Yield, Quality and Nodulation Studies of Kersting's Groundnut [*Macrotyloma geocarpum*, (Harms) Merachal and Baudet] in the Coastal Savannah Agro-Ecological Zone of Ghana

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DECLARATION

This thesis is the result of research work undertaken by Gloria Anyesom Adazebra in the school of Nuclear and Allied Sciences of the Department of Nuclear Agriculture and Radiation Processing, University of Ghana (Legon), under the supervision of Dr. Harry M. Amoatey, Dr. Daniel Asare and Mr. Emmanuel Ofori-Ayeh.

I hereby affirm that except for references which have been duly cited, this work is a result of my own research and that it has not been presented in part or whole for any other degree in this University or elsewhere.

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DEDICATION

This work is dedicated to the Almighty Father for bringing me to where I am today. It is also dedicated to my late father, Mr. Clement Adazebra Ayimbire and to all my family for their support.

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ABSTRACT

Two investigations were carried out in the field and laboratory to assess variation in yield and nodulation potential as well as differences in the types of *Rhizobia* nodulating some local accessions of Kersting's groundnut (*Macrotyloma geocarpum* Harms) Marechal and Baudet in the Coastal Savannah Agro-Ecological Zone of Ghana. The aim was to obtain information relevant to important yield and nodulation attributes of Kersting's groundnut under prevailing agro-ecological conditions and thereby determine the suitability or otherwise of growing the crop in the Coastal Savannah Agro-Ecological Zone. Ten local accessions of Kersting's groundnuts were obtained from the University for Development Studies (UDS) Nyankpala, Tamale in the Northern Region of Ghana and were evaluated under field conditions at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) research farms in the Greater Accra Region. Significant variations were found in most of the quantitative characters that were measured for all the ten accessions. Yield studies conducted identified the Kersting's groundnut accession T8 to be the highest in both shoot dry matter production and grain yield per plot with values of 35.09 t ha^{-1} and 0.84 t ha^{-1} respectively. Nodulation studies also identified accessions T5 and T3 to be the best in %N content of roots and shoots with values of 1.43% and 3.05% respectively. The nitrogen yield was, however, highest in Kersting's groundnut accession T7 for both roots and shoots with values of 12.29 kg ha^{-1} and 1,178 kg ha^{-1} respectively. Again, accession T7 was superior in the total plant nitrogen yield with a value of 1190 kg ha^{-1}. Correlation analysis revealed perfect association (r=1.0) between grain yield and dry seed and a nearly perfect association (r=0.99) between total plant nitrogen yield and nitrogen yield of shoots. Harvest index was highly

positively correlated (r=0.72) with dry pod yield. Polymerase Chain Reaction (PCR) analysis was conducted for nine (9) accessions of Kersting's groundnut (due to lack of nodulation from one of the accessions) using three (3) arbitrary primers (ERIC 1and 2, RPO4 and RPO5)and one (1) specific primer (RPO1) to generate different amplification profiles for twenty (20) nodule bacteria isolates for each accession. The amplification profiles generated showed different banding patterns in which some ranged below 300bp to 10,000bp. The specific primer RPO1 which was a 20 nucleotide sequence primer was the most effective in generating amplification profiles. Cluster analysis was also done on the different bands that were generated to identify similarities and differences in the bacteria isolates within each accession and also between 45 nodule bacteria isolates of the different accessions. The inter-accessional cluster analysis grouped the bacteria isolates into two (2) major clusters at genetic similarity of 0.7%. Both similarities and differences were observed within the accessional isolates and between the inter-accessional isolates. A number of isolates proved to be the same entities as they could not be differentiated beyond a genetic similarity of 95%. The study suggests that, Kersting's groundnut can be successfully cultivated in the Coastal Savannah Agro-Ecological Zone, with grain yield and nodulation comparable to what pertains in the Guinea Savannah Agro-Ecological Zone where the crop is traditionally grown in Ghana.

CHAPTER ONE

INTRODUCTION

1.1 Background

Legumes constitute a major component of sustainable cropping systems due to their biological nitrogen-fixing ability (Ennin *et al.*, 2004). The domestication and cultivation of legumes, generally, have helped a great deal in solving the problem of balanced diet particularly in developing countries where their consumption has greatly increased due to increase in population (Adelusi *et al*., 2006). Legume seeds are rich in proteins and starch and are now receiving increasing attention in human nutrition due to the positive effects of polysaccharides on the physiology of the digestive tract as well as on the metabolism of glucose and lipids. The inclusion of legumes as an essential component of cropping systems is an inexpensive, feasible and sustainable intervention to supplement inorganic fertilizers (Ennin *et al.*, 2004; Asibuo and Osei-Bonsu, 2000).

Kersting's groundnut [*Macrotyloma geocarpum* (Harms) Maréchal and Baudet] is one of the lesser known leguminous crops, yet contributing significantly towards rural nutrition, livelihood and sustainable development. Other common names of this legume are Hausa groundnut, ground bean and geocarpa groundnut. It is an annual herbaceous legume which is grown in the arid and semi-arid regions of West Africa (Bampuori, 2007; Aremu *et al.*, 2006). The crop is cultivated in both forest and savannah areas in tropical Africa (Echendu *et al.*, 2009; NAS, 1979).

The second part of Kersting's groundnut's common name— groundnut— not only reflects its familial relationship to common groundnut (*Arachis hypogaea* L.) but also

its subterranean fruiting habit. The centre of origin of the crop is unknown but it is suggested to have originated from northern Togo or Central Benin. The crop is in cultivation in the West African savannah zone from Senegal to Nigeria (Dako and Vodouhe, 2006). In Ghana, the production of Kersting's groundnut is concentrated in the Northern and Upper West regions (Bayorbor *et al.*, 2010; Bampuori, 2007). The crop is an important source of carbohydrates, proteins and minerals and is, thus, used to supplement household diets (Dako and Vodouhe, 2006).

The genetic diversity of crops in Africa was naturally preserved by the traditional cropping system. However in recent times, there has been a rapid deterioration of natural ecosystems resulting in loss of genetic diversity due to pressure on the land as a result of livestock grazing, deforestation, industrialization and urbanization (Amujoyegbe *et al.*, 2007).

Biological nitrogen fixation (BNF) through *Rhizobia*-legume symbiosis has been recommended for the sustenance of traditional farming due to the declining soil nitrogen status in most soils in West Africa (Postgate, 1998; Peoples *et al.*, 1995). One way of improving this decline is to inoculate legumes with native strains of *Rhizobia* which are effective and competitive (Ampomah *et al.*, 2008) and also rely on legume crops with high nodulation effectiveness and N fixation efficiency as well as with high biomass yield. Studies along such lines on several legume crops including Kersting's groundnut, therefore, are necessary.

There is a wide range of methods that have been used in ecological studies of *Rhizobia* species in soil which are in association with plants. However, attention is now focused on molecular polymerase chain reaction techniques used with random

sequence primers. These methods have been shown to be effective in differentiating complex bacterial DNA genomes (Richardson *et al.,* 1995).

1.2 Problem Statement

Inadequate supplies of protein food throughout the world, but more especially in developing countries, have necessitated the search for new sources of food (protein) to supplement or substitute the existing sources (Ahmed and Abdallah, 2010). Most African countries are facing a serious food crisis manifested in the inadequate protein intake. The low protein intake has been attributed to the high cost of traditional sources of animal protein. The search for alternative sources of inexpensive protein has led to the cultivation of more Fabaceous crops because of their advantage in worldwide distribution and their high protein content (Obasi, 1993). Unfortunately, this attention on the Fabaceous crops has not been extended to all legumes, therefore, resulting in the underutilization and neglect of others. One of such legumes is the Kersting's groundnut [*Macrotyloma geocarpum* (Harms) Marechal and Baudet] which is an indigenous legume crop grown by small scale farmers in the Northern part of Ghana (Bampouri, 2007). Despite its numerous potential benefits, it has been neglected by both government and researchers and has the potential of being extincted as it is now cultivated by mostly elderly farmers.

1.3 Relevance and Justification

Despite the numerous benefits that can be derived from *M. geocarpum*, it has not received the desired attention in terms of governmental support and scientific research to promote its usage and also to prevent its extinction. It is currently a subsistence crop, with cultivation restricted to local farmers in rural areas of Northern regions of Ghana. Ecologically vulnerable and research-neglected species like Kersting's groundnut would be completely lost, together with their associated cultural information if simple and readily available techniques are not used to facilitate studies on their diversity (Bayorbor *et al.*, 2010). The crop has some nitrogenase activity and a good nutritional composition into which much research has not been carried out. A study of the nodulating ability, nitrogen yield, the rhizobial diversity as well as the general performance of *M. geocarpum* in the coastal savannah agro-ecological zone of Ghana will bring to light the potential of this crop for cultivation in the southern part of Ghana.

1.4 Overall Objective

The overall objective of the study is to determine the nodulation and yield potential as well as rhizobial diversity of *M. geocarpum* in the Coastal Savannah Agro-Ecological Zone of Ghana, and thereby indicate the suitability or otherwise of growing the crop within this ecology.

1.5 Specific Objectives

1. To study the extent and nature of nodulation among ten local accessions of Kersting's groundnut in the coastal savannah agro-ecological zone of Ghana.

2. To determine the total nitrogen and biomass yield of each accession under the prevailing conditions.
3. To determine the seed yield and harvest index of each accession under the prevailing conditions.
4. To evaluate the protein quality of the legume forage for livestock feeding.
5. To determine the genetic similarity or diversity of *Rhizobia* nodulating the different accessions of the crop, using PCR amplification profiles of *Rhizobia* DNA.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and Distribution of Kersting's Groundnut

Kersting's groundnut [*Macrotyloma geocarpum* (Harms) Maréchal and Baudet] is a leguminous crop cultivated on small-scale in the western part of Africa (Pasquet *et al.*, 2001). The centre of origin of Kersting's groundnut is precisely unknown, but it is believed to have originated from West Africa especially, in northern Togo or central Benin (Dako and Vodouhe, 2006). Harms Kersting, a German colonial civil servant, first named it *Kerstingiella geocarpa* Harms from domesticated material collected in Togo during the years 1905 and 1907 (Harms, 1908). Two years later, Chevalier described it as *Voandzeia poiaaoni* A. Chev. from materials collected in Benin. But, a few months later, Chevalier recognized that his plant was identical to the one described by Harms. Kersting's groundnut was found to have co-generic characters with *Macrotyloma* and, therefore, given a new name *Macrotyloma geocarpum* by Marechal and Baudet (Amujoyegbe *et al.*, 2007).

In its native West Africa, *M. geocarpum* is cultivated in Mali, Nigeria, Burkina Faso, Ghana, Togo and Benin with low morphological diversity, manifested only in seed colour (Pasquet *et al.*, 2001). Outside West Africa, it is cultivated in Tanzania, Mauritius and in Fiji (Dako and Vodouhe, 2006).

2.2 Botany

M. geocarpum is an annual herbaceous plant with prostrate rooting system. The leaves are alternate trifolialate. Flowers are in pairs or solitary in the leaf axils and almost sessile. The pods contain one, two or rarely three seeds. Pods containing more than one seed have constrictions in between the seeds (Fig 2.1). Seed germination is epigeal phanerocotyly with the reserve cotyledons abscising in about 2–3 days after germination. Kersting's groundnut has similar geocarpic character just like Bambara groundnuts (*Vigna subterranea*) and groundnuts (*Arachis hypogaea*) as the pods mature on or below the soil surface (Amujoyegbe *et al.*, 2007; Dako and Vodouhe, 2006). The pods are indehiscent and usually divided by 1 or 2 constrictions into 2 or 3 joints. Seeds are oblong to oblong ovoid, about 0.6–1.3 cm long, kidney-shaped with white hilum, white, red, black or mottled in colour. They ripen as the leaves turn yellowish (Adelusi and Akamo, 2006).

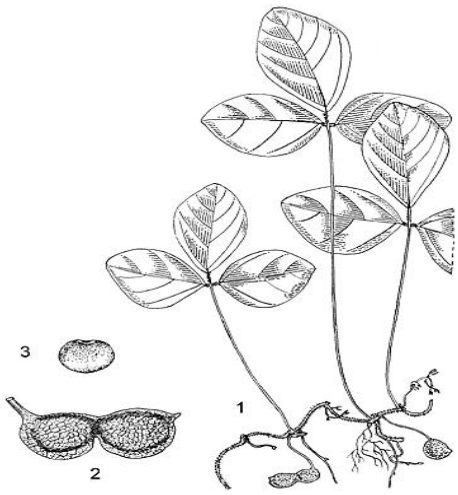

Figure 2.1.Kersting's groundnut plant; 2, fruit and 1, seed

(Source: http://www.prota4u.org/prota)

2.3 Classification

2.3.1 Botanical Classification

Kersting's groundnut belongs to the family Fabaceae, subfamily Faboideae, and tribe Phaseoleae. *Macrotyloma geocarpum* (Harms) Maréchal and Baudet is the botanically valid name for Kersting's groundnuts (Marechal and Bauduet, 1977). *Kerstingiella geocarpa* Harms is a widely used botanical synonym in which the genus name *Kerstingiella* honours Harms Kersting whilst the specific epithet *geocarpa* denotes its below-ground fruit production (Fig. 2.1).

2.3.2. Morphology and Physiology

The genus *Macrotyloma* contains several species of which three are currently used in agriculture. The species *axillare* and *uniflorum* are used as forage plants while *geocarpum* is a food legume for human beings (Blumenthal and Staples, 1993).

The wide clinal variation in Kersting's groundnut is most probably because of the wide inter-annual climatic variation that exists in its geographical range of cultivation. Seed colour is reported as one of the major sources of accessional differences. Duke *et al*., (1977) noted the existence of white, mottled and black seeds among accessions. Although phenotype is determined by the interaction between genotype and environment (G × E), morphological characters which are mainly phenotypical have largely been used for genetic characterization until the development of molecular markers (Mignouna *et al*., 1996; Patterson and Weathercup, 1984). Morphological characters are still in use, especially as basis for selecting different landraces and accessions for breeding purposes. Morphotypes have been identified in Northern

Ghana based on traits such as plant height, canopy spread, leaf area index, nodule count, pod number per plant, and grain yield (Bayorbor *et al*., 2010). Any positive correlation between nodule count, plant height and number of pods per plant would be particularly desirable for selection.

In a study conducted by Pasquet *et al*., (2001) to find the genetic diversity in *M. geocarpum*, it was found that no diversity existed within the accessions in both the domesticated accessions and between the wild accessions that were used for the study. It was therefore suggested that, an inbred breeding system of the chasmogamous flowers might have reduced the diversity of the domesticated crop over a long period of time.

2.4 Kersting's Groundnut Production in Ghana

In Ghana, cultivation of *M. geocarpum* is only limited to the northern part of the country, especially, the Upper West and Northern regions where it is mainly grown by small-scale farmers (Bampuori, 2007; Bayorbor *et al.*, 2010).

Local landraces of *M. geocarpum* are usually grown in small plots at the end of May or the beginning of June in pure stands or intercropped with yam, cowpea, cassava or other crops on mounds, beds or ridges and they mature within 4-5 months after planting as the leaves turn yellowish (Dako and Vodouhe, 2006; Duke *et al.*, 1977). The pods are hand-picked and later beaten with sticks or in a mortar when dry to remove the seeds from the pods. The average seed yield is about 500kg/ha (Duke *et al.*, 1977).

Reliable production statistics for the crops are not available mainly because it is grown for local consumption (Dako and Vodouhe, 2006) and yields are generally low (Obasi, 1993), limiting widespread cultivation. Apart from screening the crop for its nutritional attributes, very limited information is available on the spread, biodiversity, agronomic characters, yield potential, nitrogen-fixation potential, incorporation into the traditional farming system, or diseases and pests incidence (Amujoyegbe *et al.*, 2007).

2.5 Nutritive Value

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, be increased (Borget, 1992). The protein content of legumes is two or three times that of cereals depending on the type of legume (Friedman, 1996). They, therefore, serve as a source of protein to a large proportion of the population in poor countries by being the least expensive and easily stored and transported, non-processed protein source for rural and urban dwellers (Rachie and Silvester, 1977). The seeds of legumes generally contain 20–30% protein and are lysine-rich, complementing the nutritional profiles of cereals and tubers in the diet (Graham and Vance, 2003). Their industrial application depends on the knowledge of their nutritional importance and functional properties (Aremu *et al.*, 2006).

M. geocarpum is a protein-rich food legume that has not been used to any great extent by humans because its nutritional importance has not been fully uncovered (Obasi, 1996). However, within its areas of cultivation, consumers prefer Kersting's

groundnut seeds to cowpea and Bambara groundnut due to their palatability and presumed high protein content (Baudoin and Mergeai, 2001). The crop is rich in nutrients such as proteins (essential amino acids such as lysine and methionine), fats, carbohydrates, ash, fibre and calcium (Adelusi *et al*., 2006). However, a factor which could limit the acceptance of Kersting's groundnut for wider utilization is the presence of anti-nutritional factors such as phytates, tannins and haemagglutinins which reduce the biological value of dietary proteins and hinder mineral absorption from the diet. These anti-nutritional factors, however, are also reported to have health benefits. Tannins, polyphenolic compounds, are reported to possess anti-oxidative activity (Amarowicz and Pegg, 2008) and most of these anti-nutritional factors can be completely eliminated or greatly reduced through processing, particularly by pre soaking the seeds and then boiling in water (Obasi, 1996).

2.6 Climatic and Soil Requirements

One of the major challenges to crop production in present time is crop failure due to climate change that results in poor performance and low adaptation of crops even in their natural environment of origin. Invariably, this will lead to a further reduction in global crop production and supply, increase in food prices, risk of exposure to hunger, malnutrition and food insecurity (IPCC, 2007; UNFCCC, 2007).

Kersting's groundnut, an indigenous West African legume crop, is drought-resistant and well adapted to the Sudano–Guinean savannah agro-ecological zone (Baudoin and Mergeai, 2001).

The crop thrives well in the savannah and rainforest-savannah zones of west tropical Africa. It requires light, sunshine and moderate rainfall and grows well on sandy loam soils rich in calcium. It is able to tolerate some amount of drought in the tropical dry forest zone (Duke *et al.*, 1977). The crop grows successfully in areas with annual rainfall of between 500–600 mm (Echendu *et al.*, 2009), but can survive under low annual rainfall of 75–150 mm. The roots grow well in fine sand or silt where phosphorus and nitrogen are available, but they do not move easily into regions of moist gravel or coarse sand even when fertilizer is applied. The extent of the root system is related to the texture and structure of the soils as well as the available nutrients (Adelusi and Akamo, 2006).

2.7 Economic Value

Some trade in Kersting's groundnut has existed between Ghana and neighbouring countries such as Togo, Benin and Nigeria for a long time. However, no statistics is available because of the low yield and poor storage ability. In general, the economic importance of Kersting's groundnut appears to have decreased considerably in recent times (Dako and Vodouhe, 2006) due to reduced cultivation and utilization of the crop.

2.7.1 Utilization in Ghana and other parts of Africa

Matured and dried seeds of *M. geocarpum* are often consumed at the family or household level. They are consumed in the same way as cowpea (Amujoyegbe *et al.*, 2007). It is used for preparing many dishes such as fried paste (koose), and steamed

paste (tubani). The seeds can also be boiled and eaten with palm or groundnut oil and ‘‘gari’’ or boiled and eaten with yam or rice (Dako and Vodouhe, 2006).

The high protein content has been valued for many years by rural communities in the Sissala area of the Upper West Region, where orphans are fed boiled seeds of this legume in the mornings (Schmidt, 1943). Also in the Upper West Region, cooking water from *M. geocarpum* seeds is used for the treatment of stomach pains, intestinal cramps and as an antidote to food poisoning because it induces emesis when ingested. Concoctions prepared from the leaves act as a febrifuge (Buah *et al.*, 2006; Stanley, 2006).

In Nigeria, seeds of *M. geocarpum* may also be boiled in soups and served to guests as a sign of honour. Roasted seeds of black-seeded types or fresh unshelled pods are sometimes boiled with salt and eaten as snacks. *M. geocarpum* seeds also play an important role in traditional customs in West Africa, particularly in Togo, where they are used in funeral ceremonies of the Kabye and Mauba people. In many areas, consumption is limited to the male members of the family, the headman in particular. Also seeds of *M. geocarpum* are a favourite dish for “voodoo” priests. Leaves of *M. geocarpum* are sometimes eaten as a vegetable or in soups. Just as it is practiced in northern Ghana, the water in which the seeds have been boiled is taken against diarrhoea in central Benin. Powdered dry seeds mixed with water or local beer (pita) is used as an emetic in case of poisoning. Leaf decoctions act as a vermifuge. The Igbo of Nigeria use the plant in the treatment of dysentery, veneral diseases, fever and diabetes (Dako and Voduohe, 2006).

2.8 Nodulation Ability and Nitrogen Fixation Capacity

Biological nitrogen fixation (BNF) is one of the most important processes for the maintenance of life on earth as it is environmentally friendly and contributes about 70% of all nitrogen required by natural and agricultural ecosystems (Fatima, 2008). Nitrogen (N) is a key element required for plant growth and the symptoms of soil N deficiency range from poor yield to crop failures.

Studies indicate that BNF in grain and pasture legumes and in N-fixing trees have the greatest potential for increasing crop yield and improving soil fertility (Danso, 1992). Inoculating with nitrogen-fixing Leguminosae nodulating bacteria strains that are highly efficient and adapted to prevailing environmental conditions is currently practiced in some countries for a few legume crop species (Fatima, 2008).

Bambara groundnut and Kersting's groundnut are both tropical legumes that nodulate with cowpea-type *Bradyrhizobia*. Although Kersting's groundnut nodulate freely with *Bradyrhizobia*, little is known about the amounts of N- fixed by these symbioses and their relative dependence on fixation for N nutrition (Dakora and Muofhe, 1995).

Many nodules are reported to form on the roots of *M. geocarpum* as Adu-Gyamfi *et al.,* (2012) and Bayorbor *et al.,* (2010) both recorded mean nodule count of between 10-27 at 6 and 8 weeks after planting and 10.3-32.5 at 7 and 9 weeks after planting respectively.

Inhibition of nodule development and nitrogenase activity by nitrate ions (NO_3^-) has been reported for many legumes. *M. geocarpum*, however, is a legume capable of nodulating and fixing N_2 optimally in soils containing large concentrations of NO_3^- and also have the ability to overcome NO_3^- ions (Dakora, 1998).

2.9 Plant Pests and Diseases

Diseases and pests are major constraints to legume production, especially in the tropics and subtropics. This, however, is not the case with Kersting's groundnuts whose seeds are buried in the soil where they are protected from attacks by flying insects that severely limit or destroy pulses such as *G. max* whose pods remain in the air (Adelusi *et al.*, 2006). In the semiarid regions, Kersting's groundnut is not subjected to serious attacks from diseases or pests. In more humid regions, fungal diseases (rust and mould) may occur.

However, harvested and stored seeds for human consumption are liable to and easily infested by insect pests, the most destructive being the bean weevil. The bean weavil infests dry pods in the field and continues to attack stored seeds. Proper post-harvest handling of legumes is necessary to prevent both qualitative and quantitative losses. Drying legumes to safe moisture level of 12–14 % ensures good storage (Fasoyiro *et al.*, 2012). In surveys conducted in Nigeria, an estimate of 18 % yield losses in *M. geocarpum* was attributed to *Piezotrachelus varium* (Dako and Vodouhe, 2006; Obasi, 1996).

2.10 Disappearance of Genetic Diversity

The genetic diversity of crops in Africa was naturally preserved by traditional cropping systems. However, in recent times there has been a rapid deterioration of natural resources resulting in loss of genetic diversity due to increased pressure on land as a result of increased human activities such as industrialization and urbanization (Amujoyegbe *et al.*, 2007). Rapid changes in land use, modernization of

agricultural practices, adoption of new varieties possessing a narrow-genetic base and deforestation have also led to the rapid disappearance of many landraces of cultivated crops and their wild relatives (Seme *et al.*, 1992).

Reasons for the disappearance of the genetic diversity of *M. geocarpum* are not different from those of other crops. The disappearance of the crop from its traditional farming system is highly significant as only older or elderly farmers take to the cultivation of this crop as a "legacy" crop. High labour demand, low yield, change in taste resulting from non-acceptance of the crop due to urbanisation, lack of research to improve the existing landraces and, most importantly, replacement by improved varieties of cowpea are all contributing factors to the disappearance of *M. geocarpum* in most farming systems (Amujoyegbe *et al.*, 2007; Bennett-Lartey and Oteng-Yeboah, 2008).

2.11 Breeding/Improvement

Conservation and utilization of underutilized crop species is one major area of difficulty in the utilization of plant genetic resources since they are less known and are of little or no commercial importance; thus, little or no incentives exist for breeders to improve them. Examples of such crops are *M. geocarpum* and fonio (*Digitaria*spp.) (Bennett-Lartey and Oteng-Yeboah, 2008). No breeding programmes of *M. geocarpum* are known to exist (Dako and Vodouhe, 2006).

