SYMBIOTIC NITROGEN FIXATION AND CONTRIBUTION TO SOIL NITROGEN BY GROUNDNUT (*ARACHIS HYPOGAEA* L) GENOTYPES IN THE NORTHERN GUINEA SAVANNA OF NIGERIA

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SEPTEMBER, 2014

DECLARATION

I declare that the work in this thesis entitled **"Symbiotic Nitrogen Fixation and Contribution to Soil Nitrogen by Groundnut (A*rachis hypogaea* L) Genotypes in the Northern Guinea Savanna of Nigeria"** has been carried out by me in the Department of Soil Science. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

AGAH, BONIFACE UNIMKE _____ _____

Name of Student Signature Date

Certification

This thesis entitled "SYMBIOTIC NITROGEN FIXATION AND CONTRIBUTION TO SOIL NITROGEN BY GROUNDNUT (*Arachis hypogaea* L) GENOTYPES IN THE NORTHERN GUINEA SAVANNA OF NIGERIA" by Boniface, Unimke AGAH meets the regulations governing the award of the degree of Master of Science in Soil Science of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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Abstract

Evaluation of existing groundnut genotypes for nitrogen fixation may be useful as selection criteria for high nitrogen fixation thereby minimizing the high cost of inorganic fertilizer and its associated environmental consequences. The objectives of this study were to determine symbiotic nitrogen fixation and contribution to soil nitrogen by groundnut (*Arachis hypogaea L*). The treatments consisting ten groundnut genotypes(SAMNUT 24, SAMNUT 22,ARRORSICGX-SM 00017/5/P_{15}/P_2, SAMNUT 10, ICIAR 7B, ARRORSICGX 000201/5/P_4P_{10}, SAMNUT 21, SAMNUT 23 and SAMNUT 14) and two rates of nitrogen fertilizer (0 and 30 kg/ha) were laid out in a split plot design with three replications under rain-fed conditions in 2011 and 2012. Nitrogen fixation parameters were measured/calculated as nodule number, nodule dry weight, plant total nitrogen, biologically fixed N, nitrogen derived from atmosphere (Ndfa) and soil nitrogen balance while agronomic data determined were shoot and root dry weights, 100-seed weight, pod and haulm yield, harvest index (HI) and nitrogen harvest index (NHI).

The difference between years was significant for most variables with 2011 statistically out-performing 2012 except for HI, pod yield and 100-seed weight which maintained similar trend in both years. ARRORS-ICGX 000201/5/P_4P_{10} (29.11 kg N/ha) and SAMNUT 23 (22.27 kg N/ha) were the best genotypes in terms of biological nitrogen fixation, nodule number, nodule dry weight, HI and NHI. SAMNUT 14 and 6AT which fixed relatively lower amount of N_2; 17.10 and 19.95 kg/ha respectively, impacted positively on the soil N balance than all other genotypes in 2011. These genotypes had lower NHI than the highest N_2 fixers which mean that most of the N derived from BNF was not translocated to the seeds but retained in other plant parts such as shoot and root.

Application of 30 kg N ha^{-1} significantly increased the agronomic and nitrogen fixation parameters. Wide variations exist in the pod yield across the genotypes. ARRORS-ICGX 000201/5/P_4P_{10} with an average pod yield of 2670 kg/ha was the best genotype in our recent finding. The result of the interaction between nitrogen rates and groundnut genotypes indicates that ARRORS-ICGX 000201/5/P_4P_{10} (2739 kg/ha) and SAMNUT 22 (3346 kg/ha) performed best even without the addition of the starter dose of nitrogen fertilizer signifying their ability to reduce the cost of production by saving cost on inorganic fertilizer. However, the N starter dose would be necessary to prevent soil N mining in view of the overall negative soil N balance obtained despite application of 30 kg N ha^{-1} to all genotypes. In conclusion, groundnut genotypes used in this study could be grouped into three distinct categories based on the amount of biologically fixed nitrogen and pod yield. ARROS-ICGX000201/5/P_4P_{10} and SAMNUT 22 are both high fixing and high yielding; 6AT was high fixing and low yielding; but contributing positively to the soil nitrogen balance; while SAMNUT 21 and ICAR 7B are low fixing. The remaining genotypes could not be classified into any of these groups. Similarly, the results of principal component analysis conducted in the measured parameters suggest that future breeding effort should be targeted at improving the efficiency of nodulation and biomass yield to enhance higher groundnut production in the northern Guinea savanna of Nigeria.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

The soils of the Northern Guinea Savanna (NGS) of Nigeria are dominated by Alfisols/Ultisols and associated Entisols and Inceptisols. The soils are inherently low in fertility and are characterized by low activity kaolinitic clays with coarse textured surfaces, slightly acid to neutral in reaction, low levels of soil organic matter (0.5 % organic carbon); low nutrient and water holding capacities (FMNAR, 1990, Odunze, 2006). The situation is further exacerbated when soils are continuously cropped without replacement of exported nutrients in harvest produce. In this condition, soil organic matter declines rapidly followed by extensive leaching of basic cations and development of soil acidity (Yusuf *et al.*, 2008).

Among the various plant nutrients in tropical soils, nitrogen (N) is the most limiting in the Northern Guinea Savanna owing to the high rate of organic matter decomposition and short fallow period pose by population increase (Wagner, 2012). The importance of nitrogen in the nutrition of crop cannot be overlooked owing to the fact that all living organisms including plants, use N in form of ammonia (NH_3) to manufacture amino acids, proteins, nucleic acids and other nitrogen-containing compounds for life (Lindemann and Glover, 2003; Agbede, 2009, Wagner, 2012).

Even though N is a key element required for plant growth, the effect of its deficiency in the soil is still widespread. This ranges from poor yields to total crop failure (Lindemann and Glover, 2003). Nitrogen fertilizers have since been used as a quick

solution to avert nitrogen deficiency in agriculture; its improper use has recently become a threat to the environment. There have been well documented reports of the negative effects of nitrogen to the environment. In a recent review by FAO (2001a) nitrogen runoff from farms has been reported to result in severe water pollution (both underground and surface) and eutrophication or build-up of nutrients in sea water which has encourage the growth of algae; that posed a serious threat to marine environment and native animal life.

1.2 **JUSTIFICATION**

In an effort to seek alternative to N based fertilizer, several biological strategies such as the use of green and farm yard manure have been encouraged among farmers. However, the quantity and/or quality of the materials used are/is not often sufficient to meet crops N demand. Hence, nitrogen deficiency is still widespread. On the other hand, biological nitrogen fixation (BNF) a microbiological process in the biosphere, which converts atmospheric dinitrogen into a plant-usable form through the microbial enzyme nitrogenase) offers another alternative of contributing most N to the ecosystem and to food production. Nitrogen fixation by legume amount to 20% of the estimated biological nitrogen fixed each year on earth far more than any other single system with figures similar to those of all the nitrogen fixed chemically by industry (Graham, 2004). The introduction of atmospheric nitrogen (N_2)-fixing legumes into the cropping system in contrast to inorganic fertilizers serve as a cheap, clean and renewable source of N to attain high crop yields (Peoples and Craswell, 1992). Total elimination of N based fertilizer of major crops has an annual benefit of US$4,484 million hence adding value to the society via the rechanneling of resources to other productive ventures that could raise the livelihood of the citizenry and, increasing the efficiency of legumes to fix

nitrogen may have an annual benefit of US$1,067 while decreasing nitrogen fertilization (Tauro, and Khurana, 1986). About US$6.67 billion that would have been spent on N based fertilizers was saved by developing countries as a result of N_2 fixed by leguminous crops (FAO, 2001a). Industrially, the manufacture of one ton of fertilizer requires an energy equivalent of 1,113litres of fuel oil (Dobereiner, 1978).

Groundnut (*Arachis hypogaea* L.) is one of the leguminous crops that can fix atmospheric nitrogen by symbiotic relationship with cowpea-type rhizobium which predominate in tropical soils. Nitrogen fixing ability in groundnut varies widely, depending on genotypes (Nambiar *et al.*; 1982, Belnap and Lange, 2001). Increase in BNF in groundnut might increase the yield of the legume since it utilizes large quantities of nitrogen to produce protein, as well as provide rather than deplete nitrogen in the soil thus allowing a grain crop to be grown in rotation with it (Tauro, and Khurana, 1986). Groundnut fixing nitrogen at the rate of 21 to 206 kg/ha N $year^{-1}$ has been reported (Yakubu *et al*., 2010).

Despite its ability to fix N_2, groundnut may suffer a period of N starvation under field conditions especially when soil N is very low until nodules begin to function (Roughley, 1976). Similarly, the plant may continue to suffer N deficiency, if effective strains of rhizobium are absent in the soil. In these conditions, complementation with low mineral nitrogen otherwise known as starter N might be justified. In the Nigerian savannas 20-30 kg/ha N has been recommended (FMANR, 1990). However, 30-40 kg/ha N or 30 kg/ha N or less (Mulongoy, 1993; Roughley, 1976) has also been recommended to be applied at planting (Mulongoy, 1993; FMNAR, 1990). This is because it stimulates the activities of the nitrogenase enzymes (Subba Rao, 2007). However, farmers in the agro-ecological zone do not usually comply with this recommendation due to high cost or unavailability of mineral fertilizers; thus, BNF is

expected to be sub-optimal. This may drastically affect soil N balance as well as crop yields such that groundnut production cannot be sustained over a long period of time. On the other hand, Yusuf *et al.*(2008) and Hardason *et al.*(1991) have shown that certain genotypes of soybean were able to make net positive contribution to soil N at very low N starter (20 kg/ha N) while others require external N addition for increased BNF, yield and contribution to soil N. No study has been conducted in the Northern Guinea Savanna of Nigeria to screen existing groundnut genotypes for tolerance to inherent soil nitrogen. Similarly, there is dearth of information on the symbiotic N fixation and contribution to soil N of the genotypes under the prevailing N fertilizer regime. This information will guide selection of groundnut genotypes according to soil and fertilizer N resources of the farmers.

1.3 Null hypothesis

The amount of nitrogen fixed by groundnut in the Northern Guinea Savanna of Nigeria is not affected by the genotype and nitrogen fertilizer.

1.4 Specific objectives of the study

The general objective of this study is aimed at evaluating the nitrogen fixation and N contribution of groundnut genotypes in an Alfisol in the Northern Guinea Savanna of Nigeria. The specific objectives are to;

1. Quantify N fixation of groundnut genotypes and their contribution to plant total N and yield.
2. Assess the influence of selected groundnut genotypes on soil N balance.
3. Determine the response of the groundnut genotypes to applied N.

CHAPTER TWO

LITERATURE REVIEW

2.1 NORTHERN GUINEA SAVANNA

2.1.1 Extent and Description

The moist Savanna agro ecological zone extends in a band across West and Central Africa (the Guinea Savanna zone) and includes the coastal lowland of East and Southern Africa (Odunze *et al*., 2004). In the West and Central Africa, the zone is divided into the Northern and Southern Guinea Savannas and Derived Savannas. The zone covers an area of approximately 56 million km which is 29% of the total crop land of sub-Saharan Africa. It supports about 33%of the population in these countries. The zone has about 42% of sub-Saharan Africa (SSA) human population with potential for increased crop and livestock production (Mc Intirea *et.al*., 1992; Winrock, 1992; Jabbar, 1995).

2.1.2 Climate

The Northern Guinea Savanna has a unimodal rainfall pattern with an annual precipitation of 900-1300mm and a growing period of 150-180 days (from June to October). The Derived Savanna has a bimodal rainfall pattern with annual precipitation of 1100 to 1500 mm and a growing period of 180 to 210 days (IITA, 1992; Tian *et al*., 1995).

2.1.3 Vegetation

Generally, the vegetation in the zone is dominated by fire-tender and fire-tolerant trees with an under storey of shrubs and grasses. In both the Northern and Southern Guinea Savanna, the tree covers varies from open woodland to light forest, while the Derived

Savanna was originally a closed forest which has been reduced as a result of farming activities. Trees commonly occurring in the moist Savanna include, *Isoberlina doka, Butryspernum paradoxi, Daniella Oliveri, Afzella Africana, Uapaca, Terminalia, ptericarpus spp* and shrubs and grasses such as *Imperata cylindrical, Andropogon spp* and *Hparrehia spp* (Kowal and Kassam, 1987; Carsky *et al*, 1998)

2.1.4 Crop and Cropping Systems

In a general survey of cropping systems in West and Central Africa from 1988 to 1990 covering: Nigeria, Benin Republic, Niger Republic, Togo, Cameroon, and Burkina Faso. Singh *et al*. (2004) identified 15 major cropping systems, in addition to several others which vary from farmer to farmer. In the forest and Guinea Savanna zones, cowpea is intercropped primarily with maize (*Zea mays* L.), cassava (*Manihot esculenta* Crantz), yam (*Dioscorea rotundata*), groundnut (*Arachis hypogaea* L.) and soybean (*Glycine max* L.). In the Northern Guinea Savanna (NGS) of Nigeria, cowpea is intercropped with maize, sorghum (*Sorghum bicolor* L.) and/or groundnut. Fallow and legumes play a vital role in the maintenance of soil fertility. Among the legumes, cowpea is the most important for food, fodder, cash, and the maintenance of soil fertility. A cereal crop may be grown in a mixture with a legume such as cowpea, groundnut, or soybean. In this system, the legume is planted 3 to 6 weeks after the cereals have been sown. This is a common or widespread practice in Northern Nigeria.

2.1.5 Soils

The moist Guinea savanna zone is dominated by four major soil groups. These are Alfisols, Entisols, Ultisols, and Inceptisols (Vanlauwe, 2002). Low activity kaolinitic clay is dominant in the clay fraction (Vanlauwe, 2002), and together with iron (Fe) oxyhydroxides form 80-90% of the constitution of the Savanna soils of Nigeria

(Vanlauwe, 2002). The soils have coarse-textured surfaces and when unprotected, the bare surface is prone to severe accelerated soil erosion (Odunze *et al*., 2004), supra-optimal soil temperature (Odunze, 2006), rapid decline in soil organic matter, reduction in biotic activities of soil fauna (Lal *et al*.; 1980), crusting and compaction (Odunze, 2006), and overall degradation of soil quality.

The soils are inherently low in organic matter content and coupled with the low clay content, the total N, S, P, exchangeable K, Cation exchange capacity (CEC), and buffering capacity are low. Native soil N is low and all cereal crops respond to N fertilizer amendments. The soils are acidic in reaction and have moderate P fixation properties (Odunze, 2006).

2.1.6 Constraint to Agricultural Production

The major constraints to agricultural production in the zone are weed infestation, erratic rainfall, and poor soil fertility (Yadav and Sachan, 1985). In the Northern Guinea Savanna for instance, unreliability of rainfall which leads to drought, increase the risk of crop production. The high evaporation rate especially in the early part of the growing season, is a major factor causing water loss in the Savanna zone (Yadav and Sachan, 1985). Unreliable rainfall early in the growing period can cause drought for crops (Adeoye, 1984).

Nutrient limitation, especially Nitrogen (N), is another major constraint to agricultural productivity in cereal dominated systems of the Savannas of Sub-Saharan Africa (SSA) (Olawale, *et al.,* 2009). Fertilizer has been identified as the main source of soil nutrients for agricultural production in the Savanna agroecological zone (Olawale, *et al.,* 2009). Weed control is a major challenge for farmers in the moist Savanna and it is caused by

scarce cash to purchase and lack of knowledge on the use of herbicides. Major weeds of the zone are *Imperata cylindrical, Cypressus spp, Striga spp, and Alectera spp* (Carsky *et al*., 1998). Most of the countries surveyed by FAO in the moist Savanna have reported substantial yield decline due to poor weed management, declining soil fertility and increase in soil erosion (FAO, 2001a).

2.2 BIOLOGICAL NITROGEN FIXATION

2.2.1 General Overview

Approximately 80 percent by volume of the atmosphere is nitrogen gas (N_2). Unfortunately, N_2 is unusable by most living organisms. Plants, animals and microorganisms can die of nitrogen deficiency, surrounded by N_2 they cannot use. All organisms use the ammonia (NH_3) form of nitrogen to manufacture amino acids, proteins, nucleic acids and other nitrogen-containing components necessary for life (Lindemann and Glover, 2003). Biological nitrogen fixation is the process that changes inert N_2 to biologically useful NH_3 (Lindemann and Glover, 2003). It is the second most important biological process on earth, after photosynthesis (Zuberer, 2003). This process is mediated in nature only by prokaryotes, including many genera of bacteria, cyanobacteria, and the actinomycetes *Frankia* (Lindemann and Glover, 2003 and Zuberer, 2005). Other plants benefit from nitrogen-fixing bacteria when the bacteria die and release nitrogen to the environment or when the bacteria live in close association with the plant. In legumes and a few other plants, the bacteria live in small growths on the roots called nodules. Within these nodules, nitrogen fixation is done by the bacteria, and the NH_3 produced is absorbed by the plant. Nitrogen fixation by

legumes is a partnership between a bacterium and a plant (Lindemann and Glover, 2003).

Biological nitrogen fixation can take many forms in nature, including bluegreen algae (a bacterium), Lichens and free-living soil bacteria. These types of nitrogen fixation contribute significant quantities of NH_3 to natural ecosystems but not to most cropping systems, with the exception of paddy rice (Subba Rao, 2007). Their contributions are less than 5 lbs of nitrogen per acre per year. However, nitrogen fixation by legumes can be in the range of 25-75kg/ha of nitrogen per acre per year in a natural ecosystem and several hundred pounds in a cropping system (Lindemann and Glover, 2003). Central to all these system is a N_2 fixing prokaryote containing the enzyme complex called nitrogenase which is responsible for the conversion of nitrogen to ammonia (Zuberer, 2005). Currently, the subject of BNF is of great practical importance because the use of nitrogenous fertilizers has resulted in unacceptable levels of water pollution (increasing concentrations of toxic nitrates in drinking water supplies) and the eutrophication of lakes and rivers (Al-Sherif, 1998., Dixon and Wheeler, 1986). Further, while BNF may be tailored to the needs of the organism, fertilizer is usually applied in a few large doses, up to 50% of which may be leached (Dixon and Wheeler. 1986.). This is not only wasted energy and money but leads to serious pollution problems, particularly in water supplies (Hamdi, 1999).

In review of the factors limiting biological nitrogen fixation, Haque and Jutzi (1984) observe that nitrogen fixation is the product of two symbiotically interdependent organisms (the host legume plant and the bacterium), it may be affected by the reaction of one or the other or both. As a broad generalisation, fixation is proportional to the vigour of the host plant and therefore is affected by the factors that affect plant growth,

i.e. water, temperature, nutrients and light. This generalisation may be upset by factors that specifically affect the activity of the rhizobium rather than the host. These may be temperature, soil pH, nutritional status (particularly N and Mo) and genetic specificity. It is not surprising, therefore, that measurements of the amounts of N fixed vary quite widely.

Four common approaches to enhance biological nitrogen fixation as described by Takishima *et al*, (1989) include; inoculation with proven strains, microbial screening for improved strains, host plant screening and breeding and adoption of cropping system and cultural practices. There are collections of effective rhizobia located at centres around the world for most, if not all legumes used in agriculture (Takishima *et al*, 1989). These strains may be screened to identify the most effective and competitive one(s) for a given agro ecosystem. Once elite strains have been identified, the legume under consideration is inoculated (Graham, *et al*., 2004). Seed inoculation using peat inoculants is the most commonly used method. However, studies are under way to assess the effectiveness of post planting inoculation as a corrective measure (Subba rao, 2007). Dual inoculation of rhizobia and mycorrhizal fungi has proven beneficial in some cases (Graham *et al*., 2004).

Breeders have developed plant varieties with promiscuous nodulation to obviate the need for inoculation with rhizobia. In some laboratories in the USA, plants that do not nodulate with indigenous rhizobia but only with introduced "super" strains are being developed (Graham *et.al*. 2004). Hence, screening of legume plants, with high N_2-fixing components can be carried out (Graham *et al*. 2004).

2.3 SYMBIOTIC NITROGEN FIXATION

Symbiotic nitrogen fixation is a component of biological nitrogen fixation (other component been associative nitrogen fixation involving species of *Azospirillum* that are able to form close associations with several members of the *Poaceae* (grasses), including agronomically important cereal crops, such as rice, wheat, corn, oats, and barley) (Wagner, 2012). According to Graham (1998), Symbiotic nitrogen fixation occurs in plants that harbour nitrogen-fixing bacteria within their tissues. The best-studied example is the association between legumes and bacteria in the genus Rhizobium. Each of these is able to survive independently (soil nitrates must then be available to the legume), but life together is mutually beneficial and only together can nitrogen fixation take place. Many microorganisms fix nitrogen symbiotically by partnering with a host plant. The plant provides sugars from photosynthesis that are utilized by the nitrogen-fixing microorganisms for the energy it needs for nitrogen fixation. In exchange for these carbon sources, the microbe provides fixed nitrogen to the host plant for its growth. The interaction between a particular strain of rhizobia and the "appropriate" legume is mediated by:

- a "nod factor" secreted by the rhizobia and
- transmembrane receptors on the cells of the root hairs of the legume.

