VARIATION IN THE PROTEIN LEVEL OF DIFFERENT ACCESSIONS OF PIGEON PEA

(*Cajanus cajan* L. Millspaugh)

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OCTOBER, 2015.

DECLARATION

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

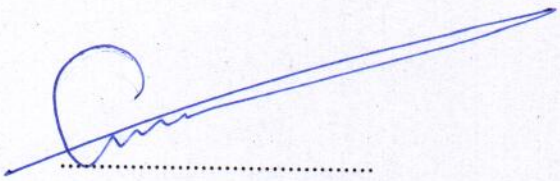
Olaiya Aderonke Eunice

Sign.....

Date: October , 2015

CERTIFICATION

I certify that this final year project was written by Olaiya Aderonke Eunice, of matriculation number BTH/11/0257 in the department of Plant Science and Biotechnology, Federal University, Oye Ekiti.



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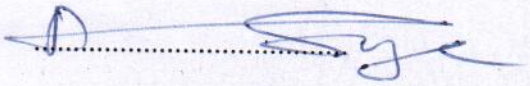

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DEDICATION

This work is dedicated to almighty God, the giver of all knowledge for the successful completion of my B.Sc programme.

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ABSTRACT

Ten accessions of Pigeon pea (*Cajanus cajan*) obtained from National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Oyo state, were assessed for their genetic and phylogenic relatedness through electrophoretic analysis of the seed proteins. 0.2g of the seeds were weighed and macerated with mortar and pestle in 0.2M phosphate buffer containing 0.133M of acid (NaH2PO4) and 0.067 of base (Na2HPO4) at Ph 6.5.Protein characterization with standard marker revealed that the seeds of the 10 accessions contained proteins (B.S.A, Oval Albumin, Pepsinogen, Trypsinogen and Lysozyme) with molecular weights ranging from 66 and above kda, 45 – 65 kda, 44 – 33 kda, 32-24 kda and 23-14 kda respectively. All the accessions had at least two proteins and two major bands in common. The study revealed intra-specific similarities and genetic diversity in protein contents among the ten accessions of pigeon pea (*Cajanus cajan*)

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW.

1.1 INTRODUCTION

Variability is an aspect of systematics. The major concern of systematics however, is to establish that variability exist among organisms. Systematics is the scientific study of biological diversity and its origins. It is the single most unifying science of life, encompassing all biological parameters and employing the most modern, cutting-edge technologies. It is not subservient to any other discipline. While it is sometimes portrayed as the mere classification of organism, in fact its range and challenge are among the greatest in biology (FAQ, 2015). Systematic studies focuses on understanding evolutionary relationship 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 geographical distributions (Society of Systematic Biologist, 2015).

In systematic studies we guess the degree of similarities among taxa and then gather data to test if these guesses are true. This is important because appearance alone cannot indicate how similar the features of the taxa are, if they are homologous, we expect that they will share many things because of their common ancestry, while if they are not, it is impossible to predict just how similar they will be. Hence, any study that asks why or how about a feature in more than one taxon, draws comparative conclusions about them, is of a systematic foundation. In this study, systematic would be employed to

ascertain that variability exists in the protein concentration of ten accessions of pigeon pea (*Cajanus cajan*).

Pigeon pea (*Cajanus cajan* (L.) Millspaugh) is the sixth most important grain legume of the world. The crop is predominantly grown in Asia, Africa and the Caribbean Islands, India has the largest area under pigeon pea (4.6 m. ha), followed by Myanmar (0.6 m ha), Kenya (0.13 m ha), Malawi (0.21 m ha), Uganda (0.1 m ha), Tanzania (0.29 m ha), Nepal (0.03 m ha) and Dominican Republic (0.02 m ha) (FAOSTAT, 2013). Pigeon pea has multiple uses, for instance, the split peas are rich source of protein (20–23%) and form an excellent combination with cereals for a balanced human diet. Additionally, it improves soil fertility by fixing atmospheric nitrogen and reducing soil erosion. Pigeon pea has a genome size of 833.07Mband is the first non-industrial food legume crop for which draft genome sequence has been developed (Varshney *etal.*, 2012). It is an often cross-pollinated diploid (2n = 2x = 22) crop and the natural out-crossing ability has been utilized to develop an efficient cytoplasmic genetic male sterility (CGMS) based hybrid system in pigeon pea (Saxena *et al.*, 2010; Varshney *et al.*, 2010).

Its seeds contain about 20-22% protein and a large amount of essential amino acid, making it an important source of dietary protein and play a vital role in meeting the protein requirements of a vegetarian population (Sexan, 2009). However, nutritional values vary slightly from one variety to another. The green pod is eaten as vegetable and dry pulse is an important source of vitamin B, carotene and ascorbic acid (Odeny, 2007 and Choudhary *et al.,* 2013). Pods and foliage of this crop is also used as livestock feed

and fodder, its woody stem is used as fuel and for construction (Mallikarjuna *et al.*, 2011), and for basket weaving and roofing in African villages.

Cultivation of pigeon pea improves soil fertility by fixing nitrogen as well as solubilizing soil-bound phosphorus (Mallikarjuna *et al.*, 2011 and Choudhary *et al.*, 2013). It is drought tolerant and have a high resistance to heat than other legumes, this traits helps the crop to regulate and maintain high temperature and low moisture by adjusting leaf osmotic, maintenance of photosynthesis under stress and their deep root systems (Subbarao *et al.*, 2000; Lopez *et al.*, 1987 and Flower and Ludlow, 1987). Its ability to enrich the soil adds more value to the crop making it an important piece in farming systems adopted by farmers in developing countries (Saxena, 2006).

In comparison with green peas, vegetable pigeon pea takes longer to cook and is not as sweet, but it is much more nutritious. It has greater edible portion, more protein, carbohydrates, fiber, fat, more minerals and vitamins. Regardless of its importance as food for both human and animals, its nutritive value and its wide range of uses, red gram still receive a relatively little research when compared to other legumes such as Soy bean (*Glycine max*), and the need to do so is of utmost importance.

1.1.1 ECONOMIC POTENTIAL OF PIGEON PEA

Crops that were once referred to as 'orphan' are now been incorporated into breeding programmes as they now show potential for ensuring world's food security. The importance of pigeon pea which is drought tolerance, enriches the soil, feed both human and animals and beneficial to the climate cannot be overlooked. According to Ryan and Spencer (2001), it is becoming an international crop, with the world facing water crisis,

its popularity has increased over the last decade. One of the main challenges facing scientist is to increase food productivity with less water, this is where drought tolerant crops like pigeon pea and other legumes become important. By the year 2050 when at least 1 in every 4 person is likely to live in a water deficient area according to current prediction, it is crops like Pigeon pea that will sustain and feed the ever increasing human population.

