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SYMBIOTIC EFFECTIVENESS AND SAPROPHYTIC COMPRTENCE OF SELECTED INDIGENOUS RHIZOBIA ISOLATES FOR GROUNDNUT INOCULATION IN NORTHERN GHANA

BY

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BSc. Agriculture (Hons)

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BSc. Agriculture (Hons)

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MASTER OF PHILOSOPHY

IN

SOIL SCIENCE

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CERTIFICATION

I hereby declare that this submission is my own work towards the MPhil degree and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Inoculation with highly effective and persistent rhizobium strains is a possible approach for enhancing the productivity of groundnut in Ghana. Therefore, a 2 × 9 factorial experiment which resulted in 18 treatments in total was set up in three farmers' fields across northern Ghana to evaluate the response of two groundnut varieties to indigenous rhizobium isolates and their persistence after a year of introduction. The persistence study was carried out under greenhouse conditions. Randomised complete block design (RCBD) and completely randomised design (CRD) with three replications were used for the field and greenhouse studies respectively. Experimental treatments included two varieties of groundnut (Samnut 22 and Chinese), five native isolates, three positive controls (Biofix, BR 3267 and +N) and a negative control without inoculation. Isolate 53e caused significant ($P \leq 0.05$) increase in nodule dry weight on Samnut 22 at both Cheshegu and Tanina, while isolate 9d was the best at Binduri. Chinese variety on the other hand produced nodules with increased ($P \leq 0.05$) weight following inoculation with isolates 9g at both Cheshegu and Tanina. At Binduri, the isolate 9d produced the highest nodule dry weight. Grain yield of Samnut 22 was significantly ($P \leq 0.05$) increased by inoculation with isolate 53e at both Cheshegu and Tanina. All except isolate 91a caused significant ($P \leq 0.05$) increase in grain yield at Binduri. The Chinese variety also produced increased grain yields following inoculation with isolate 9g at Cheshegu and Tanina. At Binduri, increased grain yield was caused by isolate 9d. Results from the persistence study indicated that the test isolates increased the native rhizobium population with subsequent improvement in N_2 fixation after the eight (8) months fallow period. Results from the study showed that the rhizobium isolates are potential elite strains which could be used for the production of groundnut inoculants upon further studies and characterisation.

DEDICATION

I dedicate this work to Prof. Robert C. Abaidoo, Prof. Samuel A. Nsiah and Dr. Andrews Opoku whose effort has brought me this far.

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CHAPTER ONE

1.0 INTRODUCTION

Low inherent soil fertility is a major concern hindering crop production in smallholder farms in Sub-Saharan Africa (SSA) (Peoples and Herridge, 1990). In situations where the nutritional demand of the crop of interest is very high, it tends to affect yields significantly. Grain legumes such as groundnut require high nitrogen for good growth (Peoples and Herridge, 1990). Agro-ecologically, groundnut is considered as one of the most important grain legumes grown in the northern savannah zones of Ghana in terms of its usage and area of cultivation (Etwise *et al*., 2013). According to Breisinger *et al*. (2008), regional contribution of groundnut to the national total production in Ghana stands as follows: Coastal zone 7.7%, Forest zone 9.5%, Southern Savannah zone 7.2%, and Northern Savannah zone 75.6%. At the farm level, grain yields of groundnut in Ghana have remained as low as 850 kg ha^{-1}comparative to the 3000 kg ha^{-1} in the developed countries (Nutsugah *et al*., 2007). This variation in yield has been ascribed to low in-built soil fertility, since the yields of groundnut have still remained low at the farm level in spite of the release of several high yielding, disease and drought tolerant groundnuts varieties through genetic manipulations (Ncube *et al*., 2009). In order to overcome this shortcoming, strategies such as application of mineral nitrogen fertilizers and rhizobium inoculants have been used by farmers (Biswas and Gresshoff, 2014). The use of the former is effective, however, their negative impact on the environment is a major concern. In addition, most mineral fertilizers are priced beyond the accessibilities of smallholder farmers (Zengeni *et al*., 2006).