- different strains of rhizobia produce different nod factors, and
- Different legumes produce receptors of different specificity (Graham, 1998).

If the combination is correct, the bacteria enter an epithelial cell of the root, then migrate into the cortex. Their path runs within an intracellular channel that grows

through one cortex cell after another. This infection thread is constructed by the root cells, not the bacteria, and is formed only in response to the infection.

When the infection thread reaches a cell deep in the cortex, it bursts and the rhizobia are engulfed by endocytosis into membrane-enclosed symbiosomes within the cytoplasm. At this time the cell goes through several rounds of mitosis without cytokinesis so the cell becomes polyploidy (Graham, 1998).

2.3.1 Assessments of Symbiotic Nitrogen Fixation

Early studies of biological nitrogen fixation were limited by the lack of techniques sensitive enough to measure nitrogen gains in plants or to measure the activity of the enzyme complex itself (Zuberer, 2005). Hence, an accurate method of measuring N_2 fixation is essential for any evaluation of usefulness of different N_2 fixing plants or different technologies, yet such a method has remained remarkably elusive (People *et al.,* 1989). As extensive literature on the subject already exists, including many recent and thorough reviews (e.g. Peoples *et al.,* 1989), only the main principles and assumptions of the different methods will be described here.

2.3.1.1 ^{15}N – Stable Isotope (Dilution method)

The best method of measuring N_2 fixation is to use an isotope of nitrogen other than ^{14}N, the isotope that make up virtually all of the N_2 in the atmosphere. Two isotope of N are useful as tracers in N_2 fixation experiments: the radioactive isotope ^{13}N which has a half-life of only 10.05 minutes; and the stable isotope ^{15}N. The short half life of ^{13}N

restricts its use to experiments lasting only a few hours, so that it is not generally useful for measuring N_2-fixation, but ^{13}N has been used for studies on the assimilation of the products of N_2-fixation in cyanobacteria (Thomas *et al*., 1977). The plant tissue or bacterial culture is incubated in an enclosed atmosphere which is enriched with $^{15}N_2$. After a period of incubation, the N in the biological materials is purified by digestion and distillation, and the proportion of ^{15}N atoms present is determined using mass spectrometry. The amount of N fixed can be calculated very precisely by the measurements of the total N and the proportion of ^{15}N in the material, if the ^{15}N enrichment of the experimental atmosphere is known. Recently, Gehringa and Vlek (2004) observed from their findings that the ^{15}N natural abundance methods failed to quantitatively estimate BNF in heterogeneous forests. ^{15}N provide means of estimating nitrogen fixation in situations where estimates based on yield or total N determinations are insufficiently specific.

2.3.1.2 N – Difference Method

This is an integrated method of measuring biological nitrogen fixation. This method has the advantage of giving a measure of the total amount of N_2 fixed over the length of the experiment and is indispensable for many laboratory-based studies. Here, it is possible to estimate N_2 fixation by comparing the yields and nitrogen content of plants grown with and without N_2 fixing bacteria (Zuberer, 2005). One example is comparing the yields of nodulated and non nodulated legumes grown under similar conditions of soil nitrogen. The nitrogen in the non fixing plant is a measure of the nitrogen acquired from soil and is substracted from the nitrogen of the fixing plant; the difference is attributed to N_2 fixation (Zuberer, 2005; Bergerson, 1980; Sprent and Sprent, 1990a; Weaver and Danso, 1994). Weaver and Danso (1994) indicated that the sensitivity of the method is

such that an increase of about 20kg N ha^{-1} would be required for detection of significant difference. The method is limited to high N fixing system (Zuberer, 2005).

2.3.1.3 N – Balance Method

In N-balance experiments the amount of N in each of the various pools (i.e. soil plants), is measured both at the beginning and the end of the experiment. Any gains unaccounted for are then attributed to N_2 fixation. Losses of N which are not measured (e.g. due to denitrification) will result in an underestimate of the amount of N_2 fixed. As the total amount of Nitrogen in the soil is generally large compared to the amount of soil N will result in a discrepancy in the estimate of N_2 fixation (Giller and Wilson, 1991).

2.3.1.4 Acetylene Reduction Assay (ARA) Measurement

The ability of the nitrogenase complex to reduce acetylene (C_2H_2) to ethylene (C_2H_4) forms the basis for the acetylene reduction assay. In this method, the system to be measure (whole plants isolated roots, soil cores, or bacterial cultures) is exposed to an atmosphere containing 11% acetylene and incubated under appropriate conditions (Zuberer, 2005). The concentration of acetylene is such that the nitrogenase complex is saturated with this substrate and dinitrogen. Samples of the gas phase is periodically collected and injected into the gas chromatograph for quantification of ethylene production from acetylene (Giller and Wilson, 1991). Application of acetylene reduction assays for measurement of N_2-fixation in soil is complicated by the effects of acetylene on other biological processes. For example, acetylene has been found to block the last steps of denitrification (Balderson *et al*., 1976) and autotrophic nitrification (Hynes and Knowles, 1978) so efficiently that it is used in experiments designed to

measure the processes. In addition, acetylene blocks bacterial oxidation of ethylene in soil so that endogenous ethylene accumulates (de Bont, 1976).

2.3.1.5 Xylem N Solutes (Ureide analysis)

The sap contained in the xylem of legumes when measured gives higher concentrations of nitrogenous solute; often with some specific enrichment of ureides or amide. In legumes, allantoin and allantoic acid are the principal ureides. These substances can be regarded as the ultimate product of nitrogen (N_2) fixation for a range of legume species and are transported in the xylem sap to support plant growth (McClure *et al.*, 1980). Both have a low C: N ratio, and so are conservative of carbon C relative to N when used in storage or transport. This approach of measuring the ureides found in nodulated legumes assumes that the rate of ureides output is direct measure of N_2 fixation where as other sources of N for the plant are presents as other compounds in the xylem sap (McClure, *et al*., 1980). Measurement of ureide and non – ureide N would, thus, permit determination of the relative contribution of fixed and absorbed N to plant development. One of the advantages of the xylem N solute is that the ureides can be readily separated and analyzed using methods which are readily automated (Atkin *et al*., 1984). The major limitations of this technique include the variation in the composition of the sap with the age of the plants and the difficulty in extraction of the sap (Peoples *et al.*, 1989).

2.3.1.6 Nitrogen Yield

This approach involves measuring increments of nitrogen in the soil/plant system over a period of time (usually a number of years) and estimating losses of nitrogen during the same period. Nitrogen fixation can be estimated as sum of both of these components.

Fundamental to this method is the need to measure accurately the different components, especially soil organic N which accounts for approximately 90% of total system N, mineral nitrogen, and rain water and plant material established (Dart, 1986). Losses in form of denitrification are also measure.

2.3.2 Significance of Symbiotic Nitrogen Fixation

The quantity of nitrogen needed for agriculture is projected to increase in the period to 2030 (Tilman, 1999) and in the USA could lead to greater environmental pollution. Reduced dependence on fertilizer N and attention to farming practices that favour the more economically viable and environmentally prudent N_2 fixation will benefit both agriculture and the environment (Vance, 2001). Since nitrogen is commonly the most limiting plant nutrient in arable farming in the tropics and also the most expensive element as a mineral fertilizer, biological nitrogen fixation (BNF) holds great promise for smallholder farmers in sub-Saharan Africa (Hardason, *et al.,* 1993). The prospect that the utilisation of legumes could be expanded substantially is anticipated by increasing demographic pressure and food demand which require the exploitation of BNF as a major source of nitrogen for plant protein (Hardason, *et al.*, 1993).

Alley farming systems which use leguminous woody species in the hedgerows can reduce or eliminate farmers' needs for commercial N fertilizer. Legumes are N-fixing systems that have long been used for biological nitrogen fixation in agriculture. The terrestrial flux of N from biological N_2 has been calculated to range from 139 to 170 t Nyr^{-1}(Burns and Hardy, 1975; Paul, 1988) It is smaller than the total soil N reserves but greater than the inputs of N fertilizers which is estimated to be $65*10^6$ $tNyr^{-1}$(Paul, 1988). While the accuracy of these figures may be open to question (Sprent and Sprent, 1990b), they do help illustrate the relative importance of BNF in

cropping and pasture systems and the magnitude of the task necessary if BNF is to be improved to replace a proportion of the 80 to 90 million tonnes of fertilizer- N expected to be applied annually to agricultural land by the end of the decade (Peoples *et al*., 1995). Much land has been degraded worldwide, and it is time to stop the destructive uses of land and to institute a serious reversal of land degradation (Burris, 1994.). BNF can play a key role in land remediation. An examination of the history of BNF shows that interest generally has focused on the symbiotic system of leguminous plants and rhizobia, because these associations have the greatest quantitative impact on the nitrogen cycle. A tremendous potential for contribution of fixed nitrogen to soil ecosystems exists among the legumes (Brockwell *et al.*, 1995a, Peoples, *et al.*, 1995, Tate, 1995). There are approximately 700 genera and about 13,000 species of legumes, only a portion of which about 20% (Burris, 1994) have been examined for nodulation and shown to have the ability to fix N_2. Estimates are that the rhizobial symbioses with the somewhat greater than 100 agriculturally important legumes contribute nearly half the annual quantity of BNF entering soil ecosystems (Tate, 1995). Legumes are very important both ecologically and agriculturally because they are responsible for a substantial part of the global flux of nitrogen from atmospheric N_2 to fixed forms such as ammonia, nitrate, and organic nitrogen. Whatever the true figure, legume symbioses contribute at least 70 million tonnes of N per year, approximately half deriving from the cool and warm temperature zones and the remainder deriving from the tropics (Brockwell *et al*., 1995a). Increased plant protein levels and reduced depletion of soil N reserves are obvious consequences of legume N_2 fixation. Deficiency in mineral nitrogen often limits plant growth, and so symbiotic relationships have evolved between plants and a variety of nitrogen-fixing organisms (Freiberg *et al*., 1997). Other recent studies by Burris, **(**1994), and Mandimba, (1995) revealed that the nitrogen contribution

of *Arachis hypogaea* to the growth of *Zea mays* in intercropping systems is equivalent to the application of 96 kg of fertilizer N ha^{-1} at a ratio of plant population densities of one maize plant to four groundnut plants.

2.4 SYMBIOTIC NITROGEN CONTRIBUTION OF LEGUMES TO SOIL N BALANCE AND ITS SIGNIFICANCE

Biological N_2 fixation represents the major source of N input in agricultural soils including those in arid regions. The major N_2-fixing systems are the symbiotic systems, which can play a significant role in improving the fertility and productivity of low-N soils. The Rhizobium-legume symbioses have received most attention and have been examined extensively (Hamdi, 1999). The Rhizobium-legume (herb or tree) symbiosis is suggested to be the ideal solution to the improvement of soil fertility and the rehabilitation of arid lands and is an important direction for future research.

Little information is available on the actual contribution of N-fixing plants to the N economy of soils. The scattered distribution and low numbers of N-fixing plants in most late seral-climax stands suggests that annual N gains on such sites would be small. Fahey *et al*., (1988) estimated that a *Lupinus argenteus* density of 1,000 plants/ha in older, south eastern Wyoming lodgepole pine (*Pinus contorta*) stands would add only 0.1 kg N/ha/yr. *Lupinus* growing in Utah aspen stands were reported to fix 0.6 kg N/ha/yr (Skujins *et al*., 1987).

The presence of N-fixing plants on a site in high numbers does not necessarily mean that significant amounts of N are being fixed. Dalton and Zobel (1977) estimated that less than 0.1 kg N/ha/yr was added to ponderosa and lodgepole pine stands in central Oregon by understories of *Purshia* (20 percent canopy cover). The amounts of light

reaching the soil surface and soil moisture levels during the growing season are critical factors in determining N-fixing plant activity (Sprent and Sprent 1990b).

2.5 NITROGEN FERTILIZATION AND GRAIN LEGUME PRODUCTION IN THE TROPICS

Plant growth in soils throughout the world is often restricted by the supply of available nitrogen and as a result, it is N supply other than the supply of any other nutrient in the soils that limits world crop production (Yusuf, 2008). Approximately 80 percent of the atmosphere is nitrogen gas (N_2). Unfortunately, N_2 is unusable by most living organisms, Plants, animals and microorganisms can die of nitrogen deficiency, surrounded by N_2 they cannot use. All organisms use the ammonia (NH_3) form of nitrogen to manufacture amino acids, proteins, nucleic acids and other nitrogen-containing components necessary for life (Lindemann and Glover, 2003). In sub - Saharan Africa, grain yield is declining in recent years (FAO, 2005). This is partly due to low N fertility of many Savannah soils especially in northern Nigeria where in addition to N, P, B and Mo deficiencies are reported (Kwari, 2005, Sandabe *et al*., 2000, Safyaru, 2004, Sandabe, 2008). With the removal of subsidies on fertilizers by the government, chemical fertilizers became unaffordable to the small holders who are the majority in the region (Yakubu, *et al*., 2010). Consequently, most of the N required for crop production comes largely from a judicious management of BNF in traditional cropping systems (Dakora and Keya, 1997). Thus, BNF is a cheaper means of improving soil fertility and productivity (Yakubu *et al*., 2010).

Generally combined N delays or inhibits nodulation and nitrogen fixation. Because of this adverse effect, N fertilisation usually is not recommended for leguminous crops (Yusuf *et al.*, 2008). However there may be situations where N has to be applied, such as to cereals in mixed cropping or rotations and then fertiliser may affect nitrogen

fixation of the legume crop (Hardason *et al*., 1991).The inhibitory effect of NO_3- on the N_2 fixation activity of legume root nodules has been under investigation for some time. Munns, (1977) and Gibson and Pagan, (1977); have recently published thorough reviews on the subject. There are two hypotheses as to the cause of this inhibition. One has been termed the photosynthate deprivation hypothesis which attributes the decrease in N_2 fixation activity to a diminished supply of photosynthate to the nodules caused by $N0_3$ reduction in the shoots (Oghorhorie and pate, 1971). The other hypothesis involves a more direct effect and attributes the inhibition to the formation of $N0_2$ in the nodules by bacteroid NO_3 reductase (Gibson and Pagan, 1977). Nitrate itself seems not to affect N_2 fixation activity in cultures of *Rhizobium spp*. 32HI but NO_2 inhibits fixation in cultures of Rhizobium spp. 32H1, *Rhizobium japonicum* bacteroid suspensions and crude *Rhizobium japonicum* bacteroid nitrogenase extracts (Kennedy *et al.*, 1975). In addition, NO_2 may form a NO compound with leghemoglobin (Subba Rao, 2007) and, thus, prevent leghemoglobin from binding O_2, which could interfere with the N_2-fixing process.

2.6 GENOTYPIC DIFFERENCES IN THE AMOUNT OF N_2 FIXED AND UTILIZED BY GRAIN LEGUMES

There is abundant information on the estimate N_2 fixed by the major species of grain legumes (Giller, 2001). However, very little studies have been reported on the genotypic variations that exist in the amount of N_2 fixed within their genotypes. This variation could be explained on the basis of the ability of the legume to form nodules with the necessary organisation and machinery for N_2 fixation. The length of time for which an annual plant can actively fix N_2 will depend, in part, on the time taken to complete its life cycle and, even in perennial plants, the period of N_2 fixation may be strongly seasonal. This in turn can be partly controlled by the environment- for instance, if the

rain end early in a seasonal climate, the maturity of annual species can be advanced. Differences in the amounts of N_2-fixation may vary enormously with the length of the growing season. Genotypes that mature in 90 days have a short time to nodulate and to fix N_2 before flowering and pod-fill occur, whereas long duration, indeterminate genotypes have a substantially longer period of N_2 fixation. The ability to form nodules and to begin fixing N_2 quickly is particularly important for short duration genotypes. In some grain legumes, such as soybean and *Phaseolus Vulgaris*, the timing of fixation will be longer if senescence of the nodules is delayed so that N_2-fixation continues during pod-fill. By contrast, no decline in rates of N_2-fixation during pod fill occurs in groundnut (Bell *et al*., 1994). Minchin *et al.*, (1996) observe that there is strong evidence that rates of N_2-fixation in legumes nodules are limited by the supply of oxygen and the strength of the sink for carbon in nodules will depend partly on the efficacy with which carbon is utilized for nitrogen fixation. Factors contributing to the overall efficiency of carbon use for nitrogen fixation include the provision of reductant and electrons to nitrogenase and the costs associated with assimilation and transport of fixed N to the shoot (Neves and Hungria, 1987).

2.6.1 Nitrogen Harvest Index (NHI)

NHI is simply the amount of nitrogen in seed as a proportion of total above ground biomass nitrogen (Bushby and Lawn, 1992). The major factor affecting NHI is the proportion of the seed N. High seed N tend to increased NHI whereas low seed N reduces it (Ayaz *et al.*, 2004). Increased NHIs in some grain legumes is often due to the low partitioning of N to leaves, roots and stem of crop; which suggest an efficient mobilisation of N in straw and leaves to seed (Ayaz *et al.*, 2004). To buttress this fact, (Rhodes, 1980) reported high NHI of 0.86 for groundnut, which he attributed to the low N concentration in the pod walls, stems and leaves. Also the results of Farrington *et al.*,

(1977) with *Lupinus angustifolius* and soybeans support this hypothesis. Also, Canvin, (1976) also observed that lodging of crops affect the NHIs by reducing it. Generally, NHIs increase with increased plant population (Ayaz *et al*, 2004). He attributed the increase to the close relationship that exists between HI and NHI. Greater NHI may be due to a greater depletion of the straw N reservoir. Selecting for high NHIs in grain legumes may also increase seed yield. The close relationship between NHI and HI of crop may allow HI to be used as an alternative selection criterion (Loffler and Busch, 1982).

2.7 BRIEF DESCRIPTION OF GROUNDNUT AND ITS IMPORTANCE

Groundnut or peanuts (*Arachis hypogeal L.*) originated in South America. It is a short herbaceous annual that produces its pods inside the soil. Groundnut was brought to West Africa through slave trade (Naab *et al.*, 2009). The crop is one of the principal oilseeds in the world. Until the mid-1980s it ranked third behind soybeans and cottonseed, but now rapeseed has passed groundnut in terms of world production, closely followed by sunflower seed. The emergence of rapeseed and sunflower seed may be attributed to health concerns by the industrial countries and the European Union's policy. Developing countries account for over 95% of world groundnut area and about 94% of total production. Production is concentrated in Asia and Africa, with Africa accounting for 35% of global area and 21% of total output mainly in Nigeria, Senegal and Sudan (RMRDC, 2004)

Groundnut high content of edible oil (50%) and protein (25%) make it a popular human food (Desire *et al.*, 2010). It is consumed either as a shelled nut or as oil, after pressing of the kernel, or in a range of other forms subject to various degrees of processing such as peanut butter, sauce, flour or confectionery items. Groundnut haulms whose feed

value is similar to that of lucerne are used as animal feed (Ahmad *et al.*, 2007). In some rural areas the empty shells are often burned on the farm or spread on the fields as a soil amendment. In addition, groundnut helps improve soil fertility through biological nitrogen fixation, and can thus contribute to significant improvements in the sustainability of cropping system (Bala *et al.*, 2011). Nutritionally, groundnut is a good source of minerals such as phosphorus (P), calcium (Ca), Magnesium (Mg) and Potassium (K) as well as the vitamins E, K and B (RMRDC, 2004).

2.8 SOIL AND CLIMATIC REQUIREMENT OF GROUNDNUT PRODUCTION

Groundnut is grown in a well drained sandy loam, or sandy clay loam soil. Deep well drained soil with a pH of 6.5-7.0 and high fertility, are ideal for groundnut. Runner and Spanish types are better suited to heavy textured soils than the Virginia types. The loss of pods is usually high in heavier soils. Putnam *et al.,* (2013) however suggest that organic matter should be maintained at a level of 1 to 2% to improve water-holding capacity of the soil and of supply plant nutrients. An optimum soil temperature for good germination of groundnut is 30^{o}C. Low temperature at sowing delays germination and increases seed and seedlings diseases (Singh and Oswalt, 1995).

