and performs better on poor soils than

groundnuts *(Tweneboah, 2000)*. Yield of bambara groundnut on low–fertility soils are generally higher than those of groundnuts grown on similar soil. Soils with a Ph of 5.0–6.5, will produce satisfactory crops *(Messiaen, 1992; RAMOLEMANA et al., 2003)*. The cultivation of bambara groundnut is of particular importance in semi – arid areas. In such regions, the crop has been found to thrive and produce yield under adverse conditions, such as limited water supply and low soil fertility *(Wassermann et al., 1983; Niogu et al., 2005)*.

1.13 DROUGHT AND HEAT TOLERANCE

Bambara groundnut is considered to be drought resistant *(Ntundu 2006; Tweneboah, 2000)*. Farmers claim that in years when groundnut fails due to low rainfall, bambara groundnut produces good returns *(Linnemann, 1990; Sticksel et al 2008)*. The reasons why bambara groundnut is apparently able to withstand greater water stress than other legumes and still produce at least some yield are unclear *(Collinson et al., 1993; Azam-Ali 2003)*. The crop will yield in unfavourable environments but there are few reports of its productivity in relation to water stress. It is generally accepted that Bambara groundnut is tolerant of drought but little research has been conducted to establish what degree of stress the crop is able to tolerate *(Linnemann, 1991)*.

The adaptations which enable the crop to tolerate drought are not well understood. Limited evidence suggests that a short growing period and deep root system are two important adaptations to a dry environment *(Begemann, 1988; Liu et al 2005)*. The well developed root system of bambara groundnut exploits the rhizosphere for moisture and the sink demand of the rather thin and much branched prostrate stem cannot offer any significant competition for assimilates relative to the developing pods *(Doku, 1996).Witcombe et al., 2006)* observed that

bambara groundnut allocated a greater fraction of its total dry weight to roots than comparable groundnut crops irrespective of available soil moisture. This strategy has clear advantage when water is scarce enabling a greater soil volume to be exploited for available water. The crop uses the available water frugally through slow leaf area development, therefore conserving water so that there is sufficient for the crop to survive through the reproductive period and produce some yield *(Muriuki, 1990; MI. Uguru 2011).*

In Africa, bambara groundnut is confined to the dry regions, between the desert and the savanna (Southern fringe of the Sahara) and adapted to growing in areas of relatively high temperatures for many leguminous crops *(Berchie et al. 2012).*

1.14 RESPONSE TO DAY LENGTH

It is important to know at what moment in their life cycle plants are sensitive to photoperiod. In the case of a flowering response to photoperiod, usually three phases are distinguished between sowing and flowering; basic vegetative phase, in which plants are not sensitive to photoperiod; an inductive phase, in which plants are sensitive to photoperiod and a post – inductive phase, during which flower buds develop into open flowers and photoperiod does not play a role anymore *(Hodges and French, 1985; GAISMA 2012).*

Bambara groundnut is not photoperiodic *(Baudion and Mergeai, 2001)* but *Tweneboah (2000)* reported it as a typical short–day plant and this agrees with *(Nrc 2006.)* who mentioned that most cultivars are adapted to short days. Fruit development has been reported to be influenced by photoperiod *(Linnemann and Azam–Ali: 1993).* Long photoperiods delay or even prevent fruit set in some cultivars. Flowering is considered day–neutral, but continuous light was

shown to delay flowering by 6–11 days in a few genotypes *(Nishitani et al., 1988; Collison et al., 2011).*

There are considerable differences between genotypes in their response to photoperiod. In many genotypes, flowering is photoperiod–insensitive, while the onset of podding is retarded by long photoperiods. In some genotypes both flowering and the onset of podding are delayed by long photoperiods *(Brink et al., 2006).* Many bambara groundnut landraces have a specific day length requirement for pod filling, that is, allocation to yield will only begin at a particular day length (*FAO, 2001).* Photoperiod usually has a stronger effect on the onset of podding than on the onset of flowering *(Linnemann and Cruafurd, 1994)*

1.15 YIELD

The highest recorded seed yield under field conditions is 4 t/ha. Average yields are 300–800 kg/ha but yields of less than 100 kg/ha are not uncommon (Brink *et al.,* 2006). Average yields of dry seeds usually range between 300 and 800 kg/ha in traditional farming and may exceed 3,000 kg in intensive farming *(Baudoin and Mergeai, 2001),* also reported yields of 500–1000 kg of dried nuts per hectare. It was observed that it yields are lower than those of groundnuts, 300–800 kg/ha being the average in most areas in the northern part of Ghana.

Bambara groundnut yields are low because the production environments are characterized by various abiotic and biotic stresses. However, even under optimal conditions the yields are variable and unpredictable and this is partly due to variability in growth and development of individual plants within landraces *(Squire et al., 1997; Holbrook et al., 2005).*

1.16 PEST AND DISEASES

The crop appears to be remarkably free from pests and diseases *(Purseglove, 1992; S.Adiku, 2005)*. This agrees with *Doku (1995; NAMET, 2009)* who mentioned that the crop is relatively pest and disease–free apart from weevil attack during storage. Scientist also observed that no serious pest or diseases are reported for this crop but damage is sometimes caused by leaf hoppers *(Hilda patruelis and Empoasca facialis)*. *Tanimu and Aliyu (1995; Tarimo 2011)* have also made similar observations that bambara groundnut is relatively free of the insect pests that plague other legumes, such as the cowpea and peanut. And on the whole, pesticides are hardly used by farmers when cultivating Bambara groundnut.

Bambara groundnut is considered to be generally less affected by diseases and pests than groundnut or cowpea, but several diseases and pests can cause serious damage to the crop. The most important fungal diseases are cercospora leaf spot *(Cercospora spp.)*, powdery mildew *(Erysiple polygoni)* and Fusaruim wilt (*Fusarium oxysporum*) (Brink *et al.*, 2006).