Rhizobia inoculation offers a better option as it has been used to increase yield in other legumes in most smallholder farms in Africa with minimal environmental effect (Mabrouk and Belhadj, 2012). Rhizobia are capable of forming symbiotic relations

with groundnut to fix nitrogen through BNF by converting atmospheric nitrogen into ammonia (NH_3), which is easily utilized by the plants (FAO, 2006). Yakubu *et al*. (2010) indicated that groundnut can fix about 65 – 100 kg N ha^{-1} $year^{-1}$ through symbiosis with rhizobia. It was shown in a field trial that 86-92 % of the nitrogen uptake of the groundnut crop comes from BNF; this is equivalent to 125-178 kg ha^{-1} of nitrogen (Kabir *et al*., 2013). Nonetheless, inoculation of groundnut with rhizobium has received little attention in Ghana. This is partly due to the unavailability of effective superior strains to formulate inoculants for smallholder farmers especially in Northern Ghana where groundnut is one of the most cultivated grain legumes.

Catroux *et al*. (2001) reported poor N-fixing ability of the native rhizobia due to poor cultivar strain interaction and suggested that N fixation could be enhanced by applying compatible foreign superior strains of rhizobia that are well suited to the local conditions. Unfortunately, the inoculation usually fails, with ineffective symbiosis being formed between the host plant and the inoculant strain. These repeated failures were attributed to the inoculant strains inability to outcompete the symbiotically ineffective native rhizobia (Thies *et al.* 1991). Indigenous strains are competitive in nodule formation, persistent and well suited to local conditions. Yakubu *et al.* (2010) reported a positive response in grain yield of groundnut following inoculation and related the positive response to the presence of indigenous rhizobium strain in the inoculant. It was therefore, necessary to bio-prospect for effective rhizobium isolates from indigenous rhizobium populations from the soils of various groundnut fields across northern Ghana to obtain elite strains to be used in inoculant formulations. Legume-rhizobium symbiosis depends on the rhizobium strain, the legume genotype, the environmental conditions and management options applied (Mathenge, 2016). Compatibility between rhizobium strain and the legume genotype in a favourable

environment is essential for successful nodulation and nitrogen fixation (Woomer *et al*., 2014).

Moreover, persistence of the rhizobium strain is vital for inoculant preparation (Ojo and Fagade, 2002). Application of persisting rhizobium strain has several advantages, including non-repeated application of inoculants and nitrogen fertilizers in between seasons (Ojo and Fagade, 2002). Fluctuating environmental conditions and management practices such as tillage, pesticides and weedicide application affect the persistence of inoculants strains and yields of legume in the field. Ojo and Fagade (2002) reported that, adoption of selected inoculant strains and understanding variations in rhizobium populations would enhance the integration of rhizobium inoculants in the preferred low-input farming system. Ojo and Fagade (2002), observed increased population sizes of rhizobia for *Leucaena leucocephala* from 360 cells g^{-1} to 8.5 x 10^4 cells$g^{-1}$of soil after ten years of fallow. Moreover, Moawad *et al.* (2005) found that two rhizobium inoculant strains (Ph 163 and CE3) for common bean persisted in clay and silt loam soils, respectively for a whole year after the first inoculation. These reports suggest that inoculation with rhizobia could improve the quality of indigenous rhizobia population in the long term.

This study therefore, seeks to identify highly effective and saprophytic competent bradyrhizobium strains for improved groundnut production in the Guinea and Sudan agro-ecological zones of Ghana.

The specific objectives were to:

i. Determine the variation in response of groundnut to promising locally isolated rhizobia strains.
ii. Evaluate the residual effect of inoculation on nodulation and shoot dry weight of groundnut after eight (8) months of fallow.