1.1.2 OBJECTIVE OF THIS STUDY

To establish that variation occurs in the protein concentration of ten accession of pigeon pea (*Cajanus cajan*) based on their molecular weight and quantity of the seeds' proteins through electrophoresis.

1.2 LITERATURE REVIEW

In the past, many researchers have made an attempt to improve this crop using different approaches. This includes mutation breeding, breeding to explore the potentiality of fitting pigeon pea in the cropping of other crops (Adjei-Nsiah, 2012), evaluation of the genetic diversity of the crop that is cracking of the genome sequence (ICRISAT, 2010), breeding to combat production constraints in specific countries such as Philippines (ICRISAT, 2010), for pests management (NCIPM, 2010), for better nutritional quality (Horbat, 1986) etc. Each of these researches have played a prominent role in the position of pigeon pea in the world legume today and contributed to the knowledge and information available on this crop.

Breeding pigeon pea has been more challenging compared to other food legumes due to various crop specific traits. Pigeon pea is an often cross pollinated crop, with an insect-aided natural out crossing range from 20 to 70% (Saxena *et al.*, 1990) that has limited the use of efficient selection and mating designs possible in self-pollinating species. Pure line breeding, population breeding, mutation breeding, and wide hybridization have been used for development of new varieties in pigeon pea and have led to incremental improvements in the yield potential of this crop. To overcome this bottleneck, two genetic male-sterility (GMS) systems were discovered in pigeon pea (Reddy *et al.*, 1978; Saxena *et al.*, 1983). Despite a 30% yield advantage over the non-hybrids, the GMS based hybrids could not be commercialized due to high cost of hybrid seed production. The yield-jump observed in the GMS hybrids encouraged the development of the alternative and more efficient cytoplasmic-genetic male-sterility (CGMS) system (Tikka *et al.*, 1997; Saxena and Kumar, 2003; Wanjari and Patel, 2003).

As a result of intensive hybrid development programme at ICRISAT in collaboration with its partners, the first CMS- based hybrid GTH-1 was released in India in 2004. Another CMS-based pigeon pea hybrid, ICPH 2671 was developed using *C. cajanifolius* (A4 cytoplasm) at ICRISAT in 2005 (Saxena, 2008), that has been released as ''Pushkal'' by Pravardhan seeds for cultivation in several states of India such as Andhra Pradesh, Karnataka, Madhya Pradesh, and Maharashtra. Continued hybrid-technology based improvements in pigeonpea yield potential, together with on going efforts to breed for resistance to biotic and abiotic stresses (Fusarium wilt, sterility mosaic, pod borer, etc.) are likely to lead to increased area under pigeon pea hybrids, contribute to increased crop returns for farmers and sustainable pigeon pea production (Varshney *et al.*, 2009).

1.2.1 ORIGIN OF PIGEON PEA

The name Pigeon pea was first reported from plants used in Barbados where it was used as pigeon feed. However, the seeds were given the name Pigeon pea in (1962) in the West Indies. There have been mixed opinion about the origin of this crop. Some historians believed that pigeon pea is a native of East indies, some said it was from Malaya while some authorities claimed that it originated from here in Africa. Other countries such as Egypt, China, India, Australia and Asia was also speculated as the primary and secondary centre of origin of this crop. However, due to the presence of several wild relatives of pigeon pea seed, archeological remains, and genetic diversity of this crop in India, it was concluded by Valvilov (1951) that 'gaundu' originated from there. Archaeological finds of pigeon pea include those from two Neolithic sites in Odisha, Gopalpur and Golbai Sassan dating between 3,400 and 3,000 years ago, and sites

in South India, Sanganakallu and Tuljapur Garhi, also dating back to 3,400 years ago (Fuller *et al.,* 2006).

Furthermore, the early Asiatic name of the species *Cajanus cajanifolius* 'kayan', which is believed to be of Indian origin (Vavilov, 1951) all the more convinced historians that pigeon pea indeed originated in India. (Agropedia, 2009). Myanmar also account for 16 species of *C. cajan.* Australia has 15 wild relatives of Pigeon pea of which 13 are endemic. *Cajanus* progenitors must have evolved along different lines in Australia and Asia because no species are common in these two continents (ICRISAT, 2010). Only one wild relative of this specie is found in Africa, the *C. kerstingii. C. volubilis* (Blanco) is found in Southeast Asia and *C. crassus* (Prain ex King), is restricted to the drier parts of the islands of Indonesia and the Philippines (Van der Maesen, 1979).

1.2.2 DOMESTICATION OF PIGEON PEA.

Historians believed that the crop travelled from India to Malaysia, then to East Africa, then up the Nile Valley to West Africa, then travelled to Zaire or Angola prior to the main slave trade. When Columbus discovered America, the people of African origin were taken as slaves and Pigeon peas travelled to the new world with the slaves. This was the time when pigeon pea started gaining popularity and was cultivated widely (ICRISAT, 2010). The crop has maintained its reputation since then, and even now it is being widely demanded (CRNIndia.com, 2008).

1.2.3 MORPHOLOGY OF PIGEON PEA.

Pigeon pea is an annual or short-term perennial shrub that is usually 1–2 meters in height, but may reach up to 4–5 meters. It is woody at the base and usually erect but with variable growth habit (ICRISAT, 2010). It has a lifespan of about five years and exhibit two types of reproduction, mostly diploid and tetraploid. The description of the main parts is given below.

Plate 1: The pigeon pea plant.

Source: ICRTISAT, 2010.

1.2.3.1 ROOT SYSTEM

Root system of pigeon pea consists of a central tap root with numerous lateral and secondary branches. The length of the lateral roots differs with the variety; usually tall, upright varieties produce longer and more deeply penetrating roots, whereas spreading types produce shallower, more spreading and deeper roots (Agropedia, 2009). It has a deep and quick growing tap root and angular stem resulting from three ribs starting from the base of each petiole (ICRISAT, 2010).

1.2.3.2 LEAVES

Leaves are trifoliately compound; central leaflet longer than lateral ones. The leaflets are entire and densely silky on the lower surface. Stipules are small; lamina hairy with the under surface grayish due to dense hairs. The intensity of the green color of the leaves differs with the variety. The total length of the leaf, as also the size, shape and texture of leaflets also differ with the varieties (Agropedia, 2009). It has pubescent trifoliate leaves alternately set in a spiral along the stem and oblong lanceolate leaflets about 5–10 cm long and 2–4 cm wide. Its lateral petioles measure about 2–3 mm and the terminal ones reach 10–20 mm while the stipules linear are 2–3 mm and stipulets filiform are 1–2 mm long (ICRISAT, 2010).

1.2.3.3 FLOWERS

The flowers of this plant are usually yellow but they may also be striated with purple streaks or plain red. The corolla is about 20–25 mm with flag 18–20 mm wide and the calyx is 10–12 mm long with 5 linear teeth (ICRISAT, 2010).