The major diseases affecting Bambara groundnut are *Fusarium* wilt, which attacks the young seedlings of all landraces in wet weather, particularly under waterlogged conditions, and Cercospora leaf spot, which also has been observed on crops under irrigation. In dry weather, pod attacks by termites also have been consistently observed, and in one particular year, 1994-95, a particularly severe attack resulted in the loss of an entire crop *(Sunley 2005)*. Root knot nematode *(Meloidogyne javanica)* also attacked the roots of the plant in sandy soils. In storage, shelled Bambara groundnut seeds were extremely susceptible to attack by bruchids *(callosobruchus maculates) (M.Neyra 2006)*. All landraces were attacked by this pest, but the black-seeded landrace was affected less severely. The unshelled seed was extremely resistant to bruchid attack. . Viral diseases are widespread in most environments, especially in areas where

other grain legumes such as cowpea are grown. Common diseases are cowpea mottle virus (CMeV) and cowpea aphid-borne mosaic virus *(AbMV) (Ng et al. 1985; Rumjanek, 2004)*. A combination of unusually heavy virus attack and Cercospora leaf spot on one particular accession (TVSU 218) resulted in zero yield during a trial at Kaboinse, Burkina *Faso (Goli et al. 1991)*.

1.17 USES

Seeds of bambara groundnut are not sold on world markets but play an important part in the diet of people in several West African countries where they are the third most important commodity after cowpea and groundnut in the national production and consumption statistics *(Baudoin and Mergeai, 2001)*. It is a rich source of protein and along with other local sources of protein could help to alleviate nutritional problems in areas where staple foods are predominantly carbohydrate sources *(Massawe et al., 2005; Okpuzor et al., 2010)*. It has been concluded that bambara groundnut seed is a useful ingredient for different food.

In Ghana, the beans used to be canned in gravy at GIHOC Cannery in Nsawam. The product was thus available throughout the year and over 40,000 cans of various sizes were produced annually *(Aremu et al.., 2006)*. The seeds are consumed either immature or in matured states, but dry seeds are hard and difficult to cook and may be ground before use (Tweneboah, 2000).

Bambara groundnut is eaten in various ways, depending on the region. They can also be processed into flour for use in soups, purées and flat cakes. The canning of bambara seeds in sauce has been reported in Ghana *(Baudoin and Mergeai, 2001)*. The crop is grown mainly for its edible protein and not as an oil crop. When dried, the seeds are very hard and can only be eaten when ground into flour. Unripe seeds can be eaten fresh but mature seeds have to be soaked and boiled before eating *(Doku and Karikari (1969; Hampson K.J 2010)* reported that in

Ghana, the nuts are boiled with pepper and salt in the preparation of "Aboboi" which, when served with "gari" (grated and roasted cassava) or "tatare" (mashed fried ripe plantain), makes a very delicious meal. In many West African countries, the fresh pods are boiled with salt and pepper, and eaten as a snack. In Côte d'Ivoire, the seed is used to make flour, which makes it more digestible. In East Africa, the beans are roasted, then pulverized, and used to make a soup with or without condiments. Bread made from bambara groundnut flour has been reported in Zambia *(Linnemann, 1990; Payne et al, 2007).* In Senegal leaf preparations are applied to abscesses and infected wounds, leaf sap is applied to the eyes to treat epilepsy, and the roots are sometimes taken as an aphrodisiac. Pounded seeds mixed with water are administered to treat cataracts. The Zybo tribe in Nigeria uses the plant to treat venereal diseases *(Brink et al., 2006).* The leaves which are rich in protein and phosphorus are used as fodder for livestock *(Drabo et al., 1995; De rock,2006).*

Bambara groundnut is essentially grown for human consumption. The seed makes a complete food, as it contains sufficient quantities of protein, carbohydrate and fat. Several workers have examined the biochemical composition of the seed *(Linnemann 1987;Yusuf et al 2008).* On average, the seeds were found to contain 63% carbohydrate, 19% protein and 6.5% oil. Despite the relatively low oil content, some tribes in Congo reportedly roasted the seeds and pounded them for oil extraction *(Karikari 1971).* The gross energy value of bambara groundnut seed is greater than that of other common pulses such as cowpea, lentil and pigeonpea *(FAO 1982; Salih 2006).* Bambara groundnut seeds are consumed in many ways. They can be eaten fresh, or grilled while still immature. At maturity, they become very hard, and therefore require boiling before any specific preparation. In many West African countries, the fresh pods are boiled with salt and pepper, and eaten as a snack. In Côte d'Ivoire, the seed is used to make flour, which makes it

more digestible. In East Africa, the beans are roasted, then pulverized, and used to make a soup, with or without condiments. Bread made from bambara groundnut flour has been reported in Zambia (*Linnemann 1990; Kunert K 2010*). Seeds can also be pounded into flour and used to make a stiff porridge, which is often kept for a long period *(Holm and Marloth 1940; Taylor PWJ 2005)*. Roasted seeds can be boiled, crushed and eaten as a relish. Another common use of bambara groundnut is to make a paste out of the dried seeds, which is then used in the preparation of various fried or steamed products, such as 'akara' and 'moin-moin' in Nigeria (*Obizoba 1983; Madamba 2009)*. Another favorite Nigerian dish is 'okpa', which is a doughy paste that is wrapped in banana leaves and boiled. In Ghana, the beans used to be canned in gravy at GIHOC cannery in *Nsawam*. The product was thus available throughout the year, and over 40 000 cans of various sizes were produced annually *(Begemann 1986a ; Sibuga, 2002)*. Recently, a trial of bambara groundnut milk was carried out which compared its flavour and composition with those of milks prepared from cowpea, pigeonpea and soybean (*Brough et al. 1993)*. Bambara groundnut was ranked first, and while all milks were found to be acceptable, the lighter colour of the bambara groundnut milk was preferred. Bambara groundnut has long been used as an animal feed, and the seeds have been successfully used to feed chicks *(Oluyemi et al. 1976; Collison et al., 2011)*. The haulm was found to be palatable *Doku and Karikari 1971a*, and the leaves were reported to be rich in nitrogen and phosphorus, and therefore suitable for animal grazing (*Rassel 1960; Jawali N 2011)*.