2.9 FERTILIZER REQUIREMENT OF GROUNDNUT

Soil fertility is among important factors that influence crop production in tropical regions (Wandahwa *et al*., 2006) such that soil productivity is hampered by the deficiencies of major nutrients. It was concluded that phosphorus is essential for improving productivity of smallholder agriculture in sub-Saharan Africa (Snapp, 1998). It is also believed that groundnut requires large quantities of phosphorus, calcium and sulphur for seed development and oil quantity. Because nutrients are removed and

consequently lost as result of cropping with crop harvests, there is need to replace lost nutrients through the application of inorganic fertilizers in order to maintain a positive nutrient balance (Buah and Mwinkaara, 2009)

2.10 RESPONSE OF GROUNDNUT TO NITROGEN FERTILIZATION

Groundnut being a legume crop meets mostly its nitrogen requirement through fixation of nitrogen in the atmosphere. So, groundnut may not respond to large application of nitrogen fertilizers (Msrivani, 2009). Excess of nitrogen could result in too much of vegetative growth at the expense of groundnut pod production. In a nitrogen trial experiment by Lanier *et al.* (2005), it was observed that nitrogen increased pod yield linearly in three of six experiments ($p \leq 0.05$). However, in early stages of plant growth nitrogen is very much in demand when the plants are in the initial stages of nitrogen fixation. A good strategy for nitrogen management in groundnut cultivation is to apply a starter dose of 15 to 20 kg N/ha, and encourage nitrogen fixation by Rhizobia inoculation to meet the nitrogen needs of plants (Msrivani, 2009). The starter dose of nitrogen is side dressed along with phosphorus and potassium application just before sowing. This basal dose of nitrogen is applied preferably as ammonium sulfate as this fertilizer also contains sulphur (16%), an important nutrient for groundnut crop. Groundnut crop may not require any top dressing with nitrogen fertilizer. However, any requirements of top dressing of nitrogen need to be assessed by examining the nodules and nodulation for efficient biological nitrogen fixation by the crop. If the nodulation and nitrogen fixation is low or poor, then the crop need to be applied with 30 to 40 kg N/ha after 30 to 45 days of sowing. The top-dressing should be done at proper moisture level in soil followed by inter-cultivation or manual weeding (Msrivani, 2009)

CHAPTER THREE

MATERIALS AND METHODS

3.1 SITE DESCRIPTION

The experiment was conducted on one of the experimental fields (S13) of the Institute for Agricultural Research (I.A.R) Samaru located at an altitude of 686m above sea level, latitude 11°11'008□N and longitude7°36'52.1"E in the Northern Guinea Savanna of Nigeria (NGS). The NGS is characterised by a monomodal rainfall pattern with a mean annual rainfall of about 1011±161mm concentrated almost entirely in the five months (May/June to September/October) of the cropping season (Oluwasemire and Alabi, 2004). Soils in the experimental area are classified as Typic Haplustalf according to the USDA soil taxonomy (Ogunwole *et al.*, 2001) and Acrisols according to FAO-UNESCO legend (1994). The soil is low in inherent fertility: organic matter, cation exchange capacity and dominated by low activity clays (Jones and Wild, 1975; Odunze, 2003).

3.2 FIELD LAYOUT, TREATMENT AND EXPERIMENTAL DESIGN

The experimental area was marked out from the field, ploughed, disc-harrowed and ridged at an inter-row spacing distance of 0.75m. The various treatments consisting of eleven genotypes of groundnut; SAMNUT 24, SAMNUT 22, ARRORS ICGX-SM 00017/5/P_{15}/P_2, SAMNUT 10, ICIAR 7B, 6AT, ARRORS ICGX 000201/5/P_4P_{10}, SAMNUT 21, SAMNUT 23, SAMNUT 14, ICGL 5 and two rates of nitrogen fertilizer (0 kg ha^{-1} and 30 kg ha^{-1}) were arranged in a split plot design. Nitrogen rates was selected to represent the main plots while the sub plots consisted of eleven groundnut genotypes replicated three times giving a total treatment combination of 66. Each replicate was divided into two block running side by side with a demarcation of 1m apart, and 2m was allowed between replicates. A basal application of 20 kg/ha K as Muriate of Potash (60% K_20), 20 kg/ha P as Single Superphosphate (18% P_2O_5). One third of 30 kg/ha N of Urea (46% N) was equally applied at two weeks after planting (2 WAP); while the remaining part (two third) was applied at eight weeks after planting (8 WAP). The fertilizers were applied by banding about 5 cm away from the seed. One seed each of groundnut genotype was sown by hand at a spacing of 20 cm by 75 cm inter and intra row spacing respectively. Each plot size measure 3m by 3m and 1m and 2m where demarcated between sub plots and replication respectively. The total plot area was 34 by 65 m^2.

3.3 SEED CHARACTERISTICS

Table 3.1 Genotypes, maturity period and origin

Genotypes	Maturity period	Days to maturity	Origin
SAMNUT 24	Extra Early	60-70	IAR
SAMNUT 22	Medium	100-120	IAR
ARRORSICGX-SM 00017/5/P_{15}/P_2	Early	75-80	IAR
SAMNUT 10	Late	140-150	IAR
ICIAR 7B	Medium	100-120	IAR
ICIAR 6AT	Medium	100-120	IAR
ARRORSICGX 000201/5/P_4P_{10}	Early	75-80	IAR
SAMNUT 21	Medium	100-120	IAR
SAMNUT 23	Early	75-80	IAR
SAMNUT 14	Early	75-80	IAR
ICGL 5	Medium	100-120	ICRISAT

3.4 OBSERVATIONS/MEASUREMENTS

(1) Biomass yield (fresh and dry weight of shoot and root) g/plant: Total biomass yield was determined at eight weeks after sowing (8WAS), it was done by randomly collecting four plant from the two outer rows, separating it into the root and shoot biomass, oven-dried at 65^{o}C for 76hrs after which the samples were weighed to obtain their dry weight before grinding prior to chemical analysis.

(2) Nodulation: Assessment of nodule number, effectiveness and mass was done at 8WAP by carefully digging up the whole plant, washed off the roots in a gushing tap water to separate adhering soils, the nodules were then manually collected from the root and weighed after which they were collected into envelopes and dried at 65^{o}C to constant weight. The dried nodules were then weighed and thereafter counted to ascertain the actual nodule number per plant. Whereas, nodule effectiveness was determined by randomly collecting ten nodules from each of the genotypes; dissect using a razor blade to determine the colour. Those with pink colours were classified as effective nodules, while those with colours other than pink where classified as ineffective nodules.

***(3) Pods, grains and haulm yield*:** Pods, grains and haulm yields were determined at full maturity depending on the genotype inherent maturity period as presented in table 3.1 and also from field observation by harvesting plants from the two inner rows (net plot) measuring 3m by 1.5m. After harvesting, the harvested part was allowed in the field to further dry after which the pods were separated from the haulms and taken away from the field for further drying in the laboratory to about 12% moisture content. The grains were obtained by manually cracking the nuts and separating the shell from the grain. The haulms yield was determined by simply weighing the sun-dried haulm.

(4) 100 seed weight: 100 randomly selected seed were collected from the shelled nuts and weighed to obtain 100 seed weight for each of the plot.

3.5 SOIL AND PLANT ANALYSIS

3.5.1 Soil Analysis

Initial soil sampling was done at a depth of 0 -15cm for physico-chemical analysis of the inherent nutrient status. An auger was used to collect a total of 20 soil samples bulked to form composite sample from which sub sample was taken for the analysis. The collected soil samples were air-dried, sieved using 2-mm mesh sieve and bagged with polythene bags in readiness for the following physico-chemical laboratory analysis; Particle size distribution was determined by the hydrometer method, as described by Gee and Or (2002), using distilled water and calgon (sodium hexametaphosphate) as dispersing agents. While textural class was obtained from the USDA soil textural triangle; Soil pH was determined electrometrically in a soil to solution ratio of 1:2.5 (Hendershot *et al.,* 1993); Total nitrogen was estimated by micro-kjeldahl digestion method (Bremmer and Mulvaney, 1982); Organic carbon was measured by the method described by Olsen and Sommers, (1982). Available phosphorus was estimated by the Bray 1 method (Olsen and Sommers,1982); Exchangeable Ca^{2+}, Mg^{2+}, K and Na^{+} were extracted with 1N ammonium acetate buffered at pH 7.0 (Chapman, 1965). Exchangeable Ca^{2+} and Mg^{2+} were determined by EDTA complexometric titration while exchangeable K^{+} and Na^{+} were estimated by flame photometry (Jackson, 1962); Exchangeable acidity was determined by titration method (McLean, 1982). The effective cation exchange capacity (ECEC) was estimated by summation method of all the exchangeable acidity and exchangeable bases. The extractable micro nutrients such as zinc (Zn), iron (Fe), manganese (Mn), and copper

(Cu) were extacted with 0.1 NHCL and determined by Atomic Absorption Spectrophotometer.

3.5.2 Plant Analysis

Plant samples were collected at eight weeks after planting in both cropping seasons (2011 and 2012) for the determination of N-accumulation in the plant. Destructive sampling was carried out on four plants, two taken from each of the outer rows. The plant samples were separated into shoot and roots, washed with distilled water to removed adhering soils, placed in envelops and oven dried at 65^{o}C to constant weight was attained. After oven drying, shoots and roots were grounds and allowed to pass through a 0.5mm mesh before analysis for total N concentration using the micro kjedahl method (Bremmer and Mulvaney, 1982).

3.6 CALCULATIONS

3.6.1 Net N Contribution of Nitrogen Fixation to Soil N Balance

The net contribution of N_2-fixation to the N balance of the soil was calculated by the method described by Peoples and Craswell, (1992). Computed as;

Net N balance = NF – NS

or

Net N balance=NF-NT

Where

NF = the amount of N_2 fixed

NT=Total N in grain+haulm

NS = Total N in the seeds

N-derived from atmosphere Ndfa: This was estimated as the ratio of total N-fix to the total plant N uptake. Computed as;

%Ndfa = NF/Total plant N uptake*100

N uptake=Shoot or root dry weight /100*Shoot or root % N

3.6.2 Harvest Index (HI) and N Harvest Index (NHI)

3.6.2.1 Harvest index

HI = GY/BY

Where,

HI= Harvest index

GY= Grain yield ($kgha^{-1}$)

BY= Total biomass yield ($kgha^{-1}$) at harvest

HY=Haulm yield.

BY= GY + HY

3.6.2.2 N Harvest Index

NHI= Ng/Nt

Where;

NHI= N harvest index

Ng= Total grain N (kg N ha^{-1})

Nt= Total plant N (kg N ha^{-1}) at harvest.

3.7 STATISTICAL ANALYSIS

Individual analysis of variance was performed for each character in each year. Error variance for the two years was tested for homogeneity by Bartletts test (Gomez and Gomez, 1984). Combined analysis was carried out for those characters having homogeneous error variance for the two years. The analysis of variance at this stage was performed using the General Linear Model (GLM) procedure of SAS; (SAS Inst., 2000) because of its high sensitivity. Graphical illustrations were used to show interaction among variables. The effects of the various treatments and their interactions was compared using standard error difference (SED). Where treatments number was less than or equal to six, means were also separated using the Least significant difference (LSD). Pearson correlation analysis was equally conducted as a test of interdependent on all the characters using SAS 2000 package. Due to the limitation pose by correlation analysis to ascertained traits that are more likely to influence the overall yield and nitrogen fixation of groundnut; a factor analysis using the method of principal component analysis (PCA) was used to that effect. In Tables and Figures, results of the T-test and F-test symbolized by *, **, and *** indicates significance at the 5%, 1% and 0.1% level of probability respectively.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 EXPERIMENTAL SOIL AND RAINFALL AND TEMPERATURE CHARACTERISTICS IN 2011 AND 2012

4.1.1 Experimental Soil Characteristics in 2011 and 2012

Some physical and chemical properties of the site before commencement of the study were determined before the establishment of the trials in 2011 and 2012 and the results obtained are as shown in Table 4.1. The results showed that the texture of the soil in both years was sandy-loam. This may be due to sorting of soil materials (such as decomposed roots, leaves etc) by biological activities, clay migration (Illuviation/elluviation) or erosion by run–off or a combination of these factors (Ojanuga, 1979).

The soil reaction was slightly acidic (5.30-6.00) but did not pose any limitation to groundnut production. The organic carbon and total N were both low in the soil in both years. However, there was a slight increase of total nitrogen in 2012 than in 2011 which could probably be due to the residual nitrogen resulting from mineralisation of below ground residues of previous groundnut crop. Similarly, decrease in organic carbon (OC) in 2012 might be due to increase in soil N which will enhance soil organic matter decomposition by soil organisms. The available P content and the exchangeable bases fall within the medium class for Nigerian soils (FMANR, 1990). The experimental soil can be classified as moderately suitable for the cultivation of most arable crops according to the modified FAO suitability classification (Young, 1976).

The exchangeable acidity and effective cation exchange capacity (ECEC) were all low in the soil. The extractable micronutrients (Zn, Fe, and Mn) were all high while the extractable Cu was medium in the soil (Odunze, 2006). Hence, the micronutrients were

considered adequate and did not limit the groundnut production (FAO, 2001b). Generally, the soils properties were typical characteristic of alfisols of Northern Guinea Savanna of Nigeria as described by Odunze, (2006) and the level of the various nutrient element fall within the ranges described for Nigeria soils (FAO, 2001b).

Table 4.1: Physical and chemical properties of the soil of the experimental site in 2011 and 2012

	Level in soil		
Soil Properties	2011	2012	Average
Sand (%)	67.10	64.00	65.55
Silt (%)	19.80	20.00	19.90
Clay (%)	13.10	16.00	14.55
Textural class	Sandy-loam	Sandy-loam	Sandy-loam
pH 1:2.5 water	5.30	6.00	5.65
pH 1:2.5 $CaCl_2$	5.10	5.15	5.13
Organic Carbon (g kg^{-1})	5.37	2.28	3.83
Total Nitrogen (%)	0.15	0.19	0.17
Available P (mg kg^{-1})	11.00	8.32	9.66
Exchangeable Ca^{2+}(Cmol/kg)	2.32	3.15	2.74
Exchangeable Mg^{2+} (Cmol/kg)	0.40	1.01	0.71
Exchangeable K^{+}(Cmol/kg)	0.20	0.16	0.18
Exchangeable acidity(Cmol/kg)	0.18	0.12	0.15
ECEC (Cmol/kg)	3.10	4.44	3.78
Extractable micronutrient (mg/kg)			
Zn	2.28	1.86	2.07
Cu	1.00	1.00	1.00
Mn	16.00	13.00	14.50
Fe	9.00	10.00	9.50

ECEC = Effective Cation Exchange Capacity

4.1.2 Rainfall and Temperature Distribution in 2011 and 2012

Results of the meteorological data generated during the period of the experiment are presented in Fig. i (a and b) and Fig. ii (a and b). These figures show that total annual rainfall was slightly higher in 2012 (1333 mm) than in 2011 (1207 mm). Although in both years, rains started in April but result shows that the rains came much earlier within the second ten days period in 2011 whereas in 2012 it was within the third ten days interval. The total rainfall in both years was greater than the long term average reported for the zone. Similarly, temperature (minimum and maximum) variation was equally observed in both years with average minimum temperatures of 18^{o}C and 19^{o}C and maximum temperature 35^{o}C and 34^{o}C observed in 2011 and 2012 respectively. The values were both above the long term mean temperature of 21.05^{o}C and 33.47^{o}C average minimum and maximum temperature reported by Oluwasemire and Alabi, (2004).

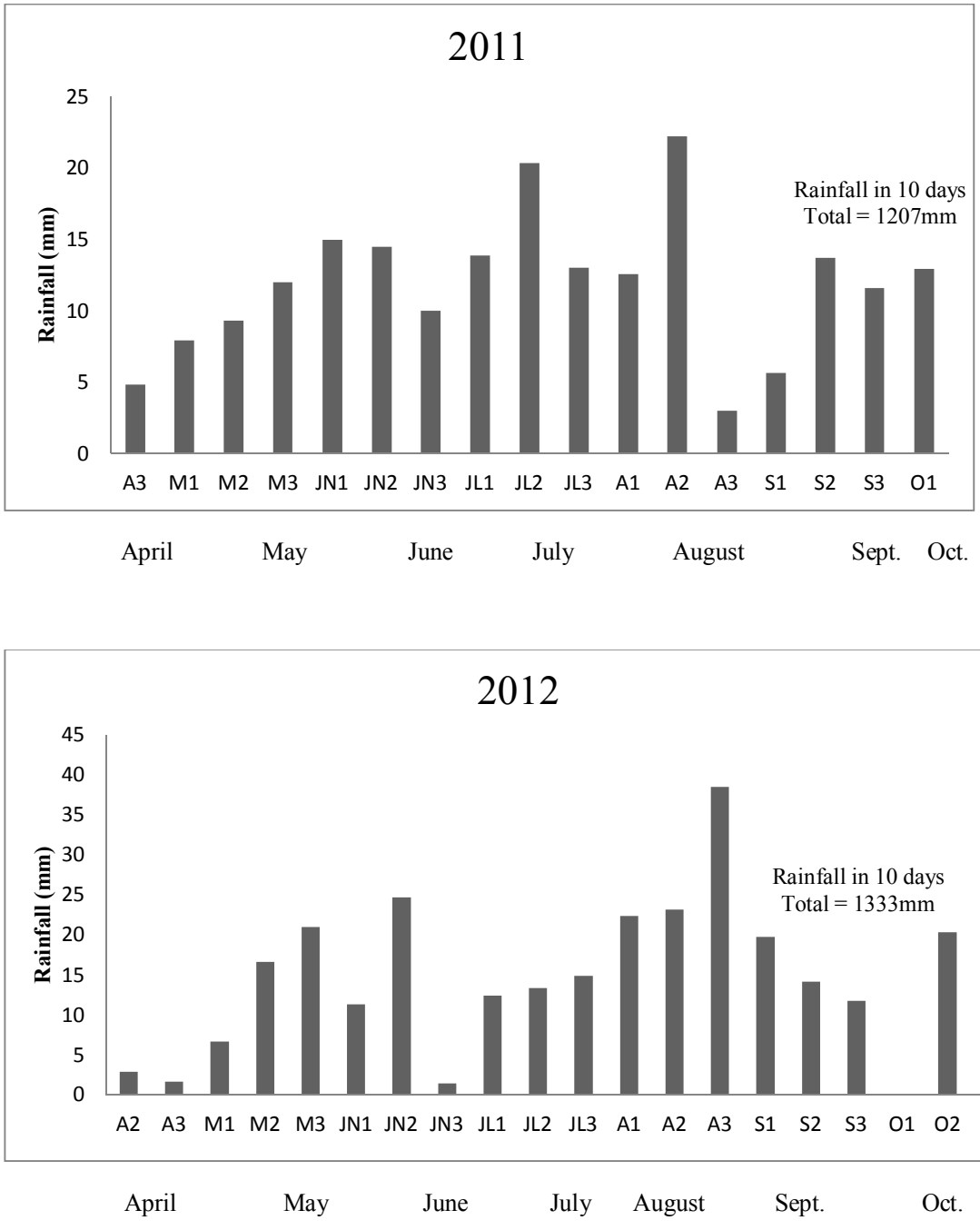

Fig. i. Rainfall patterns in Samaru in 2011 and 2012

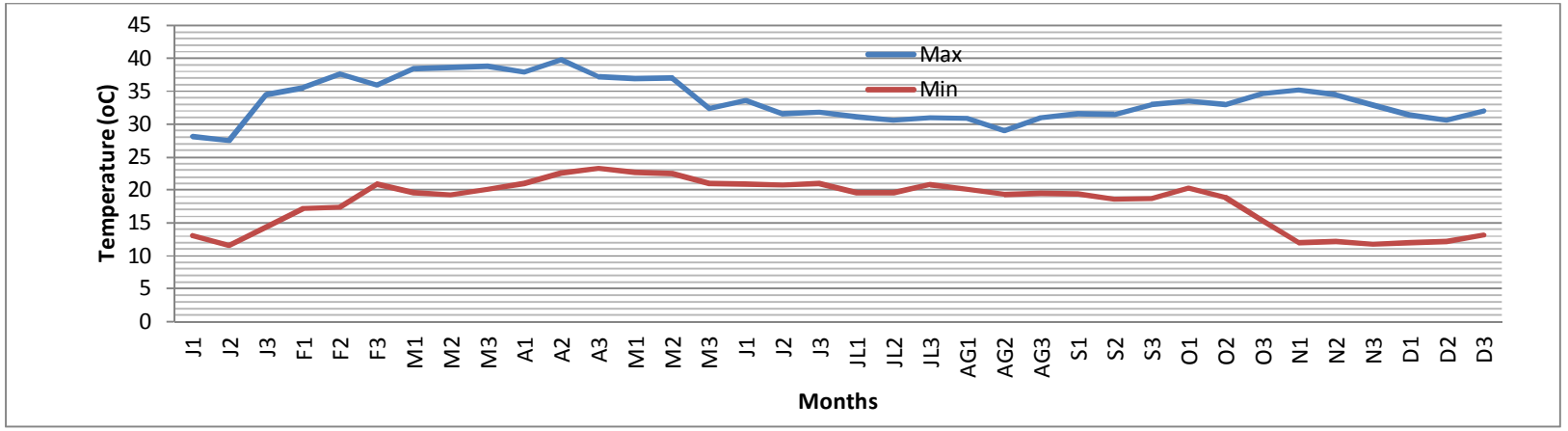

Fig. ii a. Ten days Mean monthly temperature distribution in Samaru (2011)

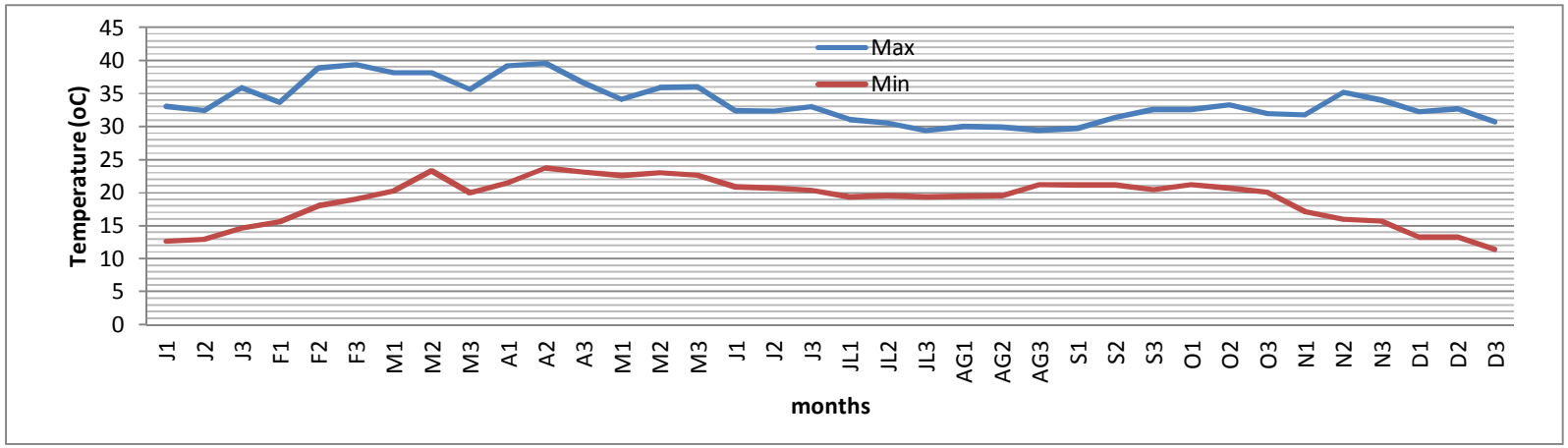

Fig. ii b. Ten days Mean Monthly Temperature Distribution in Samaru (2012)

Definition of abbreviation: J, F, M, A, M, J, JL, AG, S, O, N and D represent January to December in that order.