2.12 Molecular Characterization and Identification of *Rhizobium* Strains

Characterization and identification of *Rhizobia* has been based on its ability to nodulate a range of legume hosts. Consequently, legumes have been grouped on the basis of mutually exchangeable symbionts. This concept, which later became known as the cross inoculation concept (Fred *et al.*, 1932) had limitations. Traditionally, *Rhizobia* have been grouped into two major groups, the fast and slow growers (Fergal and Shanmugam, 1978). Characteristics, which form the minimum criteria for describing new *Rhizobia* isolates, include cultural, morphological and symbiotic traits (Graham *et al.*, 1999). Also, colony morphology has been used to distinguish *Rhizobia* (Zhang *et al.*, 1991). Genotypic characterization by the use of molecular methods has been recommended. Diversity studies of rhizobial natural populations require rapid and reliable techniques to differentiate between isolates and characterize at the strain level (Barnett, 1991).

Assessment of rhizobial genotypic diversity, characterisation and identification for ecological use has been achieved through the analysis of DNA fingerprint patterns generated with the polymerase chain reaction (PCR) using arbitrary or sequence specific primers (Lupski and Weinstock, 1992). Also, PCR techniques used in conjunction with short arbitrary oligonucleotide primers of random sequence have been shown to be an effective means of differentiating complex genomes (Caetano-Anolles *et al.*, 1991; Welsh and McClelland, 1990; Williams *et al.*, 1990). Arbitrary primers have also been used to generate randomly-amplified polymorphic DNA (RAPD) fragments (Williams *et al.*, 1990) which when analysed by gel electrophoresis, can provide a fingerprint profile for any particular target genome. PCR used together with two arbitrary primers (RPO4 and RPO5) of 10 nucleotide

length and a directed (sequence- specific) primer (RPO1) of 20 nucleotide length have been used to differentiate a diverse collection of *Rhizobium meliloti, R.leguminosarum bv. trifolii* and *R. l. bv. vaciae* strains. The amplification profiles can be used to generate a wide range of *Rhizobium* species and these profiles can be used to effectively differentiate *Rhizobium* at the strain level (Richardson *et al.*, 1995).

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CHAPTER THREE

Yield and Nodulation Studies of Kersting's Groundnut in the Coastal Savannah Agro-Ecological Zone of Ghana

3.1 Introduction

Low and declining productivity of many tropical soils are some of the major constraints limiting the realization of the improvement of genetic potential of crops (Tanimu *et al.*, 2007). While production of cereals and other crops might be improved with N fertilizer application, most farmers lack the means to purchase such inputs (Zahir *et al.*, 2004). Diversification of cropping systems and an improved supply of biologically fixed N through *Rhizobia*-legume symbiosis has therefore been proposed and advocated for in the sustenance of traditional agriculture and stabilizing food production (Ampomah *et al.*, 2008; Zahir *et al.*, 2004).

A major benefit derived from using legumes in cropping systems is the fixation of atmospheric nitrogen through the formation of nodules on roots and root hairs (Ennin *et al.*, 2004). Kersting's groundnut is reported to nodulate well with cowpea-type *Bradyrhizobia* (Dakora and Muofhe, 1995). It is, therefore, not surprising that some farmers in the Upper West region of Ghana intercrop *M. geocarpum* with late maturing maize, millet or sorghum to supply the cereals with the fixed nitrogen (Bampuori, 2007).

Although *M. geocarpum* produces palatable and nutritious seeds, it is an under-exploited crop. Currently, yield is low and this is attributed to inappropriate agronomic practices and lack of improved varieties (Adu-Gyamfi *et al.*, 2012). The crop is out of favour in production due to the introduction of improved varieties of

cowpea and is therefore fast becoming endangered in many communities including the main producing areas. Genetic resources are likely to disappear with useful associated information if steps are not taken to intensify research on *M. geocarpum.* Cultivation of this crop is also limited to the Guinea Savannah Zone in Ghana where the rainfall is erratic and sometimes not sufficient for optimal crop growth and production. It is therefore expected that, this study will reveal the full potential yield as well as the nodulating ability of this crop grown in a coastal savannah environment.

3.1.1 Objective of the Study

This study seeks to evaluate *M. geocarpum* for its potential yield and nodulating ability in the Coastal Savannah agro-ecological zone of Ghana.

Materials and Methods

3.2.1 Experimental Site

A field experiment was conducted on the research farm of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission at Kwabenya near Accra. The farm is located at 76 m above sea level on latitude 05 40' and longitude 0 13' W. It lies within the coastal savannah zone with an annual rainfall ranging between 750 mm to 1000 mm (Morris *et al.*, 1999). The predominant soil type found at the site is a well drained Savannah Ochrosol (Ferric Acrisol, locally called Haatso series, sandy loam), derived from quartzite schist (FAO/UNESCO, 1994). Some of the physical and chemical properties of the soil at the experimental area are presented in Table 3.1

Table 3.1 Some soil physical and chemical properties of the experimental area

	Soil profile depth (cm)					
Soil property	0-20	20-40	40-60	60-80	80-100	100-120
pH (H20) (1:1)	7.33	7.39	7.83	7.99	7.79	7.85
Org.C (%)	1.06	0.50	0.50	0.39	0.36	0.23
Total N (%)	0.36	0.34	0.31	1.26	0.42	1.13
AvaiP (cmol/kg)	11.07	6.79	4.28	3.89	2.40	2.10
K (cmol/kg)	0.41	0.30	0.25	0.19	0.21	0.22
Pb (kgm^{-3})	1.34	1.22	1.41	1.33	1.47	1.38
Sand (%)	41.41	40.43	45.31	47.99	46.31	55.82
Silt (%)	43.17	44.68	43.75	41.06	42.95	36.39
Clay (%)	15.42	14.89	10.94	10.95	10.74	7.79
Textural class	SL	SL	SL	SL	SL	SL

Source: Frimpong (2010)

3.2.2 Land Preparation and Planting Material

Prior to planting, the land was cleared of its vegetative cover which comprised mainly tall grasses with scattered shrubs and trees, mainly neem. It was then ploughed and harrowed for better soil aeration and porosity. Seeds of the ten accessions of Kersting's groundnut were obtained from Agronomy Department of the University for Development Studies (UDS) Nyankpala (Tamale) Campus in the Northern Region of Ghana were used for the study. Kersting's groundnut accessions used were, Najung black (T1), Funsi mottle (T2), Nakpanduri white (T3), Boli white (T4), Heng mottled (T5), Nakori mottled (T6), Puffeun black (T7), Sigiri mottled (T8), Dowie mottle (T9) and Gbangu Black (T10).

3.2.3 Experimental Design and Planting

The field experiment was laid out in a randomized complete block design (RCBD) with four replications. The total field size was 42m x 24m with a block size of 24m x 9m and a space of 2m between blocks. Each accession was planted on a subplot of 4m x 4m with inter row and intra row spacing of 0.8m x 0.4m respectively. Two seeds were sown per hill but no thinning out was carried out. The seeds were planted on the 6th of June and harvested on the 3rd of October, 2012. The experiment relied solely on rainfall to maintain the soil moisture and weeds were controlled three times throughout the experiment by manual weeding with a hoe and cutlass. No pesticides or fertilizers were applied.

3.2.4 Nodulation Studies

3.2.4.1 Nodule Number, Fresh and Dry Nodule Weight per Plant

Three plants randomly selected from each plot were carefully dug out for the nodule count and also to determine fresh and dry nodule weight at six weeks after planting (6 WAP) and eight weeks after planting (8 WAP). The process consisted of carefully loosening the soil around the plants, making sure that the roots were not disturbed. The plants were then put into polythene bags and taken to the laboratory. The roots were gently washed under running water to remove all the soil particles on them. The nodules were then removed, counted and then weighed.

3.2.4.2 Effective versus Non-Effective Nodules

The nodules counted were further separated into effective and non-effective nodules. The effectiveness or non-effectiveness of the nodules was determined using the size and the colour of the nodules. Nodules that were 2 mm or below with a creamy whitish colour were considered to be non-effective, while nodules above 2 mm with a bright or dark pink colour due to the presence of high concentration of leghaemoglobin were considered to be effective. After determining the effective and non-effective nodules, they were then combined and the total fresh nodule weight taken on a scale and then kept in white envelops and oven-dried at 65 ℃ for 72 hours. They were again weighed at the end of the drying period to determine the nodule dry weight per plant.

3.2.5 Total Shoot Dry Matter (Biomass)

Total shoot dry matter (TSDM) was determined at six and eight weeks after planting (WAP).

For each sampling period (6 WAP or 8 WAP), three plants were randomly selected from each plot for all the accessions and replications. The roots were removed from the shoots to determine the shoot dry matter at 6 WAP and 8 WAP. The total fresh shoot and sub-sample shoot weight were taken. The shoot samples were then kept in large brown envelops and oven-dried at a constant temperature of 65°C for 72 hours. The dried samples were again weighed to determine the dry weight. The shoot dry weight per hectare was determined as:

$$\text{DMY (kg/ha)} = \text{TFW (kg)} \times \frac{10000\ (\text{m}^2/\text{ha})}{\text{H}(\text{m}^2)} \times \frac{\text{SDW (kg)}}{\text{SFW (kg)}}$$

IAEA, (2001)

Where:

DMY is the dry matter yield

TFW is the total fresh weight

SFW is the subsample fresh weight

SDW is the subsample dry weight

3.2.6 Grain Yield and Yield Components

Matured pods of the accessions were harvested at 18 weeks after planting (8th October, 2012). The average number of pods per plant was obtained by sampling three plants on each plot and counting the number of pods. The fresh pod weight was determined by weighing the total harvested pods. The weighed pods were sun dried in open air for a period of 2 weeks. The dry weight of the pods was again determined and later threshed to obtain the seeds. The threshed seeds were weighed to obtain the total seed weight per plot. Lots of 100 seeds from each plot were replicated 3 times, weighed and the average weight for the 100 seeds calculated. Other parameters calculated included grain yield, harvest index and threshing percentage. The grain yield per hectare for each treatment was determined as:

$$\text{GYF (kg/ha)} = \frac{\text{GYM (g)}}{\text{H (m}^2\text{)}} \times \frac{10000\ (\text{m}^2/\text{ha})}{1000\ (\text{g/kg})}$$

IAEA, (2001)

Where:

GYF is the final grain yield,

GYM is the grain yield adjusted with respect to grain moisture content ranging between 13 -15 %

H is the area from which plant sample was harvested.

The harvest index for each treatment was also determined as:

$$\text{Harvest index} = \frac{\text{Dry matter of economic harvest (yield)}}{\text{Total dry matter (Biomass) at physiological maturity}} \times 100$$

IAEA, (2001).

3.2.7 Nutrient Analysis

3.2.7.1 Determination of Nitrogen Content

Shoots and roots of three plants from each accession were cut at ground level at 6 WAP. They were placed in brown envelops and dried in an electric oven at 65°C for 48hrs. They were then removed and milled with a hand blender to a fine powder and packaged separately as shoots and roots.

The Kjeldahl Digestion Method was employed in the determination of shoot and root N. A quantity of 0.01 g of the milled shoots and roots were used in the analysis. Hydrogen peroxide was used as an oxidation agent in the digestion of the milled materials with concentrated sulphuric acid, and selenium as a catalyst was added to accelerate the process. An aliquot was pipetted and distilled for percent total N by steam distillation of the ammonium liberated by adding 30 % sodium hydroxide (NaOH). The titration was carried out on the distillate using boric acid solution as an indicator with a known concentration of sulphuric acid.

3.2.7.2 Determination of Total Nitrogen Yield and Protein Quality

The total plant N yield was calculated as:

$$\text{TPN (kg/ha)} = \text{DMY(kg/ha)} \times \frac{\%\ \text{N of samples}}{100}$$

IAEA, (2001).

Where;

TPN is the Total plant N yield

DMY is the Dry matter yield in kg/ha

%N is percentage nitrogen

The crude protein for both the shoots and roots were also determined as:

$$\text{Crude protein} = \text{Total plant N yield} \times 6.25$$

IAEA, (2001).

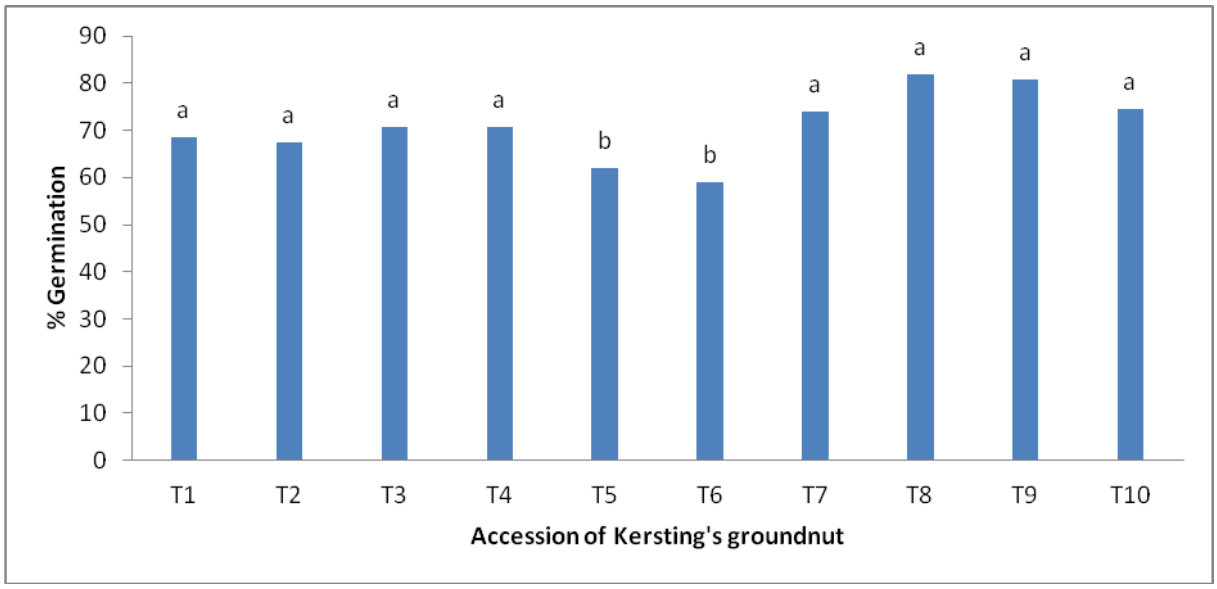
3.2.8 Statistical Analysis

Data collected on yield and nodulation parameters were subjected to analysis of variance (ANOVA) based on the randomized complete block design (RCBD). Significantly different ($p \leq 0.05$) means were separated using the Duncan multiple range test (DMRT). The GENSTATS statistical package version 12 edition was employed in the analysis of the data. The correlation analysis, using the Microsoft EXCEL was also employed to determine the relationship between the yield parameters and also between the nodule parameters.

RESULTS

3.3.1 Germination Percentage

In general, accessions T1, T2, T3, T4, T7, T8, T9 and T10 were statistically similar in terms of germination percentage (GP) with values for GP ranging between 67.4 % and 81.8 %. These GP values were significantly different ($p \leq 0.05$) from values obtained by T5 and T6 both of which recorded GP values of 62 % and 59.1 % ,respectively. Accession T8 recorded the highest GP of 81.8 %, followed by accession T9 with a GP of 80.7 %. However, the accession T6 had the least germination percentage of 59.1 % (Figure 3.1).



.Figure 3.1 Germination Percentage of Ten Accessions of Kersting's Groundnut

Bars with identical letters are not significantly different at 5% significance level by Duncan's Multiple Range Test

3.3.2 Mean Fresh and Dry Shoot Weights

Mean fresh and dry shoot weights of all accessions are presented in Table 3.2.

Accessions T1, T2, T4, T5, T6, T8, T9 and T10 recorded statistically similar mean fresh shoot weight values ranging from 97.7 g to 121.3 g that were significantly lower ($p \leq 0.05$) than those of T3 and T7 at 6 WAP. Accession T7 had the highest mean fresh shoot weight of 178.8 g, followed by T3 with 135.0 g fresh shoot weight. There were no significant differences ($p \geq 0.05$) in the fresh shoot weights of the accessions at 8 WAP, though the highest and lowest values of 338.0 and 205.0g were recorded for the fresh shoot weights of accessions T7 and T5, respectively, at 8 WAP (Table 3.2).

At 6 WAP, the Kersting's groundnut accession T7 recorded the highest mean dry shoot weight of 31.2 g which was significantly higher ($p \leq 0.05$) than values obtained for all the remaining accessions. On the other hand, accessions T1 and T3 produced statistically similar values of 22.9 g and 23.8 g respectively of dry shoot weights which were significantly different ($p \leq 0.05$) from those of T2, T4, T5, T6, T7, T8, T9 and T10. However, no significant differences ($p \geq 0.05$) were observed among the accessions in their dry shoot weights at 8 WAP, though accessions T7 and T5 recorded 60.8g and 37.7g respectively (Table 3.2).

Table 3.2 Mean Fresh Shoot Weight (FSW) and Dry Shoot Weight (DSW) per plant of Ten Accessions of Kersting's groundnut at 6 WAP and 8 WAP.

Mean Fresh Shoot Weight (g)			Mean Dry Shoot Weight (g)	
Acessions	6 WAP	8 WAP	6 WAP	8 WAP
T1	113.3c	238.0	22.9b	43.7
T2	104.1c	318.0	19.2d	52.4
T3	135.0b	245.0	23.8b	43.7
T4	121.3c	279.0	20.4c	51.6
T5	102.7c	205.0	20.1cd	37.7
T6	102.2c	269.0	18.7d	56.0
T7	178.8a	338.0	31.2a	60.6
T8	108.9c	276.0	19.3cd	50.6
T9	114.8c	252.0	19.9cd	45.0
T10	97.7c	246.0	18.5d	45.3
%CV	20.2	26.2	13.2	24.5

Note: Means with identical letters in the same column are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test.

3.3.3 Mean Fresh and Dry Root Weights

Mean fresh and dry root weights are presented in Table 3.3.

Results of the fresh root weight at 6 WAP showed some significant differences ($p \leq 0.05$) as well as similarities among the accessions. Accession T7 recorded the highest fresh root weight value of 4.88 g which was significantly different ($p \leq 0.05$) from all the remaining accessions. Also, accessions T1, T2, T5 and T6 had statistically similar fresh root weight values, ranging between 3.18 g and 3.31 g which were significantly lower ($p \leq 0.05$) than values obtained for T4, T8, T9 and T10. In addition, accession T3 produced the least value of 2.61 g for fresh root weight which was also significantly different from the rest of the accessions (Table 3.3). However, the fresh root weights recorded for the ten accessions were not significantly different ($p \geq 0.05$) from each other at 8 WAP, though the accession T8 had the highest fresh root weight of 5.51g whilst accession T5 had the lowest value of 3.95g fresh root weight (Table 3.3).

No significant differences ($p \geq 0.05$) were observed in the mean dry root weight at both 6 WAP and 8 WAP. However, at 6 WAP, accession T7 had the highest dry root weight of 1.30 g which was closely followed by accession T8 with a value of 1.29 g. Also, accessions T3 and T6 had the lowest values of 0.85 g and 0.93 g respectively for dry root weight. At 8 WAP, accessions T7 and T8 recorded the highest values of 1.60 g and 1.62 g, respectively for dry root weight while T5 and T10 recorded the lowest dry root weight of 1.18 g and 1.20 g respectively (Table 3.3).

Table 3.3 Mean Fresh Root Weight (FRW) and Dry Root Weight (DRW) on Ten Accessions of Kersting's Groundnut at 6 WAP and 8 WAP

	Mean fresh root weight (g)		Mean dry root weight (g)	
Accession	6 WAP	8 WAP	6 WAP	8 WAP
T1	3.18def	4.13	1.02	1.22
T2	3.30de	5.10	1.15	1.47
T3	2.601g	4.00	0.85	1.23
T4	4.04b	4.65	1.25	1.44
T5	3.31d	3.95	1.05	1.18
T6	3.10ef	4.84	0.93	1.47
T7	4.88a	5.14	1.30	1.60
T8	4.36b	5.51	1.29	1.62
T9	3.68c	4.70	1.03	1.40
T10	3.83bc	4.12	1.18	1.20
%CV	19.2	24.8	20.6	24.8

Note: Means with identical letters in the same column are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test.

3.3.4 Shoot Dry Matter Yield

No significant differences ($p \geq 0.05$) existed in the dry matter yield of shoots, though, accession T8 recorded the highest shoot dry matter of 35.09 t ha^{-1} which was closely followed by accessions T6 and T7 with values of 35.01 t ha^{-1} each. Accession T5, however, recorded the lowest shoot dry matter of 23.54 t ha^{-1} as shown in Figure 3.2.

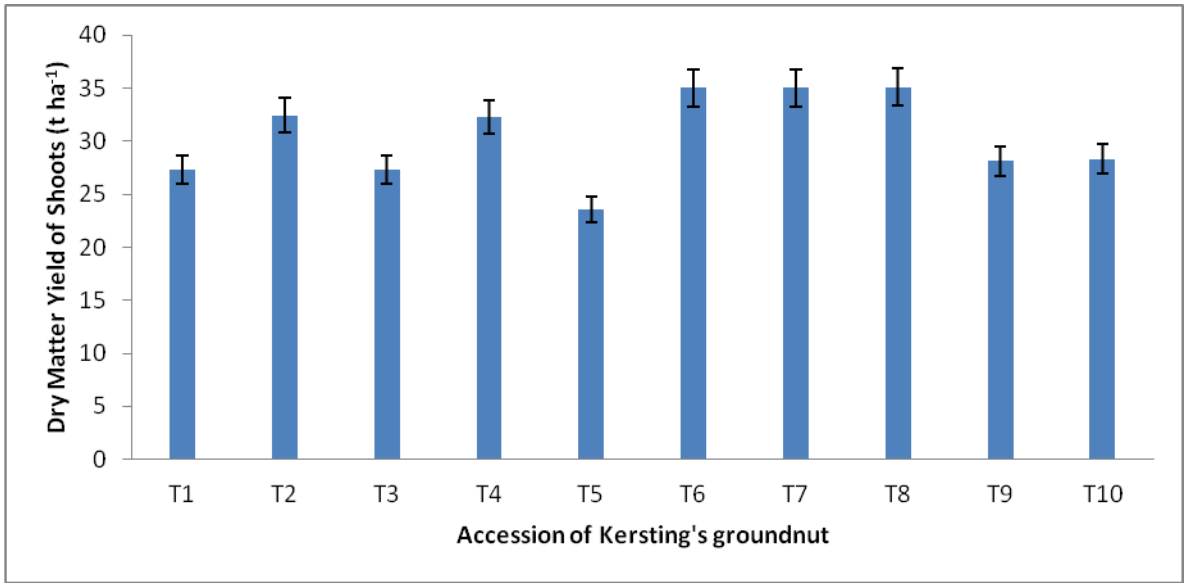


Figure 3.2 Shoot Dry Matter Yield of Ten Accessions of Kersting's groundnut

3.3.5 Mean Number of Pods per Plant

There was no significant difference ($P \geq 0.05$) in the mean number of pods per plant (Table 3.4). However, the highest mean pod number per plant was 118.2 whilst the lowest mean pod number was 85.2 for accessions T3 and T4 respectively. The remaining accessions T1, T2, T5, T6, T7, T8, T9 and T10 had number of pods which ranged between 98.5 and 107.1 (Table 3.4). Similarly, there were no significant

differences ($p \geq 0.05$) among accessions for fresh pod weight per plot, dry pod weight per plot and dry seed weight per plot (Table 3.4).

Table 3.4 Mean Number of Pods, Fresh Pod Weight (FPW), Dry Pod Weight (DPW) and Dry Seed Weight (DSW) of Ten Kersting's Groundnut Accessions.

Accession	Mean number of pod/plot	FPW/plot (kg)	DPW/plot (kg)	DSW/plot (kg)
T1	105.5	3.16	2.44	1.33
T2	98.8	3.14	2.11	1.14
T3	118.2	3.45	2.37	1.21
T4	85.2	2.42	1.74	1.27
T5	107.1	2.74	2.26	1.32
T6	99.5	2.72	2.03	1.26
T7	105.2	2.84	1.93	1.3
T8	105	2.93	2.28	1.34
T9	100.5	3.26	2.3	1.28
T10	98.5	3.1	2.26	1.32
%CV	11.1	23.4	26.7	8.5

Means are not significantly different ($p \leq 0.05$) according to Duncan Multiple Range Test.

3.3.6 Fresh Pod, Dry Pod and Dry Seed Weight per Plant

Results for fresh pod, dry pod and dry seed weight are indicated in Table 3.4.