1.18 NUTRITONAL VALUE

Bambara groundnut is the only legume whose seeds are referred to and used as complete food because they contain protein, carbohydrate and fat in sufficient proportions to provide a nutritious food *(Poulter and Caygill, 1980; Nepolo et al 2010).* The seed makes a complete food, as it contains sufficient quantities of protein, carbohydrate and fat *(Goli, 1995; Phansak et al 2005).* The ripe seeds contain on average 10% water, 15 - 20% protein, 4 - 9% fat, 50 - 65% carbohydrate and 3 - 5% fibre *(Baudoin and Mergeai, 2001).*

Brough and Azam – Ali (1992; Fell JW 20004) indicated that the mature seeds are a rich source of protein (16-25 % DM) and carbohydrate (42–60% DM) but, in comparison with groundnut, the lipid content is low (5-6% DM). The gross energy value of bambara groundnut seed is greater than that of other common pulses such as cowpea, lentil and pigeon pea *(FAO, 1982; Hampsonky 2009).* Purseglove (1992) also reported that the ripe seeds contain: protein 16-21%; fat 4.5-6.5%; carbohydrate 50-60%; thus providing a completely balanced food. *Brink et al. (2006)* mentioned that dried leaves for fodder contain crude protein 15.9%, crude fibre 31.7%, ash 7.5% and fat 1.8%.

1.19 PRODUCTION AND INTERNATIONAL TRADE

Bambara groundnuts are cultivated throughout tropical Africa and in Madagascar. It is also found on the continents of America (Brazil, Paraguay and Surinam); Asia (India, Indonesia, Malaysia, the Philippines and Sri Lanka) and Oceania (Northern Australia and New Caledonia). About 45–50% of world production comes from West Africa *(Baudoin and Mergeai, 2001).* Reliable production figures for the crop are difficult to obtain, because the crop is grown mainly for home consumption and sale at local markets. The major producers are Burkina Faso,

Chad, Cote d'Ivoire, Ghana, Mali, Niger and Nigeria. The main exporting countries are Burkina Faso, Chad, Mali, Niger and Senegal; they supply markets in Benin, Ghana, Nigeria and Togo (Brink *et al.,* 2006). The crop does not enter world trade because they are cultivated in the drier regions of tropical Africa mainly for local consumption, but seldom on a large scale. The mostextensive production is in Zambia *(Purseglove, 1992;Massawe et al 2002). Gibbon and Pain (1985; Oosthuizen et al 2006)* also reported that production is on a small scale for home consumption and the largest areas are to be found in Zambia.

1.20 GENETIC RESOURCES AND DIVERSITY

The evaluation of available genetic diversity is a pre-requisite for genetic improvement in crop plants, especially in underutilized crops such as bambara groundnut *(Olukolu et al., 2012).* Investigation of genetic diversity in both wild and domesticated species is equally important. Wild populations are known to be a potential source of useful genes and traits which could be introduced into the domesticated gene pool; in particular, genes responsible for adaptation to stressful environmental conditions *(Cattan-Toupance et al., 1998;Singh et al 2009).* Wild populations in centers of diversity or domestication constitute the initial gene pool of crops species. Crop failures and dispersal of germplasm within the centre of origin or limited introduction or isolated locations ('Founder Effects') could lead to reduced genetic diversity in particular breeding populations, which could shave long-term negative consequences for production *(Trethowan and Mujeeb-Kazi, 2008).* By focusing on commercial and elite germplasm the breeder may further reduce the genetic diversity of the domesticated gene pools *(Rauf et al., 2010; Yi et al., 2008).* Studies of genetic diversity can help to guide the exploitation of wild relatives in a breeding program to retrace or enhance gene flow between wild and

domesticated populations which may increase the genetic diversity in domesticated gene pools *(Gepts and Papa, 2002)*. Estimating the genetic diversity of crop species can be achieved using different marker methods, including; morphological, trait/agronomic, biochemical and molecular. The latter has several advantages over conventional phenotypic markers, as they can be used efficiently regardless of the developmental stage of the plant under investigation *(Mondini et al., 2009)*.

2.0 MATERIAL AND METHOD

2.1 REAGENT USED

Tris-base, tris-HCl, ethanol, phosphoric acid and coomassie 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. Phosphate buffer [Na2HPO4 and NaH2PO4].

2.2 METHODS

TABLE 1: SAMPLE IDENTIFICATION AND WEIGHT

SAMPLE	SAMPLE IDENTIFICATION	WEIGHT
NGB 01494^{K5}	BN1	2.3
NGB 01497^{K5}	BN2	3.1
NGB 01499^{K5}	BN3	2.8
NGB 01501^{K5}	BN4	3.0
NGB 01245	BN5	2.5
NGB 01489^{K5}	BN6	3.5
NGB 01311^{K5}	BN7	2.7
NGB 01496^{K5}	BN8	3.1
NGB 01495^{K5}	BN9	2.9

2.2.1 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.

2.2.1.1 PREPARATION OF BUFFER SOLUTION

Preparation of 0.2m phosphate buffer of PH 6.5 using Henderson law.

PH=pKa + log [A]/ [HA]

6.5=pKa + log [A]/[HA]

6.5= 6.8+log [Base]/[Acid]

6.5-6.8=log [Base]/[Acid]

0.3=log[Base]/[Acid]

[Base]/[Acid]= Antilog of -0.3

Antilog [-0.3]= [Base]/ [Acid]

0.501/1*[Base]/[Acid]

[Base]=0.501[Acid].....(1)

A law states that concentration of Acid+ Concentration of base equals total concentration of solution or the molarity of the buffer.