The numbers 1, 2 and 3 represent first, second and third ten days of each month.

4.2 EFFECT OF N FERTILIZER AND GENOTYPES ON NODULATION OF GROUNDNUT

4.2.1 Nodule Number in 2011, 2012 and Combined

The number and weight of nodules are commonly used as the criteria of effective complementary interaction between legume and micro- symbionts; thereby correlate on the whole with the rate of atmospheric nitrogen fixation (Datsenko *et al.*, 1997, Tahir *et al*, 2009).The number of nodule differed significantly depending on the genotype in both 2011 and 2012 cropping seasons as shown in Table 4.2. The result of the combined analysis of variance show a significant difference in nodule number across the two years with higher nodule number found in 2011 over 2012. Similarly, a highly significant difference was found among the groundnut genotypes in both years. In 2011 for instance, the highest nodule number (greater than 100 per plant) was observed in SAMNUT 22 and SAMNUT 24. These were followed by ARRORS ICGX-SM 00017/5/P_{15}/P_2 and ARRORS ICGX 000201/5/P_4P_{10} while all the remaining genotypes had less than 100 nodules per plant. This trend was somehow maintained in 2012 where the best genotypes in terms of nodule number were ARRORS ICGX 000201/5/$P_4P_{10,}$ SAMNUT 24, SAMNUT 22 and SAMNUT 23. The least nodule number in both 2011 and 2012 was found in SAMNUT 21.

Application of 30 kg N/ha significantly reduced nodule numbers compare to the control (0 kg N/ha). This result was consistent for both years. Generally, the significant difference observed in the N rates whereby high nodule number was observed in the control over the 30 kg N/ha is not surprising but confirming the result of numerous researches on nitrogen fertilizer and nodulation that concluded the suppressing effect of nitrogen fertilizer on nodulation of legumes. This is often the case particularly when applied in relatively higher quantity (Tahir *et al.* 2009 and Munns, 1977). Meanwhile,

the inhibitory effect of NO_3 on nodules has been recognised by Rhizobiologist and has been under investigation. People *et al.* (1989) and Takishima *et al.* (1989) in their separate findings attributed the effect of nitrogen on nodulation to the inhibition of the rhizobium infection process via the impairment of the recognition mechanism by nitrates. The result on the interaction between nitrogen and genotypes shows a significant ($P<0.05$) increase in nodule number of ARRORS ICGX 000201/5/P_4P_{10} even at 30 kg N/ha (Fig. iii). This trend was only found in 2011.

The number of nodules formed by promiscuous legume genotypes depends on the prevailing environmental conditions and the population of indigenous rhizobia during the process of nodulation (Yusuf *et al.*, 2008, Subba Rao, 2007, and Zuberer, 2005). Though low soil fertility has been reported by various author to inhibit initiation of nodules, but our results show high nodule number at low soil nitrogen level (0 kg N/ha) relative to improve soil N (30 kg N/ha) condition. The promotion of high nodule number at low soil N could be attributed to the considerable energy that the root must expend to move the nitrogen through the cell membranes from the soil into the roots. Once the nitrogen is inside the plant, more energy is needed to convert it to a form that can be metabolized by the plant. This stress condition will tend to force the legumes to produce more nodules relative to when soil N is readily available (Biederbeck *et al.*, 2005). However, the increase in nodulation by ARRORS ICGX 000201/5/P_4P_{10} at 30 kg N/ha could be attributed to the tolerance of the nitrogenase enzymes, mediated by a non – covalent inhibitory mechanism in the bacteria presence in the nodules (Pope *et al.,* 1985), although not confirmed in our present study and may also be due to the fact that the 30 kg N/ha was not sufficient enough to suppress nodulation completely due to the relatively low soil nitrogen characterising the experimental site. This is true occasioned by the fact that lower nodulation was recorded in the second trial following groundnut

rotation which could be as a result of the residual effect of BNF to soil nitrogen pool. Tahir *et al.* (2009), however attributed the decrease in nodulation at higher nitrogen dosage to a decrease in the activities of rhizobium.

Apart from the widely reported effect of nitrogen on nodulation, environmental stress could play a significant role on nodulation. Typical environmental stresses faced by the legume nodules and their symbiotic partner (*Rhizobium*) may include photosynthate deprivation, water stress, salinity, temperature, heavy metals, and biocides (Walsh, 1995). A given stress may also have more than one effect: e.g., salinity may act as a water stress, which affects the photosynthetic rate, or may affect nodule metabolism directly. The most problematic environments for rhizobia are marginal lands with low rainfall, extremes of temperature, acidic soils of low nutrient status, and poor water-holding capacity (Bottomley, 1991).

Table: 4.2: Effect of N fertilizer and genotypes on nodulation of groundnut

Genotype (G)	Nodule number plant^{-1}			Nodule fresh weight (mg/plant)		
	2011	2012	Combined	2011	2012	Combined
SAMNUT 24	138.00	66.00	102.00	173.33	70.83	122.08
SAMNUT 22	140.00	52.00	96.00	150.00	74.17	112.09
ARRORS ICGX-SM 00017/5/P_{15}/P_2	114.00	48.00	81.00	158.33	70.83	114.58
SAMNUT 10	61.00	48.00	55.00	89.33	50.00	69.67
ICIAR 7B	90.00	33.00	62.00	115.33	25.00	70.17
6AT	78.00	48.00	63.00	100.33	46.25	73.29
ARRORS ICGX 000201/5/P_4P_{10}	113.00	71.00	92.00	169.17	92.93	131.05
SAMNUT 21	51.00	19.00	35.00	89.33	21.67	55.50
SAMNUT 23	84.00	53.00	69.00	124.00	50.42	87.21
SAMNUT 14	59.00	48.00	54.00	120.50	62.50	91.50
SE±	13.71**	8.67**	8.83	17.12*	11.90**	10.53
Nitrogen (N) (kg/ha)						
0	114.00	56.00	85.00	153.30	57.40	105.35
30	71.00	36.00	54.00	104.63	46.09	75.36
Mean	93.00	46.00	66.00	128.97	51.75	88.30
SE±	4.60**	4.02**	3.93	7.66*	5.52**	5.30
Interactions						
G*N	*	NS	NS	NS	NS	NS

NS=Not significant at 5% level of probability, *Significant at 5% level of Probability, **Significant at 1% level of probability.

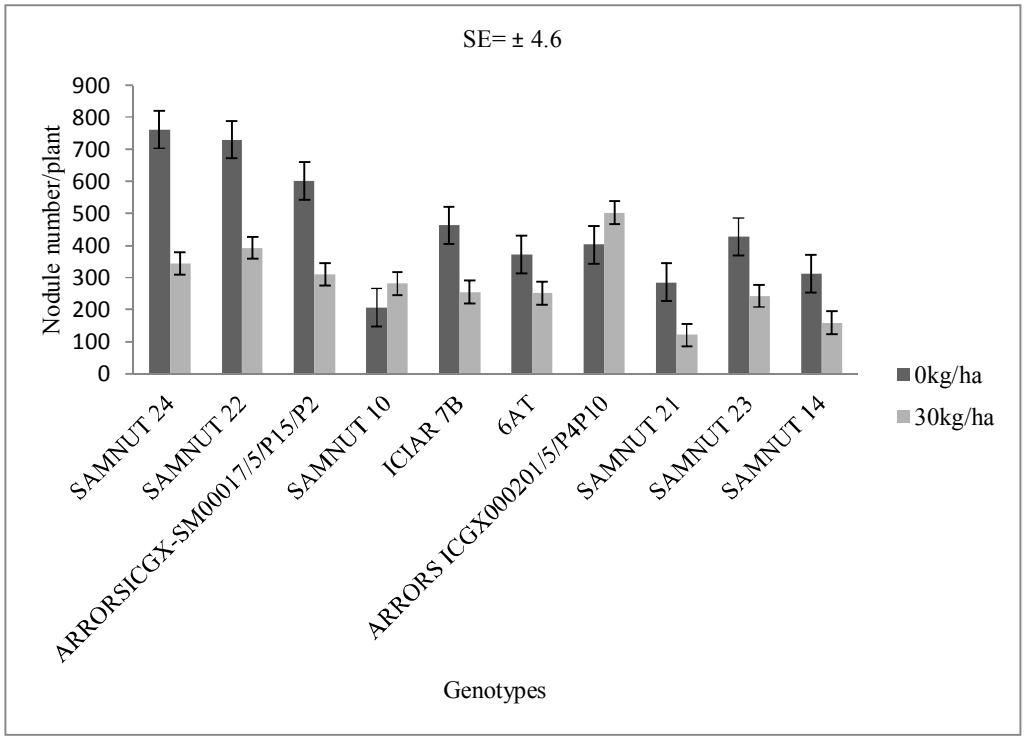

Fig. iii: Interaction of genotypes and nitrogen rates on nodule number of groundnut at 8WAP in 2011.

4.2.2 Nodule Weight in 2011, 2012 and Combined

The results on nodule weight as presented in Table 4.2 above shows a significant ($P< 0.05$) difference among the genotypes in both years. Higher nodule mass was recorded in 2011 than in 2012. The highest and lowest nodule weight was recorded in SAMNUT 24 and SAMNUT 21 respectively in 2011 and ARRORS ICGX 000201/5/P_4P_{10} and SAMNUT 21 in 2012. The trend was similar as in nodule number where genotype with higher nodule number recorded higher weight and vice versa. Interaction between genotype and N rate shows no significant ($P>0.05$) difference in both years. But a highly significant ($p<0.001$) difference was observed between the N rates in 2011 with the control recording higher nodule weight than the 30 kg N/ha. The increase could be attributed to the high nodule number recorded. Okito *et al.* (2004) attributed the

variation in nodulation by legumes to the inherent morphological differences existing among groundnut genotypes. The lack of significant difference observed in 2012 could be due to improved soil N balance of the experimental area arising from the contribution of BNF in the residues of the previous groundnut crop.

4.3 EFFECT OF GENOTYPE AND N FERTILIZER ON BIOMASS ACCUMULATION OF GROUNDNUT

4.3.3 Shoot Dry Matter Yield in 2011, 2012 and Combined

From the combined analysis of variance, the effects of genotypes and nitrogen fertilizer on shoot biomass was significant. The results clearly showed that significant difference ($p<0.05$) exists among genotype in both 2011 and 2012 cropping season (Table 4.3). Among the genotypes, SAMNUT 22 gave an outstanding performance relative to SAMNUT 23 and SAMNUT 14 with the least in ICIAR 7B. Increasing the rate of N fertilizer significantly increased the shoot biomass in 2011 but the trend was not maintained in 2012 where increased nitrogen rate does not translate to higher shoot biomass. Generally, the results of the combined analysis of variance further point to the fact that application of a starter dosage of nitrogen fertilizer is still justifiable for increased shoot biomass production in groundnut. Year effect was significant with higher average shoot biomass recorded in 2011 than in 2012. Interaction between genotype and nitrogen rate was significant for shoot biomass in 2012 but was not the case in 2011 where consistently higher shoot biomass yield was observed in the control of SAMNUT 22, ARRORS ICGX-SM 00017/5/P_{15}/$P_{2,}$ 6AT and SAMNUT 23 relative to the 30 kg/ha N (Fig. iv). The response of the groundnut genotype at increase N application rate as shown by our result is in agreement with Rego and Seeling (1996) who failed to attribute the variation to increased N-availability as reflected by our findings; considering the fact that yield was different only at low N fertilizer levels

where N availability was a limiting factor. Factors such as pest and disease pressure could equally amount to differences in shoot dry matter among groundnut genotypes. Meanwhile, the lack of significant difference occurring at the nitrogen rates of application in 2012 could be an indication of the mineralisation of the fallen leaves and roots mass resulting from previous cropping system which tends to retained part of the N in the soil organic N pool. This could be the most probable reason for the significant interaction observed in the shoot dry matter in 2012 in agreement with Rego and Seeling (1996). More so, differences in biomass production has been attributed to genetic variability among groundnut genotype by Pimratch *et al*. (2008)

4.3.4 Root Dry Matter Yield in 2011, 2012 and Combined

Root dry matter of the groundnut genotypes at mid-podding stage is presented in Table 4.3. Differences in root dry matter among genotype were significant ($P< 0.05$) in both 2011 and 2012 and also when data for the two years were combined and analyzed. However, the overall mean separation shows that increasing the rate of N fertilizer from 0 kg/ha to 30 kg/ha significantly increased root dry matter across all the genotypes in 2011 but was not sustained in 2012 where no significant difference was observed among the nitrogen rate of application. SAMNUT 23 was the best, though not significantly different from ARRORS-ICGX-00017/5/P_4P_{10} in 2011 closely followed by SAMNUT 24 which was not significantly different from ARRORSICGX-SM00017/5/P_{15}/P_2, 6AT, ICIAR7B, SAMNUT 22 and SAMNUT 14, with the least recorded in SAMNUT 10. The trend was consistent over the two years duration as shown by the combined analysis.

The result of the interaction between genotype and N rates was highly significant ($P<0.01$) for root dry matter as shown in Fig. iv. below. Increasing the N rates result to an increased root biomass of all the genotype. However, greater effects was observed in the genotype SAMNUT 23 and SAMNUT 24, closely followed by ARRORS ICGX-SM00017/5/P_{15}/P_2 and ARRORS ICGX000201/5/P_4P_{10}, with the least effect observed in SAMNUT 10 and SAMNUT 21 which tend to remain unaffected even with increasing level of N fertilizer. Genotype *N rate interaction was significant ($p<0.05$) in 2011 whereby SAMNUT 21 tend to performed better in the control over the 30 kg/ha N rates. The 2012 results however was in agreement with the work conducted by Kaushik *et al.* (1995) who observed a poorly developed root system of pigeon pea (*Cajanus cajan*) after applying up to 90 kg/ha of nitrogen fertilizer which further resulted to a reduction in the shoot and root dry weight. The increase in crop root biomass in 2012 over 2011 could be as a result of reduction in soil bulk density as a result of continous cultivation and increase in soil moisture availability occasioned by higher rainfall in 2012 in agreement with Simmonds and Azam-Ali. (1989).

Table 4.3 Effects of genotype and N fertilizer on biomass accumulation of groundnut

Treatment	Shoot dry weight/plant (g)			Root dry weight/plant (g)		
Genotypes (G)	2011	2012	Combined	2011	2012	Combined
SAMNUT 24	23.48	15.47	19.48	13.63	3.34	8.49
SAMNUT 22	27.29	21.42	24.36	9.63	2.59	6.27
ARRORS ICGX-SM 00017/5/P_{15}/P_2	22.63	19.47	21.05	11.56	1.42	6.49
SAMNUT 10	20.41	14.65	17.53	5.20	1.54	3.37
ICIAR 7B	17.09	16.94	17.02	10.58	2.4	6.49
6AT	25.14	17.91	21.53	11.55	2.59	7.07
ARRORS ICGX 00201/5/P_4P_{10}	24.64	20.91	22.78	14.08	2.54	8.31
SAMNUT 21	21.29	14.92	18.11	4.81	1.51	2.34
SAMNUT 23	21.83	18.72	20.28	14.92	2.74	8.83
SAMNUT 14	25.58	16.37	20.98	9.44	2.64	6.04
ICGL5	22.83	9.67	16.25	6.95	1.52	4.24
SE±	1.69**	1.07**	1.03**	1.02*	0.35**	0.56**
N rates (kgha^{-1})						
0	19.41	17.13	18.27	6.73	3.2	4.79
30	26.45	16.77	21.61	13.69	3.06	8.38
Mean	22.93	16.94	19.93	10.21	3.13	6.67
SE±	0.72**	0.50^{NS}	0.46**	0.43**	0.16^{NS}	0.25**
G *N						
Significance	NS	**	*	*	NS	**

NS=Not significant at 5% level of probability, *Significant at 5% level of Probability, **Significant at 1% level of probability.

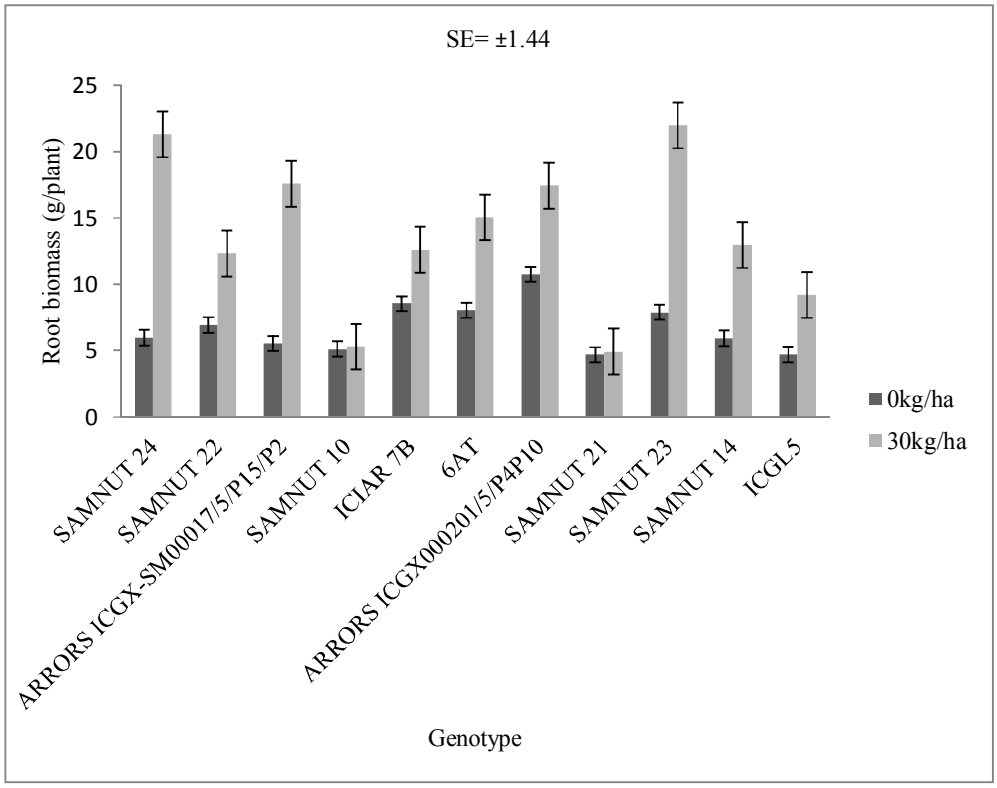

Fig iv: Interaction of genotype and nitrogen rate on root dry weight of groundnut at 8WAP in 2011 cropping season.