The specific objectives above were formulated to test the null hypotheses that:

i. Differences between groundnut varieties and the isolates will not influence nodulation, N fixation, growth and yield of groundnut.

ii. Introduced rhizobia isolates will persist in sufficient numbers and perform effectively with groundnut to avoid the need for re-inoculation of the same fields in the subsequent season.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of legumes in soil fertility improvement

An established characteristic of legumes is their capability to form root nodules that can fix atmospheric N in symbiosis with compatible rhizobia; a phenomenon known as biological nitrogen fixation (BNF). For this symbiosis to be established there must be a complex relationship between host (legume) and rhizobia (micro- symbiont) (Ahemad and Kibret, 2014). Biological nitrogen fixation provides sustained nitrogen input into an agro-ecological system. About two – thirds of the nitrogen fixed globally are supplied by BNF (Rubio and Ludden, 2008). Nodule forming legumes have the capability to provide the entire nitrogen needed for their growth, thus influencing the nitrogen balance of the soil and its accessibility by other subsequent crop (Giller, 2001). Leguminous crops turn to reduce the production cost as well as contamination of water resource by N; with respect to pulses, grains of high nutritional value are produced (Hardarson and Atkins, 2003). More than 20 million tons of fixed N are supplied to agriculture each year by grain legumes (Herridge *et al*., 2008). Giller (2001) reported that if only seeds were considered at harvest, as much as 140 kg N ha^{-1} could be accumulated as net soil N from the incorporation of residue or remains of grain legume depending on the legume. Nitrogen in Legume residues has a tendency to be released quickly when incorporated into the soil and can contribute to substantial increase in yields of subsequent crops. In Africa, this is highly significant since it exceeds the recommended 50 kg nutrient ha^{-1} fertilizer used across sub – Saharan Africa reported by Africa Heads of States at the fertilizer summit held in 2006 in Abuja, Nigeria (Africa Fertilizer Summit, 2006).

Legume crops are identified to be important globally because they grow in the tropics, subtropical and temperate regions. Groundnut is considered as one of the most important grain legumes in Northern Ghana in terms of area of cultivation and its usage. It is considered as the second and the fourth most important oil seed crop in Ghana and the world, respectively. It contains 48 – 50 % oil and 26 – 28 % protein, and also a rich source of dietary fibre, minerals and vitamins (Etwise *et al*., 2013). This crop also has the capability to convert atmospheric nitrogen for its use as well as for the profit of subsequent crops in rotation thus serving as an important input in integrated soil fertility management systems as this legume may fix up to about 250 kg N ha^{-1} $year^{-1}$ and not usually fertilized (Mohamed and Abdalla, 2013). Nitrogen fertilizer is normally or preferentially applied at planting to these legumes when grown on sandy or low organic matter soils to supply nitrogen to the plant before nitrogen fixation starts (Abdelmalik *et al*., 2015). Groundnut best perform under optimum temperature that ranges between 25 – 35 °C; pH ranging from 5.5 – 6; and soils whose topsoil have low clay content (less than 20 %) (Etwise *et al*., 2013). Groundnut production in Ghana increased in average yields from about 840 kg ha^{-1} in 2005 to 1,200 kg ha^{-1} in 2012 (FAOSTAT, 2013). Despite the increase, average yields still remain low when compared to yields of 2,500 kg ha^{-1} obtained in developed countries (Nutsugah *et al*., 2007; FAOSTAT, 2013). Abdulmalik *et al*. (2015), reported that different legume species or cultivar of a legume species respond differently to different rhizobium strains under different environmental and soil conditions. Moreover, genetic variability between legume species or cultivar of legume species influences the amount of nitrogen that can be fixed (Joint, 1998).

2.2 Rhizobia

Rhizobia are gram – negative microorganisms that are motile, non-sporulating and rod shaped (Mohammed *et al*., 2016). They are capable of symbiotically living with leguminous plants forming nodules and fixing atmospheric N for the host plant. Rhizobia are broadly categorized or grouped as fast or slow- growing based on their growth on laboratory media. Further categorization may be done according to their compatibility with particular legume group (host range). Several bacterial species fall within the family of *Rhizobiacae* in the alpha – proteobacteria and are in the *Bradyrhizobium, Rhizobium, Azorhizobium, Sinorhizobium, Allorhizobium* and *Mesorhizobium* (Weir, 2008). In addition, research has shown that there are other several species of rhizobia to these ones. In certain situations, these new species have arisen through lateral gene transfer of symbiotic genes (Giller, 2001; Weir, 2012). Weir (2008) reported that of all the above mentioned bacterial species, *Bradyrhizobium* is the one that normally form symbiosis with groundnut in the fixation of atmospheric nitrogen.