The mean fresh pod weight was highest in accession T3 with a value of 3.45 kg while T4 had the least value of 2.42 kg for fresh pod weight but with no significant differences ($P \geq 0.05$) existing among the accessions (Table 3.4). For the dry pod weight, accession T1 recorded the highest value of 2.44 g and again, T4 recorded the lowest value of 1.74 kg. The highest dried seed weight of 1.34 kg was recorded by accession T8 while the accession T2 had the lowest dry seed weight of 1.14 kg. Statistically, no significant differences ($P \geq 0.05$) were found among the ten accessions of Kersting's groundnut for both dry pod and dry seed weight (Table 3.4).

3.3.7 100-Seed Weight

Values for the mean 100-seed weight showed some significant differences ($p \leq 0.05$) as well as similarities among the ten Kersting's groundnut accessions. The Kersting's groundnut accession T7 which recorded the highest mean 100-seed weight of 14.46 g which was significantly different from all the others. Accessions T3, T5, T6, T8 and T10 had statistically similar values of 100-seed weight ranging from 13.27 g to 13.89 g but were significantly higher than values for accessions T1, T2, T4, T9 and T10 which were also statistically similar (Figure 3.3).

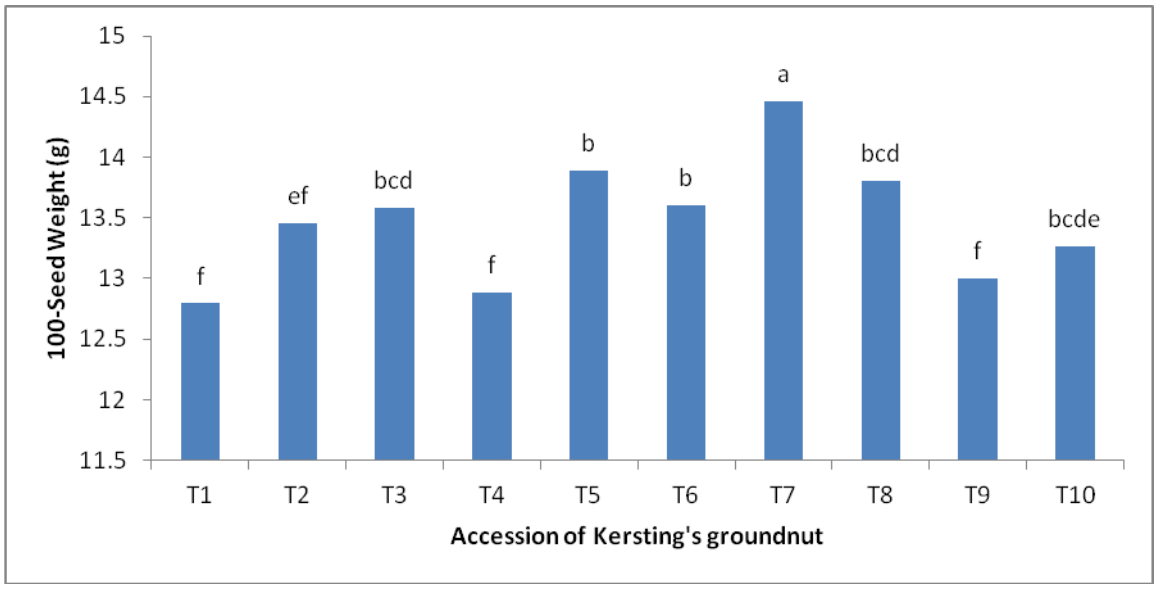


Figure 3.3 100- Seed Weight of Ten Accessions of Kersting's Groundnut.

Note: Bars with identical letters are not significantly different ($p \leq 0.05$) significance according to Duncan's Multiple Range Test.

3.3.8 Grain Yield per Plot

Mean grain yield per plot is presented in figure 3.4.

Values for the grain yield of the ten accessions of Kersting's groundnut were not significantly different ($p \geq 0.05$) from each other (Figure 3.4). The yield was however, highest in accession T8 at of 0.84 t ha^{-1} and was closely followed by accessions T10 and T1 with values of 0.83 t ha^{-1} each. The lowest yield was recorded by accession T2 with a value of 0.71 t ha^{-1}. The remaining accessions, T3, T4, T5, T6, T7 and T9 had yield values that ranged between 0.76 t ha^{-1} and 0.82 t ha^{-1} (Figure 3.4).

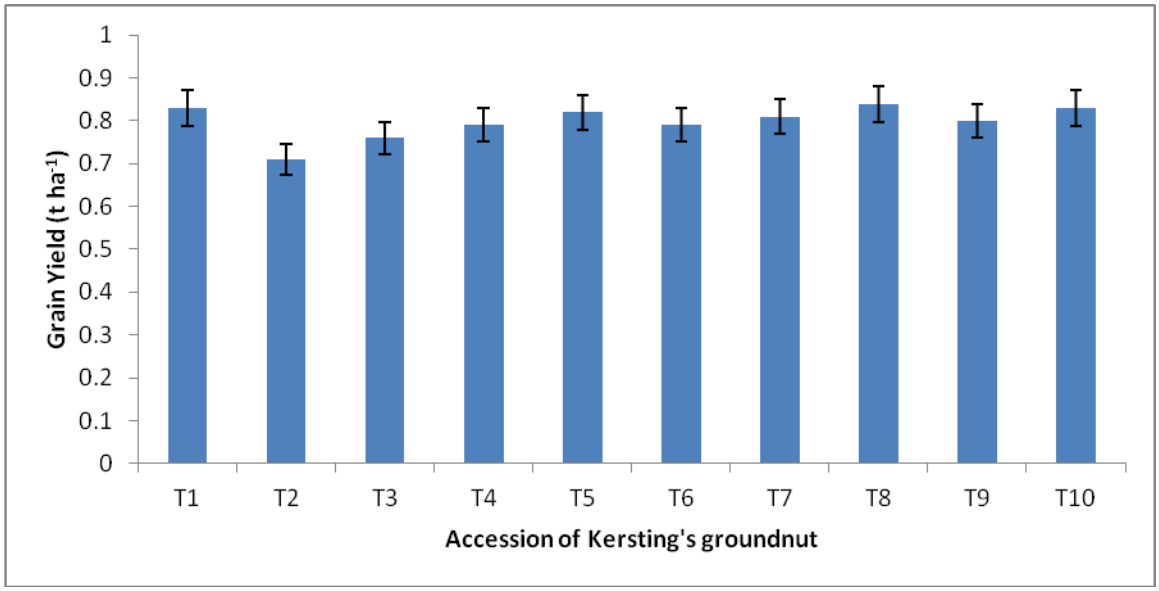


Figure 3.4 Grain Yield of the Ten Accessions of Kersting's Groundnut

3.3.9 Harvest Index

There were no significant differences ($p \geq 0.05$) existed in harvest indices of the accessions. However, accessions T1 and T5 recorded the highest harvest indices of 3.33 % and 3.30% respectively. The lowest harvest index of 2.23 % occurred in accession T4 with the rest of the accessions (T2, T3, T6, T7, T8, T9 and T10) having harvest index that ranged between 2.33 % and 2.98 % (Figure 3.5).

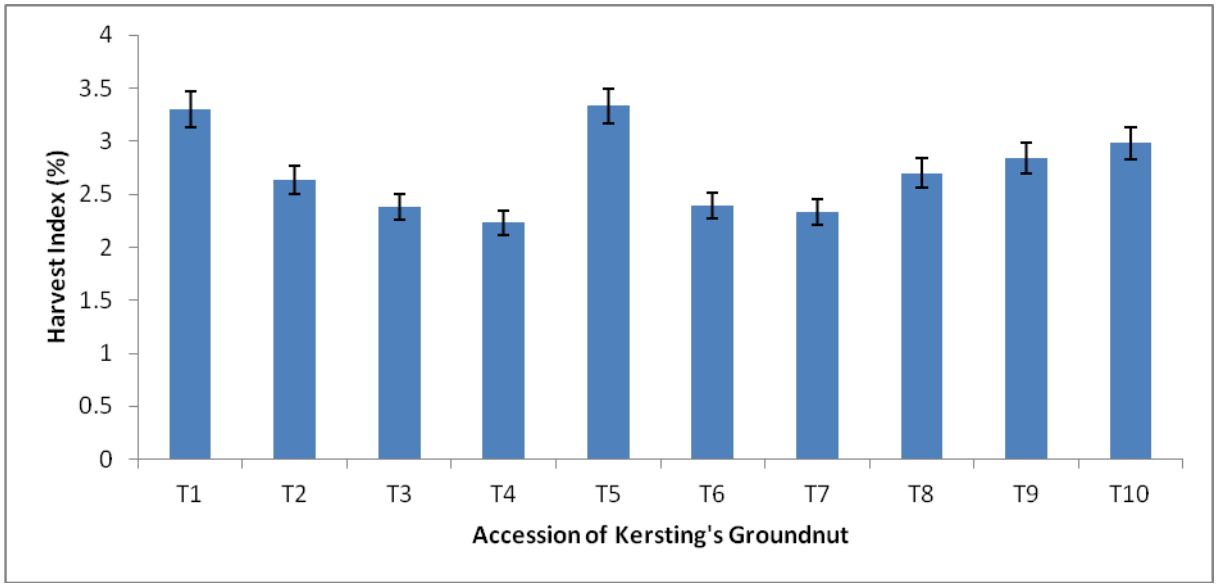


Figure 3.5 Harvest Indices of Ten Accessions of Kersting's Groundnut

3.3.10 Nodulation Studies

3.3.10.1 Mean Number of Nodules per Accession

Mean number of nodules per accession are presented in Figure 3.6.

Results obtained showed no significant differences ($p \geq 0.05$) in the mean nodule number among the ten Kersting's groundnut accessions at 6 WAP though accession T8 produced the highest mean nodule number of 40.2 whilst accession T6 recorded the least mean nodule number of 11.8. Accessions T1, T2, T3, T4, T5, T7, T9, and T10 recorded mean nodule numbers ranging from 15.8 to 32.5 at 6 WAP (Figure 3.6). However, there were significant differences ($p \leq 0.05$) and similarities in the mean number of nodules among the Kersting's groundnut accessions at 8 WAP. Accessions T4 and T7 recorded statistically similar number of nodules of 32.0 and 29.8 respectively that was significantly different ($p \leq 0.05$) from those of the remaining accessions (Figure 3.6). In addition, accessions T1 and T9 scored similar number of nodules that was also closely similar to values for T2, T3 and T8 as well as T5, T6

and T10 ranging from 6 to 20 with accession T5 recording the lowest mean nodule number of 6 (Figure 3.6).

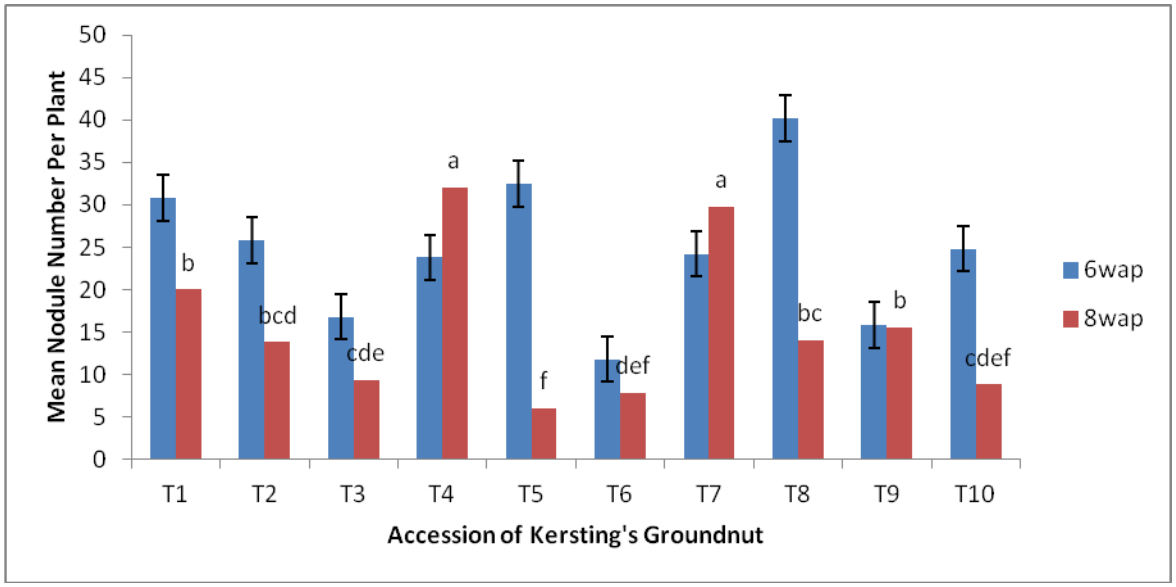


Figure 3.6 Mean Number of Nodule per Accession of Ten Accessions of Kersting's groundnut at 6 WAP and 8 WAP.

Note: Bars with identical letters are not significantly different ($p \leq 0.05$) significance according to Duncan's Multiple Range Test.

3.3.10.2 Mean Number of Effective and Non Effective Nodules

There were no significant differences ($p \geq 0.05$) observed in the mean number of effective nodules among the ten Kersting's groundnut accessions at 6 WAP, though accession T1 recorded the highest value of nine (9) effective nodules whilst accession T6 recorded the least effective nodule number of 3.3. The remaining accessions, T2, T3, T4, T5, T6, T7, T8, T9 and T10 had mean values of effective nodules that ranged from 5 to 7.5 (Table 3.5). Significant differences were however, observed in the mean number of effective nodules among the ten Kersting's groundnut accessions at 8 WAP. Accessions T7 and T4 recorded the highest mean effective nodule numbers of 10.3 and 8.0 respectively that were significantly different from those of the remaining

accessions. The mean number of effective nodules for Kersting's groundnut T1, T3, T6 and T9 clustered within a narrow range of 3.0- 4.8. Accessions T5 and T10 recorded the least values of 2.3 and 2.0 respectively, for effective nodules (Table 3.5).

The mean numbers of non-effective nodules at 6 WAP were statistically not significant ($p \geq 0.05$) among the ten accessions. The highest value of 34 non-effective nodules was recorded by accession T8, followed closely by accession T5 with a value of 32.2. The Kersting's groundnut accession T6 had the lowest number (9.5) of non-effective nodules (Table 3.5) However, significant differences ($p \leq 0.05$) were observed at 8 WAP in the mean number of non-effective nodules for the ten accessions. Accessions T4 and T7 had statistically similar values of 23.8 and 20.8 respectively, for non-effective nodules that were significantly different ($p \leq 0.05$) from values for T1, T2, and T9 (14.25, 14.5 and 14.25, respectively) which were also significantly different ($p \leq 0.05$) from the least values of 5.75, 4.5, 4.5, 7.5 and 6.5 for non-effective nodules for accessions T3, T5, T6 and T10 respectively (Table 3.5).

Table 3.5 Mean Number of Effective Nodules (EN) and Non-Effective Nodules (NEN) of Ten Accessions of Kersting's Groundnut at 6 WAP and 8 WAP

Mean Number of Effective Nodules			Mean Number of Non-Effective Nodules	
Accession	6 WAP	8 WAP	6 WAP	8 WAP
T1	9.00	4.75cd	25.20	14.25b
T2	6.50	5.50b	25.80	14.50b
T3	6.00	4.00cde	19.00	5.75c
T4	5.50	8.00a	18.20	23.75a
T5	7.75	2.25de	32.20	4.50c
T6	3.25	3.00cde	9.50	4.50c
T7	5.00	10.25a	25.50	20.75a
T8	6.25	5.25bc	34.00	7.50c
T9	5.00	4.00cde	18.80	14.25b
T10	7.00	2.00e	17.80	6.50c
%CV	43.6	38.0	39.8	19.7

Means with identical letters in the same column are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test.

3.3.10.3 Mean Fresh and Dry Nodule Weight per Plot

Statistical differences ($p \leq 0.05$) were observed in the mean fresh nodule weights of the ten Kersting's groundnut accessions at 6 WAP. Though accessions T1, T5 and T8 produced statistically similar values of 0.323 g, 0.221 g and 0.28 g respectively, they had significantly higher mean fresh nodule weights per plot than the rest of the accessions at 6 WAP. Also, accessions T2, T3, T4, T7 and T10 had statistically similar mean fresh nodule weight ranging from 0.14 g to 0.164 g and were closely followed by accessions T6 and T9 with the least values of 0.056 g and 0.099 g, respectively, for mean fresh nodule weight (Table 3.6). A similar trend was observed for the mean fresh nodule weight per plot at 8 WAP. Thus, accessions T4 and T7 scored the highest mean nodule weights of 0.188 g and 0.204 g, respectively, which were significantly different from values for the remaining accessions. However, accessions T2, T8 and T9 recorded statistically similar mean fresh nodule weight of 0.10 g, 0.087 g and 0.074 g, respectively, whilst T3, T5, T6 and T10 had the least values of mean fresh nodule weight ranging from 0.031 g to 0.053 g (Table 3.6).

At 6 WAP statistically significant differences ($p \leq 0.05$) in the mean dry nodule weight, observed among the accessions. The highest and lowest values of 0.073 g and 0.018 g mean dry nodule weights were observed for accessions T1 and T6, respectively, which were both significantly different ($p \leq 0.05$) from values for the other accessions at 6 WAP. Also, accessions T4, T5, T7, T8 and T10 had statistically similar values of mean dry nodule weight, ranging from 0.040 g to 0.064 g, followed by accessions T2, T3 and T9 which had mean dry nodule weight of 0.029 g, 0.035 g and 0.024 g, respectively at 6 WAP (Table 3.6).

Table 3.6 Mean Fresh Nodule Weight (FNW) and Dry Nodule Weight (DNW) of Ten Accessions of Kersting's Groundnut at 6 WAP and 8 WAP

Mean Fresh Nodule Weight per Plot (g)			Mean Dry Nodule Weight per Plot (g)	
Accession	6 WAP	8 WAP	6 WAP	8 WAP
T1	0.323a	0.118b	0.073a	0.012f
T2	0.153bcd	0.100c	0.029d	0.022d
T3	0.149bcd	0.050def	0.035cd	0.016def
T4	0.145bcd	0.188a	0.040bcd	0.033b
T5	0.221ab	0.036ef	0.048bc	0.014efg
T6	0.056d	0.031f	0.018g	0.010g
T7	0.164bc	0.204a	0.044bcd	0.046a
T8	0.280a	0.087cd	0.064b	0.021de
T9	0.099cd	0.074cde	0.024d	0.024c
T10	0.140cd	0.053def	0.040bcd	0.014efg
%CV	47.0	35.5	49.5	26.3

Means with identical letters in the same column are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test.

At 8 WAP, accession T7 recorded the highest mean dry nodule weight of 0.046 g, followed by T4 with 0.033 g and T9 with 0.024 g which were all significantly different ($p \leq 0.05$) from mean dry nodule weight of the remaining accessions. However, accessions T2, T3 and T8 recorded statistically similar values of 0.022 g, 0.016 g and 0.021 g respectively, of mean dry nodule weight followed by accessions T1, T5, T6 and T10 that also had similar values of mean dry nodule weight that ranged between 0.010 g and 0.014 g and being the least values of mean dry nodule weight recorded at 8 WAP (Table 3.6).

3.3.10.4 Percent Root and Shoot Nitrogen Yield

The percent nitrogen yield for both roots and shoots of the ten Kersting's groundnut accessions showed significant differences among them. Accessions T5 and T9 had the highest root N of 1.43 and 1.37 % respectively, and were significantly different ($p \leq 0.05$) from the root N of the remaining accessions. The accessions T1, T2, T3, T4, T6, T7 and T10, recorded similar values of root N which ranged between 1.16 % and 1.33 % N. In addition, accession T8 produced the lowest % N of the root at1.13 % (Figure 3.7). For % shoot N, accession T3 recorded the highest value of 3.05 % and was followed closely by accessions T7 and T9 that had statistically similar shoot N of 3.01% and 2.9 %, respectively. In addition, accessions T1, T2, T4, T6 and T10 had shoot N that ranged between 2.53 % and 2.79 % whilst accessions T5 and T8 recorded the least values of 2.41 % and 2.28 % respectively (Figure 3.7).

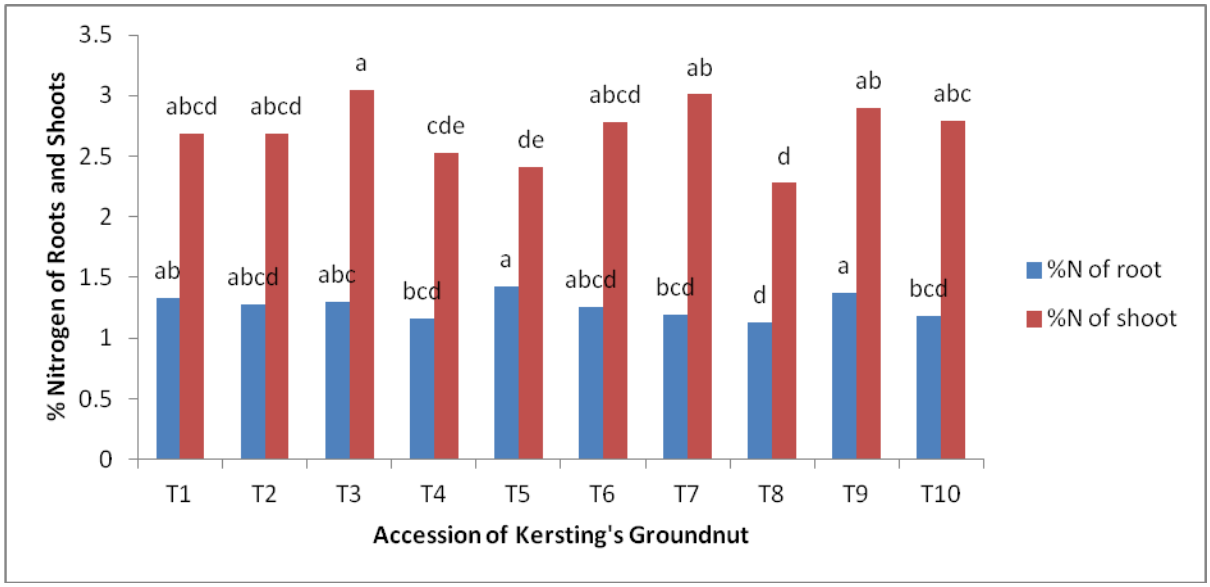


Figure 3.7 Percent Root and Shoot Nitrogen of ten accessions of Kersting's groundnut

Bars with identical letters are not significantly different ($p \leq 0.05$) significance according to Duncan's Multiple Range Test.

3.3.10.5 Contribution of Root and Shoot N to Total % Nitrogen of Plants

There were no significant differences ($p \geq 0.05$) in the N contribution of roots and shoots of the Kersting's groundnut accessions. However, accession T5 scored the highest N contribution of 37.2% whilst accession T7 had the lowest N contribution of 28.3 % in terms of N contribution of the roots. The remaining accessions T1, T2, T3, T4, T6, T8, T9 and T10 scored between 29.7% and 33.1 % for N contribution of the roots (Table 3.7).

Table 3.7 Estimates of N percentage of total plant %N of ten accessions of Kersting's groundnut

Accession	% N contribution of roots	%N contribution of shoots	Total %
T1	33.1	66.9	100
T2	32.2	67.8	100
T3	39.9	70.1	100
T4	31.4	68.6	100
T5	37.2	62.8	100
T6	31.2	68.8	100
T7	28.3	71.7	100
T8	33.1	66.9	100
T9	32.1	67.9	100
T10	29.7	70.3	100
%CV	17.1	8.4	

The % N contribution of the shoots to the whole plant was highest in accession T7 with a value of 71.7% whilst accession T5 scored the lowest value of 62.8 %. The remaining accessions T1, T2, T3, T4, T6, T8, T9 and T10 recorded values that ranged from 66.9 % to 70.3 % of the total N contribution of the shoots (Table 3.7).

3.3.11 Nitrogen Yield Of Roots and Shoots and Total Plant Nitrogen

There were no significant differences ($p \geq 0.05$) in the N yield of the roots of the accessions, though accession T7 recorded the highest root N yield of 12.3 kg ha^{-1} while accession T10 recorded the lowest N yield of 8.7 kg ha^{-1}. The remaining accessions T1, T2, T3, T4, T5, T6, T8 and T9 had root N yield values ranging between 10.0 kg ha^{-1} and 11.8 kg ha^{-1} (Table 3.8).

However, N yield for the shoots showed significant differences ($p \leq 0.05$) among the accessions. The Kersting's groundnut accession T7 again had the highest shoot N yield of 1,178.0 kg ha^{-1} which was significantly different from shoot N yields of the remaining accessions.

Accessions T5 and T8 had statistically similar values of 593.0 kg ha^{-1} and 622.0 kg ha^{-1} shoot N yield respectively, being the least shoot N values that were also significantly different from those of the rest of the accessions (Table 3.8).

In terms of total plant N yield, again significant differences ($p \leq 0.05$) were observed among the accessions. The trend in total plant N is similar to that observed in shoot N for the Kersting's groundnut accessions with accession T7 having the highest total plant N yield of 1,190.0 kg ha^{-1} which was significantly different from total plant N yield of the other accessions.