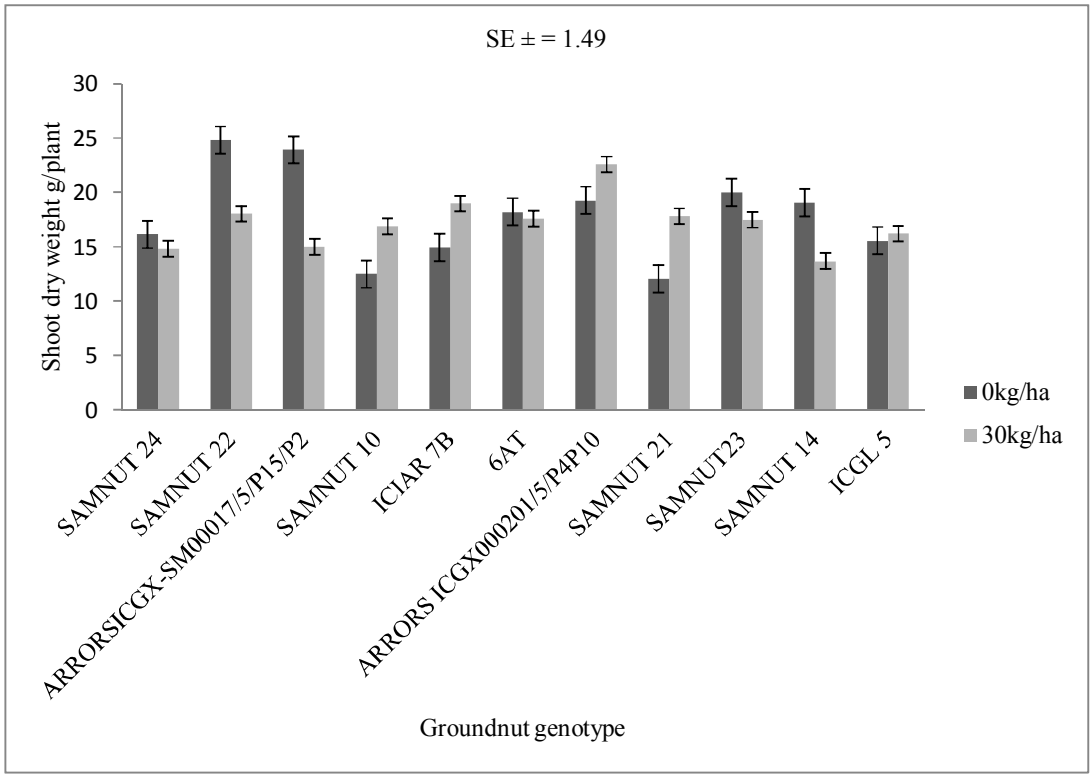

Fig v: Interaction of genotype and nitrogen rate on shoot dry weight of groundnut at 8WAP

4.4 EFFECT OF N FERTILIZER AND GENOTYPE ON SYMBIOTIC N_2 FIXATION OF GROUNDNUT

4.4.1 Amount of N_2 Fixed in 2011, 2012 and Combined

The amount of N_2 fixed by groundnut genotypes as determined by the nitrogen difference method ranged from 8.99 to 34.93 kg/ha in the 2011 planting season with a mean nitrogen fixation value of 20.71 kg/ha while in 2012, the genotypes fixed between 3.45 to 23.29 kg/ha with a mean BNF value of 11.24 kg/ha. ARRORS ICGX000201/5/P_4P_{10} fixed the highest N of 34.93 kg/ha which was consistent even in 2012; though at a lower rate of 23.29 kg/ha but it was significantly higher than SAMNUT 10 which fixed a meagre 8.99 kg/ha N in 2011 (Table 4.4).

Okito *et al*. (2004) reported a mean BNF value of 40.9 kg/ha for groundnut in his latest finding which was less than the estimated value of 96 kg/ha N by Burris, (1994); and both greater than the highest value of 34.93 kg/ha N recorded in this finding which is more than the 31.79 kg/ha N recorded by Yakubu *et al*. (2010) in south-eastern Nigeria. However, the amount of N fixed fall within the range of 32 to 134 kg/ha N estimated by Dakora (1997). Generally, there was no significant interaction between the genotype and N rate of application in 2011 but a significant difference however exist in 2012 season (Fig.vi and Fig. vii). The higher values recorded by those researchers could be attributed to the prevalence of effective strain of rhizobium, conducive environmental conditions, compatibility of the indigenous rhizobium strain and the host legumes and/or a combination of these factors (Tahir *et al*. 2009). The higher amount of nitrogen fixation recorded by ARROR ICGX 0001/5/P_4P_{10} could be attributed to the higher and effective nodules found on the genotype compared to others. However, the highly significant difference among the genotype to nitrogen fixation confirmed results of Patterson and La Rue (1983) and Hardarson *et al*. (1984) who earlier found significant

variation in N_2 fixation between various maturity groups of soybean genotypes. These disparities in fixation could be attributed to the variation in the host plant characteristic which is control at a whole by the nitrogenase enzyme which is reserved only for prokaryotes and responsible for BNF. Similar trend was also observed for N rates with the application of 30 kg/ha N significantly outperforming the control in 2011 planting season but was not significant in 2012. This could be due to the fact that the application of 30 kg/ha N boosts nitrogen fixation by impacting positively on the activities of nitrogenase enzyme complex responsible for nitrogen fixation in legumes in agreement with previous researchers notably; Patterson and La Rue (1983) and Hardarson *et al*. (1984). The high amount of N fixed in the first year over the second year could probably be due to the high amount of nitrate build up in the soil profile during the first season resulting in the suppression of nodule number in agreement with Wetselaar *et al*. (1972) which has been shown to correlate positively with BNF (Okito *et al*., 2004) and/or probably due to the diversion of photosynthates toward assimilation of nitrates as a result of improved soil nitrogen which tend to equally suppress nodulation in agreement with People *et al*. (1989).

The differences observed in the amount of N_2 fixed by the groundnut genotypes could be attributed to the number of days required to attain maturity. This was confirmed during the course of our findings where higher amount of N was found in medium (90-110 days) maturing genotypes such as ARROR ICGX 0001/5/$P_4P_{10,}$ SAMNUT 21, SAMNUT 22, ICIAR 6AT and SAMNUT 14 all fixing within the range of 17.00 to 29.11 kg/ha N. Our result is in line with that of Yusuf *et al*. (2008); although contrary to the findings of Sanginga *et al*. (2002) and Yusuf *et al*. (2008) who reported higher BNF for longer duration soybean genotypes.

It is a well established fact that combined nitrogen significantly reduce BNF, but the fixation of high nitrogen even in the presence of combined nitrogen provide an important criteria in selecting legumes genotypes most suitable in a mixed or multiple cropping system (Danso and Eskew, 1994). However, research findings on cowpea and soybean genotypes by Patterson and La Rue (1983) and Hardarson *et al*. (1984) has further shown that certain genotypes can fix high BNF to quite similar extents at both low and high soil inorganic N levels while others can fix high amount of N_2 only when the soil N levels are low all depending on the inherent genotypic trait existing among them. For instance, Hardarson *et al*. (1984) observed from their research with eight nodulating lines of soybeans that genotypes such as Ada, Altonia and Kalitur fixed higher N at 20 kg/ha N rates than at higher rates of 100 kg/ha N; whereas, genotypes such as Dunadja shows no significant difference in their amount of nitrogen fixed at both rates of nitrogen fertilizer.

Apart from most of the earlier mentioned physiological process that influence BNF in groundnut, several environmental conditions are limiting factors to the growth and activity of the nitrogen fixing plants. In the rhizobium legume symbiosis, which is a nitrogen fixing system, the process of nitrogen fixation is strongly related to the physiological state of the host plant (Brockwell *et al*., 1995b). Unlike in most soil-plant system, the principle of limiting factor is equally at play in this regard. Therefore, a competitive and persistent rhizobial strain is not expected to express its full capacity for nitrogen fixation if limiting factors (e.g., salinity, unfavourable pH, nutrient deficiency, mineral toxicity, temperature extremes, insufficient or excessive soil moisture, inadequate photosynthesis, plant diseases, and grazing) impose limitations on the vigour of the host plant.

Table 4.4 Effects of N Fertilizer and Genotype on Symbiotic N_2 Fixation of groundnut and Nitrogen Derived from Atmosphere

	Nitrogen fixation (kg/ha)			% Ndfa		
Genotype (G)	2011	2012	Combined	2011	2012	Combined
SAMNUT 24	14.13	3.45	8.78	27.11	16.36	21.74
SAMNUT 22	22.80	18.32	20.56	36.50	50.48	43.49
ARRORS ICGX-SM00017/5/P_{15}/P_2	21.72	8.77	15.25	38.93	30.76	34.83
SAMNUT 10	8.99	5.72	7.36	19.96	23.43	21.70
ICIAR 7B	10.20	8.65	9.43	22.68	32.62	27.65
6AT	26.87	13.03	19.95	43.09	41.59	42.34
ARRORS ICGX000201/5/P_4P_{10}	34.93	23.29	29.11	51.17	53.17	52.17
SAMNUT 21	11.91	8.04	9.98	27.83	28.01	27.92
SAMNUT 23	31.44	13.11	22.28	44.95	42.40	43.68
SAMNUT 14	24.17	10.03	17.10	41.12	35.41	38.27
SE±	12.55**	1.94**	2.28**	7.41^{NS}	4.06**	4.10
Nitrogen (kg/ha)						
0	10.06	11.56	10.81	23.19	35.35	29.35
30	31.37	10.92	21.15	47.48	35.50	41.49
Means	20.71	11.24	10.98	35.34	35.43	35.42
SE±	5.61**	0.87^{NS}	3.32**	3.32^{**}	1.81^{NS}	1.82**
Interactions						
G*N	NS	*	NS	NS	**	NS

NS=Not significant at 5% level of probability, *Significant at 5% level of probability, **Significant at 1% level of probability.

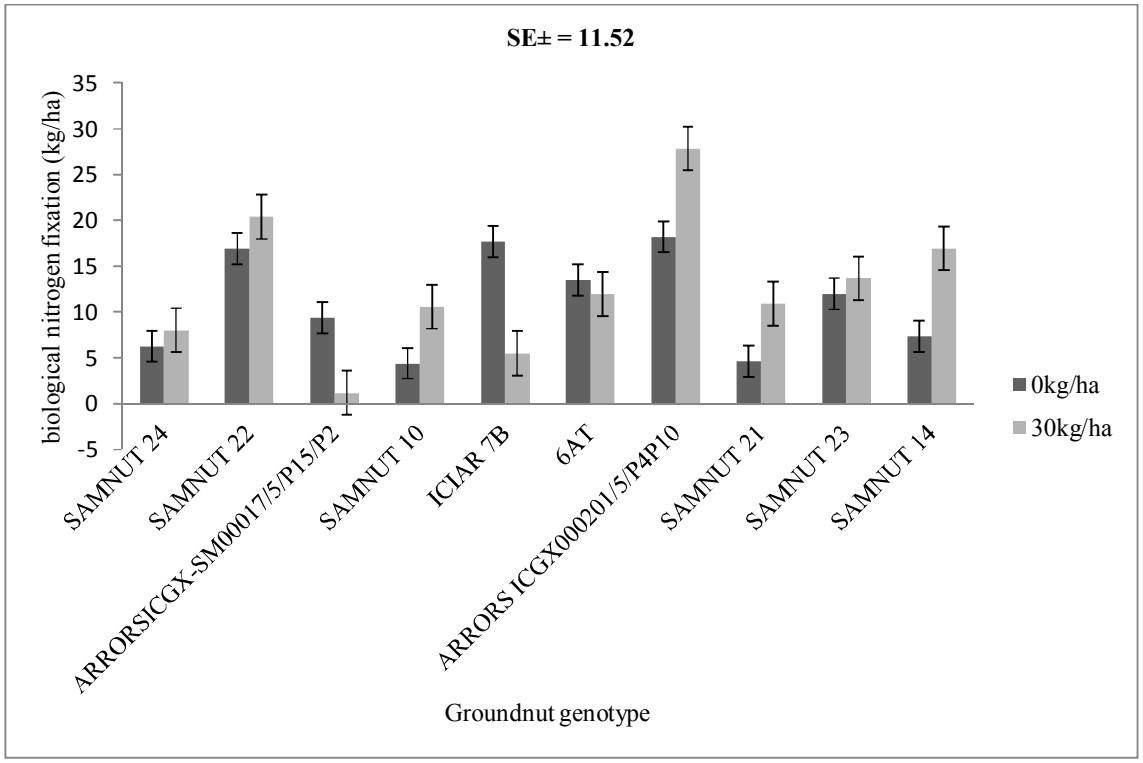

Fig. vi: Interaction between genotype and N-rates on BNF in 2012

4.4.2 Proportion of N Derived from Atmosphere (%Ndfa) in 2011, 2012 and Combined

This is a measure of the proportion of N derived from BNF in plants (Okito *et al.*, 2004, Yusuf, *et al*., 2008). The groundnut genotype shows wide variation in their proportion of Ndfa in both years. The result shows an average %Ndfa of 35.34% and 35.43% respectively in 2011 and 2012 trial respectively. The genotypes used did not have significant difference in their proportion of Ndfa ($p< 0.05$) in 2011. In 2012, however, the highest and lowest values were recorded in ARRORS ICGX000201/5/P_4P_{10} (53.17%) and ARRORS ICGX-SM 00017/5/P_{15}/P_2 (18.58%) respectively. However, a highly significant difference ($p< 0.01$) was observed in 2011 among the N rates with the application of 30 kg/ha N rates contributing more nitrogen from the BNF over the control (Table 4.4). Genotype * Nitrogen interaction was not significant in 2011, but not in 2012 where most of the groundnut genotypes increased their proportion of nitrogen under the control than the 30 kg/ha N-rates (Fig. vii).

The mean values of the proportion of Ndfa for both years were slightly lower than 52.4% recorded by Okito *et al*. (2004) but fall within the range of 28-81% reported by Ganry (1992) and Badiane and Gueye (1992) in the moist Guinea Savanna of West Africa. The major contribution of grain legumes to soil fertility lies in their ability to fix atmospheric nitrogen. Therefore genotype that derived high proportion of their N from fixation will be highly desirable especially in soils with low N status (Yusuf, *et al*., 2008). The result shows that less than half of the plant total N was derived from the atmosphere indicating that none of the genotype will be able to meet its N requirement for growth and development. In conformity with the above hypothesis, the application of 30 kg/ha N as starter dose was still justified in order to enhance BNF in groundnut genotype. However, further investigation into more genotypes of groundnut in view of

discovering genotype(s) with promising N fixing potential, without necessarily including starter dose of N fertilizer is highly desirable considering the high cost of mineral fertilizer and its negative impact on the environment.

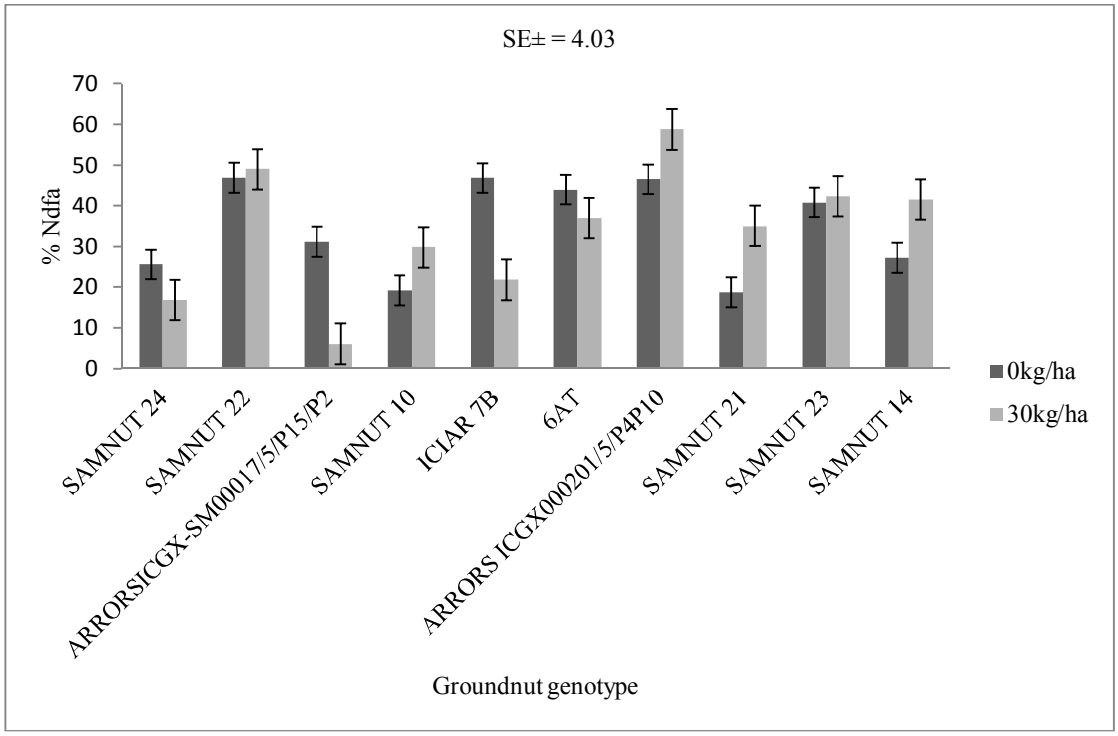

Fig. vii: Percentage Ndfa of groundnut genotypes in 2012 cropping season

4.5 EFFECT OF GENOTYPE AND N FERTILIZER ON YIELD AND YIELD COMPONENTS OF GROUNDNUT

4.5.1 Pod Yield in 2011, 2012 and Combined

The effect of genotype and nitrogen rates on pod yield showed a highly significant difference in both years (2011 and 2012) as shown in Table 4.5. However, higher pod yield was observed in 2012 than in 2011. Result of analysis of variance further showed that the highest pod yield was recorded in groundnut genotype SAMNUT 22 in 2012 which was not significantly different from ARRORS ICGX000201/5/P_4P_{10}, ARRORS 1CGX-SM00017/5/$P_{15}P_2$ and SAMNUT 21. However in 2011 ARRORS ICGX000201/5/P_4P_{10} was the best closely followed by SAMNUT 22 and SAMNUT 23 which was not statistically different from ARRORS ICGX-SM 00017/5/P_{15}/P_2 and the two genotypes tend to maintain their consistency in high pod yield across the two year duration. Among the nodulating lines, ICIAR 7B and SAMNUT 14 consistently recorded the least yield in both the 2011 and 2012 season. Nitrogen rates effect was significant in both 2011 and 2012; with the 30 kg/ha N outperforming the control.

The increase in yield with inorganic nitrogen over the control could be due to the ability of fertilizer to provide growing plants with nutrients in readily available form (Mengel and Kirkby, 1987, Agbede, 2009). More so, Dashiell *et al.* (1998) reported similar trend with respect to the effect of nitrogen rates which he attributed to the low soil nitrogen at the experimental site which resulted to rapid response of the genotype to soil added nitrogen in conformity with People and Craswell (1992) hypotheses that the benefit of nitrogen application are generally thought to be higher if nitrogen is a limiting factor; or deficient in the soil because of high nitrogen demand of high yielding cultivars. Genotype * Nitrogen interaction was not significant ($p<0.05$) in both 2011 and 2012, but physical observation shows that SAMNUT 22, ARRORS 1CGX-SM00017/5/$P_{15}P_2$,

ARRORS ICGX000201/5/P_4P_{10} and SAMNUT 23 yield were slightly better in the control over the 30 kg/ha N rate in 2012 representing 32%, 14%, 15% and 17% respectively increased in the control over the 30 kg/ha N rate of application (Fig viii).

The improved yield in the second year over the first could be due to the high N-fixed resulting from legume-legume mono-cropping, increased moisture during the pod filling stage and according to Bogino *et al*., (2006) higher plant population posed by the uniform establishment of the field. Physical observation however shows that genotype which flower earlier seem to record high pod yield in agreement with Ngo Nkot *et al*. (2011) who observe similar trend while working in same agro-ecological zone with same genotypes of groundnut.

Environmental factors such as rainfall and temperature have been reported to have significant influence on groundnut yield. Thus the little variation in yield during the two year period could be attributed to the even distribution of rainfall during the flowering and pod filling stage which is considered critical to groundnut growth. Putnam *et al*.; (2013) from his maiden research at Minnesota has indicted temperature and rainfall as the major environmental factors influencing the yield of groundnut. For instance, a peanut crop will not reach optimum maturity for a marketable yield to justify commercial production in areas with fewer heat units during the growing season. Little if any growth and development can occur at temperature below 20^{o}C and 30^{o}C (Putnam *et al*., 2013). Similarly, average yield increased from 1000 to 1450 kg/ha was obtained with increased moisture availability during the most critical growth period (flowering and pod filling) (Putnam *et al*., 2013)

Similarly, results of some preliminary work by Ngo Nkot *et al*., (2011) in different land use system in Cameroun indicate that, soil infertility and low density of indeginous Rhizobium could be one of the causes of low pod filling of groundnut

Table 4.5 Effects of Genotype and N Fertilizer on Pod Yield in 2011, 2012 and Combined

Treatment	Pod (kg/ha) 2011	2012	Combined
Genotypes (G)			
SAMNUT 24	1108	1106	1107
SAMNUT 22	2179	2963	2571
ARRORSICGX SM 00017/5/P_{15}/P_2	2070	2738	2404
SAMNUT 10	1249	1433	1341
ICIAR 7B	1038	614	826
6AT	1088	674	881
ARRORSICGX 000201/5/P_4P_{10}	2801	2540	2670
SAMNUT 21	1025	2473	1749
SAMNUT 23	2122	1678	1900
SAMNUT 14	1138	669	904
ICGL5	479	525	502
Mean	1482	1547	1514
SE±	178**	266***	160**
N rates (kg/ha)			
0	1313	1619	1466
30	1650	1475	1563
Mean	1482	1547	1515
SE±	76**	113*	68.12^{NS}
Interaction			
G*N			
Significance	NS	**	NS

NS=Not significant at 5% level of probability, *Significant at 5% level of probability, **Significant at 1% level of probability.