[Acid]+[Base]=0.2m.....(2)

Substitute (1) into (2)

[Acid] +0.501 [Acid]=0.2m

1 +0.501 [Acid] =0.2m

1.501=0.2m [Acid] 0.2m/1.501 =0.133m

Concentration of Acid = 0.133m

[Acid] + [Base] =0.2m [Base]= 0.2m –[Acid]

0.2m-0.133m =0.067m in mol/dm3

To calculate the mass conc[mol/dm3] =conc g/dm/Molar mass

Conc = Reacting mass/ molar mass

Reacting mass =conc * molar mass

Conc of acid in mol/dm3 =conc in g/dm3/ 156.01

=156.01*0.133m/dm3

Conc in g/dm3 =20.75/2 =10.375

Concentration of base mol/dm3/1=conc of base in g/dm3/M.M

0.067m *177.99 =11.925 in g/dm3

Therefore in 500ml =20.75/2 =10.375g/dm3 of acid and

in 500ml of base conc =11.925/2 =5.963g/dm3

hence, preparation of 0.2m phosphate buffer of PH 6.5

=10.375g of acid and 5.963g 0f base.

2.2.1.2 PREPARATION OF BRADFORD REAGENT

Add 0.1g of commasie brilliant blue 6-250 in a 50ml of 99% ethanol and 1000ml of 85% [W/V] of phosphoric acid make the solution up 1ml, then the mixture is filtered to remove impurities which can hinder viewing.

2.2.2 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 10 µl of the sample solutions and 1.0ml of Bradford reagent (Bradford, 1976). The absorbance was read at 595nm.

2.2.3 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, 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 intrapolated from the curve.

CHAPTER 3

3.0 RESULTS

All the accessions of Bambara groundnut *(vigna subterranean)* revealed considerable intra specific variation and overlap in most of their banding patterns table 2 present the result of protein determination at optical density 595nm

TABLE 2: RESULT FOR DETERMINATION OF PROTEIN AT OPTICAL DENSITY (O.D) OF 595nm, in which the sample with the highest protein concentration is BN_1 with 0.066mg/ml and sample with the lowest concentration is BN_2 with 0.053mg/ml

S/N	PROTEIN CONCENTATION (mg/ml)
BN1	0.066
BN2	0.053
BN3	0.065
BN4	0.060
BN5	0.062
BN6	0.062
BN7	0.063
BN8	0.061
BN9	0.061

To get the protein concentration, according to the standard graph at every optical density of 0.4, protein concentration =5.0mg/ml

Hence, at 0.4 =5.0mg/ml

O.D =X

5.0/0.4 * O.D new

i.e every O.D new *12.5 gives the protein concentration

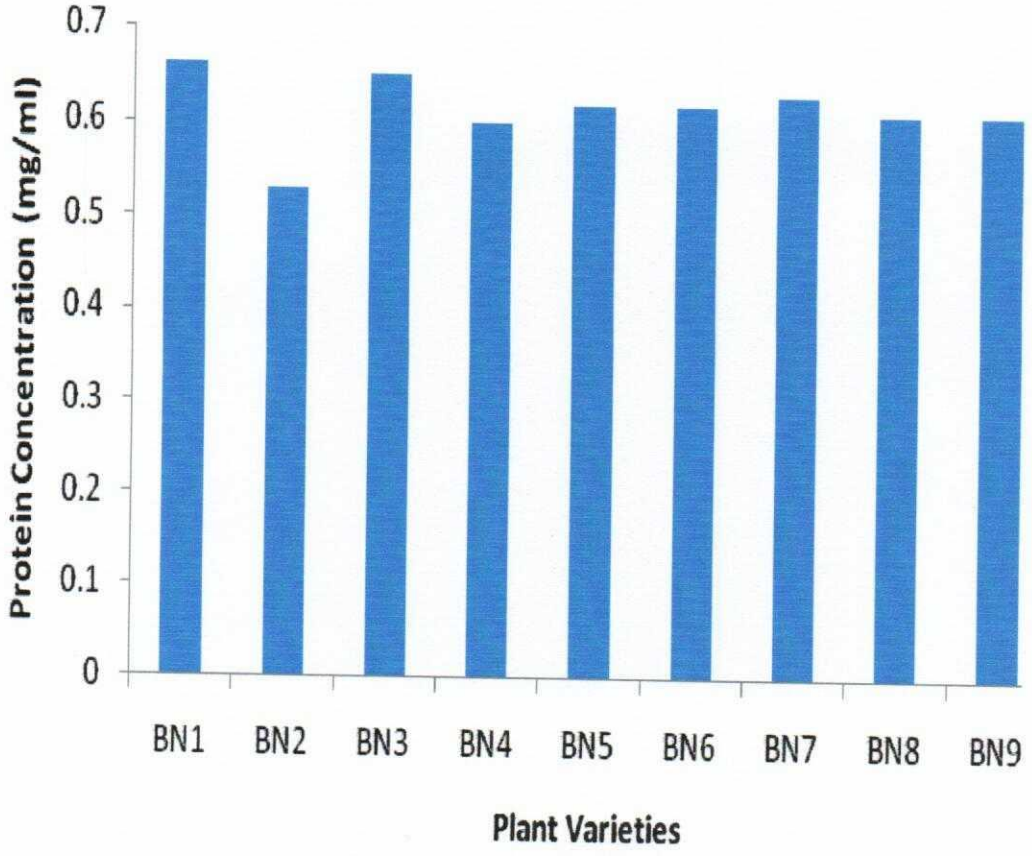
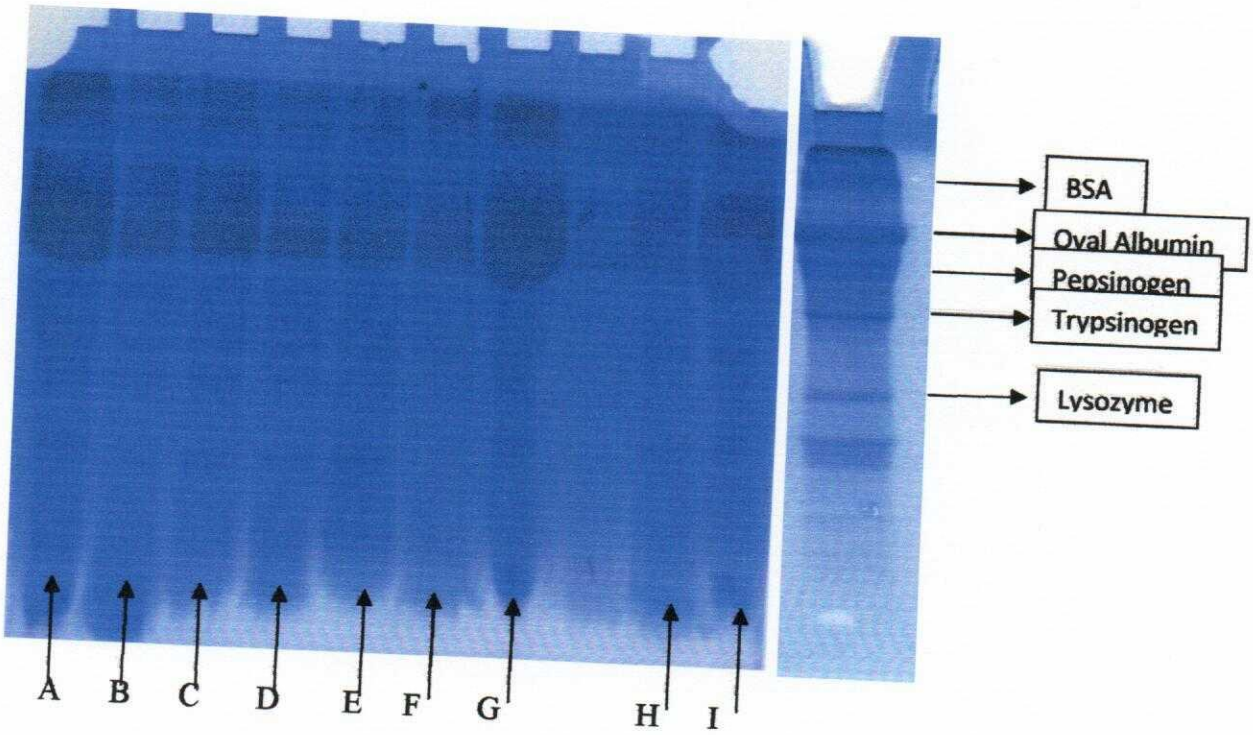