The lowest two values of 663.0 kg ha^{-1} and 628.0 kg ha^{-1} total plant N yield were recorded by accessions T5 and T8, respectively (Table 3.8).

Table 3.8 Nitrogen (N) yield of root and shoot and total plant nitrogen (kg ha^{-1}) of ten accessions of Kersting's groundnut

Accession	N yield of root (kg/ha)	N yield of shoot (kg/ha)	Total plant N yield (kg/ha)
T1	10.02	733efg	743ef
T2	11.65	845cde	857cde
T3	11.13	826cdef	837cdef
T4	10.21	856cd	868cd
T5	10.11	593h	663g
T6	11.45	972b	984b
T7	12.29	1178a	1190a
T8	11.65	622h	628g
T9	11.81	724efgh	735efg
T10	8.71	919bc	928bc
%CV	27.2	11.9	11.1

Means with identical letters in the same column are not significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test.

3.3.12 Protein Quality of Shoots and Roots

The protein quality of the roots showed statistically significant differences ($p \leq 0.05$) among the accessions. Notably, accessions T2, T5 and T9 recorded similar values of 8.79 %, 8.97 % and 8.53 % that were the highest recorded values and also significantly different from the protein quality of the remaining accessions. These were closely followed by accessions T1, T3 and T6 with similar values of 8.32 %, 7.59 % and 7.88 % protein quality of roots. The least values of 7.27 %, 7.49 %, 7.05 % and 7.34 % of protein quality were observed by accessions T4, T7, T8 and T10 respectively. These values were also statistically similar but significantly lower than values for the other accessions (Figure 3.8).

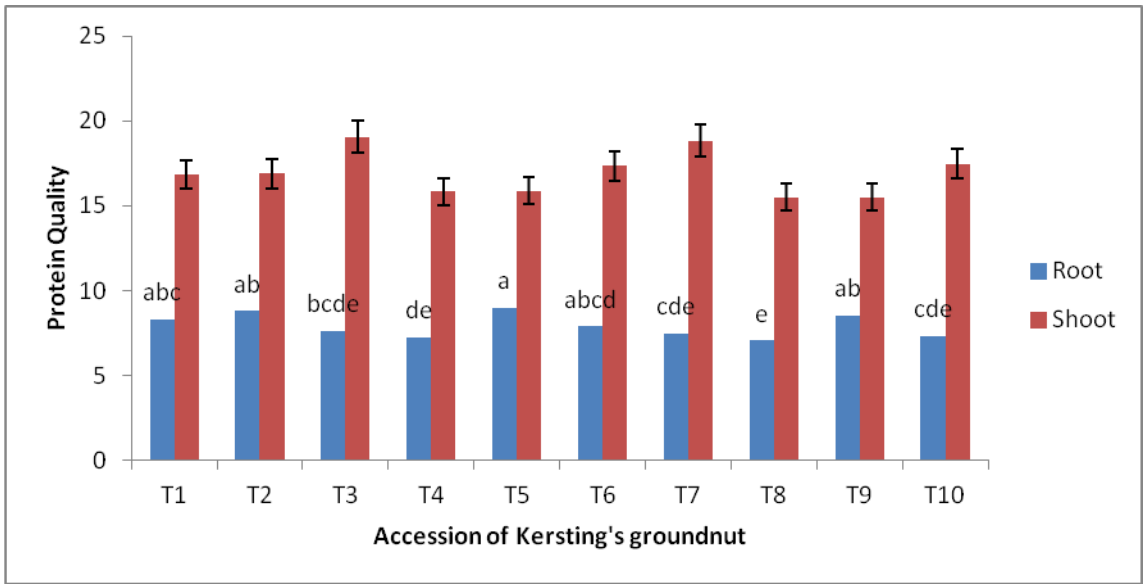


Figure 3.8 Protein quality of roots and shoots of ten accessions of Kersting's groundnut.

Bars with identical letters are not significantly different ($p \leq 0.05$) significance according to Duncan's Multiple Range Test.

However, no significant differences ($p \geq 0.05$) were found in the protein quality of the shoots among the ten accessions though accession T3 recorded the highest value of 19.05 % while accessions T8 and T9 recorded the least protein quality of 15.51 % each. The remaining accessions T1, T2, T4, T5, T6, T7 and T10 had protein quality that ranged between 15.84 % and 18.84 % (Figure 3.8).

3.3.13. Correlation Analysis of Growth and Yield Parameters of Kersting's Groundnut Accessions.

The fresh shoot weight at 6 WAP positively correlated with four yield parameters namely, fresh shoot weight at 8 WAP, dry shoot weight at 6 WAP, dry shoot weight at 8 WAP and 100-seed weight (Table 3.9). However, the fresh shoot weight at 6 WAP demonstrated zero and negative correlations with the dry seed weight and grain yield, respectively (Table 3.9). The fresh shoot at 8 WAP was also positively correlated with six yield parameters which included, dry shoot weight at 8 WAP, fresh root weight at 6 and 8 WAP, dry root weight at 6 and 8 WAP and dry matter yield (Table 3.9). The fresh shoot weight at 8 WAP on the other hand correlated negatively with dry seed weight and grain yield (Table 3.9). The dry shoot weight at 6 WAP correlated positively with 100-seed weight with $R^2 = 0.53$ (Table 3.9). Also, dry shoot weight at 8 WAP correlated positively with fresh root weight at 6 WAP, fresh root weight at 8 WAP, dry root weight at 8 WAP and dry matter yield with R^2 values of 0.54, 0.74, 0.84 and 0.94 respectively (Table 3.9). However, the dry shoot weight at 8 WAP, negatively correlated with mean number of pods per plant, fresh pod weight, dry pod weight, dry seed weight, harvest index and grain yield (Table 3.9). Fresh root weight at 8 WAP, dry root weight at 6 and 8 WAP and dry matter yield all

correlated positively to fresh root weight at 6 WAP with R^2 being 0.61, 0.91, 0.65 and 0.53 respectively. The fresh root weight at 8 WAP correlated positively with dry root weight at 6 WAP (R^2= 0.59), dry root weight at 8 WAP (R^2= 0.97) and dry matter yield (R^2= 0.88). However, the dry root weight at 6 WAP only correlated positively with dry root at 8 WAP (R^2= 0.58), whilst the dry root weight at 8 WAP also correlated positively with dry matter yield with R^2= 0.91 (Table 3.9).

The mean number of pods per plant was positively correlated with fresh pod weight (R^2= 0. 66) and dry pod weight at (R^2= 0.70). However, the mean number of pods per plant negatively correlated with the dry matter yield while it poorly correlated with the rest of the parameters. The fresh pod weight only correlated positively with the dry pod weight at (R^2= 0. 78) but was negatively correlated with dry seed weight, 100-seed weight, dry matter yield and the grain yield (Table 3.9). Also, the dry pod weight correlated positively with the harvest index with R^2 being 0.72. It was, however, negatively correlated with 100-seed weight and dry matter yield but poorly correlated with dry seed weight and grain yield (Table 3.9). The dry seed weight perfectly and positively correlated (R^2= 1.00) with grain yield, whilst it negatively correlated with dry matter yield and poorly correlated with 100-seed weight and harvest index (Table 3.9). The 100-seed weight correlated positively but weakly with dry matter yield (R^2= 0.32). Dry matter yield was negatively correlated with harvest index and grain yield whilst the harvest index also correlated poorly with the grain yield with R^2= 0.46 (Table 3.9).

Table 3.9 Correlation analysis among 15 Yield Parameters of Ten Accessions of Kersting's Groundnut

	FSW 6 WAP (g)	*FSW 8 WAP (g)*	*DSW 6 WAP (g)*	*DSW 8 WAP (g)*	*FRW 6 WAP (g)*	*FRW 8 WAP (g)*	*DRW 6 WAP (g)*	*DRW 8 WAP (g)*	*MNPPP*	*FPW (kg)*	*DPW (kg)*	*DSeW (kg)*	*HSW (g)*	*DMY(t/ha)*	*HI %*	*GY (t/ha)*
FSW 6 WAP (g)																
FSW8WA(g)	***0.56***															
DSW6 WAP(g)	***0.96***	0.45														
DSW8 WAP(g)	***0.50***	***0.91***	0.39													
FRW6 WAP(g)	0.48	***0.56***	0.39	***0.54***												
FRW8 WAP(g)	0.22	***0.78***	0.08	***0.77***	***0.61***											
DRW6 WAP(g)	0.25	***0.56***	0.18	0.46	***0.91***	***0.59***										
DRW8 WAP(g)	0.38	***0.81***	0.23	***0.84***	***0.65***	***0.97***	***0.58***									
MNPPP	0.25	-0.27	0.36	-0.34	-0.37	-0.29	-0.50	-0.28								
FPW (kg)	-0.01	-0.16	0.05	-0.36	-0.44	-0.25	-0.47	-0.34	***0.66***							
DPW (kg)	-0.33	-0.61	-0.18	-0.70	-0.48	-0.42	-0.50	-0.54	***0.70***	***0.78***						
DSeW (kg)	0.00	-0.39	0.09	-0.22	0.44	-0.12	0.29	-0.10	0.01	-0.27	0.20					
HSW(g)	***0.53***	0.39	***0.53***	0.40	0.38	0.34	0.25	0.40	0.43	-0.13	-0.18	0.03				
DMY(t/ha)	0.30	***0.82***	0.16	***0.94***	***0.53***	***0.88***	0.49	***0.91***	-0.35	-0.37	-0.60	-0.14	0.32			
HI%	-0.50	-0.69	-0.29	-0.75	-0.25	-0.51	-0.17	-0.64	0.24	0.26	***0.72***	0.47	0.24	-0.71		
GY (t/ha)	-0.01	-0.40	0.07	-0.21	0.42	-0.12	0.27	-0.10	0.03	-0.23	0.23	**1.00**	0.02	-0.13	0.46	

FSW = fresh shoot weight, DSW =dry shoot weight, FRW =fresh root weight, DRW = dry root weight, MNPPP =mean number of pods per plant, FPW = fresh pod weight, DPW =dry pod weight, DSeW =dry seed weight, HSW =100-seed weight, DMY=dry matter yield, HI=harvest index, GY=grain yield, 6 WAP= Six weeks after planting and 8 WAP=Eight weeks after planting.

3.3.14. Correlation Analysis of Nodulation and N-Yield Parameters of Kersting's Groundnut Accessions.

The mean nodule number correlated well and positively with fresh nodule weight and dry nodule at 6 WAP with R^2 values of 0.86 and 0.82 respectively. However, with the exception of mean nodule number and fresh nodule weight at 8 WAP which it correlated positively but poorly with, the mean number of nodules at 6 WAP correlated negatively with the rest of the parameters. Similarly, at 8 WAP mean nodule number correlated highly and positively with fresh nodule weight with R^2=0.97 and dry nodule weight with R^2= 0.77. At 8 WAP, however, the mean nodule number negatively correlated with root N, protein quality of roots and protein quality of shoots and positively but poor correlated with N yield of roots, N yield of shoots and total plant N with R^2= 0.19, 0.41 and 0.39 respectively (Table 3.10).

The fresh nodule weight at 6 WAP only correlated well and positively with dry nodule weight at 6 WAP with R^2 being 0.97. Again, at 8 WAP the fresh nodule weight positively correlated with only dry nodule weight at 8 WAP R^2 of 0.81 and positively but poorly correlated with N yield of roots, N yield of shoots and total plant N yield with R^2 of 0.22 , 0.43 and 0.41 respectively. The dry nodule weight correlated negatively with all the other parameters at 6 WAP. The dry nodule weight however, correlated positively with N yield of roots, N yield of shoots and the total plant N yield with R^2 values of 0.54, 0.60 and 0.60 respectively, at 8 WAP. Again, at 8 WAP the dry nodule weight negatively correlated with root N and protein quality of the roots, whilst it correlated positively but weakly with shoot N and protein quality of shoots with R^2 of 0.32 for both parameters (Table 3.10). The root N correlated positively with the protein quality of the roots with R^2 of 0.88. There was also a

negative correlation between the root N and the protein quality of shoots, N yield of roots, N yield of shoots and the total plant N yield. the shoot N correlated well and positively with the protein quality of the shoots, N yield of the shoots and total plant N yield with R^2 of 0.77, 0.66 and 0.65 respectively. However, shoot N correlated negatively with the protein quality of the roots and weakly correlated with N yield of roots with R^2 0.22.

The protein quality of roots correlated negatively with protein quality of shoots, N yield of shoots and total plant N yield. The protein quality of the shoots positively correlated with N yield of shoots and total plant N yield with R^2 of 0.71 each. However, there was a poor correlation between N yield of roots and N yield of shoots and the total plant N yield with R^2 of 0.22 and 0.20 respectively (Table 3.10).

Table 3.10 Correlation Analysis of Nodule Parameters among Ten Accessions of Kersting's Groundnut

	MNN (6 WAP)	*MNN (8 WAP)*	*FNW (6 WAP)*	*FNW (8 WAP)*	*DNW (6 WAP)*	*DNW (8 WAP)*	*%N of roots*	*%N of shoots*	*Protein quality of roots*	*Protein quality of shoots*	*N yield of roots*	*N yield of shoots*	*Total plant N yield*
MNN (6 WAP)													
MNN (8 WAP)	0.06												
FNW (6 WAP)	**0.86**	0.10											
FNW (8 WAP)	0.19	**0.97**	0.20										
DNW (6 WAP)	**0.82**	0.12	**0.97**	0.22									
DNW (8 WAP)	-0.05	**0.77**	-0.12	**0.81**	-0.11								
%N of roots	-0.17	-0.44	0.01	-0.46	-0.09	-0.43							
%N of shoots	-0.76	0.07	-0.53	0.03	-0.48	0.32	0.13						
Protein quality of roots	-0.04	-0.34	0.01	-0.31	-0.15	-0.35	**0.88**	-0.04					
Protein quality of shoots	-0.42	-0.03	-0.25	0.04	-0.12	0.32	-0.12	**0.77**	-0.25				
N yield of roots	-0.20	0.19	-0.21	0.22	-0.36	**0.54**	-0.03	0.22	0.07	0.10			
N yield of shoots	-0.52	0.41	-0.52	0.43	-0.41	**0.60**	-0.43	**0.66**	-0.37	**0.71**	0.22		
Total plant N yield	-0.51	0.39	-0.53	0.41	-0.41	**0.60**	-0.38	**0.65**	-0.32	**0.71**	0.20	**0.99**	

MNN =Mean number of nodules, FNW =Fresh nodule weight, DNW = Dry nodule weight, 6 WAP= Six weeks after planting and 8 WAP= Eight weeks afterplant

DISCUSSION

3.4.1 Germination Percentage of *M. geocarpum*

Germination, which was observed from the third day, was completed by the seventh day. Lack of germination in some accessions could be attributed to insect damage after sowing since seeds of *M. geocarpum* are highly susceptible to insect infestation (Badii *et al.*, 2011).

The percent germination was highest in accessions T8 and T9 but lowest for T5 and T6. Significant differences observed among accessions may be due to differences in adaptation to environmental conditions in the coastal savannah agro-ecological zone. This is supported by Salifu *et al.*, (2007) who noted that the rate of seed emergence depends on seed characteristics (seed type or genotype and size), soil type, environmental conditions and depth of sowing. Thus, the environmental conditions appeared to have favoured accessions T7, T8, T9 and T10, all of which had high germination percentages compared to the rest of the accessions.

3.4.2 Fresh and Dry Shoot Weight per Plant

Generally, there was an increase in both the mean fresh and dry shoot weight per plant from 6 WAP to 8 WAP. Significant differences ($p \leq 0.05$) were found in the mean fresh and dry shoot weight at 6 WAP and this agrees with findings of Bayorbor *et al*., (2010) but contrasts with findings of Adu-Gyamfi *et al*., (2012). However, accession T7 had the highest mean fresh and dry shoot weight at 6 WAP with values of 178.8 g and 31.2 g, respectively. At 8 WAP, there was no significant difference among the mean fresh and dry shoot weight per plant of the ten Kersting's groundnut accessions, which corroborates results of Adu-Gyamfi *et al*., (2012). Again accession T7 recorded

the highest mean fresh and dry shoot weights at 8 WAP with values of 338.0 g and 60.6 g respectively, suggesting being superior in shoot yield at 8 WAP. The high performance of accession T7 may be due to favourable genotype and environment interactions. The higher mean fresh and dry shoot weights of the ten accessions at 8 WAP as compared with the lower values obtained at 6 WAP, may be due to increased metabolic activities in the shoots at 8 WAP (Adelusi and Akamo, 2006).

3.4.3 Fresh and Dry Root Weight per Plant

Both mean fresh and dry root weight at 6 WAP were lower than those recorded at 8 WAP. The higher root weight observed at 8 WAP compared to 6 WAP could be as a result of continuous growth of the roots after 6 WAP. This observation agrees with Adelusi and Akamo, (2006) who reported that plant root biomass increases with plant age. The dry root weight observed in this study are lower than values obtained by Gueye, (1986) who recorded dry root weight values ranging from 1.2 to 3.7 g representing the lowest and highest dry root weights respectively.

3.4.4 Shoot Dry Matter Yield

Total plant dry matter is an indicator of resources use strategy by crops (Garnier *et al*., 2001; Wilson *et al*., 1999). While variations were not observed in the dry matter yields, accessions T6, T7 and T8 had the highest dry matter yield while T5 had the least dry matter yield. The high dry matter yield observed in accessions T6, T7 and T8 could be as a result of their ability to efficiently use intercepted solar radiation for biomass production. Indeed, the accessions T7, T8 and T9 all had higher germination percentages resulting in more plant density per plot than the other accessions, and therefore comparatively higher biomass. This observation agrees with findings of

Egli, (1988), Nakagawa *et al.*, (1988) and Funnah and Matsebella, (1985) who reported that dry matter yields in soybeans increase with a plant density. Though accession T6 had the lowest germination percentage, it was among those that recorded high dry matter yield. This suggests that, even though, accession T6 was lowest in plant density due to low germination percentage, this was compensated by extensive above ground biomass production resulting from interception of higher percentage of solar radiation for dry matter production, as dry matter accumulation is directly related to the amount of solar radiation intercepted by plants (Chevula, 1991).

3.4.5 Number of Pods per Plant, Fresh, Dry Pod Weight and Dry Seed Yield per Plot

In general, the mean pod number per plant was high in all accessions. Values obtained range from 85.25 to 118.25, which compare favourably with those obtained by Adu-Gyamfi *et al.*, (2012). Also, the result obtained for the mean pod number per plant agrees with those reported by Adu-Gyamfi *et al.*, (2012) and Bayorbor *et al.*, (2010) both of whom observed similarities among the accessions in the mean pod number per plant.

The Kersting's groundnut accession T3 produced the highest mean number of pods per plant with a value of 118.25 and also the highest fresh pod weight. The highest dry pod weight was, however, by accessionT8. The high pod weight recorded by accession T3 can be attributed to the high pod number per plant. However, accession T3 did not produce the highest dry pod and dry seed weight. The high mean number of pods produced in this accession did not translate into high seed yield as some pods not bearing seeds.

Accession T4 recorded the least pod number per plant, fresh pod weight, dry pod weight and dry seed weight per plot. This poor performance by T4 with respect to yield components could be due to poor adaptation to environmental conditions of the Coastal Savannah Agro-Ecological Zone.

3.4.6 Mean 100-Seed Weight per Plant

Significant differences were observed among the Kersting's groundnut accessions with respect to 100-seed weight and this observation agrees with findings of Adu-Gyamfi *et al.,* (2012). It however, contrasts findings of Bayorbor *et al.,* (2010) who reported no significant differences in the 100 seed weight of among the accessions. These differences observed could be a result of differences in the environmental settings where the experiments were carried out.

3.4.7 Grain Yield per Plot

Grain yield of the ten accessions of Kersting's groundnut was high, ranging from 0.71 t ha^{-1} to 0.84 t ha^{-1} (i.e. 710 kg ha^{-1} and 840 kg ha^{-1}). Adu-Gyamfi *et al.,* (2012), reported yields of 192 kg ha^{-1} and 688 kg ha^{-1} representing the lowest and highest yields, respectively, while Duke *et al.,* (1977), also reported yields of 500 kg ha^{-1} or lower. Bampouri, (2007), who reported mean yields of 178, 124 and 250 kg ha^{-1} for the white, mottled and black seeded cultivars, respectively, of *M. geocarpum* in the Upper West region of Ghana, attributed the lower yields obtained to the practice of weeding only once during cultivation. Yield results, however, disagree with findings of Bayorbor *et al.,* (2010) in that, no significant differences were found to exist among the accessions. The higher grain yield recorded also goes to confirm the report

by Stanley, (2006) who indicated that *M. geocarpum* seeds sown in June gave a high grain yield.

The higher yields obtained in this study could be attributed to the differences in the environmental conditions (such as sunlight, rainfall and the topography of the land) and also due to better weed control as weeding of the plots was done three times. Adelusi and Akamo, (2006) reported that weeds constitute a major limiting factor to grain legume production and is also a major yield depressing factor as they compete for nutrients, moisture, light and space.

3.4.8 Harvest Index

Harvest indices observed for all the ten accessions of Kersting's groundnut were low. This observation is similar to results obtained by Ghafoor *et al.*, (1993) who also observed low harvest indices in Bambara groundnut as compared with cereals and Malik *et al.*, (1986) who also observed low harvest indices in Mungbean. The low harvest indices observed in this study could be attributed to high moisture content which favoured dry matter production. Larry *et al.*, (2012), reported higher dry matter production but low harvest indices under ≥70% soil moisture content but higher harvest indices under 30-40 % soil moisture content. This was attributed to overnight rehydration of plants under low soil moisture content which enhance the whole plant senescence and therefore improve remobilization of pre stored carbon reserves. It is therefore assumed that, the low soil moisture could have made the plants to efficiently utilize carbon reserves and water, resulting in enhanced harvest indices (Zhang and Yang, 2004).

3.4.9 Total Nodule Number, Effective and Non Effective Nodules

Total nodule numbers were similar among the accessions at both 6 WAP and 8 WAP. The results obtained indicate that *M. geocarpum* is capable of nodulating in the Coastal Savannah of Ghana. Adu-Gyamfi *et al.,* (2012) and Bayorbor *et al.*, (2010) had reported that it nodulates well in the Guinea Savannah of Ghana. Significant differences were not detected among the accessions with regard to total nodule number and this agrees with findings of Adu-Gyamfi *et al.,* (2012) and Bayorbor *et al.,* (2010). However, results obtained indicated a decrease in nodule number from 6 WAP to 8 WAP whilst Adu-Gyamfi *et al.,* (2012), reported an increase in nodule number from 6 WAP to 8 WAP.

The results also showed no significant difference in the number of effective and non effective nodule at both 6 WAP and 8 WAP. The non effective nodules however, were higher than the effective nodules at both 6 WAP and 8 WAP. This could mean that there was continuous utilization of effective nodules and formation of new ones as the plants matured. This is supported by Lindemann and Glover, (1990) who reported that, nodules on annuals are short-lived and are constantly replaced during the growing season. Lindemann and Glover, (1990) also reported that at the time of pod fill, nodules on annual legumes generally lose their ability to fix nitrogen because the plant feeds the developing pod and seed rather than the developing nodule. This may explain why the non-effective nodules were more than the effective nodules at both 6 WAP and 8 WAP and also why the numbers of effective nodules at 6 WAP was more than that at 8 WAP.

3.4.10 Fresh and Dry Nodule Weight per Plot

The fresh nodule weight per plot ranged from 0.099 g to 0.323 g at 6 WAP while at 8 WAP it ranged from 0.031 g to 0.204 g. Variations among accessions for fresh nodule weight at the 8 WAP could be attributed to the variations observed in the number of nodules at 8 WAP. Dry nodule weights on the other hand ranged between 0.018 g and 0.073 g at 6 WAP and between 0.01 g and 0.046 g at 8 WAP and are similar to findings by Bayorbor *et al.,* (2006).

3.4.11 Nitrogen concentration in Roots and Shoots per Plant

Assessment of the root biomass for N content revealed that the roots of *M. geocarpum* contain some amount of N though small, ranging from 1.13 to 1.43 % representing 33.1 % and 37.2 % of the total plant N. The concentrations of N in the roots were statistically significant ($p \leq 0.05$) among accessions. This observation agrees with Cadisch *et al.*, (2002) who reported that below ground N vary largely between species, ranging between 9 and 61 %. The results however, contrasts the those of Unkovich *et al.*, (1994) that, root biomass of legumes at crop maturity has smaller mass than that of the shoots and therefore have low N content which comprises only about 10-15 % of the total plant N content.