1.2.3.4 INFLORESCENCE

The plant's inflorescence is composed of racemes with 5–10 flowers on top of an axillary and slightly divided peduncle (ICRISAT, 2010). The size of inflorescence varies in different types. The flowers are distinctly papallionaceous. In the late maturing varieties, the flowers are usually grouped together at the ends of the branches, but in early maturing varieties, the flowers are produced at several points along the branches. Usually flowers open at a time on the same inflorescence, but the process of flowering continuous in each plant almost up to the time of harvest. The flowers are self pollinated, pollination takes place before the flowers open. Cross fertilization may also occur to some extent (Agropedia, 2009)

1.2.3.5 PODS

The fruit of pigeon pea is a pod.The pods are about 5–9 cm long x 12–13 mm wide, flat, of variable color, pubescent, acuminate tip and contain 2–9 seeds in shades of brown, white, red or black. The husks bear deep, oblique furrows underlining the septa between the seeds (ICRISAT, 2010). The seed with in the pod may vary in number, but there are usually four to five in each pod in late maturing varieties and two to three in early maturing varieties (Agropedia, 2009)

1.2.3.6 SEEDS

Seeds are differing in great deal in size, shape and color. Seeds are round or lens shaped, the color of the seeds coat being dirty white to silver white, light brown to chestnut brown, dark mottled brown and pinkish black and the cotyledons yellow coloured (Agropedia, 2009).

1.3 TAXONOMY OF PIGEONPEA.

Pigeon pea belongs to subtribe Cajaninae of tribe Phaseoleae under sub-family Papilionoideae of family Leguminosae. *C. cajan* is the only domesticated species under sub-tribe Cajaninae. Within Phaseoleae, Cajaninae is well distinguished by the presence of vesicular glands on leaves, calyx, and pods. Currently, 11 genera are grouped under Cajaninae. The members of the earlier genus *Atylosia* closely resembled the genus *Cajanus* in major vegetative and reproductive characters but they were relegated to two separate genera, mainly on the basis of the presence or absence of seed strophiole. Based on growth habit, leaf shape, hairiness, structure of corolla, pod size, and presence of strophiole, Van der Maesen (1980) grouped the genus *Cajan* into six sections. The revised genus *Cajanus* currently comprises of 18 species from Asia, 15 species from Australia, and one species from West Africa. The 18 erect species were placed under three sections: seven species in *Atylosia*, nine species in section *Fruticosa*, and two species in section *Cajanus* that consists of the cultivated species along with its progenitor, *C. cajanifolius*. Eleven climbing and creeping species were arranged in two sections, Cantharospermum (5) and Volubilis (6) and the remaining three trailing species were classified under Rhynchosoides. Three *Cajanus* species have been further subdivided into botanical varieties; *C. scarabaeoides* into *Var. pedunculatus* and *Var. scarabaeoides; C. reticulatus* into *Var. grandifolius, Var. reticulatus,* and *Var. maritimus;* and *C. volubilis* into *Var. burmanicus* and *Var. volubilis* (Varshney *et al.,* 2009).

1.4 ECOLOGY OF PIGEON PEA.

Pigeon pea is a very resistant crop; it grows even in unimaginable areas and on soil like gravel, heavy clay, and on well-drained medium. This crop can survive and produce seeds in extreme conditions with limited amount of rainfall and also during the dry season, but less suited in humid areas due to its low tolerance of water and flood. However, rain during flowering stage causes poor pod set and permits the attack of pod borers. On the other hand, the growth of pigeon pea at high altitude with low temperature is slow and the plant is susceptible to water logging and frost. Therefore, the most suitable condition for its growth is an annual precipitation of 600-1000 mm with moist conditions for the first two growing months, drier conditions during f lowering and at 18-30oC during harvest time (ICRISAT, 2010).

The growth and development of this crop is influenced by photoperiodism, especially the traditional cultivars and landraces which are highly sensitive to photoperiod. This and temperature plays a great role in the determination of the time of flowering in Pigeon pea. Studies suggest that there is a need to fully understand the influences of photoperiod and temperature on flowering in the genotypes of different maturity groups as this might help breeders to manipulate flowering of the sensitive types by adjusting pre- and post-floral initiation temperatures and photoperiod conditions (ICRISAT, 2010).

1.5 PRODUCTION OF PIGEON PEA.

Earliest cultivation was in India, Africa, Asia and ancient Egypt during the pre-historic times and was later introduced to America. Within 1979 to 2009, 'red gram' recorded a 78% increase in production from 2.14 to 3.8 million tons owing to a 57% increase in cultivated areas. India is the largest producer of this crop with over 100 cultivars, cultivated on 3.38 million hectares of land thereby accounting for 90% of the world's production. Followed by Myanmar with 580 thousand hectares, then China where it is grown mainly for soil conservation and food with 150 thousand hectares and Nepal with 21,360 hectares of land. Production in East and South Africa is low, cultivation is on 0.82 million hectares and it is grown majorly for consumption and export. In Asia, it is grown on 4.33 million hectares and total production is 3.8 million tons, Pigeon pea production currently account for 5.2 million hectares in this rain fed areas (ICRISAT, 2010).

1.6 NUTRITIONAL COMPOSITION OF PIGEON PEA

Pigeon pea seeds contain about 20-22% of protein and essential amino acids making it an important diet for the vegetarian population (Sexan, 2009). It also contains a wide range of vitamins and minerals. The cotyledons are rich in carbohydrates (66.7%) while a major proportion (about 50%) of seed protein is located in embryo. About one-third of seed coat is made up of fiber. The quantities of important sulfur-containing amino acids such as methionine and cystine range around 1% and they are present in cotyledons and embryo; while calcium is pre-dominantly present in seed coat and embryo (Sexan *et al.,* 2010). The

nutritional composition varies from the immature to mature seeds. Due to its high nutritive values, its seeds are cooked as food, eaten as vegetables and as side dishes.

The difference in the nutrient composition can be seen in the table below.

Table 1: Nutritional content of pigeon pea, mature, raw.

Amount per 100gms	
Calories	343
	% Daily Value*
Total fat	1.49 g
Sodium	17 mg
Total Carbohydrate	62.78g
Dietary fiber	15 g
Sugar	n/a
Protein	21.7 g
Thiamin (Vitamin B1)	0.643 mg(56%)
Riboflavin (Vitamin B2)	0.187 mg (16%)
Niacin (Vitamin B3)	2.965 mg (20%)
Pantothenic acid(B5)	1.266 mg (25%)
Vitamin B6	0.283 mg (22%)
Folate (Vitamin B9)	456 µg (114%)
Choline	0.00 mg (0%)

Vitamin C	0 mg
Vitamin E	0.0 mg
Vitamin K	0.0 µg
Calcium	130 mg (13%)
Iron	5.23 mg (40%)
Magnesium	183 mg (52%)
Manganese	1.791 mg (85%)
Phosphorus	367 mg (52%)
Potassium	1392 mg (30%)
Sodium	17 mg (1%)
Zinc	2.76 mg (29%)
Per cent Daily Values are based on a 2,000 calorie diet.	