FIGURE 1: Shows the bar chart presentation indicating the protein concentration in the different accession.



Samples: A, B, C, D, E, F, G, H, I.

BNI=A; BN2=B; BN3=C; BN4=D BN5=E; BN6=F; BN7=G; BN8=H; BN9=I

PLATE 3: RESULT OF SDS PAGE AND STANDARD GEL MARKER

This result indicate bands showing the protein concentration in each sample in which sample A,B,F,G and H have a total bands 6 and the crude protein is found in abundance in band 3, while band C and E has a total bands of 7

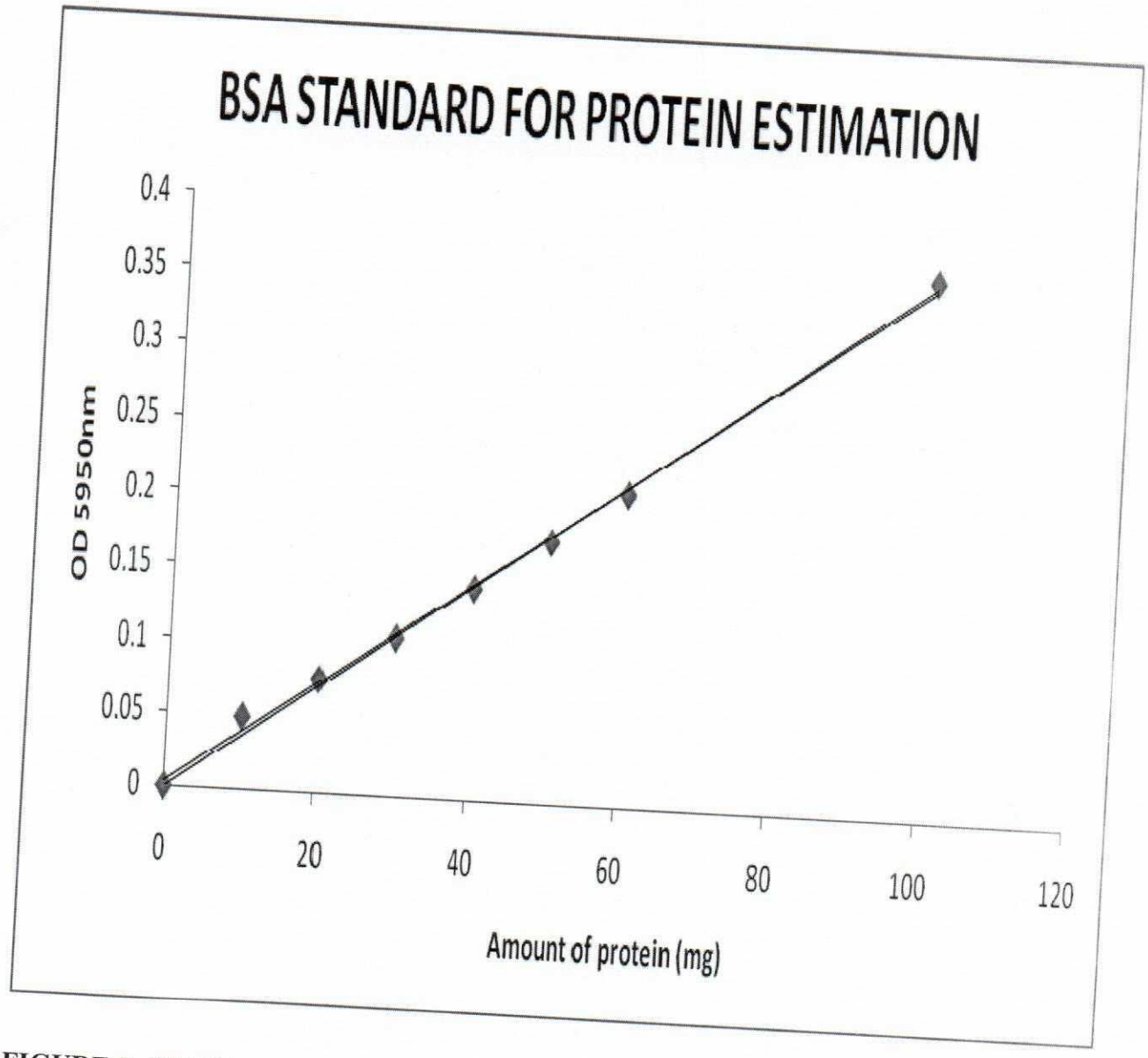


FIGURE 2: STANDARD PROTEIN GRAPH

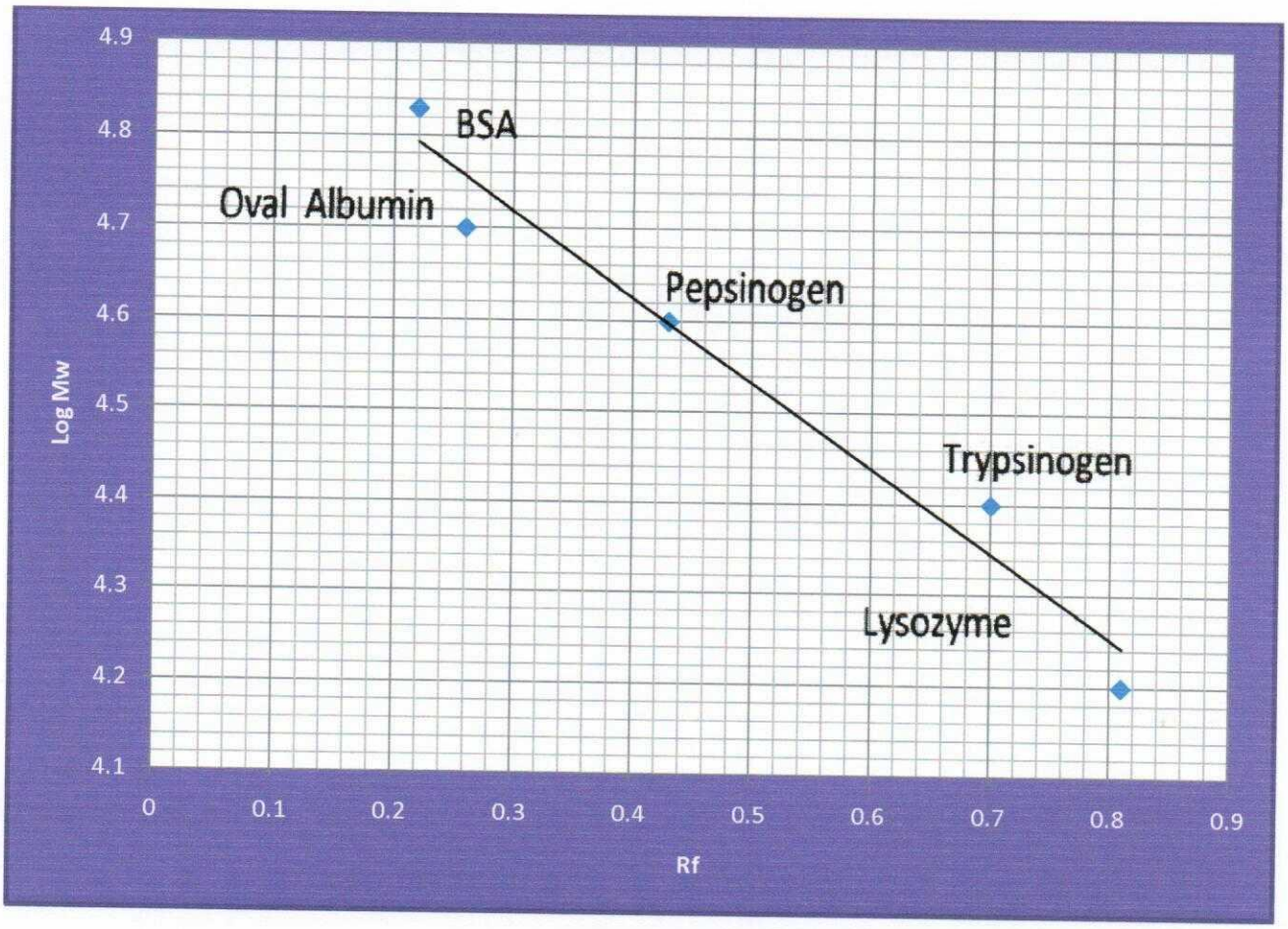


Figure 3: Graph showing Log M_w versus R_f for the Estimation of Molecular Weight of Protein

Source: Weber and Osborn (1976)

TABLE 3: CHARACTERIZATION OF PROTEIN SAMPLE BASED ON THEIR MOLECULAR WEIGHT IN KILODALTON

SAMPLE	TOTAL BANDS	BSA(66 AND ABOVE)	OVAL ALBUMIN (45 TO 65)	PEPSINOGEN (33 TO 44)	TRYPSINOGEN (24 TO 32)	LYSOZYMES (14 TO 23)
BN_1	6	2	1	2	1	0
BN_2	6	2	1	2	1	0
BN_3	7	2	1	2	0	2
BN_4	8	2	1	2	0	3
BN_5	7	2	1	2	0	2
BN_6	6	2	1	1	2	0
BN_7	6	2	1	2	1	0
BN_8	6	2	1	2	1	0
BN_9	5	1	1	2	1	0

4.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1 DISCUSSION

The relationship of a group of specie/ accession can be determined directly through electrophoresis, which deals with proteins that are 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.