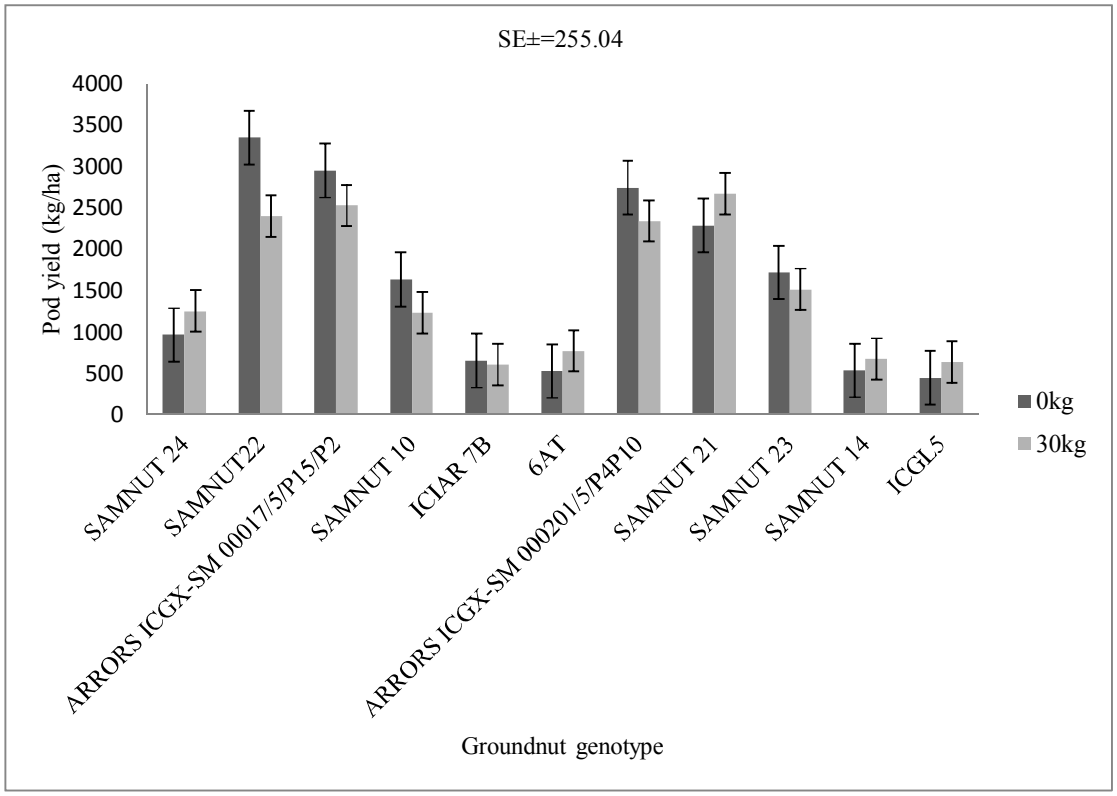

Fig. viii: Interactions between genotype and N rates on pod yield of groundnut in the 2012 trial.

4.5.2 Effect of Genotype and N Fertilizer on Haulm Yield in 2011, 2012 and Combined

Results of haulm yield followed similar trend with those of the pod yield as presented in Table 4.6. Results obtained in both 2011 and 2012 shows a significant difference between the years and genotype. Mean haulm yield was higher in 2012 than in 2011. The highest and lowest haulm yield in 2011 were recorded in SAMNUT 21 (3890 kg/ha) and SAMNUT 24 (1221 kg/ha) respectively whereas, in 2012 the highest and lowest haulm yield were recorded in SAMNUT 23 (3778 kg/ha) and SAMNUT 24 (2556 kg/ha) respectively. The high haulm yield recorded in SAMNUT 23 and SAMNUT 21 could be due to their profuse branching habits. However, longer maturity period which tends to prolong the process of nitrogen fixation and allocation of carbon/assimilates to vegetative parts could also explained the probable reason for the high haulm yield reported in SAMNUT 21 than SAMNUT24; which is a longer maturing genotype. Genotype * nitrogen rate interaction was significant for haulm yield in 2012 but not in 2011 (Fig ix). Result shows that although the highest haulm yield was recorded in the nitrogen treated but some genotypes notably ARRORS ICGX SM 00017/5/P_{15}/P_2, SAMNUT 22, SAMNUT 10, ARRORS ICGX 000201/5/ P_4P_{10} and SAMNUT 23 were relatively better in the control over the 30 kg/ha nitrogen rate. This increase could probably be attributed to the improved soil N of the experimental site due to the effect of biological nitrogen fixation (BNF) arising from groundnut mono-cropping. Differences in sowing dates has also been shown to bring about variation in haulm yield of groundnut by shortening its vegetative cycle (Morthy and Rao, 1986) which was the case in our research where sowing in 2011 was delayed by one month over those of 2012. Hence, the average haulm yield was higher in 2012 than 2011. The significant interaction observed between the nitrogen treated plots and the control in

2012 is in agreement with Bala *et al*. (2011) who also found that the application of NPK fertilizer to soil with high residual nitrogen could not influence haulm yield. Meanwhile, differences in haulm yield among the groundnut genotypes in our present study is not surprising occasioned by the fact that varietal differences in haulm yield per hectare has earlier been reported in two groundnut genotype in India (Patel *et al.*, 2005).

Table 4.6 Effects of Genotype and N Fertilizer on haulm Yield in 2011, 2012 and Combined

	Haulm (kg/ha)		
Treatment	2011	2012	Combined
Genotypes (G)			
SAMNUT 24	1221	1961	1591
SAMNUT 22	3330	2672	3001
ARRORSICGX SM 00017/5/P_{15}/P_2	2232	2539	2386
SAMNUT 10	3460	3411	3436
ICIAR 7B	1987	2100	2043
6AT	1980	2144	2062
ARRORSICGX 000201/5/P_4P_{10}	2852	2833	2843
SAMNUT 21	3890	3011	3450
SAMNUT 23	2275	4056	3165
SAMNUT 14	2109	2322	2216
ICGL5	1968	2228	2098
Mean	2482	2662	2572
SE±	324**	158**	177*
N rates (kg/ha)			
0	2382	2532	2457
30	2582	2791	2687
Mean	2482	2662	2572
SE±	138**	67**	76**
Interaction			
Significance	NS	**	NS

NS=Not significant at 5% level of probability, *Significant at 5% level of probability, **Significant at 1% level of probability.

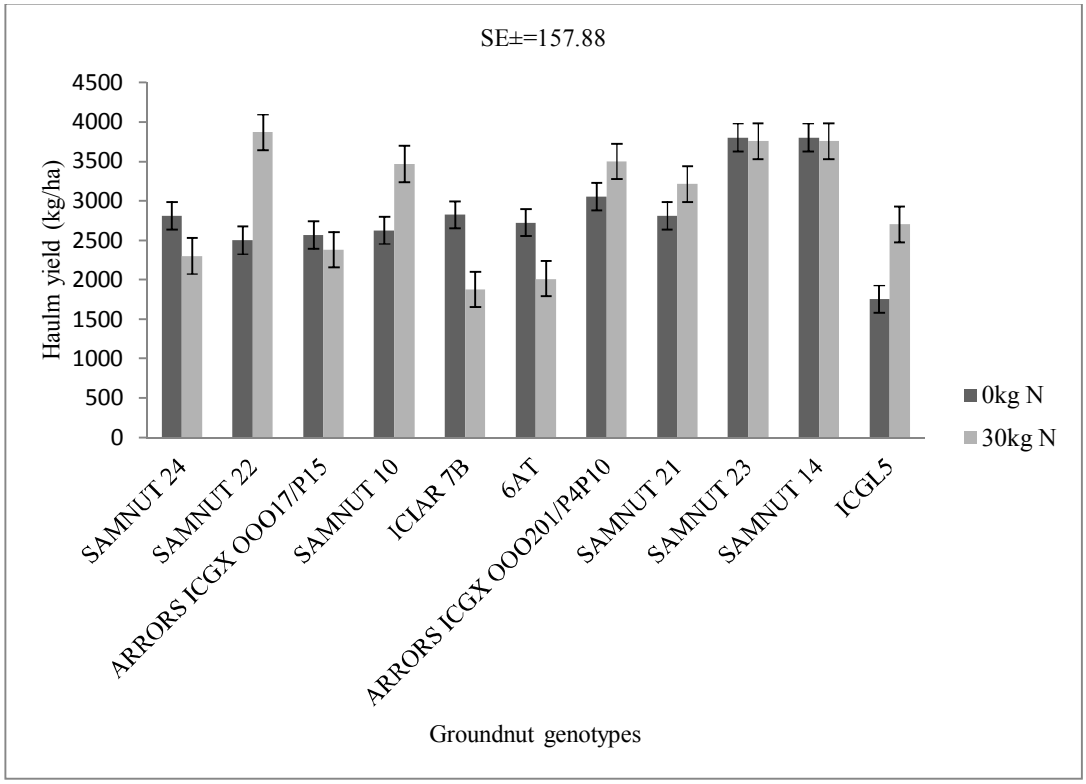

Fig. ix: Interactions between genotypes and N-rates on haulm yield 2012

4.5.3 100 seed weight in 2011, 2012 and Combined

The results of 100 seed weight as presented in Table 4.7 below shows significant variations among the various genotypes in both 2011 and 2012. The 100 seed weight ranges between 28.35 to 48.75 g with a mean of 39.68 g in 2011 and 28.33 g to 49.83 g and with a mean of 39.36 g in 2012 across the genotype. The highest was consistently recorded in groundnut genotypes ARRORS ICGX000201/5/P_4P_{10} which was not significantly different from ARRORS1CGX SM00017/5/$P_{15}P_2$ while the lowest mass was recorded by SAMNUT 14. The effect of nitrogen rates on 100 seed weight was significant in both years with the 30 kg/ha N significantly outperforming the control. The difference between years was equally not significant. This differential response of

groundnut genotypes to nitrogen fertilizer could be attributed to the inherent variability among the genotypes. The genotype * N-rates interaction was not significant in both years.

Table 4.7 Effects of Genotype and N Fertilizer on 100 seed Weight in 2011, 2012 and Combined

Treatment	100 seed (g) 2011	2012	Combined
Genotypes (G)			
SAMNUT 24	37.41	37.33	37.37
SAMNUT 22	43.26	42.00	42.63
ARRORSICGX SM 00017/5/P_{15}/P_2	48.16	47.00	47.58
SAMNUT 10	43.39	41.83	42.61
ICIAR 7B	37.68	37.50	37.59
6AT	31.52	32.17	31.85
ARRORSICGX 000201/5/P_4P_{10}	48.75	49.83	49.29
SAMNUT 21	42.85	43.50	43.18
SAMNUT 23	41.72	41.67	41.69
SAMNUT 14	28.35	28.33	28.34
ICGL5	33.41	31.83	32.62
Mean	39.68	39.42	39.52
SE±	0.99**	1.09*	0.74**
N rates (kg/ha)			
0	38.41	38.05	38.22
30	40.95	40.79	40.87
Mean	39.68	39.42	39.55
SE±	0.43**	0.46*	0.31*
Interaction			
Significance	NS	**	NS

NS=Not significant at 5% level of probability, *Significant at 5% level of probability, **Significant at 1% level of probability.

4.5.4 Effect of Genotype and N Fertilizer on Seed Yield in 2011, 2012 and Combined

Result of the analysis of variance shows a significant difference among genotypes, nitrogen rates and years in terms of seed yield (Table 4.8). The overall mean seed yields were 839 and 843 kg/ha in the 2011 and 2012 planting season respectively. In 2011, the highest and lowest seed yield was recorded in ARRORS ICGX 000201/5/P_4P_{10} and SAMNUT 14 respectively; whereas, in 2012 SAMNUT 22 and SAMNUT 14 recorded the highest and lowest seed yield respectively. SAMNUT 14 maintained its trend as the lowest yielding in both years but was not the case for the high yielding genotypes. SAMNUT 22 which was among the best performers in 2011 equally tend to maintain the trend and even outperforming the best genotype of 2011 to become the best in 2012; although not significantly different from ARRORS ICGX 000201/5/P_4P_{10}. Nitrogen rates effect was significant in both years with 30 kg/ha N rates outperforming the control.

Difference in seed yield cannot be separated from those of pod yield since both are influenced by the same growth condition; hence, similar factors as earlier highlighted for pod yield in addition to differences in moisture content of seed at the point of weighing could contribute to these variations among genotypes and years.

Table 4.8 Effects of Genotype and N Fertilizer on Grain Yield in 2011, 2012 and Combined

Treatment	Seed yield (kg/ha) 2011	2012	Combined
Genotypes (G)			
SAMNUT 24	547	749	648
SAMNUT 22	1104	1770	1437
ARRORSICGX SM 00017/5/P_{15}/P_2	1242	1539	1391
SAMNUT 10	717	869	793
ICIAR 7B	696	329	513
6AT	704	359	532
ARRORSICGX 000201/5/P_4P_{10}	1651	1283	1467
SAMNUT 21	685	1043	864
SAMNUT 23	980	619	800
SAMNUT 14	468	389	429
ICGL5	434	322	378
Mean	839	843	841
SE±	113	173	103**
N rates (kg/ha)			
0	722	824	773
30	956	862	909
Mean	839	843	841
SE±	48**	74^{NS}	44*
Interaction			
Significance	NS	**	NS

NS=Not significant at 5% level of probability, *Significant at 5% level of probability, **Significant at 1% level of probability.

4.5.4 Effect of Genotype and N Fertilizer on Harvest Index of Groundnut in 2011, 2012 and Combined

Results of Harvest Index (HI) show a significant difference between genotypes across the two years and the combined as shown in Table 4.9. The overall mean harvest index (HI) was significantly higher in 2011 (0.26) than 2012 (0.24). ARRORS ICGX 000201/5/P_4P_{10} (0.37) had the highest harvest index which was not significantly different from ARRORS 1CGX-SM 00017/5/P_{15}/P_2 (0.35) in 2011. In 2012 however, there was an improvement in HI among some genotypes, notably among which is SAMNUT 22; whereby genotype which were group next to the best drastically improved to become the best. Nitrogen effect was not significant in 2012 probably due to previous N fixation benefit. In 2011, 30 kg/ha N rates significantly outperformed the control. Genotype * N-rates effect was significant in 2011 but not in 2012 (Fig. x). 6AT tends to perform better in the control than the 30 kg/ha N rate which was consistent over the two years.

Harvest index is the ratio of total grain yield to total biomass yield. Here total biomass yield takes into account grain yield as well as vegetative parts of crop plants above the soil surface. Thus, the ratio of economic yield to total plant yield gives HI (Ahmad *et al*. 2007). It can be used as a measure of reproductive efficiency of crops (Ahmad *et al*. 2007). The index can also be used to estimate crop carbon (C) balances by applying it to grain yield statistics to determine total shoot dry matter and then calculating crop residues as the difference between shoot C and grain C. Factors such as pest and diseases attack, delayed sowing, harsh environmental condition could result to variation in HI (Ahmad *et al*., 2007).

Table 4.9 Harvest Index as Influenced by Groundnut Genotype and N Fertilizer on Groundnut in 2011, 2012 and Combined

	HI		
Genotypes	2011	2012	Combined
SAMNUT 24	0.31	0.28	0.30
SAMNUT 22	0.26	0.38	0.32
ARRORS1CGX-SM00017/5/$P_{15}P_2$	0.35	0.38	0.37
SAMNUT 10	0.16	0.20	0.18
ICIAR 7B	0.31	0.14	0.22
6AT	0.30	0.14	0.22
ARRORSICGX 000201/5/P_4P_{10}	0.37	0.32	0.35
SAMNUT 21	0.16	0.26	0.21
SAMNUT 23	0.30	0.13	0.22
SAMNUT 14	0.18	0.14	0.16
SE±	0.04*	0.03**	0.03**
Nitrogen(kg/ha)			
0	0.25	0.24	0.25
30	0.29	0.23	0.26
Means	0.27	0.24	0.26
SE±	0.02^{NS}	0.01^{NS}	0.01^{NS}
Interactions			
G*N	*	NS	NS

NS=Not significant at 5% level of probability, *Significant at 5% level of Probability, **Significant at 1% level of probability

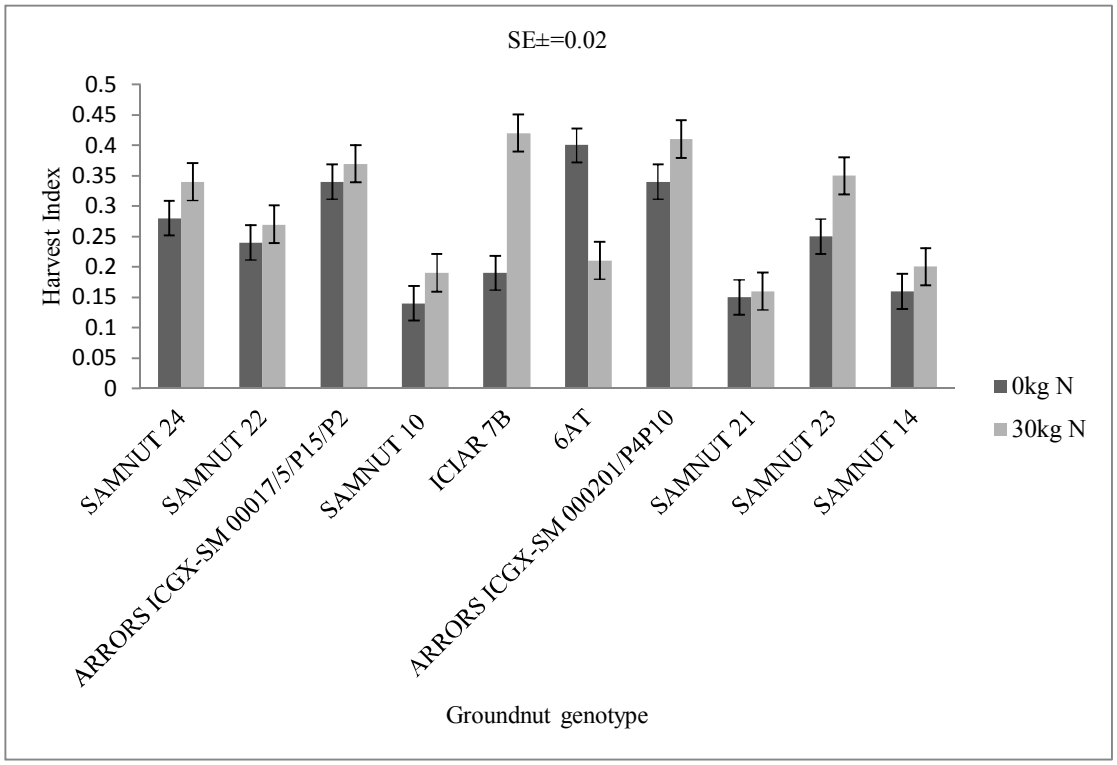

Fig. x: Interactions between genotypes and N-rates on HI of groundnut in 2011.

4.6 EFFECT OF GENOTYPE AND N FERTILIZER ON CONTRIBUTIONS OF GROUNDNUT GENOTYPE AND N FERTILIZER TO SOIL N

4.6.1 Effect of Genotype and N Fertilizer on N Uptake in 2011, 2012 and Combined

Significant difference ($P<0.05$) among genotypes and nitrogen rates of application was observed in both 2011 and 2012 as well as the combined (Table 4.10). The total nitrogen uptake across the genotypes ranges from 23.84 to 52.96 kg/ha N with a mean of 38.37 kg/ha N. SAMNUT 22, 6AT, SAMNUT 23 and SAMNUT 14 which were significantly similar were next ranked to the best genotypes and were significantly different from all other genotypes, with the least values found in SAMNUT 10 among the nodulating lines but generally better than the non-nodulating reference genotype (ICGL 5).

Application of N had a significant effect on N uptake with the application of 30 kg/ha N significantly increasing uptake over the control. Significant difference exists between total uptake and genotypes over the two years duration with higher uptake being observed in the 2011 (48.89 kg/ha) than 2012 (26.86 kg/ha). But shoot uptake which although not reflected here was significantly higher in 2012 than 2011. According to Moll *et al.* (1982), variation in N uptake could be separated from grain yield variation. Lee *et al*. (2004) indicated that N uptake was positively correlated with plant dry matter, leaf area index and leaf nitrogen content.

The significant increase in the total N uptake in the 30 kg/ha N rates of application than the control could be as a result of the relatively low soil N recorded (0.15 gkg^{-1}) at the onset of the field trial which tend to increase crop nitrogen uptake and utilization since crops respond most readily to the most limiting nutrient element to satisfy their physiological need (Sowers *et al*. 1994). This result also suggests that the 30 kg/ha N resulting in a mean total plant N uptake of 37.88 kg/ha was just sufficient enough for

groundnut N utilization/uptake. According to Sowers *et al*. (1994), the application of high N rates may result in poor N uptake and low NUE due to excessive N losses. Similar observation was recorded recently by Majid, *et al.* (2010). From his finding, he discover that, N Uptake of wheat decreased with each incremental addition of N fertilizer and the lowest N Uptake in each rotation was for maximum N rates.