Source: IPGA, 2014

Table 2: Nutritional content of pigeon pea, immature, raw.

Amount per 100gms	
Calories	136 Kcal
	% Daily Value*
Total fat	1.64 g
Sodium	5 mg
Total Carbohydrate	23.88 g
Dietary fiber	5.1 g
Sugar	3 g
Protein	7.2 g
Thiamin (Vitamin B1)	0.4 mg (35%)
Riboflavin (Vitamin B2)	0.17 mg (14%)
Niacin (Vitamin B3)	2.2 mg (15%)
Pantothenic acid(B5)	0.68 mg (14%)
Vitamin B6	0.068 mg (5%)
Folate (Vitamin B9)	173 µg (43%)
Choline	45.8 mg (9%)
Vitamin C	39 mg
Vitamin E	0.39 mg
Vitamin K	24 µg
Calcium	42 mg (4%)
Iron	1.6 mg (12%)

Magnesium	68 mg (19%)
Manganese	0.574 mg (27%)
Phosphorus	127 mg (18%)
Potassium	552 mg (12%)
Sodium	5 mg (0%)
Zinc	1.04 mg (11%)
Per cent Daily Values are based on a 2,000 calorie diet.	

Source: IPGA, 2014

1.7 UTILIZATION OF PIGEON PEA

Pigeon pea as an important source of dietary protein is used mainly as food both for human and animal and as forage for livestock. Other part of the plant such as the woody stem is used for construction and as fuel in villages. It is also used medicine and industries (ICRISAT, 2010)

1.7.1 FOOD.

The matured dry seeds are dehulled and split as *dal,* then boiled and eaten as a pulse just like any other similar edible dried beans. Dehulling greatly reduces cooking time and improves the appearance, texture, palatability, digestibility and nutritional quality of the seeds (Faris and Singh, 1990). In the Caribbean region, there is a persistent demand for vegetable pods whether canned or frozen green peas because this pea is usually combined with rice or served in a soup. In India, it may be ground and used in a variety of meat and vegetable dishes. In Africa, it is usually served as a stew (www.gracefoods. com/site/gungopeas). Dry seeds of pigeon pea also have other uses, such as in the preparation of *tempe*, a traditional Indonesian food prepared by fermenting the legume seeds with *Rhizopus* and ketchup, snacks .as finger foods and to produce wine (ICRISAT, 2010)

Plate 2: Noodles made from pigeon pea starch in China. (Photo: KB Saxena)

Source: ICRISAT, 2010.

1.7.2 FUEL

Since pigeon pea has strong woody stems that grow up to 4m tall and branch freely, its spindly stalks are extensively used as a cooking fuel in energy short villages of several African countries and in India, Nepal and Sri Lanka. Historically, the stalks were employed to make the charcoal used in gunpowder. Farmers in Africa grow pigeon pea for its wood instead of its grain (ICRISAT, 2010)

1.7.3 SOIL CONSERVATION

In addition to food uses, pigeon pea (*Cajanus cajan*) has outstanding soil amelioration and conservation properties. The growth habit of this crop facilitates soil protection as the canopy continues to expand for 4 months after other crops are harvested. For more than 100 years, the legume symbiosis as shown by pigeon pea was known to be the most efficient way of transforming atmospheric nitrogen into plant nutrients (Alam and Manzoor, 2005). Leaf fall at maturity adds to the organic matter in the soil and

provides additional nitrogen. The root system is reported to break plough pans, thus improving soil structure, encouraging infiltration, minimizing sedimentation and smothering weeds. The crop nodulates with wide ranges of Rhizobium and consistently fixes 20 to 140 kg/ha of nitrogen in fertile soil (Anderson *et al.,* 2001; ICRISAT, 2010)

1.7.4 INTERCROP

Pigeon pea has been used successfully in coffee plantations as a cover crop to improve soil properties, reduce weed competition as well as act as a food source for predators (Venzon *et al.,* 2006). Maize yields have been increased by 32.1% in West Africa by using pigeon pea as a cover crop (Sogbedji *et al.,* 2006). Pigeon pea is used in alley cropping, and being perennial, it can be rationed successfully, that is it can be harvested without cutting the root and the lower part of the plant. Thereby making the crop mature early and decreases the cost of preparing the field and planting (Sharma *et al.,* 1978; ICRISAT, 2010)

1.7.5 MEDICINE

According to Morton (1976), Duke (1981) and Van der Maesen (2006), pigeon pea finds wide application in traditional medicine. Diarrhoea, gonorrhoea, measles, burns, eye infections, earache, sore throat, sore gums, toothache, anaemia, intestinal worms, dizziness and epilepsy are treated with leaf preparations. Root preparations are taken to treat cough, stomach problems and syphilis. Stem ash is applied on wounds, and stalks and roots are chewed against toothache. Powdered seeds serve as a poultice on swellings.

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

Although the medicinal value of pigeon pea in Africa has not been fully exploited, leaf decoction is diuretic and is used to control nervous breakdown, pulmonary troubles, stomach troubles, nasopharyngeal affections, smallpox, chicken-pox and measles. The roots can be used are used to cure venereal diseases and the seeds as sedatives. Pigeonpea leaves have been used to treat malaria in Nigeria (Aiyeloja and Bello, 2006; ICRISAT, 2010).

The following are the natural benefits and curative properties derived from this plant.

- Baldness - A fine paste made of this pulse is highly useful in bald patches. It should be applied regularly.
- Jaundice - The expressed juice of the leaves given, with a little salt, is highly beneficial in the treatment of jaundice. 60ml of this juice should be taken daily in this condition.

- Checking breast milk secretion - The pulse and leaves ground into a paste, warmed and applied over the mamma, has the effect of checking the secretion of breast milk.
- Inflammation - The leaves of the plant are effective in all inflammatory conditions. A poultice made with the seeds will also reduce swelling.
- Piles (Haemorrhoids) - Paste of the leaves, mixed with a teaspoonful of paste of neem leaves, is highly beneficial in the treatment of piles and itching in the anus. It should be taken once daily for a week.

(best-home-remedies.com/herbal_ medicine/grains&pulses/pigeon_pea ; ICRISAT, 2010)

1.8 DISEASES, PESTS AND MANAGEMENT

1.8.1. DISEASES

1.8.1.1 WILT

This disease is caused by a fungus, *Fusarium oxyporum f. sp. Udum*, which survives in off season on plant trashes in the soil. The leaves of the affected plants become yellowish in color, then drop and finally the whole plant dry out. These types of symptoms can be easily confused with shortage of moisture in the soil though there is plenty of moisture in the soil where these symptoms develop. The disease in fact can be diagnosed by seeing the black streaks on the wood after removing the outer epidermal strip from the major roots (Agropedia, 2009).