2.3 Role of rhizobia in nitrogen fixation

The most well-known primary symbiotic fixer of nitrogen of bacteria are rhizobia (Souza *et al*., 2015). They infect roots of legumes, resulting in the development of nodules where N fixation takes place (Gage, 2004). The host legume obtains constant source of reduced nitrogen from the system of bacterium's enzyme and the legume also furnishes nutrient and energy for the activities of the bacterium. Rhizobium can nodulate above 90 % of legumes (Gage, 2004). Free living rhizobium in the soil feed on remains of dead organisms. They cannot fix nitrogen and have different shape from the root nodule bacteria. They are regular in shape, appearing as straight rod; in root nodules the nitrogen- fixing form exists as irregular cells called bacteriods which are

often club and Y–shaped (Burdass, 2002). Rhizobia symbiotically interact with legume roots to convert atmospheric N_2 to NH_3 with the aid of the nitrogenase enzyme. Hence, the use of rhizobia is a natural and environmentally–friendly way to fertilize plants. Through symbiotic nitrogen fixation process plants benefit from a limitless source of nitrogen from the atmosphere. Current works have revealed that rhizobia may encourage plant growth through mechanisms other than nitrogen fixation (Glick, 2014). For example, the activity of the presence of 1-aminocyclopropane-1-carboxylate (ACC) deaminase in some strains of rhizobia encourage plant growth through lowering the levels of ethylene in plants (Glick, 2014). Furthermore, rhizobia contribution to increased resistance against plant pathogens has been reported (Glick, 2014).

2.4 Mechanisms of biological nitrogen fixation

Legumes secret chemical compounds called flavonoids from their roots, these chemical compounds trigger the bacteria to produce nodulation (nod) factors (Brencic and Winans, 2005). The sense of nod factor by the root, leads to a number of changes which occur biochemically and morphologically within the roots. Nodule initiation is triggered by cell division, and the root hair growth is redirected to fold – around the bacteria multiple times until it fully engulfs one or more bacteria (Brencic and Winans, 2005). The engulfed bacteria divide several times, forming a micro colony. Through an infection thread growing in the root hair the bacteria from this micro colony enter the developing nodule into the basal part of the epidermis cell, through to the root cortex (Brencic and Winans, 2005). They are then engulfed by a plant-derived membrane and differentiated into bacteroids that convert nitrogen gas from the air into ammonium, a form usable by the host plant (Brencic and Winans, 2005). In return, the host plant provides the rhizobia with products of photosynthesis such as sugars and

carbohydrate that can be used as fuel by the bacteria in the BNF process (Hubbell and Kidder, 2009; Barnish and Spinelli, 2011). The equation below explains the nitrogen fixing reaction by which nitrogen is fixed from the atmosphere and thus becomes available for the plant (Urzua, 2005)

$$N+16ATP + 8e + 8H \longrightarrow 2NH + 8H + 16ATP$$

2.5 Types of nodules formed by groundnuts

Nodules formed by legumes can be classified into two groups (indeterminate and determinate) based on their mode of development (Drew *et al.*, 2012). The type of root nodule formed is only dependent on the legume plant, regardless of the rhizobia species. However, groundnut form determinate nodules (Drew *et al.,* 2012). These nodules are mostly spherical, less than 5 mm in diameter and is deficient of distinct internal zones. They develop from cell divisions within the outer or middle cortex of the root, lack persistent meristem and tend to be spherical in shape (Drew *et al*., 2012). They do not exhibit any clear developing gradient; infected plant cells enlarge to lodge or accommodate the invading and dividing bacteria, with fixation of nitrogen starting simultaneously throughout the infected plant cells leading to one homogenous N_2-fixing zone (Drew *et al*., 2012). Symbiosomes form determinate nodules usually containing two or more bacteriods of similar size to free–living bacteria. Rather than pink if the interior colouration of these nodules is white or green, there is a possibility of them not to fix nitrogen.