Despite the similarities observed in the binding patterns, a close examination reveled that differences still abound among the Bambara groundnut accession. These similarities are expected and the differences noticed are understandable because both ''nature and nurture'' determine the phenotype of an organism. The differences observed might be due to the impact of environment (nurture). It follows quite logically that different accession belonging to the same specie are expected to be more phylogenetically related.

The results of the electrophoresis of crude protein from the nine (9) accessions of Bambara Groundnut studied are shown in Plates 1. Table 2 shows the list of the accession used for the electrophoresis and the protein concentration observed for each accession. Table 3 shows characterization of the proteins based on their molecular weight. 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 the observation made by (Agbolade et al, 2013). Table 4 shows the result for determination of protein at optical density (O.D) 595nm which gives the concentration of protein in each accession. BN_1 has the highest protein concentration while BN_2 has the lowest. Similarities and differences in protein composition of the accession are represented in table 3 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 has a high degree of BSA present in their bands with molecular weight ranging from 66kilodalton and above.

Sample A with sample number NGB 01494^{K5} and Sample identification of (BN_1) with average protein concentration of 0.066 at optical density of 595nm with length of gel 7.1 which contains total bands of 6 of which two (2) of the bands falls under Bovine Serum Albumin with RF value of 0.042 and 0.085 respectively and molecular weight of 66kilodaltons and above and band 3 falls under Oval Albumin with RF value of 0.324 and molecular weight 45- 65kilodaltons, band 4 and 5falls under protein pepsinogen with RF value of 0.451 and0.507 and molecular weight of 33-44kilodaltons, the last band, band 6 falls under protein Trypsinogen with RF value of 0.648 and molecular weight of 24-32kilodaltons, but lack the presences of lysozymes.

Sample B with Sample number NGB 01497^{K5} and Sample identification of (BN_2) with average protein concentration of 0.053 at optical density of 595nm with length of gel 7.3 which consist of 6 bands , 3 thick bands and 3 thin bands, in which 2 of these falls under Bovine Serum Albumin with RF value of 0.041 and 0.082 and molecular weight of 66kilodaltons and above, and band 3 falls under the Oval Albumin of RF value of 0.288 and molecular weight of 45-65kilodaltons, band 4 and 5 falls under the protein pepsinogen with RF value of 0.438 and 0.506 with molecular weight of 33-44kilodaltons and the last bands 6 falls under the protein Trypsinogen with RF value of 0.644 molecular weight 24-33kilodaltons, but lack the presence of lysozyme.

Sample C with Sample number NGB 01499^{K5} and Sample identification of (BN_3) with average protein concentration of 0.065 at optical density of 595nm with Gel length 7.0 has a total number of 7bands, 5 thick bands and 2 thin bands and 2 of these bands falls under the Bovine Serum Albumin with RF value of 0.057 and 0.086 and molecular weight of 66kilodaltons and above, band 3 falls under the Oval Albumin of RF value of 0.314 and molecular weight of 45-

65kilodaltons, bands 4 and 5 falls under the protein pepsinogen with RF value of 0.457 and 0.514 and molecular weight of 33-44kilodaltons and band 6 falls under protein lysozyme with RF value of 0.657 and molecular weight 14-23kilodaltons,but lack the presences of Trypsinogen.

Sample D with Sample number NGB 01501^{K5} and Sample identification of BN_4 with average protein concentration of 0.060 at optical density at 595nm with length of gel 6.8 which consist of 8 bands, with 6 thick bands and 2 thin bands, 2 of which falls under the BSA with RF value of 0.044 and 0.074 and molecular weight of 66kilodaltons and above, band 3 occurs in the region of 45-65kilodaltons regarded as the Oval Albumin with RF value of 0.309, band 4 and 5 also falls in the region of protein pepsinogen with RF value of 0.426 and 0.470 and molecular weight of 33-44kilodaltons,while bands 6,7 and 8 falls under the Lysozymes with RF value of 0.676, 0.750 and 0.809 with molecular weight of 14-23kilodaltons,this accession of all has the highest protein concentration and band 2 and 3 contains the highest concentration of the crude protein.

Sample E with Sample number NGB 01245 and Sample identification of BN_5 with average protein concentration of 0.062 at optical density at 595nm with length of gel 6.6 which consist of 7 total bands, with 5 thick bands and 2 thin bands, 2 of which fall under the Bovine Serum Albumin with RF value of 0.045 and 0.091 and molecular weight of 66kilodaltons and above, bands 3 occurs in the region of 45-65kilodaltons regarded as the Oval Albumin with RF value 0.318. band 4 and 5 also occurs in the region of protein pepsinogen with RF value 0.469 and 0.530 molecular weight of 33-44kilodalton, while bands 6 and 7 occurs under the Lysozymes with RF value of 0.682 and 0.818 respectively. Band 1,2 and 3 contains the highest concentration of the crude protein.