MANAGEMENT

It is difficult to control the disease due to the soil borne nature of the causal fungus. However, its incidents can be reduced considerably by taking certain precautions. These include following a three to four year crop rotation, taking a mixed crop of jowar and arhar and collecting and burning the plant trasnes left after harvesting. Best control is to plant disease resistant varieties like Amar, Azad, Asha (IPCL-87119), Maruthi, C-11, BDN-1, BDN-2, NP-5 etc. (Agropedia, 2009).

1.8.1.2 STEM ROT

This disease is caused by fungus *Phytophthora dreschsleri* var. *cajani.* The disease affected plants show formation of brown to dark brown lesions on the stem near the soil surface. Theses lesins rapidly girdle the whole stem because of which the plant starts drying. It may be noted that the symptoms can be confused with symptoms of the wilt disease. But this disease can be differentiated from wilt by examining the roots which remain healthy in this case. Also, plants affected by stem rot cannot be easily pulled out (Agropedia, 2009).

MANAGEMENT

Planting resistant varieties, plants should be protected from stem injury and good drainage system in the fields should be maintained. (Agropedia, 2009).

1.8.1.3 CANKERS

Several types of cankers are found on red gram. These are caused by fungi like *Dilopodia cajani, Colletotrichum cajani* and *Macrophoma cajanicola.* The disease affected plants show formation of cankers on stem and twigs. The plant part may break at such places (Agropedia, 2009).

MANAGEMENT

In case of severe intensity of this disease, the crop should be sprayed with Mancozeb 75 WP at the rate of 2.5 kg per hectare. A suitable crop rotation should be followed if the cankers are a problem in the same field every year (Agropedia, 2009).

1.8.1.4 STERILITY MOSAIC

It is caused by sterility mosaic virus. It is an important disease of pigeon pea. The virus is spread from plant to plant under field conditions through *Eriophyid mite.* The affected plants become light greenish in color which can be easily differentiated from dark green healthy plants. Leaves are reduced in size. Affected plants remain stunted and branch profusely, as a result of which they appear bushy. No flowers and fruits are borne on such affected plants resulting in total loss of yield. Sometimes only a few branches in the plant are affected others

remaining healthy. In such cases the yield reductions are patial. The virus is not seed borne (Agropedia, 2009).

MANAGEMENT

Plant resistant varieties like Pusa-885, Asha, Sharad (DA-11), Narendra-Arhar-1, Bahar etc. Control mites by spraying 0.1% Oxydemton methyl (Metasystox). Start spraying as soon as first affected plants are seen in the field. Three to four sprays are needed to control the mites (Agropedia, 2009).

1.8.2 INSECT PESTS

Pigeon pea crop is attacked by a number of insect pests. The important ones are given below:

1.8.2.1 POD BORER

This is widely distributed and is the most injurious pest of early and medium maturing varieties. The larvae, after hatching, feed on tender leaves and twigs but at pod formation they puncture pods and feed on developing grains. The caterpillars are green with dark brown, grey lines along the sides of the body (Agropedia, 2009).

MANAGEMENT

The caterpillar should be picked by hand after shaking the plants and destroyed in the early stages of attack. Spray the crop with 1.5 litre Endosulfan 35 EC or Monocrotophos (Nuvacron) 36 per hectare in 1000 litres of water (Agropedia, 2009).

1.8.2.2 TUR POD FLY

It is an important pest of Pigeon pea causing more severe damage in medium and late maturing types. The eggs are laid in tender pods. As the larvae grow and feed on the seeds, damage becomes more conspicuous and distinct. Stripes can be seen on the surface of the affected grains, while the attacked pods are somewhat twisted of deformed. In case of severe damage, as many as 80 per cent pods and 60 per cent grains may be damaged (Agropedia, 2009).

MANAGEMENT

The pests can be controlled by spraying the crop with 1.5 liters of Endosulfan 35 EC or Monocrotophos (Nuvacron) 36 EC in 1000 liters of water per hectare (Agropedia, 2009).

1.8.2.3 PLUME MOTH

This is a serious pest of Pigeon pea. The larvae damage seeds as well as cause flowers, buds and pods to drop. The caterpillar is greenish-brown in color and fringed with short hairs and spines. It also enters into the pod and feeds on developing grains (Agropedia, 2009).

MANAGEMENT

Spray the crop with Endosulfan 35 EC (1.5 milliliter in 1 liter of water) at the rate of 800-1000 liters per hectare (Agropedia, 2009).

1.8.2.3 HAIRY CATERPILLAR

Three species of hairy caterpillars may cause damage to early crop of arhar by eating away the green water of the leaves. The adult moth of these caterpillars laid eggs in large clusters and the young larvae are also congregated. The red hairy caterpillar may damage the crop at seedling stage (Agropedia, 2009).

MANAGEMENT

The eggs and young larvae of the caterpillar should be collected and destroyed. The young caterpillars can also be killed by dusting 2% Mythyl parathion at the rate of 25-30 kg per hectare. For full grown caterpillars spray Endosulfan #% EC at the rate of 1.5 liters in 1000 liters of water per hectare (Agropedia, 2009).

1.8.2.4 LEAF HOPPER

The adult and nymphs of this green hopper suck the juice from the larvae. Generally the insects sucks sap from the lower surface of the leaves but also occasionally from the upper surface. As a result of sucking the sap, the leaves turn brown and curl from the edge. In severe cases, they show symptoms of 'hopper burn' and ultimately dry up (Agropedia, 2009).

MANAGEMENT

Give basal application of Phorate (Thimet) 10 % granules at the rate of 10 kg per hectare or Disulfortan 5% granules at the rate of 20 kg per hectare at the time of sowing. Spraying with Monocrotophos 36 EC (1 millilitre in 1 litre of water) also controls the insect effectively (Agropedia, 2009)

1.8.2.5 BEAN FLY

It is a sporadic type of pest. The larva enters into the stem and causes plants to wilt or young plant to die. In case of severe infestation, there may be considerable damage (Agropedia, 2009).

MANAGEMENT

Application of systemic soil insecticide as used in case of leaf hopper provides adequate protection to the crop (Agropedia, 2009).

1.8.2.6 GALERUCID BEETLE

It is an important pest of Pigeon pea. It avoids sunlight and causes more damage during dusk and night. It hides under debris and loose soil during the day time. The adult beetle stipples the leaves with small and more or less circular holes. In severe cases, the leaf area is very much destroyed on account of feeding by the beetles and this adversely affects the vigor and growth of the plant (Agropedia, 2009).

1.9 CONSTRAINTS TO PIGEON PEA PRODUCTION.

Pigeon pea production has remained nearly static over the years and this can be partially attributed to unavailability of good materials and the effects of the ever changing climatic condition of our environment.