%Nitrogen concentration in the shoots also showed significant differences ($p \leq 0.05$) among the different accessions. The lowest and highest N recorded were 2.28 % for accession T8 and 3.05 % for T3, respectively, representing 66.9 % and 70.1 % of the total plant N. This shows that, the shoots contributed more N in the plants than the roots. Also the variations observed in the N of both the roots and shoots suggests that, the different accessions have different abilities in %N fixation. The high N associated

with T3 in both shoots and roots, indicates the superior ability of the accession to attract more effective nodulating *Rhizobia*. Elkan, (1990), stated that, the ability to fix high amounts of nitrogen involves control by the cultivar. Therefore symbiotic compatibility between the *Rhizobia* and the host plant is an important step.

3.4.12 Total Plant N Yield of Root and Shoots

There were no significant differences ($p \geq 0.05$) among accessions with respect to the N yield of the roots suggesting that, even though variations existed in the N content of the various accessions in their roots, the N yield (kg/ha) was similar for all accessions. The N yield of the shoots, however, showed significant differences ($p \leq 0.05$) among the accessions where the total plant N yields were different among the accessions studied. Values for the total plant N yield ranging from 663 to 1,190 kg ha^{-1}, were recorded. These values are higher than values of 95 kg N ha^{-1}, 170 kg N ha^{-1} and 255 kg N ha^{-1} for *Canavalia ensiformis*, *Mucuna cochinchinensis* and *Crotalaria anageroides* respectively at 120 days reported by Becker *et al.*, (1996). The high total N yield of *M. geocarpum* could be attributed to the higher nodule number recorded and the favourable soil nutrient composition at 0-20cm soil sub-stratum (Frimpong, 2010) where most of the plants' roots were found

3.4.13 Protein Quality/Crude Protein of Shoots and Roots per Plant

Results obtained for protein quality of the roots suggests that, the roots of all ten accessions of *M. geocarpum* have similar protein quality. The significant differences ($p \leq 0.05$) could also be as a result of the differences observed in the %N concentrations. Shoot protein quality was similar among accessions even though accessions T3 and T7 proved to be leading in the protein quality. These accessions also recorded the highest N content, proving to be superior to the rest of the accessions. The ability of *M. geocarpum* to produce high crude protein in the shoots means that it qualifies to be grown in a grass/legume mixture or in pure stock for livestock feeding.

3.4.14 Correlation among Growth and Yield Parameters

The correlation analysis revealed that, even though, there were positive associations between some of the yield parameters and grain yield, there were equally negative correlations between other parameters and grain yield. This suggests that, for a number of the yield parameters could assist in the selection for grain yield of Kersting's groundnut. With the exceptions of fresh root weight at 6 WAP, dry root weight at 6 WAP and dry pod weight, which weakly but positively correlated with the dry seed weight, all the other parameters did not correlate or were negatively correlated with the dry seed weight. Hence, the selection based on seed yield attributes may not be appropriate. This is emphasized by Benedict *et al.*, (2011) who stated that, seed yield is a polygenic trait and is greatly influenced by its component characters, therefore, direct crop selection on the basis of yield is often misleading. However, the positive and high correlation between the fresh shoot weight at 8 WAP, dry shoot weight at 8 WAP, fresh root weight at 6 WAP, fresh root weight at 8 WAP

and dry root weight at 8 WAP with that of the dry matter yield suggests that these parameters can therefore be used as a selection criteria when dry matter production is desired for forage production. The perfect correlation between dry seed weight (kg) and grain yield (t ha^{-1}) suggests that selecting for one of these traits will result in selection for the other trait. Therefore for the improvement of yield, the information on these direct and indirect effects of the yield attributes could provide a realistic basis for a successful breeding programme (Achakzai and Bangulzai, 2006).

3.4.15 Correlation among Nodule and Nodulation Parameters

The correlation studies involving nodulation parameters revealed that, there were strong correlations among the parameters that were measured. Dry nodule weight at 8 WAP, which correlated well with N yield of roots, N yield of shoots and total plant N yield, could be used as a selection criterion when better N yielding accessions of Kersting's groundnut are desired. Also, N content of roots correlated strongly and positively with the protein quality of the roots suggesting that, for a high protein quality of the roots, N of the roots could be used as a selection criterion. The nearly perfect correlation between the N yield of shoots and total plant N yield is an indication of the significance of N yield of shoots in the total plant N yield. This also means that, an increase in the N yield of the shoots will also result in a direct increase in total plant N yield and vice versa.

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CHAPTER FOUR

Molecular Diversity Studies of Nodule *Rhizobia* in Kersting's Groundnut Accessions Using the Polymerase Chain Reaction

4.1 Introduction

The ability of prokaryotic organisms to reduce atmospheric nitrogen to forms that can be utilized by plants has been known for a long time. It is estimated that biological nitrogen fixation (BNF) accounts for about 175 million tonnes of fixed nitrogen per year (Elkan, 1992). Soil bacteria, *Rhizobia*, can enter into a symbiotic association with legumes which leads to formation of N- fixing root nodules (Ampomah e*t al.*, 2008). Successful management of these symbiotic associations between legume plants and their bacterial endo-symbionts (*Rhizobia* or *Bradyrhizobia* species) requires that specific strains of the bacteria are identified reliably (Richardson *et al.*, 1995). There are several methods that have been used, traditionally, in ecological studies of *Rhizobia* species in association with legumes. Much more attention is now drawn to the use of molecular based techniques that utilize the polymerase chain reaction (PCR) (Mullis and Falloona, 1987).

Polymerase chain reaction (PCR) techniques, used together with short arbitrary oligonucleotide primers of random sequence, have been shown to be an effective means of differentiating complex rhizobial genomes (Richardson *et al.*, 1995). Kersting's groundnut (*Macrotyloma geocarpum* Harms) is a geocarpic legume indigenous to Africa and capable of nodulating with cowpea type *Bradyrhizobia* (Dakora and Muofhe, 1995). Characterization of rhizobial strains naturally associated with the roots of legumes has been recommended as an effective approach to successful management of the legume-*Rhizobium* symbioses (Fening *et al.*, 2004; Mpepereki *et al.*, 1997; Richardson *et al.*, 1995). Although, African soils are believed

to contain a large diversity of rhizobial populations, information on the diversity of *Rhizobia* is limited. In Ghana, the diversity of rhizobial populations of different legumes has not been examined. Since Kersting's groundnut grows over a wide range of soil, its symbioses with the root nodule bacteria will differ from one locality to another. It is therefore, expected that, this study will be able to identify the level of diversity that exist among *Rhizobia* nodulating the crop.

4.1.1 Objective of the Study

To determine the level of similarities and diversity existing among *Rhizobia* bacteria nodulating Kersting's groundnut in the Coastal Savannah Region of Ghana.

Materials and Methods

4.2.1 Experimental Setting

The field experiment was carried out at the research farm of the Biotechnology and Nuclear Agricultural Research Institute (BNARI), Ghana Atomic Energy Commission (GAEC) as previously described in chapter three. The laboratory experiment was conducted at the Molecular Biology Laboratory of BNARI.

4.2.2 Kersting's Groundnut Germplasm

Nine accessions of *M. geocarpum* were obtained from the Agronomy Department of the University of Development Studies (U.D.S), Nyankpala, Tamale, (Table 4.1) in the Northern Region of Ghana. Seeds were planted on the 10th of December, 2012 and nodules harvested on the 4th of February, 2013 (8 weeks after planting).

Table 4.1 Accessions of *Macrotyloma geocarpum* (Harms) used for the study

Mottled (I. L)	Black (I. L)	White (I. L)
Funsi (T2)	Nayung (T1)	Nakpanduri (T3)
Heng (T5)	Puffeun (T7)	
Nakori (T6)	Gbangu (T10)	
Sigiri (T8)		
Dowie (T9)		

I. L is the Identification Label

4.2.3 Nodule Harvesting, Sterilization and Preparation of Template DNA

Kersting's groundnut plants were dug up from the soil and roots cut off from the shoots. The roots were washed thoroughly with water to remove soil particles from the nodules. Intact nodules were then collected and kept in cryovals and labelled according to accessions and stored in the refrigerator at 0^{o}C for one month. The nodules were later subjected to surface sterilization by immersing them in 70 % ethanol for 5 seconds. They were then removed using forceps and transferred into a diluted 3.5 % solution of sodium hypochlorite and soaked for 5 minutes. The nodules were again removed and rinsed in six changes of distilled water. The forceps were sterilized by quickly dipping in 70 % ethanol and then flamed. Each nodule was then picked with the forceps into well labelled eppendorf tube and sterile distilled water added to each tube to the 0.5 ml mark. The forceps were again used in crushing each

nodule after which it was sterilized before using for the next nodule. The crushed nodule suspensions were kept in the refrigerator at 4^{o}C till the next day.

The sterilization process was carried out in the laminar flow hood at the microbiology laboratory of the compost plant in BNARI.

4.2.4 Preparation of PCR Reaction Mix and DNA Amplification

Polymerase Chain Reaction (PCR) amplifications were performed on crude extracts from crushed nodules. Four well labelled eppendorf tubes representing four primers were placed on ice and 40 *ul* of eppendorf master mix pippetted into each tube. 20 *ul* of each of the previously designed primers was pippetted and added to its master mix with 170 *ul* of MilliQ water added to each tube, making a total volume of 230 *ul*. Again 20 well labelled eppendorf tubes (for each primer) representing each nodule were used and a volume of 11.5 *ul* of the PCR mix pippetted into each tube. One micro litre (1 *ul*) of *Rhizobia* bacteria DNA extract from each crushed nodule suspension of the nine accessions of *Macrotyloma geocarpum* was added to its PCR mix in each tube to make a total volume of 12.5 *ul*. All the laboratory activities were done under microbiologically axenic conditions including wearing hand gloves to prevent contaminations of the PCR mix. The primers used were obtained from Metabion International AG, Deutschland and the reference sources of the primers are shown in Table 4.2.

Table 4.2 Primers used for the amplification and detection of *Rhizobia* bacteria DNA obtained from root nodules of *M. geocarpum*

Primer	Type	Reference
ERIC (1 and 2)	Arbitrary oligonucleotide	Ampomah *et al.*, (2008)
RPO1	Specific nif-directed	Richardson *et al.*, (1995)
RPO4	Arbitrary oligonucleotide	Richardson *et al.*, (1995)
RPO5	Arbitrary oligonucleotide	Richardson *et al.*, (1995)

The PCR was performed in a 96 well eppendorf thermal cycler (Nexus, Wagtech). The DNA amplification protocol used for all the primers was initiated at a temperature of 95°C for initial denaturation for 7 minutes. This was followed by 30 cycles of denaturation at a temperature of 94°C for 1 minute, primer annealing at 52°C for 1 minute and primer extension at 65°C for 8 minutes. In all, 30 cycles were carried out for a period of 6 hours. This procedure was a modified protocol of de Bruijn, (1992).

Table 4.3 PCR Reaction Mix

Reagent	1X mix (micro litres)	20X mix (micro litres)
Master Mix	2.0	40.0
Primer	1.0	20.0
MilliQ water	8.5	170.0
DNA template	1.0	20.0
Total reaction volume	12.5	250.0

4.2.5 Agarose Gel Electrophoresis

Gel electrophoresis was done using a modified protocol of Ampomah *et al.*, (2008). Three percent (3 % W/V) of agarose gel was prepared by weighing 2.7 g of gel-grade agarose (sigma) into 90 ml of 1 X TAE buffer. The mixture was then boiled in a microwave oven until a clear solution was obtained before allowing it to cool to about 55°C. For every 50 ml of agarose solution, 40 *ul* of ethidium bromide solution was added. The solution was then poured into a horizontal gel electrophoresis tray mounted in a gel casting tray and filled with a 20-tooth comb and allowed to solidify. The tray was then removed from the gel caster and placed in an electrophoresis tank filled with 1 X TAE buffer to about 2 mm above the top of the gel. The combs were then removed gently from the mounted wells. 10 *ul* aliquots of the PCR reaction mix were then loaded into the wells and run at a constant 90 V for 50 minutes. The gel was subsequently visualized under a high performance ultraviolet trans-illuminator (UVP, Cambridge, UK) and images captured with the aid of a UVP Life Science Software (Doc-It.Ls Image Acquisition). Based on the results obtained from the nine (9) accession of the Kersting's groundnut with the different primers, five (5) nodules from each accession were selected and used together with the RPO1 primer to determine the similarities or diversity among the different accessions of Kersting's groundnut. The procedures used were the same as previously described in sections 4.5 and 4.6.

4.2.6 Scoring for Bacteria DNA fingerprint polymorphism and statistical analysis

The DNA bands that were obtained were converted into one (1) as being present and zero (0) as being absent for the different base pairs (bp) that were obtained through the amplification. Cluster analysis using the hierarchical method of complete link and the Euclidean coefficient was performed to generate dendrograms of the different accessions of Kersting's groundnut using the Genstat software version 12.

RESULTS

4.3.1The Arbitrary Primer RPO4

Following PCR amplification profiles, figures 4.1a and 4.1b were generated from *Rhizobium* bacteria DNA using the arbitrary primer RPO4 in accession T2. The Rhizobium strains 1, 2, 4, 5, 6, 7, 9, 12 and 16 were strongly amplified.

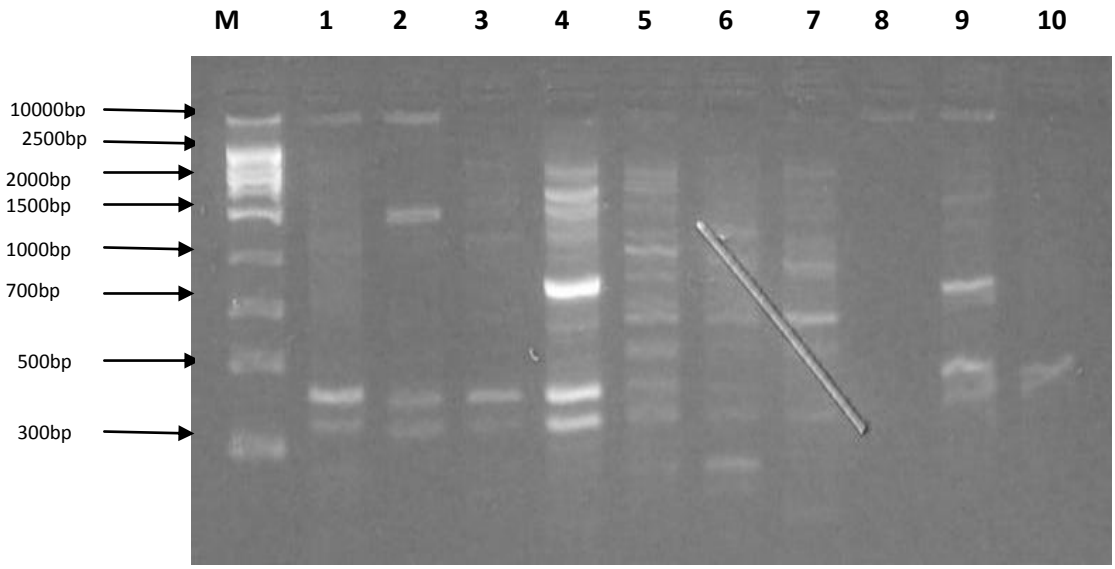

Figure 4.1a. Gel electrophoresis of PCR amplification of *Rhizobium* DNA using the arbitrary primer RPO4. M is the DNA ladder and 1-10 represent *Rhizobium* strains from squashed nodule extracts of Kersting's groundnut accessions following sterilisation.

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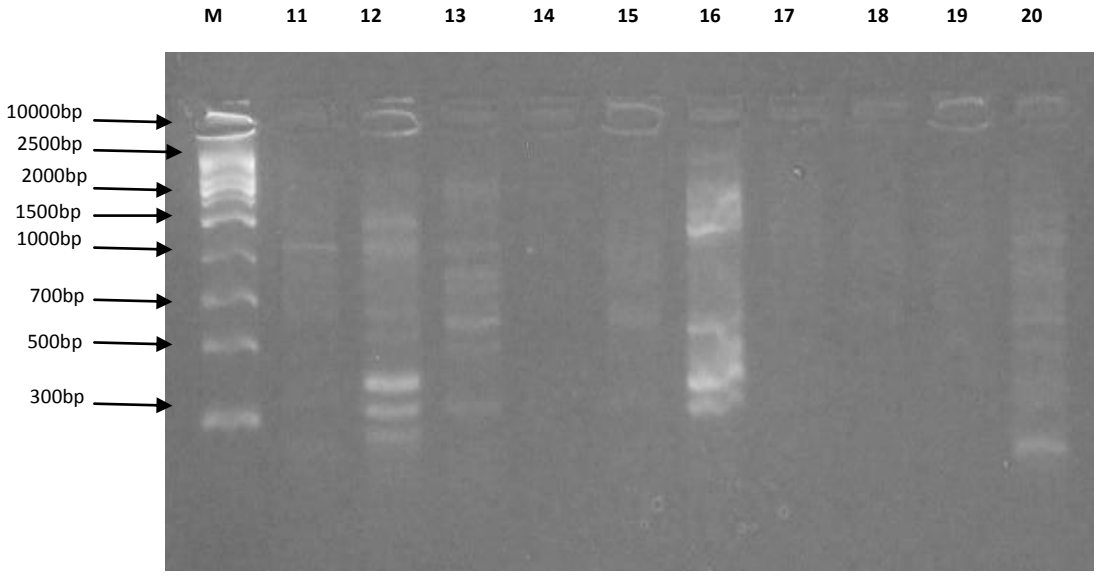

Figure 4.1b. Gel electrophoresis of PCR amplification of *Rhizobium* DNA using the arbitrary primer RPO4. M is the DNA ladder and 11-20 represent *Rhizobium* strains from squashed nodule extracts of Kersting's groundnut accessions following sterilisation.

These were followed by strains 3, 10, 11, 13, 15, 19 and 20 which were weakly amplified whilst no amplification profiles were found in strains 8, 14, 17 and 18. The amplification profiles generated ranged from less than 300bp to 10,000bp.

4.3.2 The Arbitrary Primer RPO5

DNA amplification profiles were generated for some of the 20 *Rhizobium* strains using the RPO5 primer. However, DNA from most of the strains was not amplified. The strains that were strongly amplified include strains 4 and 13.

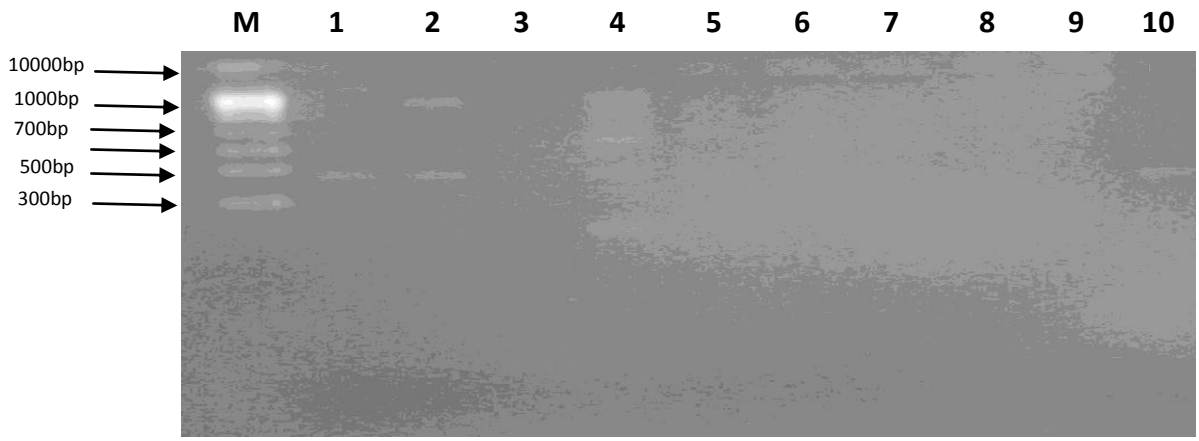

Figure 4.2a. Gel electrophoresis of PCR amplification of *Rhizobium* DNA using the arbitrary primer RPO5. M is the DNA ladder and 1-10 represent *Rhizobium* strains from squashed nodule extracts of Kersting's groundnut accessions following sterilisation.

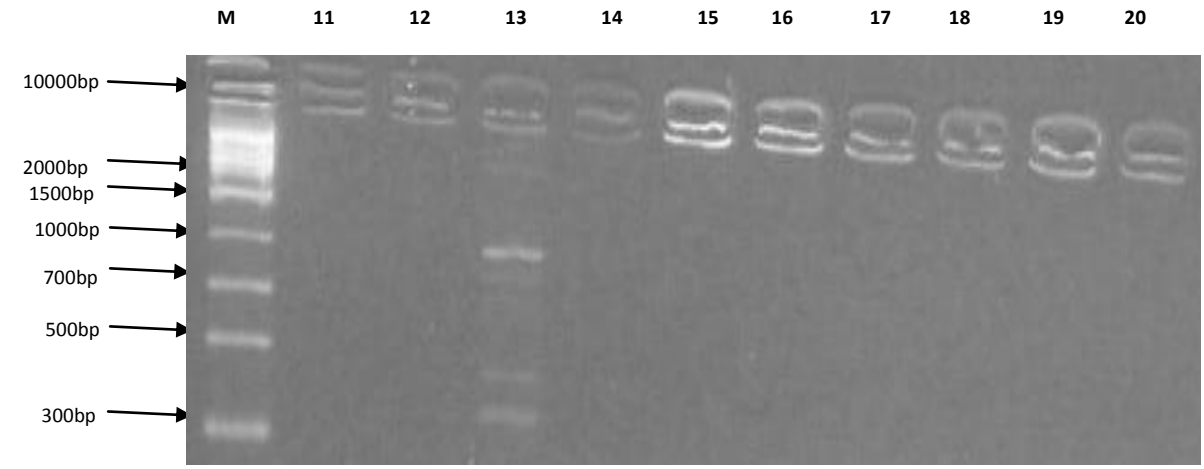

Figure 4.2b. Gel electrophoresis of PCR amplification of *Rhizobium* DNA using the arbitrary primer RPO5. M is the DNA ladder and 11-20 represent *Rhizobium* strains from squashed nodule extracts of Kersting's groundnut accessions following sterilisation.

Only 10,000bp was generated for DNAs in strains 11, 12, 14, 15, 16, 17, 18, 19 and 20. No amplification profiles were detected for strains 1, 2, 3, 5, 6, 7, 8, 9 and 10 (Figures 4.2a and 4.2b).

4.3.3The Arbitrary ERIC 1 and 2 Primers

The ERIC 1 and 2 primers were also able to generate DNA bands from some of the *Rhizobium* strains extracted from nodules of Kersting's groundnut accession T2. The level of amplification was, however, low since most of the DNA extracts did not show any bands. However, strain 3 was strongly amplified whilst strains 6, 7, 9, 17, 18 and 19 were weakly amplified. No amplification profiles were generated for strains 1, 2, 4, 5, 8, 10, 11, 12, 13, 15, 16 and 20 (Figures 4.3a and 4.3b).

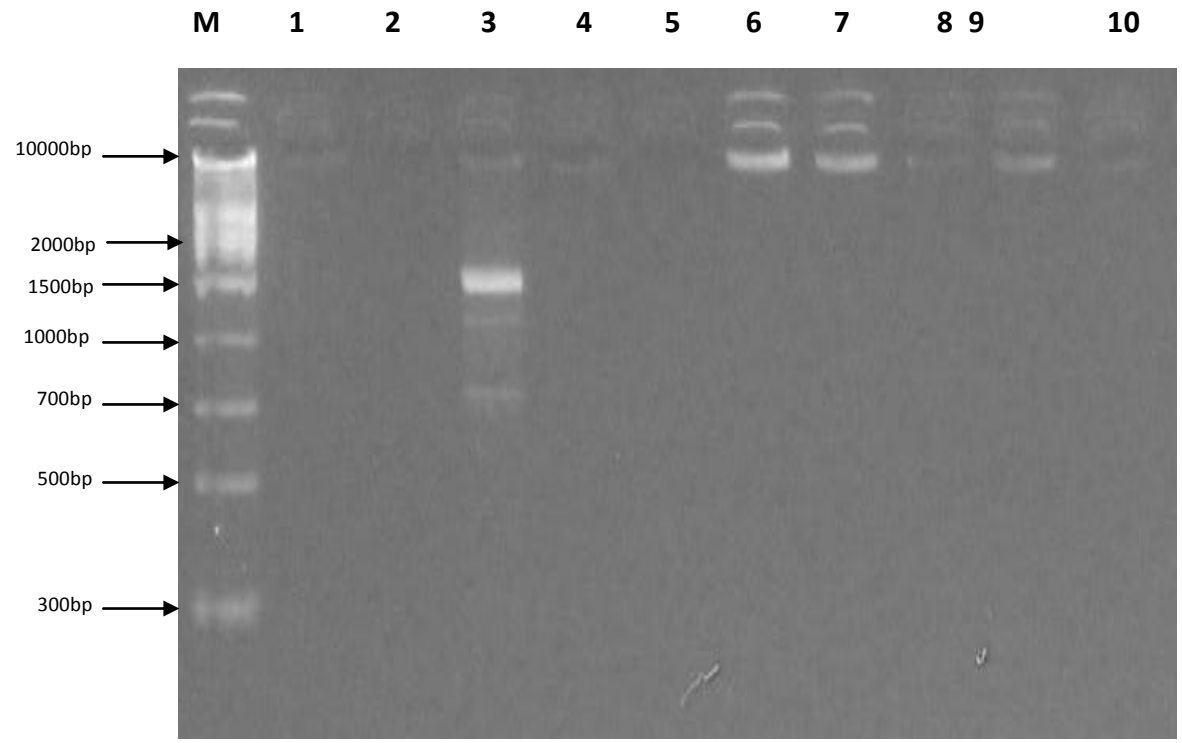

Figure 4.3a. Gel electrophoresis of PCR amplification of *Rhizobium* DNA using the arbitrary primer ERIC. M is the DNA ladder and 1-10 represent *Rhizobium* strains from squashed nodule extracts of Kersting's groundnut accessions following sterilisation.