Sample F with Sample number of NGB 01489^{K5} and Sample identification of BN_6 with average protein concentration of 0.062 at optical density of 595nm with Gel length of 6.8, which consist of 6 clear bands , with 4 thick bands and 2 thin bands, 2 of which are found under the Bovine Serum Albumin with molecular weight of 66kilodaltons and above and RF value of 0.029 and 0.072 respectively, band 3 has an RF value of 0.333 and occurs in the region of Oval Albumin of molecular weight of 45-65kilodalton, band 4 with RF value of 0.449 and molecular weight of 33-44kilodalton, while band 5 and 6 occurs in the region of protein Trypsinogen with molecular weight of 24-32kilodalton with RF value of 0.493 and 0.623 respectively, in which bands 1, and 3 has the highest concentration of the crude protein.

Sample G with Sample number of NGB 01311^{K5} and Sample identification of BN_7 with the average protein concentration of 0.063 at optical density of 595nm with length of gel 6.9, which has a total band of 6, with 4 thick bands and 2 thin bands, the highest concentration of the crude protein is found in bands 1,2 and 3. Two of these bands occur in the BSA region with RF value of 0.029 and 0.072 and molecular weight of 66kilodaltons and above, bands 3 falls under the Oval Albumin with molecular weight of 45-65kilodalton and RF value of 0.333. Band 4 and 5 occurs in the region of protein pepsinogen with molecular weight of 33-44kilodalton with RF value of 0.449 and 0.493 respectively, while band 6 falls under the region of protein Trypsinogen with RF value of 0.623 and molecular weight of 24-32kilodalton.

Sample H with Sample number of NGB 01496^{K5} and Sample identification of BN_8 with the average protein concentration of 0.061 at optical density of 595nm with Gel length of 6.7, which has a total bands of 6,with 4 thick bands and 2 thin bands, the highest concentration of the crude protein is found in band 1,2 and 3. Two of these bands occurs in the region of the Bovine Serum Albumin with RF value of 0.029 and 0.075 with molecular weight of 66kilodalton and above,

band 3 with RF value of 0.313 and molecular weight of 45-65kilodalton which occurs to be Oval Albumin, band 4 and 5 with RF 0.463 and 0.507 and molecular weight of 33-44kilodalton is said to be protein pepsinogen, while the last bands for this sample occurs in the region of the protein Trypsinogen with RF value of 0.642 and molecular weigth of 24-32kilodalton.

Sample I with Sample number NGB 01495^{K5} and Sample identification of BN_9 with the average protein concentration of 0.061 at optical density of 595nm with Gel length of 7.0 which has a total band of 5, 3 thick and 1 thin band. Band 1 occurs in the region of the BSA with molecular weight of 66kilodalton and above and RF value of 0.071, while band 2 with RF value of 0.314 and molecular weight of 45-65kilodalton occurs in the region of the Oval Albumin and band 3 and 4 with RF value of 0.428 and 0.471, which falls under the protein pepsinogen with molecular weight of 33-44kilodalton. In this region of bands 1 and 3 has the highest concentration of the crude protein, while the last band with RF value of 0.600 falls under Trypsinogen with molecular weight of 24-32kilodalton.

The proteins characterized in these samples are of great importance to science and humanity. Bovine Serum Albumin with molecular weight 66kda and above is a protein with high negative charge and It binds water, salts, fatty acids, vitamins and hormones, and carries these bound component between tissues and cells, It is also effective scavenger removing toxic substances and also a stabilizers for other solubilized protein.(wiki/ Bovine serum Albumin 2014). While Oval Albumin works effectively a selective insulin binding agents. It potential applications are used in the area of oral drug delivery and also as functional food supplement. (wiki/ oval albumin 2014). Pepsinogen, this is a product of pepsin in an inactive precursor form by the chief cell. It helps in digestion of protein (wiki/pepsinogen, 2014).While also Trysinogen a precursor of trypsin functions as Protein activation at appropriate location (wiki/Trypsinogen

2014) and lastly the lysozyme in which Children who fed with foods lacking lysosome have 3 times the rate of diarrheal disease. According to *Philips Mechanism (1965),* lysozyme is used as a binding agent.(wiki/ Lysozyme 2014). It is worthwhile to emphasize that optimately and logically differences in electrophoresis mobility of protein fractions obtain from 2 sources are of greater importance for taxonomic purpose than the similarities of mobility. The possibility of 2 dissimilar protein having identical electrophoretic mobility is known [Gottlieb, 1971], yet the assumption is made that bands derived from 2 difference accession that migrate the same distance in Bambara groundnut are considered to be produced by gene(s) common to both accession.

The result of the electrophoretic banding patterns of the studied accessions of Bambara groundnut reveals same 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, Massawe et al 2002, and Modini et al 2006, who employed comparative electrophoretic protein banding pattern of different species and accession in establishing relation among various taxa.

4.2 CONCLUSION

The similarities and differences that occurs in this protein profile of all the accessions of *vigna subterranean* are indicatives of genetic protein content and thus may be useful in the taxonomic delimitation of the different accession belonging to this specie. And furthermore, the electrophoresis of the seeds protein appear to demonstrate close relationship and distinctiveness of the different accession put into consideration and could therefore be important in genetic delimitation .And despite the fact that they possesses the same phylogenic characteristics, there

are still some little differences and similarities in the protein concentration The seeds as a well balanced food it can be used as an excellent supplement in helping to achieve a balanced diet and overcome malnutrition especially among children. Extracts like boiled seeds, ethanol extract, boiled immature seeds of bambara groundnut are used to treat various ailments such as: anemia, diarrhea, ulcers, gonorrhea impotence and colon cancer.

4.3 RECOMMENDATION

Although Bambara groundnut is largely unexploited, cheap and given little or no priority, there is an urgent need for awareness campaigns on its uses, nutritional benefits, and as a matter of urgency it should be a cash crop. Again the nut should not be seen and cultivated as subsistence-female crop; rather it should be seen as a crop that is relevant to food security.

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

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