Odeny (2006) identified six constraints which can be worked upon to improve the productivity of the crop.

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

(ii) Environmental conditions such as extreme drought, poor water holding capacity of the soil etc. As well as lack of roads and market infrastructures are also factors hindering better production of the crop. With the increasing water stressed areas of the World from 28-30 countries today to 50 countries with billions of people (Postel, 2000), there is a crucial need to increase both salinity and drought tolerance in legumes. For countries coping with the above stated environmental condition, for example Africa, it should be made known to them that pigeon pea cultivation is the best solution to enhancing food security in such areas.

(iii) Insect, pests and diseases also inhibits 'guandu' production. Pigeon pea were less infected in the past but in recent times, diseases like *Fusarium wilt* , leaf spot and powdery mildew are of economic concern. (Kelly *et al.,* 2003) stated that pests and

diseases are more prevalent in third world countries. Fallow period to reduce vector population, plowing to bury infected plant tissues, biological and chemical control as well as chemical applications are methods of control (Beaver *et al.,* 2003, Coyne *et al.,* 2003)

(iv) Crop's long life cycle and complex genome structure makes breeding slow and particularly expensive. The establishment of the International Crops Research Institute for Semi-Arid Tropics (ICRISAT) in 1972 created a new focus and research interest leading to the recent development of cytoplasmic male sterile (CMS) lines for commercial hybrid breeding of pigeon pea (Mallikarjuna and Saxena, 2005).

(v) Various breeding methods should be incorporated by breeders to produce more wild relatives of pigeon pea which have been found to posses traits like resistance to pests and diseases (Sharma *et al.*, 2003), salinity tolerance (Subbarao *et al.,* 1991), and high protein content (Saxena *et al.,* 2006)

(vi) Cultivation of pigeon pea in the same area using the same technology will not enhance its production. Pigeon pea production can be improved by using modern method of breeding with better market prices.

Rusike and Dimes (2006) cite marketing, institutional and policy failures as the major constraints to expanded production of Pigeon pea in Africa. Despite an effective market demand regionally and internationally, farmers remain very poor (Jones *et al.,* 2002). Their lack of access to market information and scattered small unit production makes it difficult to form an association for collective bargaining. Markets should therefore be strengthened and inappropriate regulations that hinder information flow and products development should be lifted. Innovations that foster

transparency in markets and institutions would further reduce transaction costs and improve competitive position of smallholder farmers and other market intermediaries (Freeman and Jones, 2000).

CHAPTER TWO

MATERIALS AND METHOD

Table 3: Sample identification and weight

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

2.1 PREPARATION OF 0.2M PHOSPHATE BUFFER OF PH 6.5

PH = PKa + log (Base) ÷ (Acid)

6.5 = 6.8 + log (Base) ÷ (Acid)

6.5- 6.8 = log (Base) ÷ (Acid)

-0.3 = log (Base) ÷ (Acid)

Antilog (-0.3) = (Base) ÷ (Acid)

0.501 = (Base) ÷ (Acid)

Therefore,

(Base) ÷ (Acid) = 0.501÷1

(Base) = 0.501 × Acid

The phosphate buffer contains both acid and base, the total volume of the buffer must therefore equal the addition of acid and base. That is concentration of acid and base must equal 0.2M.

For the concentration of Acid

(Acid) + 0.501 × Acid = 0.2M

1.501 × Acid = 0.2M

Divide both sides by 1.501

0.2M ÷1.501 = (Acid)

0.133M = (Acid).

For the concentration of Base

(Acid) + (Base) = 0.2M

0.133 + (Base) = 0.2M

(Base) = 0.133 – 0.2M

(Base) = 0.067M.

2.1.1 CALCULATION OF THE MASS OF ACID AND BASE IN THE BUFFER

Acid (NaH_2PO_4) has a molecular mass of 156.01 and a concentration of 0.133M while Base (Na_2HPO_4) has a molecular mass of 177.99 and a concentration of 0.067M.

Concentration of acid in mol/dm^3 = Concentration of acid in g/dm^3

Molar mass

Cross multiply

Concentration of acid in g/dm^3 = 0.133M/dm^3×156.01 g/mol = 20.75g/dm^3.

Therefore; Concentration of acid in 1000ml is 20.75g/dm^3; in 500ml = 20.75g/dm^3 ÷ 2

= 10.375g/dm^3.

While

Concentration of base in mol/dm^3 = Concentration of base in g/dm^3

Molar mass

Cross multiply

Concentration of base in g/dm^3 = 0.067M/dm^3 ×177.99g/mol = 11.92533g/dm^3

Therefore; concentration of base in 500ml as calculated for acid = 11.92533g/dm^3 ÷ 2

=5.963g/dm^3.

NOTE: The above calculations implies that to prepare 0.2M Phosphate buffer of Ph 6.5, 10.375g/dm^3 of acid (NaH_2PO_4) and 5.963g/dm3 of base (Na_2HPO_4) is needed.

2.2. SAMPLE PREPARATION.

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

2.3 PROTEIN DETERMINATION

Protein concentration was determined by the method of Bradford (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.

Mathematically,

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

Therefore, at 0.4 = 5.0mg/ml

$$O.D_{new} = X$$

$$\frac{5.0 \times O.D_{new} = 12.5 \times \text{every } O.D_{new}}{0.4}$$

2.3.1 PREPARATION OF BRADFORD REAGENT

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

2.2.4 ELECTROPHORESIS ON SDS-PAGE

The molecular weight range of the samples was determined by SDS polyacrylamide gel electrophoresis as described by Weber and Osborn (1976). Standard proteins were as contained in Sigma Molecular Weight Markers Calibration Kit for SDS polyacrylamide gel electrophoresis (Daltons Mark VII-L, Molecular Weight Marker Range 14,000-70,000). The vial was reconstituted in 1 ml of sample buffer, mixed propers 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).