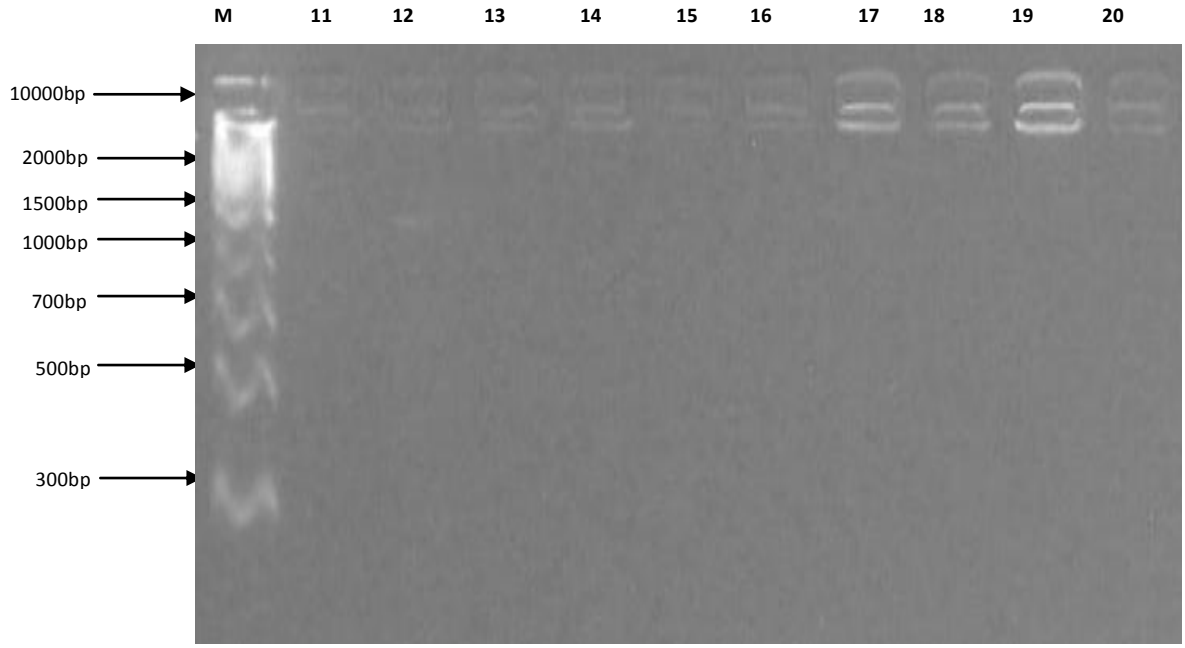

Figure 4.3b. Gel electrophoresis of PCR amplification of *Rhizobium* DNA using the arbitrary primer ERIC. M is the DNA ladder and 11-20 represent *Rhizobium* strains from squashed nodule extracts of Kersting's groundnut accessions following sterilisation.

4.3.4 The nif-Directed PrimerRPO1

Figures 4.4a and 4.4b show amplification profiles from 20 *Rhizobium* strains in accession T6 of Kersting's groundnut using the nif-directed primer RPO1. With the exception of strains 10, 12, 13, 18 and 19 which did not generate any amplification profiles, all others were amplified. The strongly amplified strains were 1, 3, 4, 5, 8, 9, 11, 14, 15, 17 and 20 whilst those that were weakly amplified included strains 2, 6, 7 and 16. The base pairs generated ranged from less than 300bp to 10,000bp (Figures 4.4a and 4.4b).

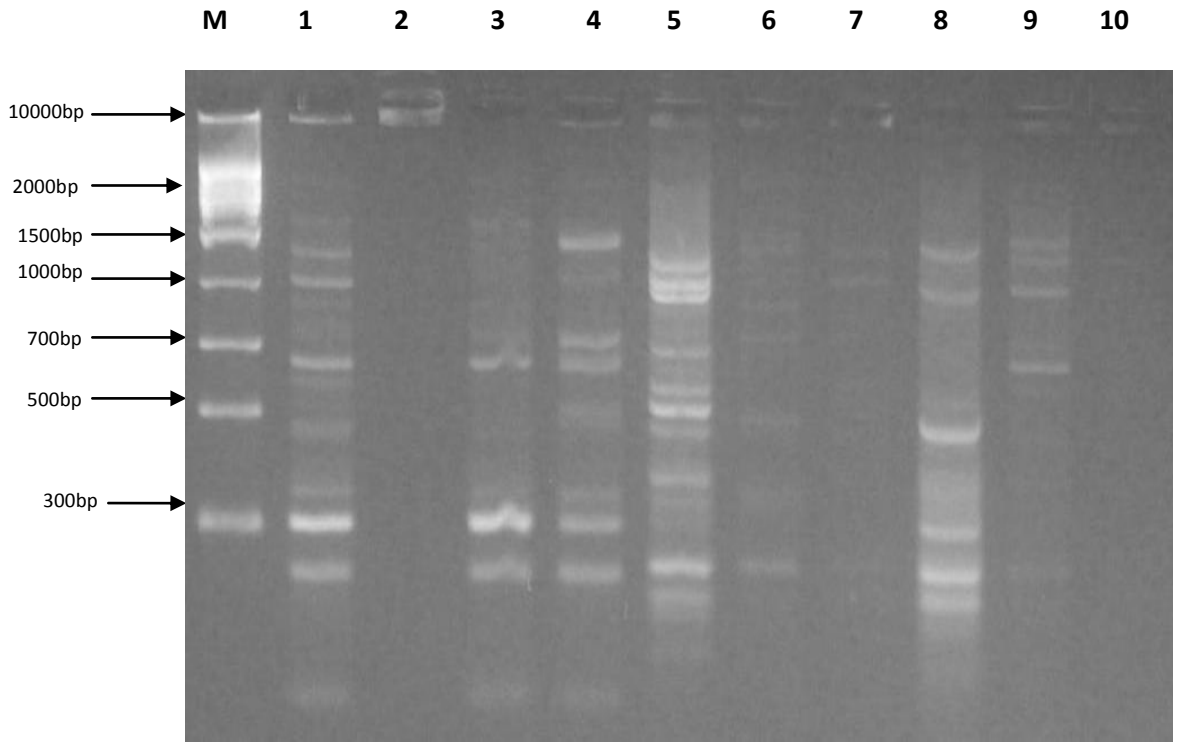

Figure 4.4a. Gel electrophoresis of PCR amplification of *Rhizobium* DNA using the specific primer RPO1. M is the DNA ladder and 1-10 represent *Rhizobium* strains from squashed nodule extracts of Kersting's groundnut accessions following sterilisation.

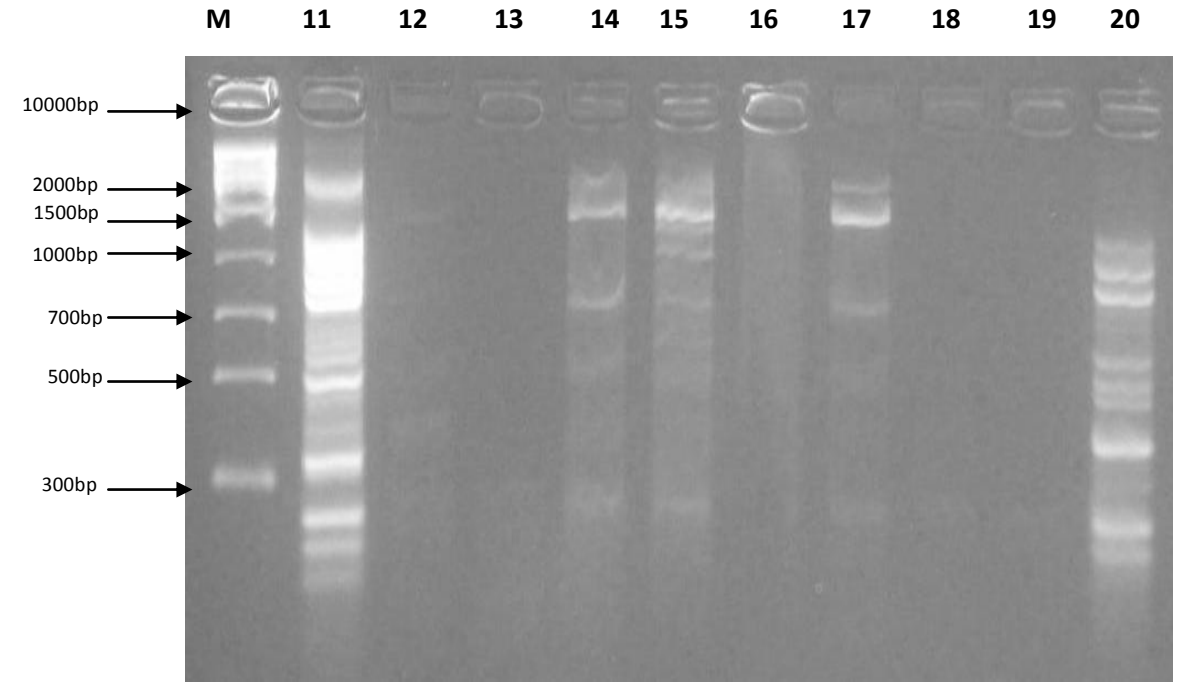

Figure 4.4b. Gel electrophoresis of PCR amplification of *Rhizobium* DNA using the specific primer RPO1. M is the DNA ladder and 11-20 represent *Rhizobium* strains from squashed nodule extracts of Kersting's groundnut accessions following sterilisation.

4.3.5 Inter-Accessional DNA Amplification Profiles Using the Nif-Directed Primer RPO1

Figures 4.5a, 4.5b, 4.5c and 4.5d show amplification profiles generated from *Rhizobium* strains from squashed nodules from all the nine (9) accessions of Kersting's groundnut using the nif-directed primers RPO1. Strong amplification profiles were generated for most of the strains and these includes, strains 3, 4, 5, 6, 9, 10, 13, 15, 16, 17, 18, 19, 20, 21, 22, 24, 25, 26, 27, 28, 30, 33, 34, 39 and 40. Weak amplifications were also generated for strains 1, 2, 7, 8, 11, 12, 23, 29, 31, 32, 35, 36, 37, 38, 41, 42, 43 and 45. There were no amplification profiles for strains 14 and 44. The base pairs generated were well below the range of 300bp -10,000bp. Accessions T5 (16-20) produced the highest DNA bands and was followed by accessions T1 (1-5), T6 (21-25) and T7 (26-30). The lowest amplification profiles produced was in accession T10 (41-45). accessions T2 (6-10), T3 (11-15), T8 (31-35) and T9 (36-40) produced weak amplifications.

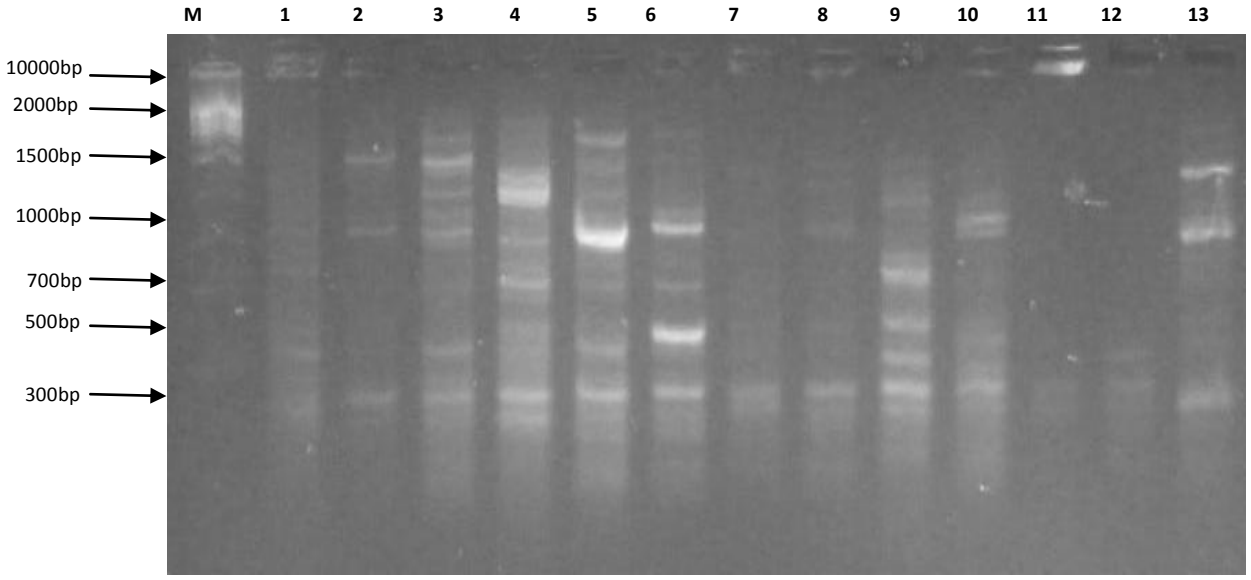

Figure 4.5a. Inter-accessional Gel electrophoresis of PCR amplification of *Rhizobium* DNA using the specific primer RPO1. M is the DNA ladder and 1-10 represent *Rhizobium* strains from squashed nodule extracts of Kersting's groundnut accessions following sterilisation.

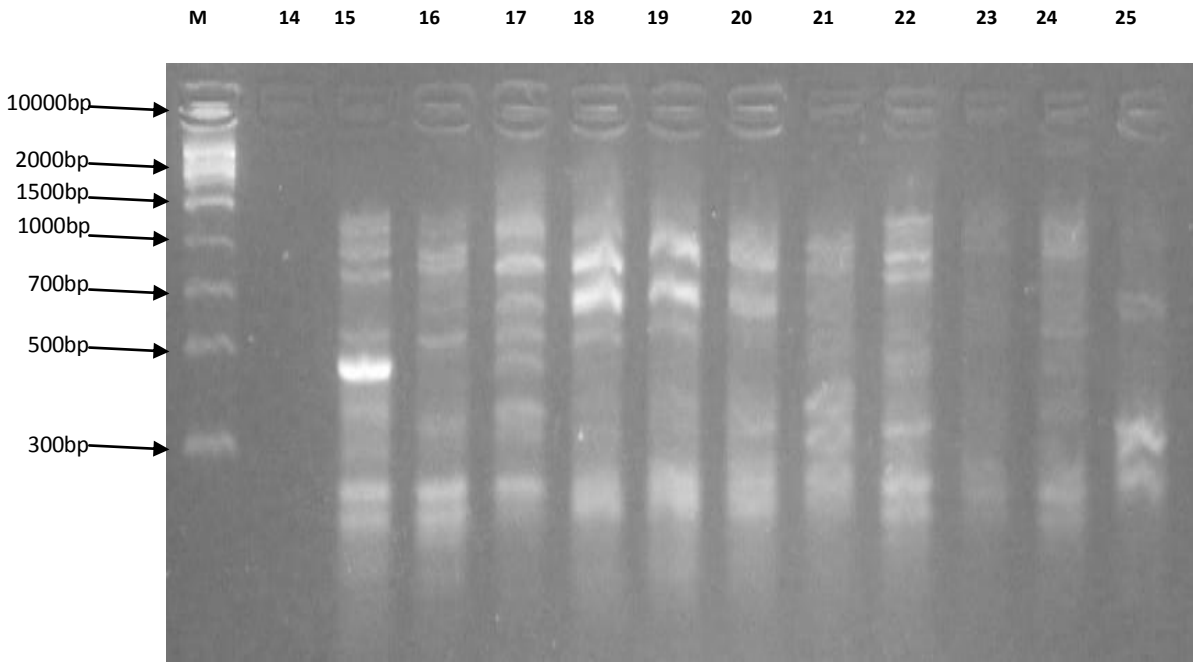

Figure 4.5b. Inter-accessional Gel electrophoresis of PCR amplification of *Rhizobium* DNA using the specific primer RPO1. M is the DNA ladder and 14-25 represent *Rhizobium* strains from squashed nodule extracts of Kersting's groundnut accessions following sterilisation.

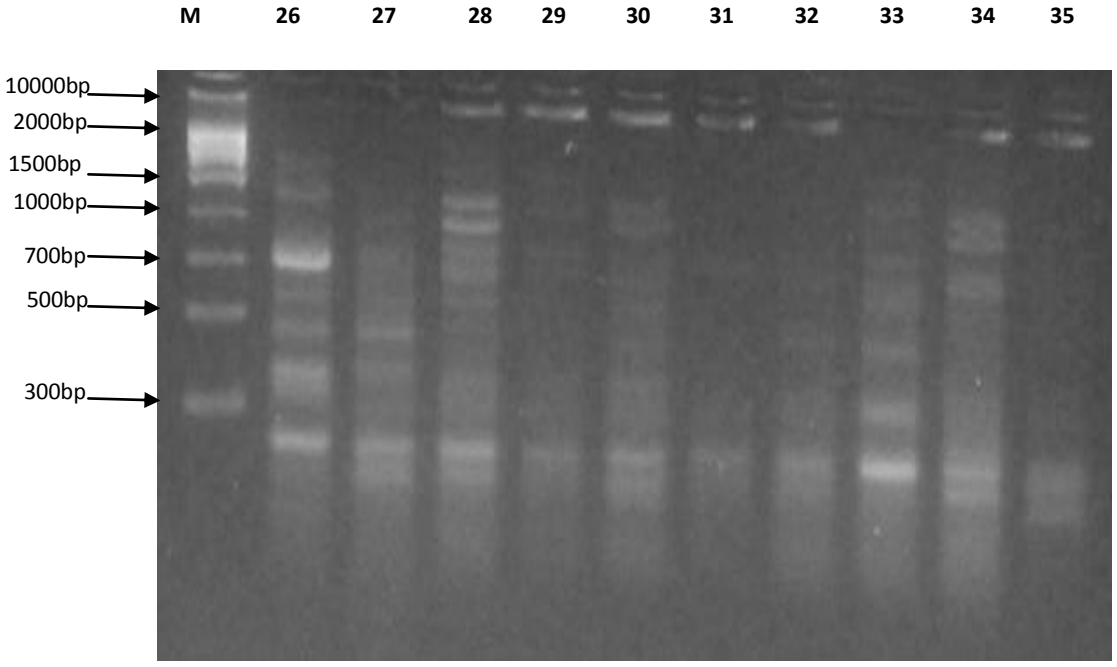

Figure 4.5c. Inter-accessional Gel electrophoresis of PCR amplification of *Rhizobium* DNA using the specific primer RPO1. M is the DNA ladder and 26-35 represent *Rhizobium* strains from squashed nodule extracts of Kersting's groundnut accessions following sterilisation.

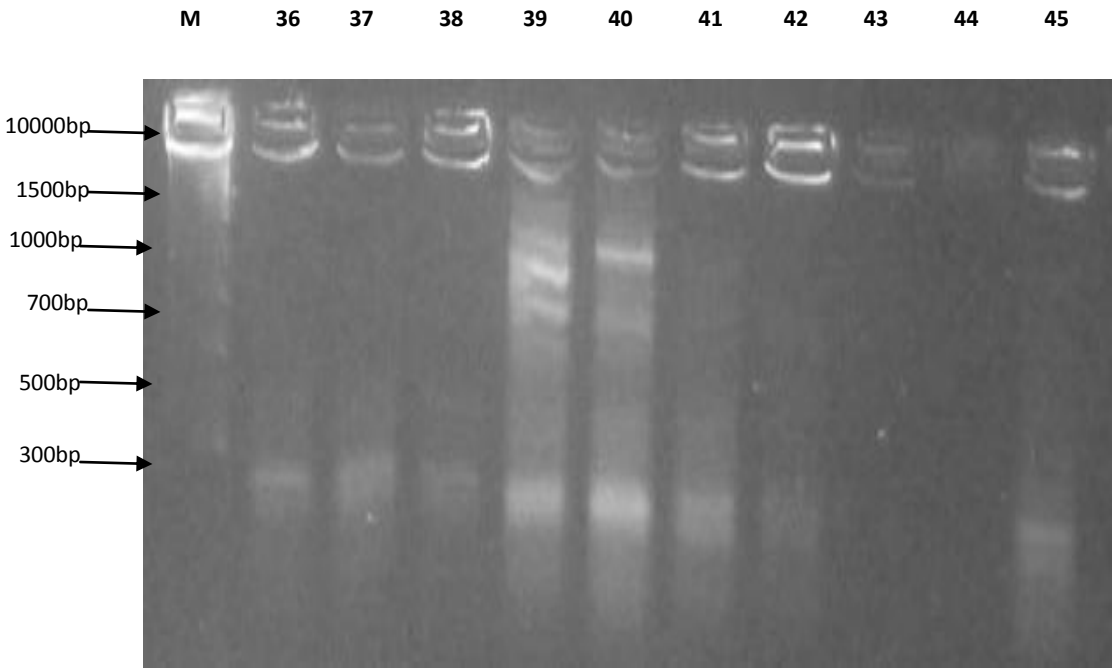

Figure 4.5d. Inter-accessional Gel electrophoresis of PCR amplification of *Rhizobium* DNA using the specific primer RPO1. M is the DNA ladder and 36-45 represent *Rhizobium* strains from squashed nodule extracts of Kersting's groundnut accessions following sterilisation.

4.3.6 Genetic Relationship among *Rhizobium* Bacteria DNA in Kersting's Groundnut Accession T1

At a genetic similarity of 50 %, three main clusters were formed (Figure 4.6). Cluster 1 was divided into two sub clusters, 1A and 1B at a genetic or similarity distance of 58 %. The sub cluster 1A consisted of *Rhizobium* strains 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14 and 16 whilst sub cluster 1B consisted of only *Rhizobium* strain 20. The main cluster 2 consisted of only strain number 11 whilst the main cluster 3 was made up of strains 15, 18, 17 and 19. At 100 % similarity distance, *Rhizobium* strains 1, 2, 3, 6, 9, 12 and 13 were identical while strains 5 and 7 were duplicates. The furthest genetic distance was between *Rhizobium* strains 1 and 19.

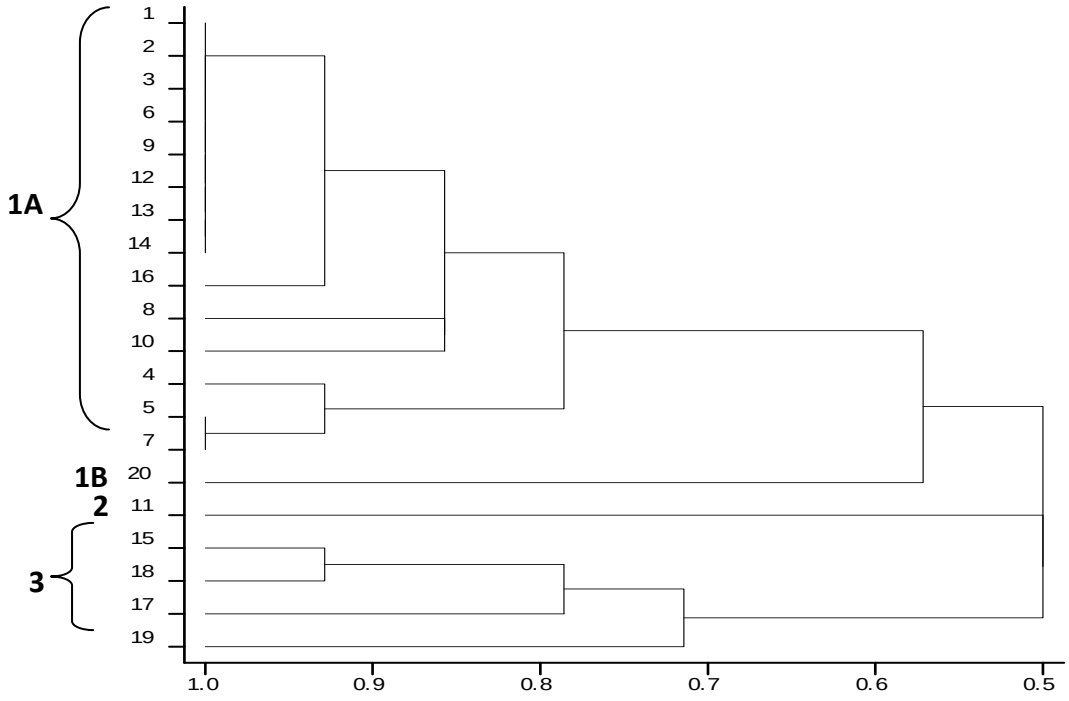

Figure 4.6. Dendrogram showing the genetic relationship among *Rhizobium* bacteria DNA extracted from 20 nodules of the Kersting's groundnut accession T1 (Nayung black) based on Euclidean coefficient using complete link similarity matrix method.