Table 4.10: Nitrogen Uptake as Influenced by Groundnut Genotype and N Fertilizer in 2011, 2012 and Combined

	Total N Uptake (kg/ha)		
	2011	2012	Combined
Treatment			
Genotype (G)			
SAMNUT 24	44.19	21.08	32.64
SAMNUT 22	52.85	35.95	44.40
ARRORSICGX-SM 00017/5/P_{15}/P_2	51.78	26.40	39.09
SAMNUT 10	59.04	23.36	41.20
ICIAR 7B	40.26	26.28	33.27
6AT	56.93	30.66	43.80
ARRORSICGX 000201/5/P_4P_{10}	64.98	40.93	52.96
SAMNUT 21	41.97	25.67	33.82
SAMNUT23	61.50	30.75	46.13
SAMNUT 14	54.23	27.66	40.95
ICGL5	30.06	17.63	23.85
SE±	4.33**	1.85**	2.39**
Nitrogen N (kg/ha)			
0	38.09	25.77	31.93
30	59.69	27.94	43.28
Means	48.89	26.86	37.61
SE±	1.85**	0.87**	1.02**
Interactions			
G*N	NS	NS	NS

NS=Not significant at 5% level of probability, *Significant at 5% level of probability, **Significant at 1% level of probability

4.6.3 N Harvest Index (NHI) by Groundnut Genotypes and N Fertilizer in 2011, 2012 and Combined

Nitrogen Harvest Index (NHI) is simply the amount of nitrogen in seed as a proportion of total above ground biomass nitrogen (Bushby and Lawn, 1992). It reflects the grain protein content and thus the grain nutritional quality (Hirel *et al*., 2007). The genotypes accumulated a range of 0.33 to 0.60 kg/ha of the total nitrogen with a mean accumulation of 0.47 kg/ha relative to the 86 kg/ha recorded by Rhodes, (1980) during the planting season (Table 4.11). The result shows that although the average NHI was greater in the 30 kg/ha N rates over the control, significant interaction between genotype and N rates was pronounced in 2011, but not in 2012 with SAMNUT 22, ARRORS ICGX-SM 00017/5/P_{15}/$P_{2,}$ and 6AT consistently maintaining high NHI in the control over the 30 kg/ ha N rates (Fig. xi). However, N rates effects on NHI was not equally significant in 2012 but was significant in 2011 with the 30 kg/ ha N outperforming the control.

The lack of significant difference observed in the two nitrogen rates was in agreement with Majid *et al*. (2010) who reported that NHI was unaffected by N rate. Lopez-Bellido and Lopez-Bellido (2004) showed that the increase in crop N uptake with rising N fertilizer rates was greater than the increase in grain yield, so there is less transfer of N to grain when N rates was increased. Increased NHIs in some grain legumes is often due to the low partitioning of N to leaves, roots and stem; which suggest an efficient mobilisation of N in straw and leaves to seed (Ayaz *et al.*, 2004). To buttress this fact, Rhodes, (1980) reported high NHI of 0.86 kg/ha for groundnut, which he attributed to the low N concentration in the pod walls, stems and leaves. The lower NHI recorded in our finding entails that more nitrogen will be returned to the soil for the proceeding crop as residual N thus justifying the lack of significant difference observed among the N

rates in 2012. However, this benefit may not be obtained if the crop residues are removed from the field.

Table 4.11 N Harvest Index (NHI) by Groundnut Genotypes and N Fertilizer in 2011, 2012 and Combined

	NHI (kg/ha)		
Genotype (G)	2011	2012	Combined
SAMNUT 24	0.51	0.67	0.59
SAMNUT 22	0.49	0.80	0.65
ARRORSICGX-SM 00017/5/P_{15}/P_2	0.59	0.81	0.70
SAMNUT 10	0.33	0.59	0.46
ICIAR 7B	0.43	0.35	0.39
6AT	0.60	0.50	0.55
ARRORSICGX 000201/5/P_4P_{10}	0.60	0.84	0.72
SAMNUT 21	0.29	0.84	0.57
SAMNUT 23	0.57	0.70	0.64
SAMNUT 14	0.38	0.63	0.51
SE±	0.08**	0.02**	0.04**
Nitrogen N (kg/ha)			
0	0.46	0.67	0.57
30	0.49	0.67	0.58
Means	0.48	0.67	0.57
SE±	0.08**	0.08^{NS}	0.02^{NS}
Interactions			
G*N	*	NS	NS

NS=Not significant at 5% level of probability, *Significant at 5% level of Probability, **Significant at 1% level of probability

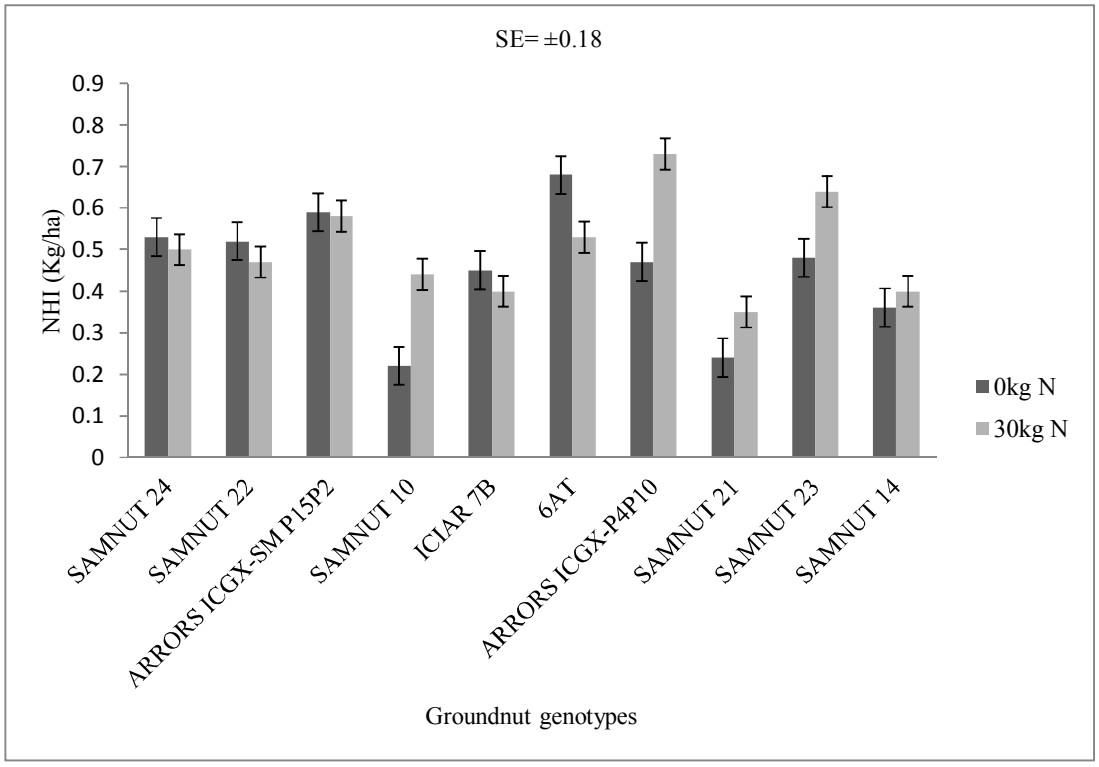

Fig. xi: Interaction between genotype and N – rate on NHI of groundnut in 2011

4.6.4 Soil N Balance as Influenced by Groundnut Genotypes and N Fertilizer in 2011, 2012 and Combined

The results obtained showed that there was a significant difference among the genotype in their contribution to soil nitrogen balance in both 2011 and 2012 planting season. The mean N balance value was significantly higher in 2011 (-7.12) than in 2012 (-20.03) (Table 4.12). However, N rate effect was not significant in both 2011 and 2012. The highest and lowest soil N balance were observed in SAMNUT 14 (9.79) and ARRORS ICGX-SM 00017/5/P_{15}/P_2 (-16.55) in 2011 while in 2012, the highest ICIAR 7B (1.78) and SAMNUT 22 (-56.07) returned the highest and lowest nitrogen balance respectively. Generally, negative N balance was predominance at both rates of N application with the 30kg/ha N rates returning significantly higher value (-1.10) than the

control (-13.23) in 2011 (Table 4.12). The result of the combined analysis of variance shows that SAMNUT 14 impacted positively on the soil nitrogen balance. Although ICIAR 7B which in 2011 was among genotypes leaving close to zero nitrogen balance could be seen imparting positively on the soil nitrogen balance in the 2012 trial indicating that continuous cultivation of such genotype could have a marked significant improvement on the soil nitrogen balance. Genotype and nitrogen interaction was significant in 2011 but the trend was not maintained in 2012 and combined (Fig. xiii). However, considering the other instance where both the haulm and grains were transported from the field, a more negative nitrogen balance was observed as shown in Table 4.13. Result of the combined analysis of variance showed that soil nitrogen balance at this stage ranges from -53.08 kg/ha to -10.95 kg/ha. The highest value was reported in SAMNUT 14 with a mean value of -10.95 kg/ha which was significantly different from all other genotypes; closely followed by groundnut genotype 6AT with a value -11.78 kg/ha while the least value was reported in ARRORS ICGX-SM 00017/P_{15}/P_2. This implies that SAMNUT 14 and 6AT was reported to have moderate depletion effect on soil nitrogen status. The result of the interaction between genotype and nitrogen rates was significant in both 2011 and 2012 (Fig xiii and xiv). However, in both 2011 and 2012, supplementary addition of 30kg N ha^{-1} significantly increased the soil nitrogen balance over the control (0 kg/ha N).

Most grain legumes irrespective of their ability to fix reasonable amount of nitrogen to the soil, impact negatively on the soil nitrogen balance (Yusuf *et al*., 2008). This could be due to the fact that the benefit of nitrogen fixation is not often realizable to the current crop but depend on whether their residue is incorporated back into the soil. For instance, Yusuf *et al*. (2008) recorded a mean range of -18.9 and 9.4 kg/ha N for

cowpea and soybeans respectively, and Sanginga, *et al*. (2002) recorded a mean range of -10.6 to 11.1 kg/ha N for 8 cowpea genotype. The positive N balance observed in SAMNUT 14 (9.79 kg/ha), 6AT (4.32 kg/ha) and SAMNUT 23 (1.50 kg/ha) in 2011 as well as ICIAR 7B (1.78) in 2012 when only the grain was exported from the field respectively could be attributed to the amount of biologically fixed N as reflected by our finding as presented in Table 4.4; but for such a positive N balance to occur, it is expected that the amount of fixed N by the legumes to the soil must be greater than the amount of soil N in the harvested grain (Giller, 2001).

From the result of the interaction between genotype and nitrogen application rate, significant difference was observed among the genotypes whereby some genotypes left a positive N balance at 30 kg/ha N rates; whereas majority were with negative nitrogen balance. However, it's interesting to know that some genotypes notably SAMNUT 14, SAMNUT 22 and SAMNUT 24 left close to zero nitrogen balance at 0 kg/ha N rates but these genotypes however responded to nitrogen fertilizer application by impacting positively on the soil nitrogen balance. Although this trend was not so pronounced for SAMNUT 21, which even though response to nitrogen application but could not attain a positive nitrogen balance. These however could be due to the fact that the maximum nitrogen rate was yet to be attained by this genotype.

The occurrence of legume benefit does not necessarily indicate a positive soil nitrogen balance of legume crops hence, higher soil mineral nitrogen content after legumes can be due to the nitrogen saving effect of legumes (Rego and Seeling, 1996). Factors such as the removal of the above ground crop residues and clear weeding could promote the predominance of negative soil nitrogen balance which could likely explain the overall negative nitrogen balance observed in our current study. In addition, soil nitrogen balance depends on the nitrogen harvest index of the total plant (including roots) and

soil nitrogen status. For instance, research findings by Kumar Rao and Dart, (1987) showed that short duration pigeon pea with a NHI of 54% had a negative nitrogen balance of -32 kg/ha N even when crop residues remained in the system in contrast to late maturity type with NHI of 21% given a positive nitrogen balance of 41 kg/ha N. Further justifying our result where negative soil nitrogen balance was observed in both years, higher soil N availability has been found to reduce the nitrogen balance of cowpea from a positive nitrogen balance of +52 kg/ha to 0 kg/ha N due to reduced nitrogen fixation (Eagleshan *et al*., 1992).

Thus, for agronomic purposes, it is important to know when and how much nitrogen from this source become available to the following crops and also from sustainability point of view, it is also important to know how soil fertility changed in the long term if legumes provide the sole means of nitrogen input into system (Rego and Seeling, 1996).

Table 4.12 Soil N Balance as Influenced by Groundnut Genotypes and N Fertilizer in 2011, 2012 and Combined (Grain removed)

	Soil N-balance (kg/ha)		
Genotype (G)	2011	2012	Combined
SAMNUT 24	-1.23	-20.44	-10.83
SAMNUT 22	-12.03	-56.07	-34.05
ARRORS ICGX-SM 00017/5/P_{15}/P_2	-16.55	-48.98	-32.77
SAMNU 10	-16.40	-21.88	-19.14
ICIAR 7B	-9.34	1.78	-3.78
6AT	4.32	-19.34	-7.51
ARRORS ICGX 000201/5/P_4P_{10}	-15.98	-33.55	-24.77
SAMNUT 21	-4.26	-8.95	-6.61
SAMNUT 23	1.50	-1.82	-0.16
SAMNUT 14	9.79	-1.11	4.34
Means	-7.12	-20.03	-7.85
SE±	6.07**	7.64**	4.87**
Nitrogen N (kg/ha)			
0	-13.13	-19.80	-16.47
30	-1.10	-20.25	-10.68
Means	-7.12	-20.03	-13.58
SE	2.71*	3.26^{NS}	2.16^{NS}
Interactions			
G*N	*	NS	NS

NS=Not significant at 5% level of probability, *Significant at 5% level of Probability, **Significant at 1% level of probability.

Table 4.13 Effect of nitrogen rates and genotypes on soil nitrogen balance (Haulm + Grain)

	Nitrogen balance (kg/ha)		
Genotypes	2011	2012	Combined
SAMNUT 24	-15.11	-32.58	-23.84
SAMNUT 22	-48.3	-54.43	-51.37
ARRORS ICGX-SM 00017/P_{15}/P_2	-42.95	-63.21	-53.08
SAMNUT 10	-62.11	-35.14	-48.62
ICIAR 7B	-36.03	-20.84	-28.44
6AT	-12.42	-11.14	-11.78
ARRORS ICGX 000201/5/P_4P_{10}	-49.57	-26.24	-37.91
SAMNUT 21	-52.56	-41.24	-46.90
SAMNUT 23	-21.25	-17.96	-19.61
SAMNUT 14	-13.01	-8.89	-10.95
SE±	7.24**	4.86**	4.36**
Mean	-34.45	-29.66	-32.06
Nitrogen rates (kg/ha)			
0	-41.05	-32.71	-36.88
30	-27.85	-26.60	-27.23
Mean	-34.45	-29.66	-32.06
SE±	3.09**	2.07**	1.89**
Interactions			
G*N	**	*	NS

NS=Not significant at 5% level of probability, *Significant at 5% level of Probability, **Significant at 1% level of probability.

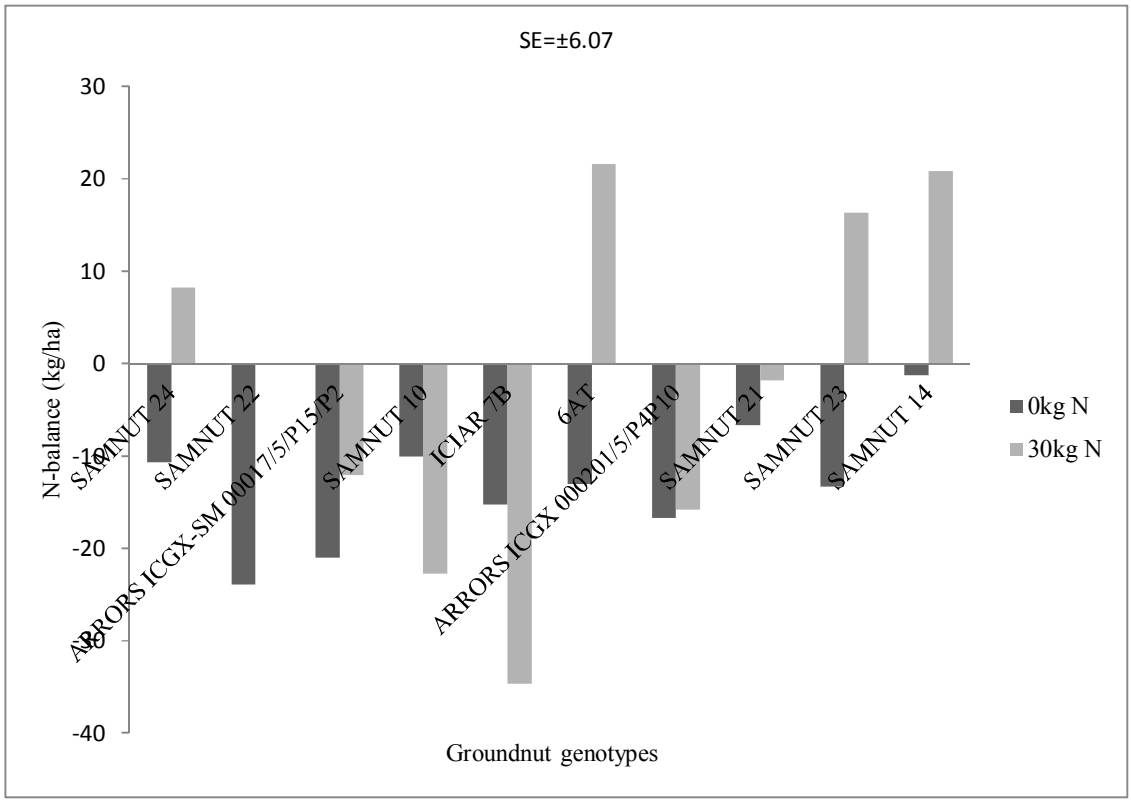

Fig. xii: Interaction between genotype and N-rates on nitrogen balance of groundnut in 2011 (Only grain removed)

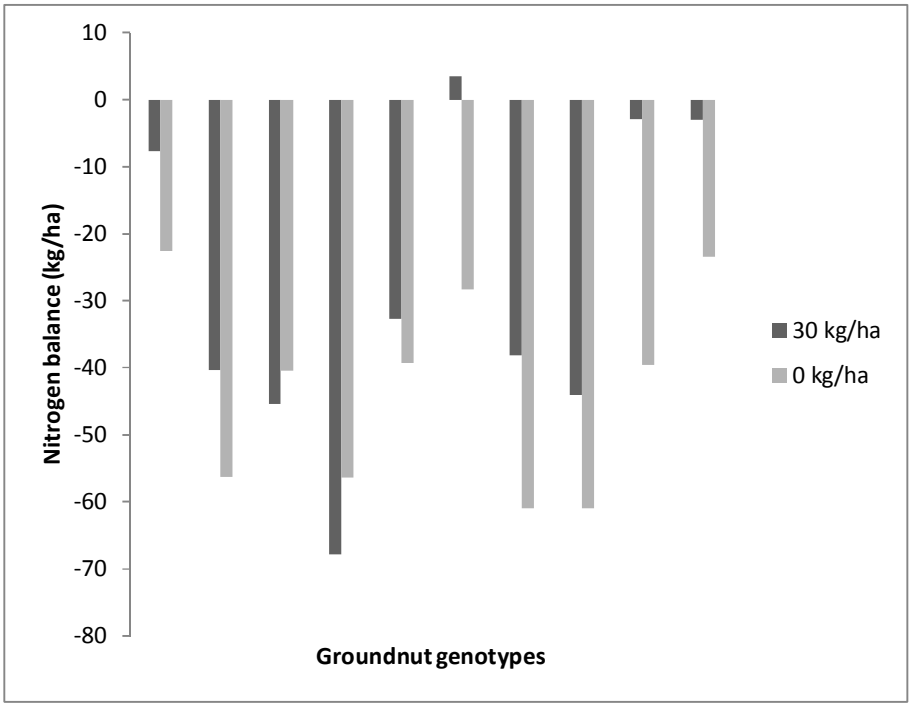

Fig xiii: Interaction effect of nitrogen rates on soil nitrogen when both the haulm and grain are removed in 2011.