CHAPTER THREE

RESULTS

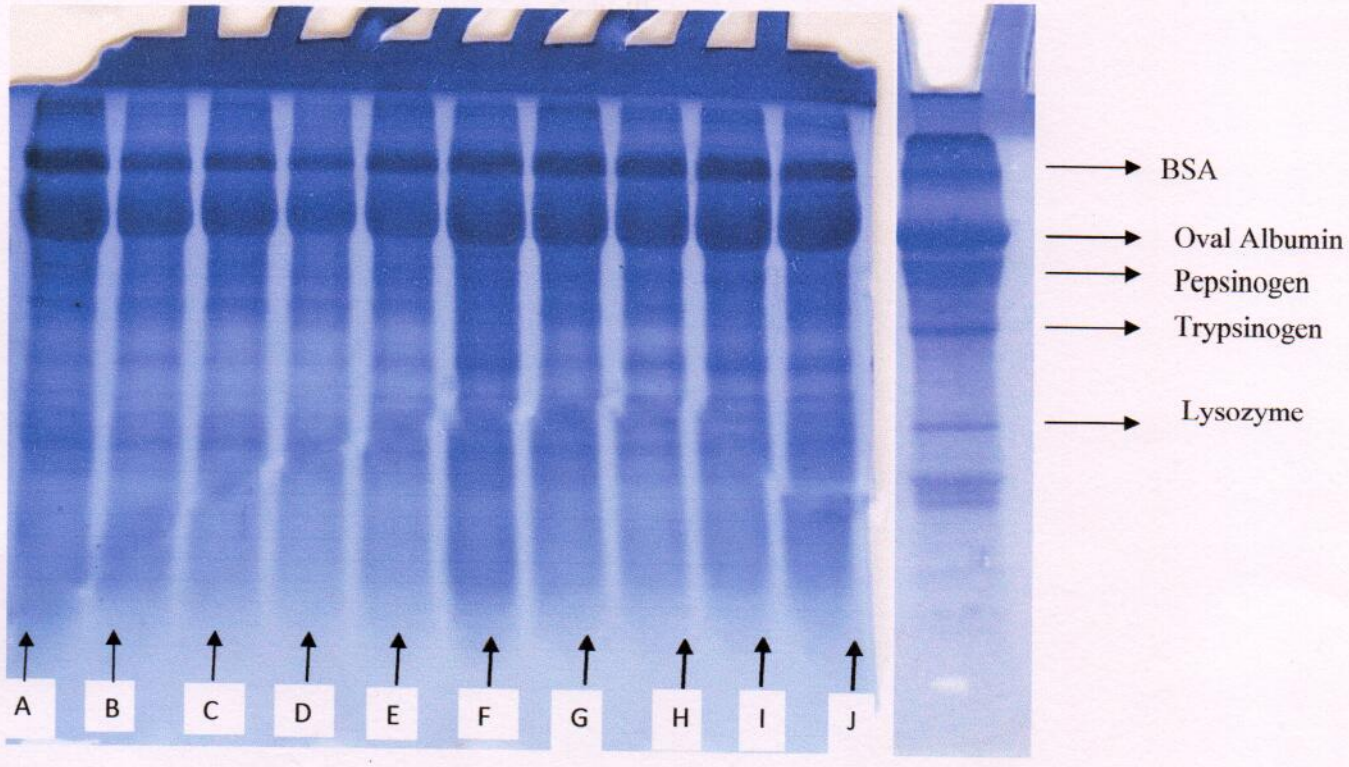
All the accessions of pigeon pea (*Cajanus cajan)* revealed considerable intra-specific variation and overlap in most of their banding patterns.

Table 4 shows the result of protein determination at optical density 595nm. The ten accessions of pigeon pea (*Cajanus cajan)* contain proteins. However, sample 5 (NG/AO/MAY/10/021) shows the highest degree of protein concentration while sample 3 (NG/AO/MAY/09/021) contains the lowest amount of protein.

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

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

Plate 3 is the result of SDS-PAGE and a standard gel. The number and thickness of band depict the quantity and the molecular weight of proteins present per sample. Each sample has at least 3 clear bands, the number of bands found in sample A to I is 7 while sample J has 8 bands. The standard gel used for the estimation of the types of proteins present in the samples.



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

Plate 3: Result of SDS-PAGE and a Standard gel.

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

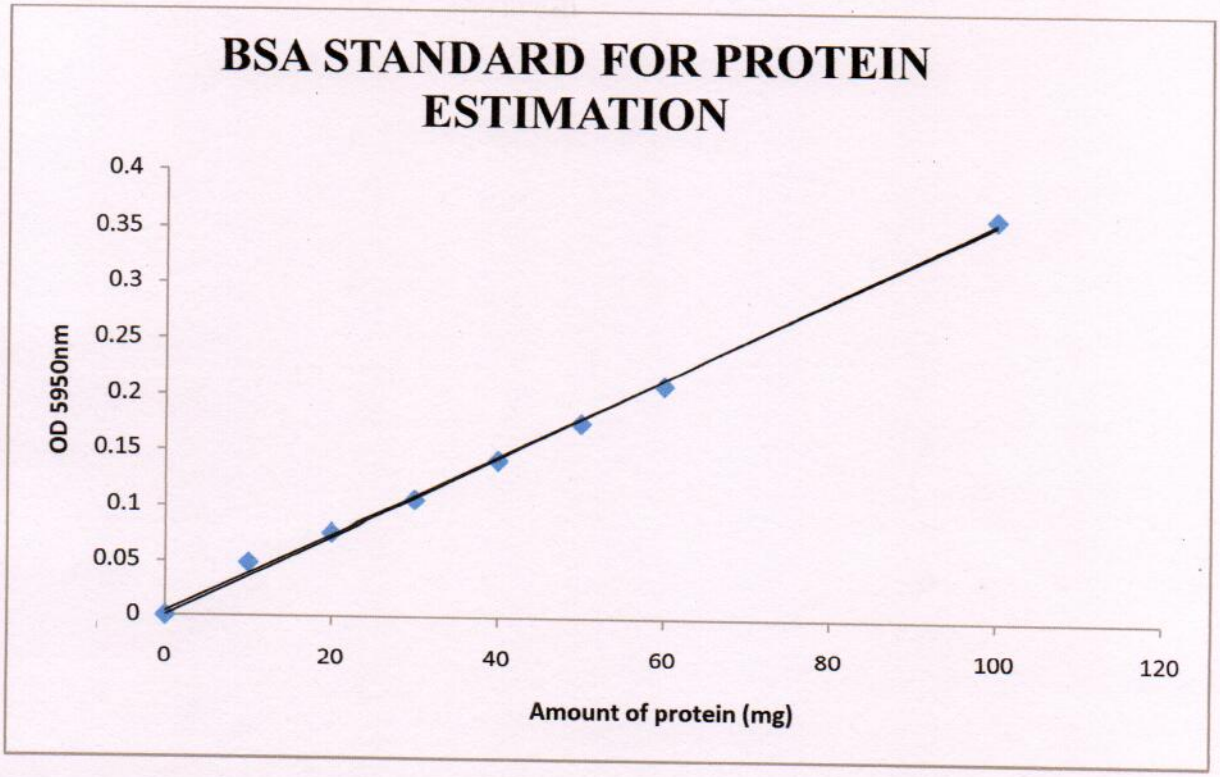


Figure 2: Standard protein graph

SOURCE: WEBER AND OSBORN (1976).

Figure 3 is a graph showing values of the standards plotted against the logarithm of their molecular weight. The molecular weight of the enzymes was interpolated from this graph.