4.3.7 Genetic Relationship among *Rhizobium* Bacteria DNA in Kersting's Groundnut Accession T2.

All the *Rhizobium* strains were clustered into two main groups at 20 % genetic similarity. The cluster 1 was further divided into two sub clusters 1A and 1B at a similarity distance of 40 %. Sub cluster 1A consisted of *Rhizobium* strains 1, 2, 8, 10, 15, 17, 18 and 19 whilst sub cluster 1B consisted of *Rhizobium* strains 3, 9, 12, 13, 14 and 16. The remaining strains 4, 5, 6, 7, 11 and 20 were found in the main cluster 2. The furthest genetic distance was between strains 1 and 20 (Figure 4.7).

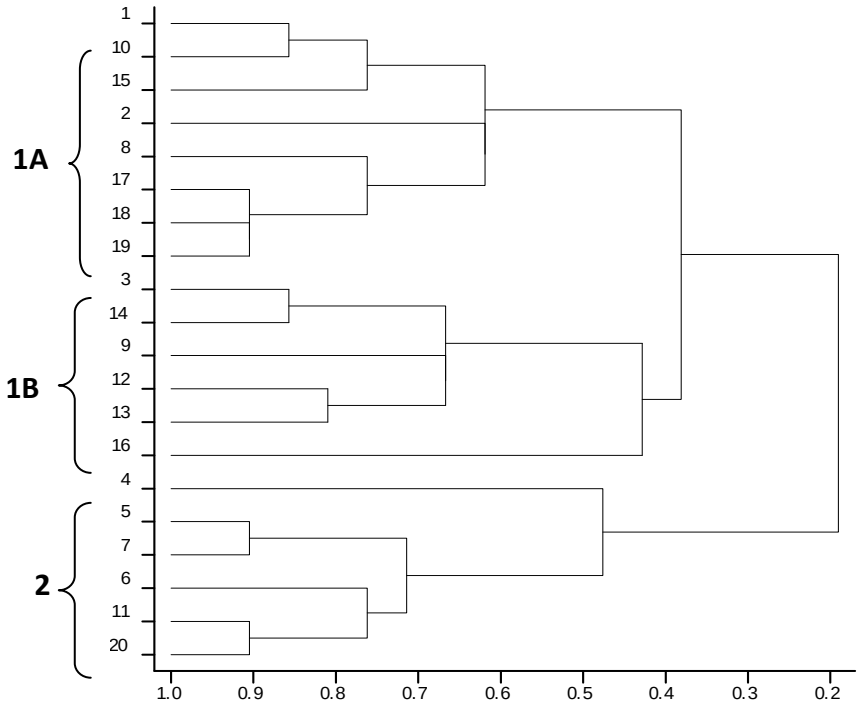

Figure 4.7. Dendrogram showing the genetic relationship among *Rhizobium* bacteria DNA extracted from 20 nodules of the Kersting's groundnut accession T2 (Funsi mottled) based on Euclidean coefficient using complete link similarity matrix method.

4.3.8 Genetic Relationship among *Rhizobium* Bacteria DNA in Kersting's Groundnut Accession T3

The dendrogram generated clustered the strains into 2 main clusters at a 20 % level of genetic similarity. The cluster 1 was divided into 2 sub clusters 1A and 1B at 50 % genetic similarity. Sub cluster 1A consisted of only *Rhizobium* strain 1 while sub cluster 1B was again divided into 3 sub clusters I, II and III at 60 % level of genetic similarity (figure 4.8). The sub cluster 1BI was made up of strains 2, 3, 13, 15, 16 and 18. The sub cluster 1BII also consisted of strains 5, 7 and 9 whilst sub cluster 1BIII was made up of *Rhizobium* strains 6, 8, 10, 14, 17, 19 and 20. The main cluster 2 consisted of *Rhizobium* strains 4, 11 and 12. At 100 % genetic distance, *Rhizobium* strains 3, 6, 13, 15, 17, 18 and 19 were similar. However, the furthest genetic distance was between *Rhizobium* strains 1 and 12 (Figure 4.8).

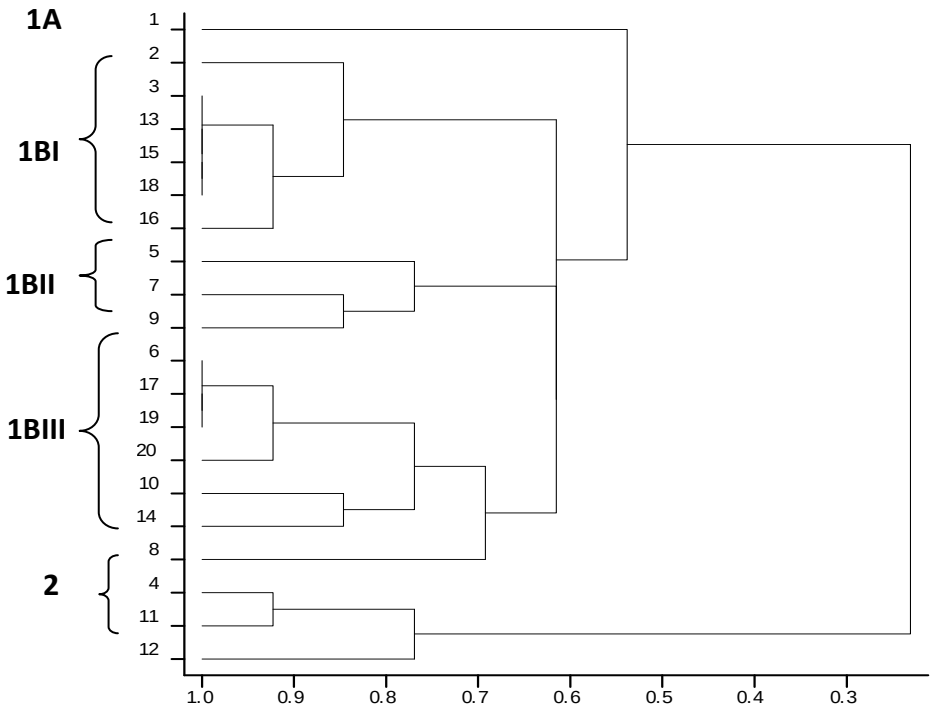

Figure 4.8. Dendrogram showing the genetic relationship among *Rhizobium* bacteria DNA extracted from 20 nodules of the Kersting's groundnut accession T3 (Nakpanduri white)based on Euclidean coefficient using complete link similarity matrix method.

4.3.9 Genetic Distance among *Rhizobia* Bacteria DNA in Kersting's Groundnut Accession T5.

Two major clusters were identified at a genetic similarity of 10 %. The main cluster 1 was again divided into two sub clusters 1A and 1B in which the sub cluster 1A comprised of *Rhizobium* strains 1, 2, 4, 6, 8, 11 and 13 while sub cluster 1B consisted of strains 10 and 15. Again the major cluster two was also divided into 2 sub clusters at a genetic similarity of 60 %. The 1st sub cluster 2A comprised of strains 3, 5, 7, 9, 12, 14, 16, 17, 18 and 20 whilst the 2nd sub cluster 2B was made up of only strains 19. At 100 % genetic similarity, strains 1, 2 and 8 were identical as did strains 16, 17 and 18. In addition, four pairs of strains namely, strains 11 and 13, 10 and 15, 3 and 14 and 12 and 20 proved to duplicate each other. The farthest genetic distance was between strains 1 and 19 (Figure 4.9).

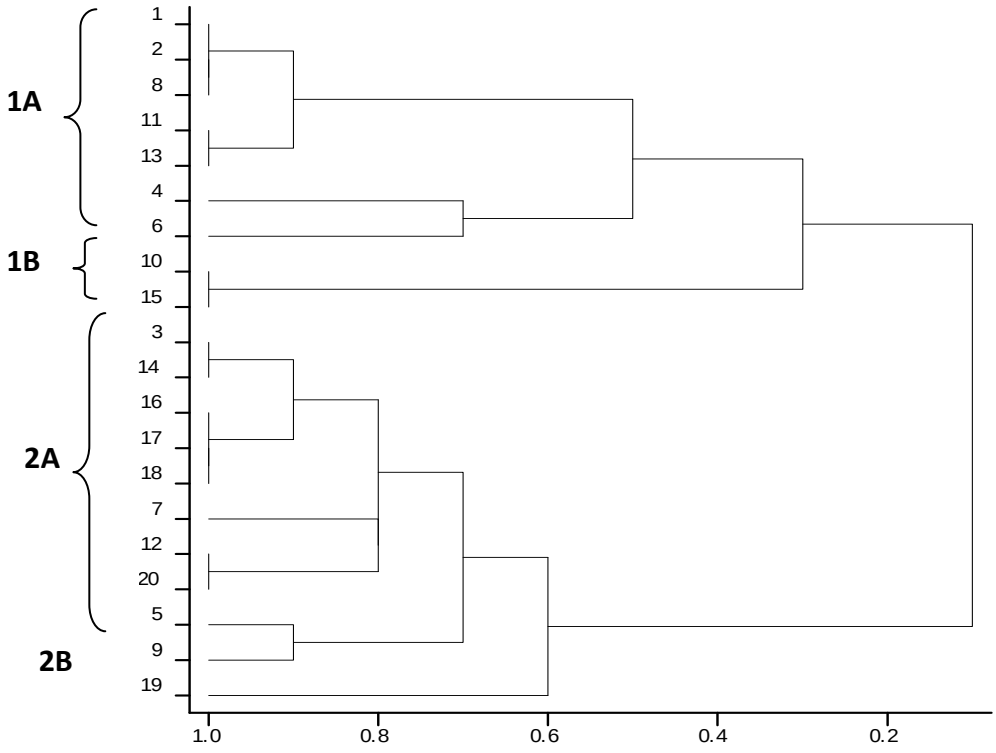

Figure 4.9. Dendrogram showing the genetic relationship among *Rhizobium* bacteria DNA extracted from 20 nodules of the Kersting's groundnut accession T5 (Heng mottled)based on Euclidean coefficient using complete link similarity matrix method.

4.3.10 Genetic Relationship among *Rhizobia* Bacteria DNA in Kersting's Groundnut Accession T6

At 8 % genetic distance, two main clusters were formed. The cluster one comprised of 5 strains namely, strains 1, 5, 11, 18 and 20 whilst the remaining strains were found in the second cluster (figure 4.10). The second cluster was further divided into two sub clusters, 2A and 2B. Sub cluster 2A contained strains 2, 7, 10, 12, 13, 14, 15, 16, 17 and 19 whilst sub cluster 2B had strains 3, 4, 6 and 8. *Rhizobium* strains 10 and 16 and also 12 and 13 were identified as duplicates at 100 % similarity whilst strains 1 and 8 were farthest apart (Figure 4.10).

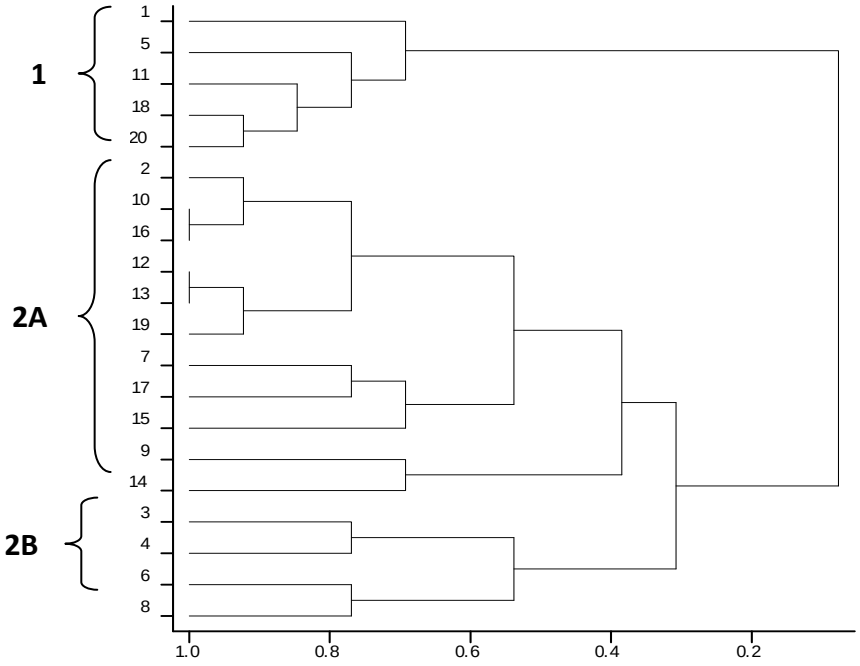

Figure 4.10. Dendrogram showing the genetic relationship among *Rhizobium* bacteria DNA extracted from 20 nodules of the Kersting's groundnut accession T6 (Nakori mottled) based on Euclidean coefficient using complete link similarity matrix method.

4.3.11 Genetic Relationship among *Rhizobium* Bacteria DNA in Kersting's Groundnut Accession T7

At a genetic distance of 0.2 %, 2 main clusters were generated. Cluster 1 consisted of *Rhizobium* strains 1, 2, 8 and 10 whilst cluster 2 consisted of the rest of the strains (figure 4.11). At 20 % similarity distance, cluster 2 was divided into two sub clusters, 2A and 2B. Sub cluster 2A was made up of strains 3, 4, 16 and 17 whilst sub cluster 2B was again divided into two other clusters I and II at a 30 % level of genetic similarity. Sub cluster 2BI comprised of strains 5, 9, 12, 15, 18 and 19 whilst sub cluster 2BII comprised of strains 6, 7, 11, 13, 14 and 20. At 100 % level of genetic similarity, strains6, 13 and 14 were identical while strains 6 and 16 as well as 15 and 19 were duplicates. The farthest genetic distance was between strains 1 and 11 (Figure 4.11).

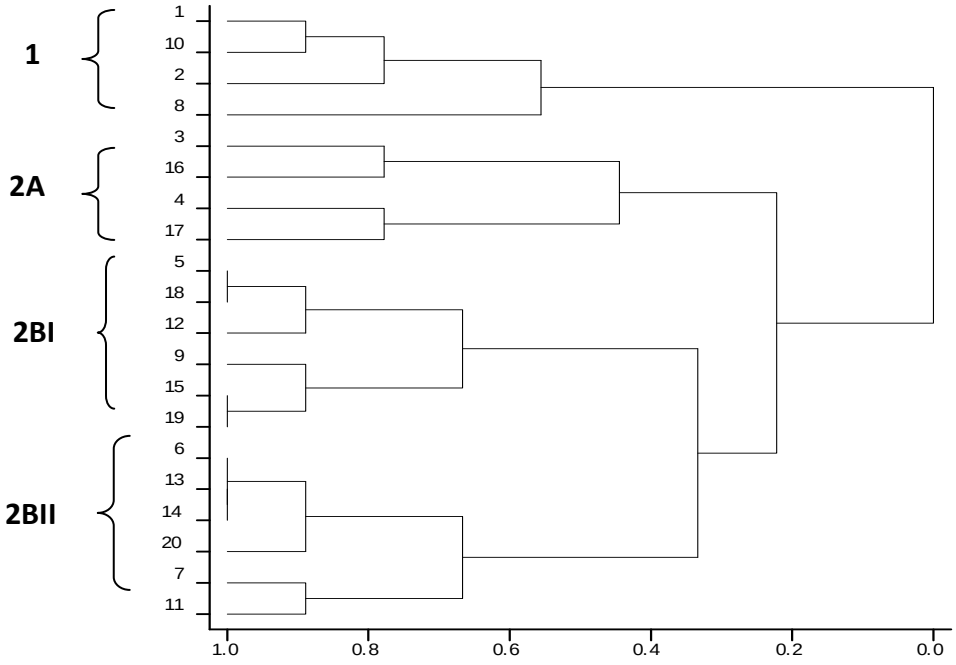

Figure 4.11 Dendrogram showing the genetic relationship among *Rhizobium* bacteria DNA extracted from 20 nodules of the Kersting's groundnut accession T7 (Puffeun black)based on Euclidean coefficient using complete link similarity matrix method.

4.3.12 Genetic Relationship among *Rhizobium* Bacteria DNA in Kersting's Groundnut Accession T8

Two major clusters, 1 and 2, were formed at a genetic similarity level of 0.7%. The cluster 1 was further divided into two sub clusters 1A and 1B. The sub cluster 1A was made up of strains 1, 2, 3, 11, 13, 15, 19 and 20 whilst sub cluster 1B was made up of strains 5, 6, 7, 12, 16, 18 and 19. The major cluster 2 was also divided into two sub clusters 2A and 2B at a similarity distance of 59 %. The sub cluster 2A was made up of strains 4, 8, 10 and 17 whilst sub cluster 2B was made up of only strain 14. At 100 % genetic similarity, strains 1, 2 and 3, 11, 13, 15 and 20, 5 and 6, and 9 and 16 were all identical. The farthest genetic distance was however, between strains 1 and 14 (Figure 4.12).

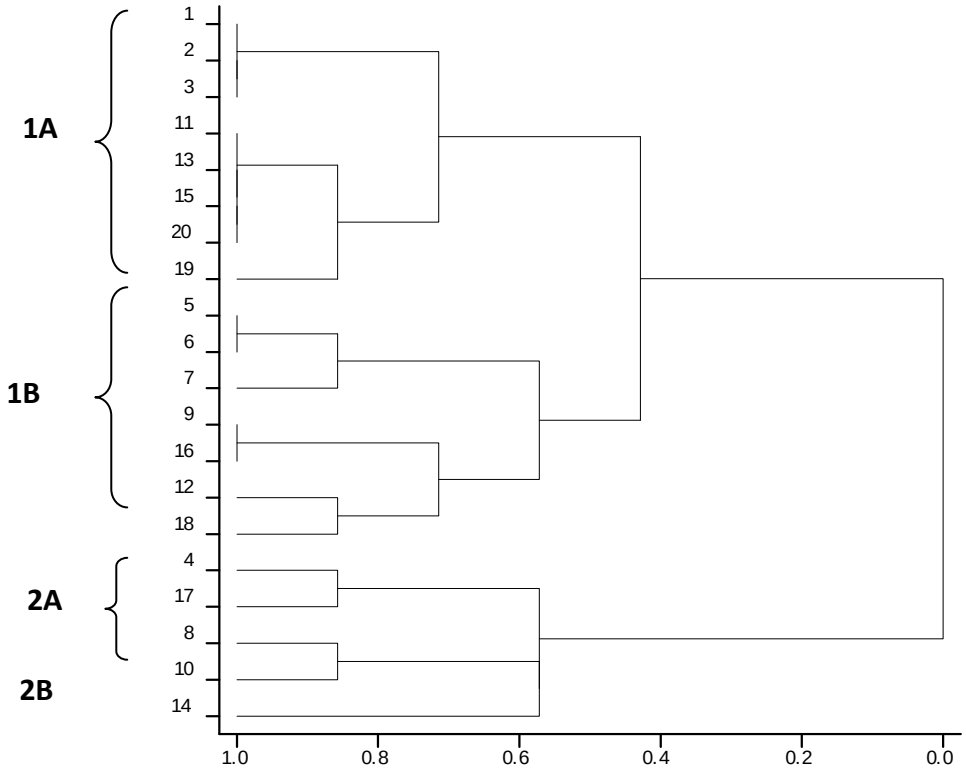

Figure 4.12 Dendrogram showing the genetic relationship among *Rhizobium* bacteria DNA extracted from 20 nodules of the Kersting's groundnut accession T8 (Sigiri mottled)based on Euclidean coefficient using complete link similarity matrix method.

4.3.13 Genetic Relationship among *Rhizobium* Bacteria DNA in Kersting's Groundnut Accession T9

At a genetic similarity of 11.7 %, two main clusters, 1 and 2, were formed. The main cluster 1 was sub divided into two clusters 1A and 1B at a similarity of 56 %. The sub cluster 1A consisted of strains 1, 2, 5, 6, 8 and 11 whilst sub cluster 1B was made up of strains 10, 12, 13 and 20. The major cluster 2 was also further sub divided into two2A and 2B at a similarity of 34 %. The sub cluster 2A consisted of strains 3, 4, 7, 9, 14 and 16 whilst sub cluster 2B was made up of strains 15, 17, 18 and 19. The farthest genetic distance was between strains 1 and 17. Strains 1, 2, 5 and 6 as well as 15, 18 and 19 were identical at 100 % genetic similarity (Figure 4.13).

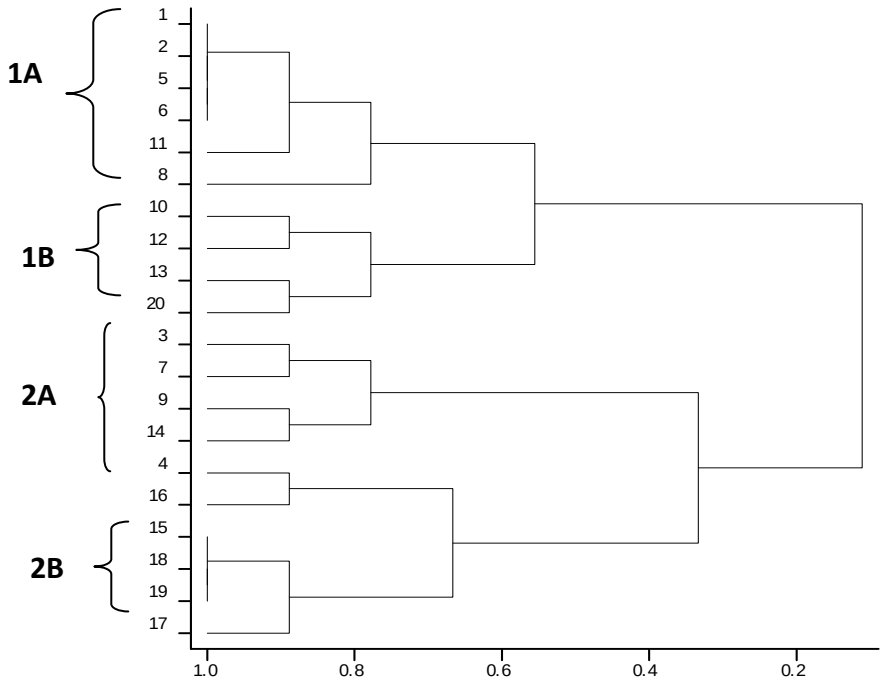

Figure 4.13 Dendrogram showing the genetic relationship among *Rhizobium* bacteria DNA extracted from 20 nodules of the Kersting's groundnut accession T9 (Dowie mottled)based on Euclidean coefficient using complete link similarity matrix method.

4.3.14 Genetic Relationship among *Rhizobium* Bacteria DNA in Kersting's Groundnut Accession T10

At a genetic similarity of 0.3 %, two main clusters were formed. The cluster 1 was divided into 2 sub clusters 1A and 1B at a genetic similarity of 43 %. Sub cluster 1B was made up of strains 10, 12, 14, 15, 18, 19 and 20. The sub cluster 1A was further divided into 3 sub clusters I, II and III at a genetic similarity of 58 %. The sub cluster 1AI was made up of strains 1, 3, 4, 5, 6 and 8 whilst sub cluster 1AII consisted of strains 2, 7 and 16 and sub cluster 1AIII was made up of strains 13 and 16. The main cluster 2 was made up of strains 9 and 17. At 100 % level of genetic similarity, strains in three sets 1, 3, 4, 5 and 6, 2, 7 and 11and 10, 12, 18 and 19 were identical. Strains 1 and 17 were the farthest apart (Figure 4.14).