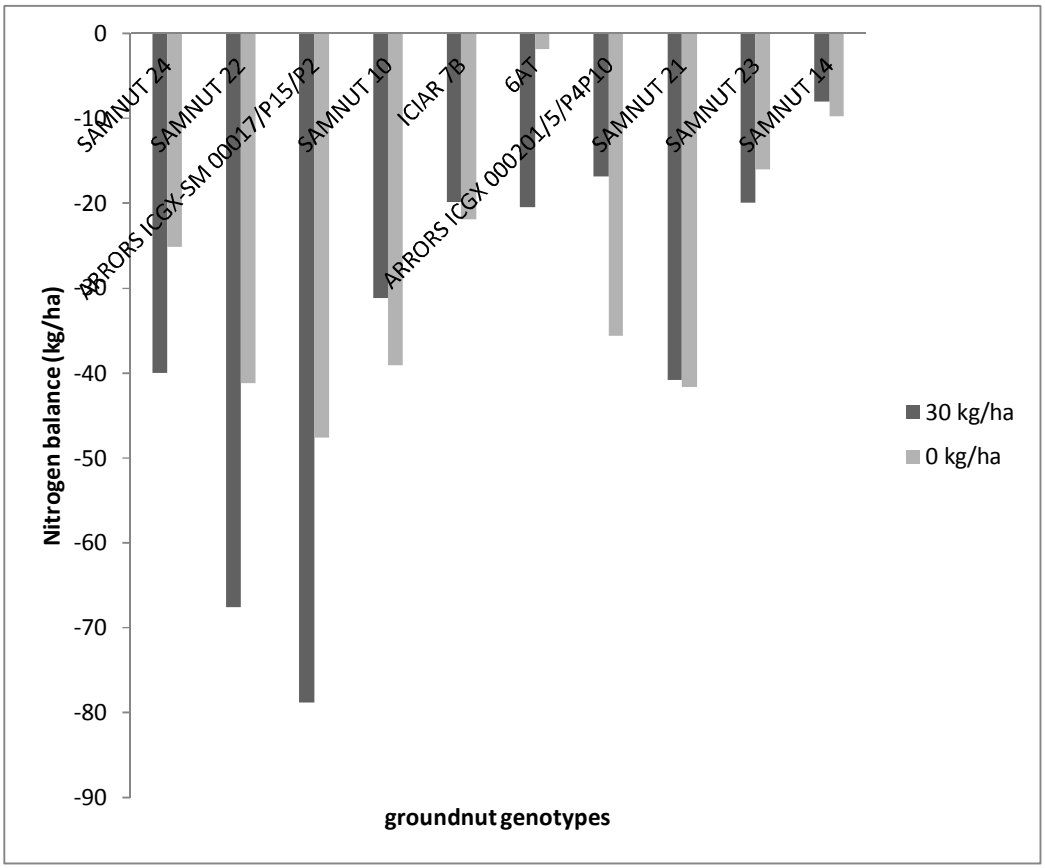

Fig xiv: Interaction effect of nitrogen rates on soil nitrogen when both the haulm and grain are removed in 2012

4.7 CORRELATION MATRIX OF SOME AGRONOMIC AND NITROGEN FIXING TRAITS IN 2011 AND 2012 TRIAL

Correlation analysis of some agronomic and nitrogen fixation properties were conducted for the two years. Result of the combined analysis shows both positive and negative correlation among the variables assayed (Tables 4.13 and 4.14). The result shows a strong negative and significant correlation between BNF and nodule number with a correlation value of (r=-0.02). Also, result shows no significant correlation between BNF and nodule mass in both 2011 and 2012 trial. However BNF was highly positively correlated with shoot biomass which was consistent over 2011 and 2012. The negative correlation observed between BNF and nodulation could be attributed to the relatively high proportion of ineffective nodules as is the case in this study which is relatively contrary to Pimratch *et al.* (2008) finding who though observed similar trend in most of the parameters, but assumed a positive correlation between BNF and nodule mass while working with seven genotypes of groundnut. It is worthy of note that the presence of nodules on groundnut roots does not necessarily mean that sufficient N_2 is being fixed for maximum benefit to the host plant (Wani *et al.*, 1995). However the strong positive correlation between shoot biomass and BNF was in agreement with Pimratch *et al.* (2008). Most of the trait tested here were in agreement with his work except for HI, which he consistently assumed a strong negative correlation contrary to our findings where we observed no significant correlation in both years. Ajeigbe *et al.* (2010) found that genotypic and phenotypic correlation of nitrogenase activities with yield was significant with phenotypic correlation coefficients range from 0.66 to 0.89 over sampling dates and environment. They also observed that correlation between nitrogenase activities and yield were low suggesting that nitrogenase had less effect on fruit weight than shoot weight which explained the lack of significant difference

observed in our findings in both 2011 and 2012 with a correlation value of 0.44 and 0.08 respectively. Strong positive correlation also exist between BNF and Ndfa (r=0.94) in 2011 but the trend was not maintain in 2012 where no significant difference was observed (r=0.24). Similarly, BNF show a significant positive correlation with soil nitrogen balance and 100 grain weight in 2011 but except for soil nitrogen balance that show a positive significant correlation with BNF in 2012 all other parameters show lack of significant correlation. Result further show that a positive and significant correlation also exists between nodule mass and nodule number which was consistence under the two year duration.

Table 4.14 Correlation Matrix of Some Agronomic and Nitrogen Fixing Trait in 2011

	Nodno.	NFW	RDW	SDW	FIX	NDFA	NBAL	NHI	HI	100	PY
Nodno											
NFW	0.83**										
RDW	0.13^{NS}	0.14^{NS}									
SDW	-0.17^{NS}	-0.03^{NS}	0.27^{NS}								
FIX	-0.10^{NS}	-0.002^{NS}	0.74**	0.65**							
NDFA	-0.16^{NS}	-0.02^{NS}	0.71**	0.56***	0.94**						
NBAL	-0.33^{NS}	0.30*	0.33^{NS}	0.54**	0.41*	0.56**					
NHI	0.37**	0.38*	0.14^{NS}	-0.08^{NS}	0.08^{NS}	0.10^{NS}	-0.47**				
HI	-0.57**	-0.70*	-0.14^{NS}	0.03^{NS}	0.03^{NS}	0.02^{NS}	-0.20^{NS}	-0.41*			
100 grain	0.38*	0.45*	0.54**	0.12^{NS}	0.38*	0.38*	-0.36*	0.61**	-0.19^{NS}		
PY	0.20^{NS}	0.32^{NS}	0.52**	0.12^{NS}	0.44^{NS}	0.47^{NS}	-0.41*	0.65**	-0.21^{NS}	-0.19^{NS}	
HLMYLD	0.29^{NS}	0.38*	0.48*	-0.09^{NS}	0.23^{NS}	0.32^{NS}	-0.27^{NS}	0.28^{NS}	-0.38*	0.48*	0.61*

Nodno=nodule number, NFW=Nodule fresh weight, RDW=Root dry weight, SDW=Shoot dry weight,
FIX=Biological nitrogen fixation, NDFA=Nitrogen Derived From Atmosphere, NBAL=Nitrogen Balance, NHI=Nitrogen Harvest Index'
HI=Harvest Index, PY=Pod yield,
HLMYLD=Haulm yield.

Table 4.15 Correlation Matrix of Some Agronomic and Nitrogen Fixing Trait in 2012

	Nodno.	NFW	RDW	SDW	FIX	NDFA	NBAL	NHI	HI	100	PY
Nodno											
NFW	0.86**										
SDW	0.05^{NS}	0.23^{NS}									
RDW	0.25^{NS}	0.26^{NS}	0.11^{NS}								
FIX	0.27*	0.35^{NS}	0.31^{NS}	0.13^{NS}							
NDFA	0.32^{NS}	0.48^{NS}	0.57*	0.20*	0.24^{NS}						
NBAL	0.01^{NS}	0.10^{NS}	0.08^{NS}	0.18^{NS}	0.13*	0.05^{NS}					
NHI	0.19^{NS}	0.16^{NS}	0.34^{NS}	-0.12^{NS}	0.12^{NS}	0.48**	-0.78**				
HI	-0.06^{NS}	-0.09^{NS}	0.06^{NS}	0.07^{NS}	0.21^{NS}	-0.10^{NS}	0.32^{NS}	0.35^{NS}			
100	0.43^{NS}	0.22^{NS}	0.05^{NS}	-0.10^{NS}	-0.25^{NS}	0.11^{NS}	0.03^{NS}	0.01^{NS}	0.02^{NS}		
PY	0.21^{NS}	0.29^{NS}	0.50*	0.23^{NS}	0.08^{NS}	0.71**	-0.16^{NS}	0.03^{NS}	0.62**	0.09^{NS}	
HLMYLD	-0.01^{NS}	-0.06^{NS}	0.31^{NS}	0.02^{NS}	0.27^{NS}	0.27^{NS}	-0.60**	0.80**	0.61**	-0.40*	0.66**

Nodno=nodule number, NFW=Nodule fresh weight, RDW=Root dry weight, SDW=Shoot dry weight,
FIX=Biological nitrogen fixation, NDFA=Nitrogen Derived From Atmosphere, NBAl=Nitrogen Balance, NHI=Nitrogen Harvest Index'
HI=Harvest Index, PY=Pod yield, HLMYLD=Haulm Yield

4.8 PRINCIPAL COMPONENT ANALYSIS (PCA) OF SOME SELECTED NITROGEN FIXING AND AGRONOMIC TRAITS

Due to the limitation of the correlation analysis to isolate trait that future research effort should be channelled to enhance the productivity of groundnut, a simple factor analysis using the method of PCA was conducted and presented in Table 4.15-4.18. Result of the factor analysis as explained by PCA shows that out of the twelve selected agronomic and nitrogen fixing traits, four major traits namely, nodule number, nodule mass, shoot and root dry weight were confirmed to account for the highest variance in both 2011 and 2012.

Generally, the cumulative percentage variance was significantly influenced by fertilizer application. Thus, higher percentage accumulation was reported in the fertilizer treated plots relative to the control in both 2011 and 2012. For instance, in 2011 the cumulative percentage of these traits accounted for about 82.33% and 77.53% in the nitrogen treated and control plots respectively. In contrast to 2011 results, 2012 returned 83.29% and 81.03% in the nitrogen treated and the control plots respectively.

However, in all cases; irrespective of nitrogen fertilizer amendment, the sequence; nodule number>nodule weight>shoot weight>root weight was reported in both 2011 and 2012. These results suggest that future breeding effort should be targeted at improving the efficiency of nodulation and biomass yield to enhance profitable output (yield) of groundnut.

Table 4.16 Total Variance Explained in 2011 (+N)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
Nodule no.	3.735	31.123	31.123	3.735	31.123	31.123	3.285	27.377	27.377
Nodule mass	2.896	24.134	55.257	2.896	24.134	55.257	2.549	21.243	48.620
Root mass	1.892	15.768	71.025	1.892	15.768	71.025	2.186	18.216	66.836
Shoot mass	1.356	11.301	82.325	1.356	11.301	82.325	1.859	15.490	82.325
N-fixed	0.625	5.205	87.530						
Ndfa	0.564	4.700	92.230						
N-balance	0.323	2.691	94.922						
NHI	0.266	2.217	97.139						
100seed	0.165	1.376	98.515						
Pod yield	0.121	1.007	99.521						
Haulm weight	0.034	0.280	99.801						
HI	0.024	0.199	100.000						

Extraction Method: Principal Component Analysis.

Table 4.17 Total Variance Explained in 2012 (+N)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
Nodule no.	3.797	31.640	31.640	3.797	31.640	31.640	3.501	29.176	29.176
Nodule mass	2.970	24.747	56.387	2.970	24.747	56.387	2.737	22.805	51.981
Root mass	2.140	17.834	74.221	2.140	17.834	74.221	2.154	17.950	69.931
Shoot mass	1.089	9.073	83.294	1.089	9.073	83.294	1.604	13.363	83.294
N-fixed	0.802	6.687	89.981						
Ndfa	0.525	4.376	94.357						
N-balance	0.251	2.095	96.452						
NHI	0.144	1.202	97.655						
100seed	0.130	1.086	98.740						
Pod yield	0.087	0.723	99.464						
Haulm weight	0.050	0.414	99.878						
HI	0.015	0.122	100.000						

Extraction Method: Principal Component Analysis.

Table 4.18 Total Variance Explained in 2011 (Control)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
Nodule No.	3.273	27.275	27.275	3.273	27.275	27.275	2.599	21.655	21.655
Nodule mass	2.564	21.366	48.641	2.564	21.366	48.641	2.536	21.136	42.791
Root mass	1.882	15.683	64.324	1.882	15.683	64.324	2.159	17.993	60.783
Shoot mass	1.584	13.204	77.528	1.584	13.204	77.528	2.009	16.744	77.528
N-fixed	0.916	7.631	85.159						
Ndfa	0.668	5.569	90.727						
N-balance	0.533	4.442	95.169						
NHI	0.264	2.200	97.370						
100seed	0.146	1.215	98.585						
Pod yield	0.089	0.746	99.331						
Haulm weight	0.062	0.519	99.850						
HI	0.018	0.150	100.000						

Extraction Method: Principal Component Analysis.

Table 4.19 Total Variance Explained in 2012 (Control)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
Nodule no.	4.719	39.322	39.322	4.719	39.322	39.322	3.982	33.182	33.182
Nodule mass	2.180	18.165	57.487	2.180	18.165	57.487	2.744	22.870	56.052
Root mass	1.711	14.259	71.746	1.711	14.259	71.746	1.869	15.577	71.630
Shoot mass	1.115	9.288	81.034	1.115	9.288	81.034	1.128	9.404	81.034
N-fixed	0.830	6.913	87.947						
Ndfa	0.609	5.073	93.020						
N-balance	0.373	3.106	96.126						
NHI	0.193	1.611	97.738						
100seed	0.115	0.957	98.695						
Pod yield	0.074	0.613	99.307						
Haulm weight	0.050	0.415	99.723						
HI	0.033	0.277	100.000						

Extraction Method: Principal Component Analysis.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 SUMMARY

This study sought to evaluate existing groundnut genotype for symbiotic nitrogen fixation and their subsequent contribution to soil N-balance in the Northern Guinea Savanna of Nigeria. Base on this, a comprehensive field trial was designed and carried out to test the hypotheses.

The result of the soil characteristics showed that the soil was sandy loam in texture with low clay content. The soil pH was slightly acidic, low inorganic carbon, total nitrogen and moderate to low available phosphorus. Iron, manganese and aluminium concentrations in the soil were below the levels considered toxic to plants in acidic soils, while exchangeable base were of medium concentrations with calcium dominating the exchange sites.

There was significant variation in both the agronomic and nitrogen fixing trait during the duration of the trial. The nitrogen fixing traits evaluated include nodulation (number, mass and effectiveness), nitrogen uptake by the shoot, root, haulm and grains. Based on these attributes however, there was significant variation in their respective level among the genotypes. For instance results on nodule mass, number and effectiveness were in favour of ARRORS ICGX-000201/5/P_4/$P_{10;}$ which was significantly different from all other genotypes. Although SAMNUT 24 and SAMNUT 22 were the best genotype in terms of nodule mass and number; but could not translate into effectiveness. The effectiveness score was however ascribed to ARRORS ICGX 000201/5/P_4P_{10}. N rate effects on nodulation were significant. Result shows that increased N-rates negatively impacted on nodulation. Thus, the control was the best in

terms of nodulation. Interaction between genotype and N – rates shows that ARRORS ICGX-000201/P4/P10 observed significantly higher nodule number even at higher N – rates.

Similarly, there were varietal differences in the amount of nitrogen fix, N-rates effects and interaction. The best genotype was ARRORS ICGX 000201/5/P_4P_{10} fixing 60kg N ha^{-1} representing 58% over the least genotype (SAMNUT 24). N rates effect shows that application of 30kg N ha^{-1} increased BNF about 44% above the control. Generally, the wide variation in the amount of nitrogen fixed could be attributed to the inherent genetic variability of groundnut genotypes, since no two genotypes could fix equal amounts of nitrogen under similar environmental condition. SAMNUT 21 fixes significantly higher N under the control than at 30 kg/ha N starter dose.

On the proportion of nitrogen derived from atmosphere (NDFA), results follows similar trend with those obtained for BNF where genotypes that fix significantly higher N equally derived greater proportion of their N-from the atmosphere. This is obvious because BNF rely solely on atmospheric (Inert) nitrogen far more than the soil available N. Hence, ARRORS ICGX 000201/5/P_4P_{10} was the best. Nitrogen effects on NDFA were significant with the 30kg/ha N significantly increasing the proportion of NDFA.

The effect of nitrogen and genotypes on soil N balance revealed a significant difference at both extreme/instances (grain and/or haulm) under consideration. At one instance where only the grain was exported from the field, a mild damage was seen to be done to the soil N reserved, that is positive N-balance predominates with SAMNUT 14, ARRORS ICGX 000201/5/P_4P_{10}, 6AT and SAMNUT 22 outperforming other genotypes. N rates effect however was significant with 30 kg/ha N leaving 36 kg/ha N representing 55% increase above the control, thus, significantly improving the soil N

status. Whereas, at the other extreme (haulm and grain), negative N balance predominate. ICIAR 6AT was the best genotype; even though SAMNUT 22 and ARRORS ICGX 000201/5/P_4P_{10} were still able to impact positively on the soil N balance but at a lower rate relative to ICIAR 6AT. Also, N-rates effect was equally significant with the 30kg/ha N improving the groundnut ability to positively influenced the soil N balance. SAMNUT 21, even at 0 kg N still left a positive N-balance at both extreme.

In terms of nitrogen uptake the best genotype was ARRORS ICGX 000201/5/P_4P_{10} and the best N-rates was 30 kg/ha.

NHI and HI were better with ARRORS ICGX 000201/5/P_4P_{10} than other genotypes in both years. The combined N rate effect was not significant for both parameters. In both cases, there was a significant interaction between genotype and N-rates in 2011 where SAMNUT 10, ARRORS ICGX 000201/5/P_4P_{10}, SAMNUT 21, SAMNUT 23 and SAMNUT 14 reported higher values of NHI at 30 kg/ha N rate relative to the control.

Root and shoot dry matter weight was significant among genotypes in both 2011 and 2012. Shoot biomass was highest in SAMNUT 22 but in contrast with root dry matter which was rather higher in SAMNUT 23. N rate effect was significant; with the 30kg/ha N significantly increasing both root and shoot dry matter in both years.

ARRORS ICGX 000201/5/P_4P_{10} was the best genotype in terms of pod, grain and 100 seed mass in contrast to SAMNUT 22, which although have the potential of increased pod, grain and 100seed mass was statistically the best in terms of haulm yield 30 kg/ha N.

Starter N significantly improved all this parameters except 100seed mass which was not significant at both N rates.

5.2 CONCLUSION

In conclusion, groundnut genotypes used in this study could be grouped into three distinct categories based on the amount of biologically fixed nitrogen and pod yield. ARRORS ICGX 000201/5/P_4P_{10} and SAMNUT 23 are both high fixing and high yielding; 6AT was high fixing but low yielding; while SAMNUT 21 and ICAR 7B are low fixing and low yielding. The remaining genotypes could not be classified into any of these groups. The study also showed that application of 30 kg/ha N is adequate to support groundnut production in the Nigerian savannas without depleting soil N. This rate could also enhance the ability of certain genotypes to fix atmospheric nitrogen. Similarly, research finding suggest that future breeding effort should be targeted at improving the efficiency of nodulation and biomass yield to enhance profitable groundnut production in the Northern Guinea Savanna of Nigeria. Similarly, even distribution of rainfall in 2011 resulted in most of the variation observed in some of the agronomic and nitrogen fixing trait existing among groundnut genotypes.

5.3 RECOMMENDATION

Information generated from this study may be limited to the particular groundnut genotypes used and the specific environmental condition in which the parameters were measured. Nevertheless, experimental results clearly demonstrate that BNF efficiency can be successfully bred into adapted genotypes of groundnut; Thus suggesting that future breeding effort be targeted at improving the efficiency of nodulation (number and mass) to achieve profitable output (Yield) of groundnut. Additional studies using various genotypes and environments however are essential to confirm the types of gene action governing the different measures of N-fixation in other groundnut genotypes and under different growth conditions and if possible their net nitrogen

contribution should be ascertained by rotating the crop with a non legume maize/cassava. In addition, it may also be necessary to vary the nitrogen rates probably to a higher dose to ascertain the nitrogen rate that could completely suppress nodulation of groundnut.

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APPENDIX 1: Proportion of ineffective nodules from 10 randomly selected nodules

	REP 1		REP 2		REP 3		Mean (kg/ha)	
Genotypes/N rates (kg/ha)	30	0	30	0	30	0	0	30
SAMNUT 24	8	8	7	5	7	7	7	7
SAMNUT 22	6	8	6	8	7	8	6	8
ARRORS ICGX-SM 00017/5/P_{15}/P_2	10	6	6	5	8	10	8	7
SAMNU 10	8	6	7	8	9	7	8	7
ICIAR 7B	6	10	10	10	9	6	8	9
6AT	6	6	8	8	8	8	7	7
ARRORS ICGX 000201/5/P_4P_{10}	10	7	6	5	7	8	8	7
SAMNUT 21	7	7	9	10	10	9	9	9
SAMNUT 23	10	5	10	7	6	6	9	6
SAMNUT 14	10	10	6	9	10	10	9	10