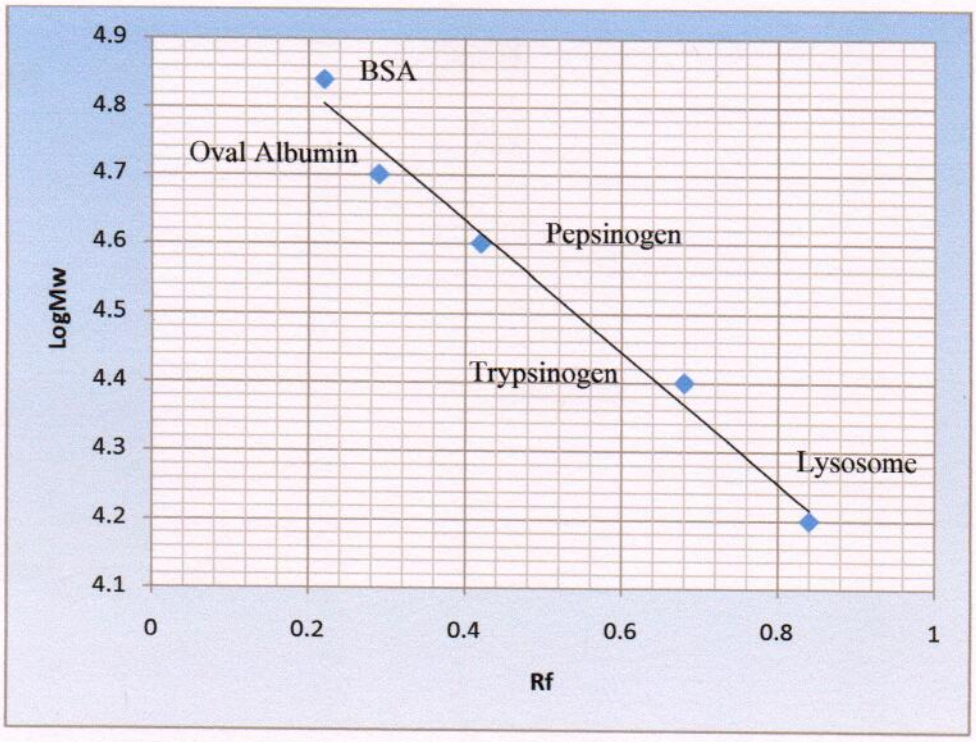


Figure 3: Log of Mw versus Rf for the estimation of the molecular weight of proteins

Table 5 below shows the characterization of the seed proteins. The proteins were characterized based on the molecular weight of the bands in comparison to the standard gel.

Table 5: Characterization of protein based on their molecular weight.

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

CHAPTER FOUR

DISCUSSION, CONCLUSION, RECOMMENDATION

4.1 DISCUSSION

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

The accessions of Pigeon pea used for this experiment are shown in Table 3. The result of electrophoresis of crude protein of the 10 accessions can be seen in Plate 1. This result shows that some samples are quite dissimilar both in terms of number and intensity of the bands while some other ones show a certain degree of relatedness as shown in Plate 6. Accessions 1 to 9 have seven bands each while the last accession has 8 bands. Sample A, B, C, and E, has about 5 clear bands and 2 faint bands while samples F, G, H, and I has 6 clear bands and 1 faints band, sample D has just 4 clear bands and 3 faints ones and sample J has the highest number of bands with 7 clear bands and 1 faint band. Sample F has the highest quantity of proteins due to the thickness of its bands while sample D has the faintest of all bands making it the less protein concentrated sample. All the samples have at least 2 major bands. Sample A has the thickest of all bands making it most concentrated with protein at this region. The thickness of the bands indicates the molecular weight of the protein, hence, the thicker the band the heavier the protein present at that region. The number of bands per sample establishes the amount of protein present in that sample. Since sample A to I have a total number of seven bands each, they contain equal number of

protein, while sample J has a total of eight bands making its protein content higher than the rest by just 1 band.

Despite the similarities observed in the banding pattern, a close examination revealed that differences abound among the pigeon pea (*Cajanus cajan)* accessions. However, this similarities and differences observed are understandable because both "nature and nuture" determine the phenotype of an organism. These differences might be due to the influence of nuture (environment). It follows quite logically that different accession belonging to the same specie are expected to be more phylogenetically related.

According to Figure 1 above, the protein concentration per sample varies. Sample 5 (NG/AO/MAY/10/021) has the highest number of protein concentration while sample 3 (NG/AO/MAY/09/O21) has the lowest concentration. The characterization of the samples protein based on their respective molecular weight done in Table 5 establish that Lysozyme according to the standard protein graph is least common in the samples, each sample has one or two Bovine Serum Albumin protein, almost equal amounts of Oval Albumin and Pepsinogen and some Trypsinogen. Bands with molecular weight lesser than Lysozyme were regarded as invalid. This is a similarity found among this specie. Despite the fact that these samples belong to the same specie and have the same physiology, there exist a certain degree of similarities and differences at the molecular level .

It is worthwhile to emphasis that optimately and logically, the difference in electrophoresis mobility of protein fractions obtain from two sources are of greater import for taxonomic purpose than the similarities of mobility. The possibility of two dissimilar proteins having identical electrophoretic mobility is known (Gottlieb, 1976), yet the assumption is made

that bands derive from two different accessions that migrate the same distance in polyacrylamide gel are considered to be produced by gene(s) common to both accessions.

The proteins observed in the samples are of great value to the health. Lysozyme is a small, stable enzyme, making ideal for research into protein structure and function. It attacks the protective cell wall of bacteria, thereby protecting us from the ever-present bacterial infection. In addition to this, lysozyme also benefits bladder health, inflammation management and wound repair (xtend-life, 2015). However, Trypsinogen and Pepsinogen functions as storage of an inactive form of trypsin and pepsin and kept in the pancreas to be release in significant amount when required for protein digestion (Wikipedia), while Oval albumin is presumed to be a storage protein, Bovine serum albumin (BSA)is used to stabilize some restriction enzymes during digestion of DNA to prevent adhesion of the enzyme to reaction tubes, pipet tips and other vessels. It is also commonly used to determine the quantity of other proteins by comparing an unknown protein to known amounts of BSA.

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

CONCLUSION AND RECOMMENDATION

CONCLUSION

Pigeon pea remains underutilized despite its wide range of uses and nutritive value. This study has contributed additional valuable information to the nutritional composition and genetics of pigeon pea. At the end of this study, it was established that despite the fact that the samples are of the same species, they still exhibit certain differences and similarities which can be seen in terms of their protein concentration which ranges from 7.312 in sample A, 9.250 in sample B, 5.713in sample C, 6.900 in sample D, 9.800 in sample E, 9.050 in sample F, 6.850 in sample G, 8.963 in sample H, 9.113 in sample I, to 9.038 in sample J. The types of protein present in the samples and the number and intensity of bands obtained from the SDS PAGE electrophoresis also shows the extent of relatedness of the accessions.

RECOMMENDATION

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

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