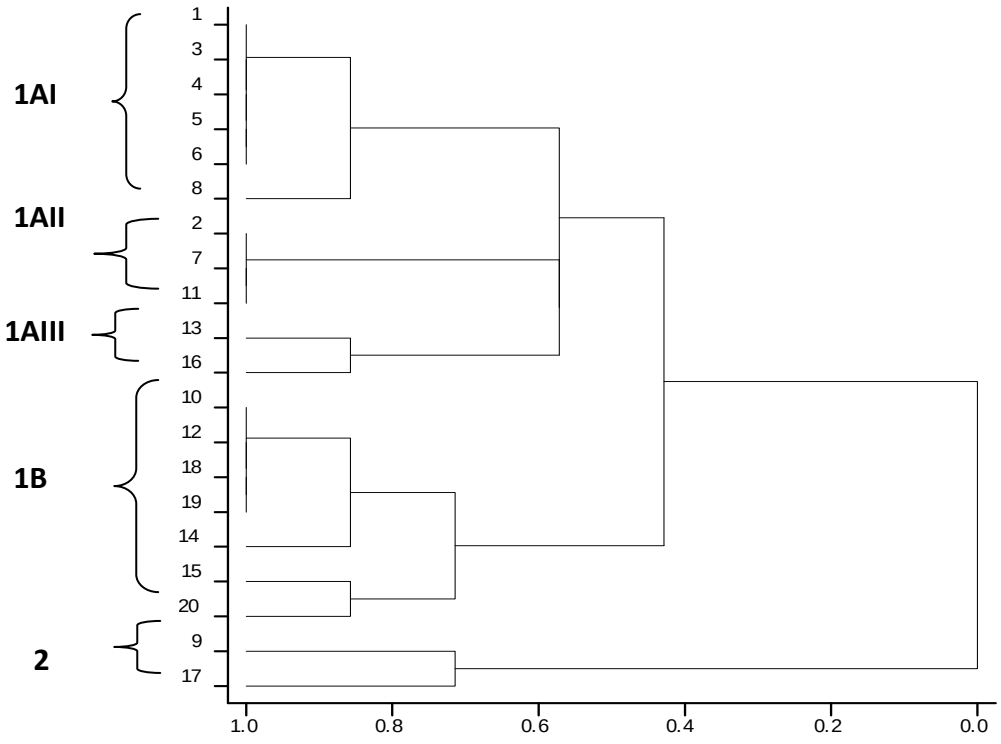

Figure 4.14. Dendrogram showing the genetic relationship among *Rhizobium* bacteria DNA extracted from 20 *Rhizobium* strains of the Kersting's groundnut accession T10 (Gbangu black) based on Euclidean coefficient using complete link similarity matrix method.

4.3.15 Genetic Relationship among *Rhizobium* Bacteria DNA in Inter-Accessions of Kersting's Groundnut

Two major clusters were formed at 0.7 % level of genetic similarity, indicating early divergence of the strains into two major groups. The cluster 1 further diverged into two sub clusters at a genetic similarity of 46%. The sub cluster 1B was again divided into three sub clusters I, II and III at a genetic distance of 55 % (figure 4.15).

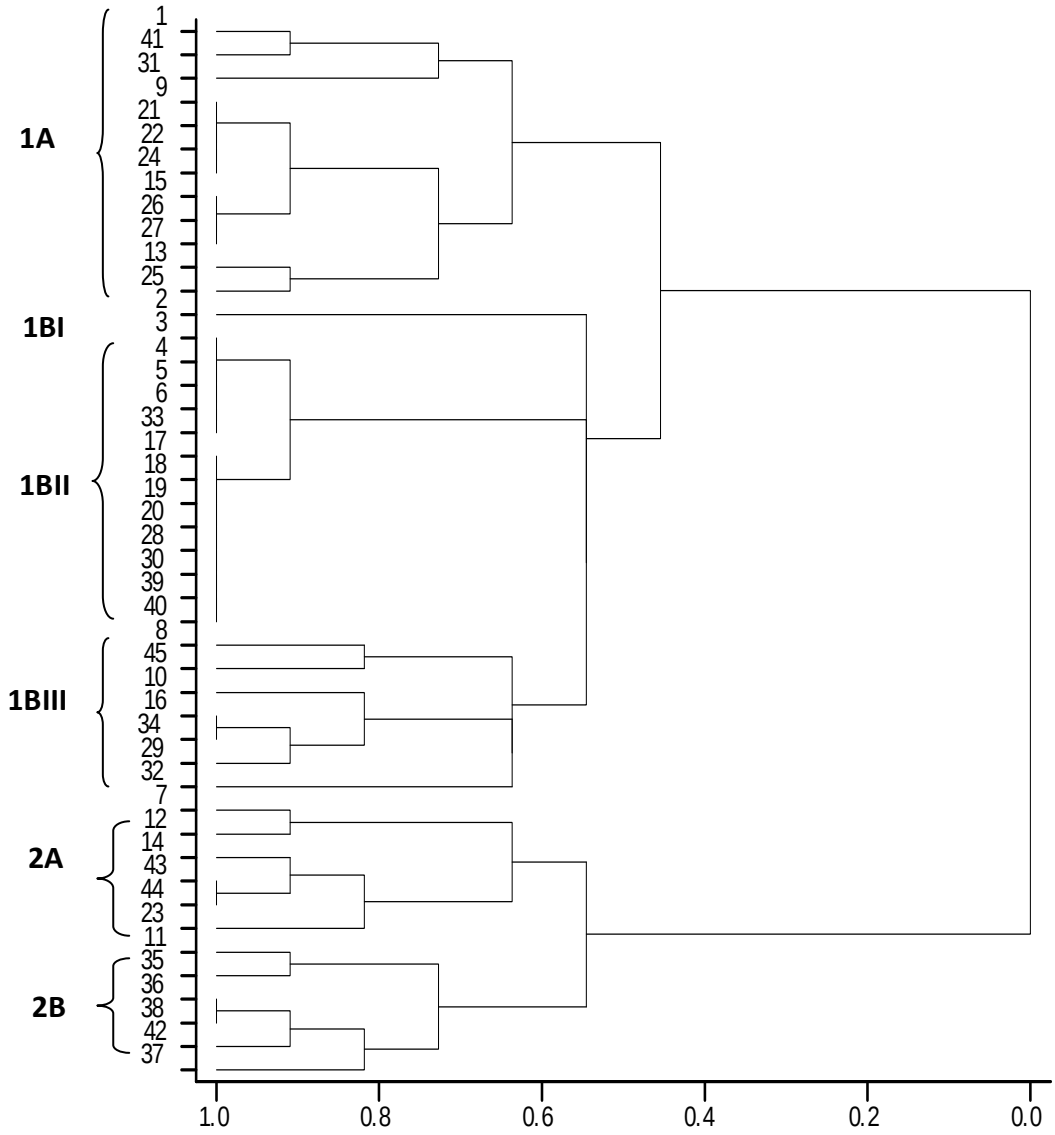

Figure 4.15. Dendrogram showing the genetic relationship among *Rhizobium* bacteria DNA extracted from 45 nodules of the nine Kersting's groundnut accessions, 1-5 (T1), 6-10(T2), 11-15 (T3), 16-20 (T5), 21-25 (T6), 26-30 (T7), 31-35 (T8), 36-40 (T9) and 41-45 (T10)based on Euclidean coefficient using complete link similarity matrix method.

The sub cluster 1BI consisted of only strain 3 (Figure 4.15) while the main cluster 2 also diverged into two sub clusters at a genetic similarity of 55 %.

At genetic similarity of 100 %, two sets of duplicates were identified, each in sub clusters 2A and 2B, comprising of strains 44 and 23 and then 38 and 42. In addition, *Rhizobium* strains 29 and 34 within sub cluster1BIII were duplicates. Furthermore, four sets of strains were each identified as identical as they showed no divergence at 100 % genetic similarity level. These are strains 15, 21, 22 and 24; 13, 26 and 27; 4, 5, 6, 17 and 33 and 8, 18, 19, 20, 28, 30, 39 and 40.

DISCUSSION

4.4.1 Differentiation of *Rhizobium* DNA Band Using Different Primers

The DNA from *Rhizobium* bacteria examined in this study was differentiated by PCR amplification using both arbitrary primers ERIC, RPO4 and RPO5 as well as the nif-directed primer RPO1. In most of the amplification profiles generated, distinct profiles were observed for most of the DNA of the bacteria by all the four primers. However, primers ERIC and RPO5 were not highly discriminatory and therefore, generated a lower degree of polymorphism on the *Rhizobium* DNA.

Primers RPO1 and RPO4 were, however, highly discriminatory and were therefore able to generate a higher degree of polymorphic DNA bands than those observed for the ERIC and RPO5 primers (Harrison *et al.*, 1992). The unique amplification profiles generated by the primers, especially primers RPO1 and RPO4 for the DNA tested is an indication that these primers could be used for differentiation of the *Rhizobium* at the strain level. The nif-directed primer RPO1 was the best in clearly amplifying the DNA profiles of the *Rhizobium* bacteria for all the Kersting's groundnut accessions. This observation agrees with Richardson *et al.*, (1995), who reported that the RPO1 primer had a wide applicability for amplification and differentiation of *Rhizobium* DNA.

Again, results obtained by Richardson *et al.*, (1995) indicated that, the RPO1, RPO4 and RPO5 primers were suitable for differentiation of *Rhizobium* species to the strain level. It is, therefore possible that the amplification profiles generated by the RPO1 primer identified the *Rhizobiuml.bv. trifolii* strain, since the sequence of this primer corresponds to a conserved region located within the *R.l.bv. trifolii* strain ANU843 nif-HDK promoter region (Richardson *et al.*, 1995). According to Schofield and Watson, (1985), that part of the sequence of the RPO1 primer (the first 9 nucleotides

at the 3' end) is the nif promoter consensus element which has been shown to be highly conserved across fast-growing *Rhizobium* species and is extensively repeated within individual strains.

Based on the results that were obtained, the RPO1 primer was selected for inter-accessional *Rhizobium* DNA amplification, as the RPO1 primer was able to amplify and differentiate a diverse collection of *Rhizobium* DNA from nodules obtained from the nine different accessions of Kersting's groundnut. The inability of the ERIC and RPO5 primers to generate more amplification profiles as compared with that of the RPO1 and RPO4 primers could be as a result of the annealing temperature which was at 52^{o}C. This corroborates work by Richardson *et al.*, (1995), who observed that the RPO5 primer failed to generate amplification fragments at annealing temperature above 45-50^{o}C.

4.4.2 Genetic Diversity and Similarity among the Nodule Bacteria Isolates in Nine Accessions of Kersting's Groundnut

The dendrogram for the nodule bacteria isolates for the Kersting's groundnut accession T1 revealed three major clusters at a genetic similarity of 50 %. This could be an indication of the diverse nature of these isolates. However, *Rhizobium* strains 5 and 7 as well as 1, 2, 3, 6, 9, 12, 13 and 14 prove to be the same entities as they were not differentiated beyond the 95 % genetic similarity.

For accession T2, two main clusters were observed in the dendrogram generated at a genetic distance of 20 % for the bacteria isolates. In this accession, possible duplicates were not identified, suggesting that, there was a high variation among the strains. This

agrees with findings of Chen *et al.*, (2000) who observed high level of genetic diversity among Paraguayan soybean *Rhizobia*.

Possible duplication of the bacteria strains were observed in the dendrogram generated for the accession T3. The possible duplications were among *Rhizobium* strains 3, 13, 15 and 18 as well as strains 6, 7 and 19 beyond genetic similarity of 95 %. These strains had common attributes such as nodule size and produced either no bands or two bands. The strains formed two major clusters at a genetic distance of 23 % such that these could be thought of as being diverse.

At 11 % similarity distance, two major clusters were formed in the dendrogram for all the bacteria isolates for accession T5. This implies early divergence among the strains to form two clusters of strains 9 and 11. It is interesting to note that, despite this early divergence in the strains, more than half of these strains could be considered as possible duplicates.

Two major clusters were identified for all the isolates in accession T6 and these were at a genetic similarity of 9 % indicating great diversity among the isolates. Few strains were identical in this accession and included strains 12 and 13, and 10 and 16 as these were not differentiated at 100 % level of genetic similarity.

High genetic diversity was observed from the dendrogram generated for the bacteria strains of accession T7 in which two main clusters were formed at a similarity distance of 1.2 %. The diversity of the sub clusters was also observed at a genetic distance of 22 %. However, possible duplicates were found among the strains which included strains 5 and 18, 15 and 19, and 6, 13 and 14 since these were linked at a similarity level of 100 %.

Even at a genetic similarity level of 0.7 %, two major clusters were identified showing the dissimilar nature of the bacteria isolates in accession T8. It is, however, worth noting that even though high diversity seems to exist, three quarters of the isolates in the main cluster 1could be considered as possible duplicates as these were linked at a similarity level of 100 %.

Genetic diversity could also be observed in the bacteria isolates of accession T9 as the dendrogram generated differentiated these strains at a genetic similarity of 12.1%. The sub clusters within the major cluster 2 also exhibited some amount of diversity as they diverged very early at a similarity level of 33.6%. Possible identical strains were also identified among strains 1, 2, 5 and 6, and then 15, 18 and 19.

High genetic diversity was again observed in the bacteria strains of accession T10. These strains diverged very early at a similarity level of 0.3 %, indicating high diversity. However, almost three quarters of the strains could be considered as possible identical entities as they were undifferentiated at a genetic similarity of 100%.

Results obtained from all the similarity matrices suggest that, the relationship between the *Rhizobium* strains were different (El-Fiki, 2006).

The high genetic diversity observed in almost all the nodule bacteria isolates for all the nine accessions studied corroborate studies by Berrada *et al.*, (2012) who observed high diversity among *Rhizobia* strains. Also, the possible duplicates observed in all the accessions with the exception of accession T2 is in accordance with Anderson *et al.*, (2007), who indicated that for accessions to be considered as possible duplicates, their genetic similarity index should be equal to or greater than 95%.

4.4.3 Genetic Diversity among Inter-Accessional Rhizobial Isolates

The dendrogram generated for the inter-accessional nodule bacteria isolates revealed two main clusters at a genetic similarity of 0.7 % indicating a high diversity among the isolates of all the accessions. This is in agreement with findings of Irum *et al.*, (2009) and Young and Cheng, (1998), who indicated that genetic differences among *Rhizobia* isolates from the same area might be attributed to their adaptive factor which is possibly modulated by the differences in root exudates composition of different plants found in the rhizosphere from which the isolates are sampled. More than half of the isolates could be considered as possible duplicates since they were linked at a genetic distance of 100 %. Studies by Rincon *et al.*, (2007) also indicate wide genotypic diversity of rhizobial nodulating bacteria from wild woody legume species even within the same given geographic area.

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CHAPTER FIVE

GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The following conclusions can be drawn from the two major experiments that were conducted on Kersting's groundnut accessions in the Coastal Savannah Agro-Ecological Zone of Ghana.

1a. Germination of Kersting's groundnut is high (67.4-81.8%) for eight of the ten accessions.

b. The mean fresh and dry shoot weights per plant as well as the mean fresh and dry root weights per plant at 6 WAP were generally lower than those recorded at 8 WAP.

2a. There were no significant differences among accessions for mean number of pods per plant, fresh pod weight, dry pod weight, dry seed weight per plant, grain yield, shoot dry matter yield as well as harvest index.

b. Significant differences in the 100-seed weight existed among accessions with T7 recording the highest 100-seed weight of 14.46 g.

3. The accessions exhibited significant variations with respect to nodulation at 8 WAP. Accessions T4 and T7 produced the highest number of nodules as well as effective nodules at 8 WAP. Accession T7 again produced the highest mean dry nodule weight at 8 WAP.

4. Percent nitrogen yield for both roots and shoots also varied significantly among the ten accessions of Kersting's groundnut, with the roots contributing on the average one-third (1/3) of the total plant nitrogen, while the shoots contributed two-third (2/3). Total plant nitrogen yield as well as protein quality of both shoots and roots also varied significantly among the accessions.

5. Dry matter yield was positively correlated with nine traits investigated but negatively correlated with three other traits. Hundred seed weight was positively correlated with ten traits, although only moderately or poorly. Harvest index was highly positively correlated (r = 0.72) with only dry pod weight while grain yield was perfectly correlated (r = 1.0) with dry seed weight, showing that, selection for each of these pair of traits will lead to a corresponding improvement in the other.

6. Fresh nodule weight was positive and highly correlated with dry nodule weight at both 6 WAP and 8 WAP. Protein quality of both shoots and roots were positive and highly correlated (r = 0.88 and r = 0.77) with percent nitrogen of roots and shoots respectively.

7a. Grain yield per hectare under prevailing conditions in the coastal savannah agro-ecological zone comparably, was higher than that obtained in the Guinea Savannah Agro-Ecological Zone of Ghana.

7b. Nodulation in the coastal savannah agro-ecological zone also compared favourably to those obtained in the guinea savannah agro-ecological zone. Hence, the crop can be successfully cultivated in the Coastal Savannah Agro-Ecological Zone of Ghana.

8. Similarities and differences were observed within the various accessions with respect to nodule bacteria strains as well as between the accessions. Possible identical strains also existed among the isolates both within (with the exception of accession T2) and between the accessions.

5.2 RECOMMENDATIONS

5.2.1 Accessions for Future Breeding Work

Based on the yield and nodule parameters that were assessed, the following are recommended:

- Based on seed germination, Kersting's groundnut accessions T8 and T9 are most suitable for the Coastal Savannah Zone of Ghana.
- For high shoot dry matter production, Kersting's groundnut accessions T6, T7 and T8 are recommended whilst the accession T8 is recommended for higher grain yield.
- Accessions T3, T5 and T3, T7 should be considered when the aim is to cultivate Kersting's groundnut for high %N content in the roots and shoots.

5.2.2 Future Research Work

1. Research to identify the best cultural and management practices should be done to improve upon the yield of the crop.
2. Further research should also be conducted to identify the specific type and strains of *Rhizobia* nodulating the crop.
3. Phenotypic, morphological and biochemical characterization of the *Rhizobia* should be the focus of future work to ascertain the attributes of the bacteria (eg. pH tolerance, salt resistance and drought resistance) associated with the crop in order to improve upon them for better nodulation.
4. Different molecular methods and sequencing of the *Rhizobia* DNA should also be employed for phylogenetic classification at the species level.

APPENDICES

1 Germination percentage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	1266.99	422.33	4.97	
Groundnuts	9	1894.79	210.53	2.48	0.033
Residual	27	2294.37	84.98		
Total	39	5456.15			

2 Fresh shoot weights at 6 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	9571.1	3190.4	5.62	
Groundnuts	9	20783.7	2309.3	4.07	0.002
Residual	27	15329.9	567.8		
Total	39	45684.8			

3 Fresh shoot weights at 8 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	8326.	2775.	0.57	
Groundnuts	9	54568.	6063.	1.24	0.312
Residual	27	131725.	4879.		
Total	39	194619.			

4 Dry shoot weights at 6 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	370.898	123.633	15.41	
Groundnuts	9	532.834	59.204	7.38	<.001
Residual	27	216.588	8.022		
Total	39	1120.321			

5 Dry shoot weights at 8 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	531.2	177.1	1.24	
Groundnuts	9	1669.6	185.5	1.30	0.280
Residual	27	3840.4	142.2		
Total	39	6041.2			

6 Fresh root weights at 6 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	24.0301	8.0100	16.46	
Groundnuts	9	16.1041	1.7893	3.68	0.004
Residual	27	13.1420	0.4867		
Total	39	53.2763			

7 Fresh root weights at 8 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	6.276	2.092	1.60	
Groundnuts	9	10.725	1.192	0.91	0.530
Residual	27	35.294	1.307		
Total	39	52.295			

8 Dry root weights at 6 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	1.76079	0.58693	11.31	
Groundnuts	9	0.84652	0.09406	1.81	0.112
Residual	27	1.40089	0.05188		
Total	39	4.00820			

9 Dry root weights at 8 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	0.5150	0.1717	1.46	
Groundnuts	9	0.9714	0.1079	0.92	0.524
Residual	27	3.1727	0.1175		
Total	39	4.6590			

10 Shoot dry matter yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	456.32	152.11	4.32	
Groundnuts	9	591.32	65.70	1.87	0.101
Residual	27	950.04	35.19		
Total	39	1997.68			

11 Number of pods

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	3935.2	1311.7	10.07	
Groundnuts	9	2528.9	281.0	2.16	0.059
Residual	27	3515.9	130.2		
Total	39	9980.0			

12 Fresh pod weights

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	14.2571	4.7524	9.80	
Groundnuts	9	3.3222	0.3691	0.76	0.652
Residual	27	13.0994	0.4852		
Total	39	30.6787			

13 Dry pod weights

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	16.0863	5.3621	15.90	
Groundnuts	9	1.6802	0.1867	0.55	0.822
Residual	27	9.1051	0.3372		
Total	39	26.8716			

14 Dry seed weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	4.63236	1.54412	130.76	
Groundnuts	9	0.13547	0.01505	1.27	0.295
Residual	27	0.31884	0.01181		
Total	39	5.08666			

15 100 seed weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	0.1934	0.0645	0.49	
Groundnuts	9	11.1786	1.2421	9.51	<.001
Residual	27	3.5269	0.1306		
Total	39	14.8989			

16 Grain yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	1.808427	0.602809	130.55	
Groundnuts	9	0.052892	0.005877	1.27	0.296
Residual	27	0.124672	0.004617		
Total	39	1.985992			

17 Harvest index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	8.2110	2.7370	6.25	
Groundnuts	9	5.6869	0.6319	1.44	0.220
Residual	27	11.8300	0.4381		
Total	39	25.7279			

18 Number of nodules at 6 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	1285.1	428.4	2.85	
Groundnuts	9	2609.6	290.0	1.93	0.090
Residual	27	4058.7	150.3		
Total	39	7953.4			

19 Number of nodules at 8 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	1423.08	474.36	25.63	
Groundnuts	9	2942.02	326.89	17.66	<.001
Residual	27	499.68	18.51		
Total	39	4864.77			

20 Number of effective nodules at 6 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	37.475	12.492	1.75	
Groundnuts	9	92.125	10.236	1.43	0.223
Residual	27	192.775	7.140		
Total	39	322.375			

21 Number of effective nodules at 8 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	72.600	24.200	7.00	
Groundnuts	9	237.600	26.400	7.63	<.001
Residual	27	93.400	3.459		
Total	39	403.600			

22 Number of non-effective nodules at 6 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	2282.6	760.9	2.84	
Groundnuts	9	2353.1	261.5	0.98	0.482
Residual	27	7239.9	268.1		
Total	39	11875.6			

23 Number of non-effective nodules at 8 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	615.275	205.092	39.14	
Groundnuts	9	1726.625	191.847	36.61	<.001
Residual	27	141.475	5.240		
Total	39	2483.375			

24 Fresh nodule weight at 6 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	0.134274	0.044758	6.77	
Groundnuts	9	0.234353	0.026039	3.94	0.003
Residual	27	0.178480	0.006610		
Total	39	0.547106			

25 Fresh nodule weight at 8 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	0.058445	0.019482	17.44	
Groundnuts	9	0.132402	0.014711	13.17	<.001
Residual	27	0.030165	0.001117		
Total	39	0.221012			

26 Dry nodule weight at 6 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	0.0068316	0.0022772	5.41	
Groundnuts	9	0.0103889	0.0011543	2.74	0.020
Residual	27	0.0113601	0.0004207		
Total	39	0.0285806			

27 Dry nodule weight at 8 WAP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	0.0036	0.0012	38.37	
Groundnuts	9	0.0043	0.0005	15.50	<.001
Residual	27	0.0008	0.00003		
Total	39	0.0087			

28 % Nitrogen of roots

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	0.10321	0.03440	2.33	
Groundnuts	9	0.33701	0.03745	2.53	0.030
Residual	27	0.39943	0.01479		
Total	39	0.83965			

29 % Nitrogen of shoots

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	1.16692	0.38897	6.60	
Groundnuts	9	2.24270	0.24919	4.23	0.002
Residual	27	1.59130	0.05894		
Total	39	5.00092			

30 Nitrogen yields of roots

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	29.429	9.810	1.12	
Groundnuts	9	43.540	4.838	0.55	0.825
Residual	27	237.538	8.798		
Total	39	310.507			

31 Nitrogen yields of shoots

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	233307.	77769.	8.05	
Groundnuts	9	1078536.	119837.	12.40	<.001
Residual	27	260960.	9665.		
Total	39	1572804.			

32 Total plant nitrogen yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	280647.	93549.	10.72	
Groundnuts	9	994701.	110522.	12.67	<.001
Residual	27	235532.	8723.		
Total	39	1510879.			

33 Protein quality/crude protein of roots

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	4.3277	1.4426	2.74	
Groundnuts	9	16.7752	1.8639	3.54	0.005
Residual	27	14.2158	0.5265		
Total	39	35.3187			

34 Protein quality/crude protein of shoots

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	3	53.463	17.821	3.31	
Groundnuts	9	59.966	6.663	1.24	0.315
Residual	27	145.496	5.389		
Total	39	258.924			

35 Composition of Eppendorf Master Mix

Taq DNA polymerase

12.5 mMMgCl_2

75mM Tris-HCl at PH 8.3

3.75mMg $(OAC)_2$

0.25% Igepal-CA630

500uM of dNTPs

Stabilizers

36 Nucleotide lengths of primers used

Primer	Sequence	Length
RPO1	5-AATTTTCAAGCGTCGTGCCA-3	20 nucleotides
RPO4	5-GGAAGTCGCC-3	10 nucleotides
RPO5	5-AGTCGTCCCC-3	10 nucleotides
ERIC 1	3-CACTTAGGGGTCCTCGAATGTA-5	22 nucleotides
ERIC 2	5-AATGAAGTGACTGGGGTGAGCG-3	22 nucleotides