2.6 Rhizobia host specificity and effectiveness

The interaction between legume and rhizobia results in the formation of a nitrogen fixing symbiosis (Ohyama *et al*., 2009). The only most important source of fixed nitrogen in agricultural systems is legume-rhizobia symbiosis (Graham and Vance,

2000). Legume-rhizobia symbiosis contributes between one-third and one-half of the total N added to agricultural land (Herridge *et al*., 2008). Generally, rhizobia and legume relation is a selective one: each rhizobia species has a diverse host range allowing nodulation of a particular set of leguminous species, and each leguminous species nodulates only with a certain range of rhizobia. Nonetheless, a complex relationship between legume and rhizobia has been found and several species of legumes can be nodulated with different rhizobia species in different geographical regions (Han *et al*., 2005). Evolution of rhizobia-legumes associations has been suggested by some authors that, it may be host driven (e.g., Kiers *et al*., 2003; Sachs *et al*., 2011). Additionally, rhizobia can nodulate legumes even if the association lead to low symbiotic nitrogen fixation (Den Herder and Parniske, 2009). Oldroyd *et al*. (2001) reported that, functional nodules development needs spatially controlled activities of genes and gene products of both partners. Specificity between legume cultivar and rhizobia strain is required for successful infection. Symbioses between rhizobia and legume plants are mainly a mutualistic interaction (Lindström and Mousavi, 2010). However, it seems that there are cases where these partnerships can also be considered as parasitic when they form ineffective symbiosis with legumes. These types of situations may occur when multiple rhizobia strains compete for the same plant and when the strains infect non-specific hosts promiscuously. Several legumes are promiscuous meaning different rhizobia species and mostly broad host range rhizobia strains can nodulate them (e.g. *Sinorhizobium* sp. NGR234). For example, rhizobia for promiscuous hosts like *Astragalus* spp, have very diverse genomic and symbiotic gene backgrounds (Zhao *et al*., 2008). Rhizobia strains can also form effective symbioses when they interact with their own specific host legumes (Denison and Kiers, 2008). For example, *Rhizobium leguminosarum* strains isolated

from nodules of native legumes in New Zealand were found to form ineffective nodules, while they still reserved the ability to form an effective symbiosis with classical host plants (pea, bean and clover) (Weir, 2006). Restrictive hosts are usually found in these genera *Cicer*, *Vicia* and *Trifolium* since they are not common (Laranjo *et al*., 2013). Particularly, *Cicer arietinum* is not nodulated by broad host range rhizobia and considered a restrictive host (Laranjo *et al.*, 2013). Cicero strains nodulate only chickpea, which however are not known to nodulate any other legumes. This compatibility is far from being understood, but it may be related to the type of flavonoid nod gene inducers the legume releases and the type of nod factors the rhizobium releases. Restrictive symbioses seem to be less ancestral than promiscuous ones which are widely spread (Laranjo *et al*., 2013); possibly the former rhizobia were wide host range strains ancestral from *Sinorhizobium* sp. NGR234 (Laranjo *et al*., 2013). Thus, ancestral nod factors would have been simpler chitin oligomers, able to interact with many plants; in the course of evolution additional substituents have been added to narrow the host range (Laranjo *et al.*, 2013). Sprent, (2007) reported that is still unclear whether the ability of legumes to be nodulated occur once or several times during evolution. The fact that nodulation genes are probably less ancient than nitrogen fixation genes, may propose that the nitrogen fixing ability is an ancient characteristic which subject rhizobia ancestors for co-evolution with legumes (Laranjo *et al*., 2013). In some situations, where there was a total absence of rhizobia able to nodulate legumes introduced in countries, generated exceptional conditions for the study of rhizobia evolution (Laranjo *et al*., 2013). Perhaps the most outstanding cases are soybean introduction in Brazil (Barcellos *et al*., 2007) and biserrula in Australia.

Failure to fix nitrogen by rhizobia often occur when they come across nonconforming hosts. Sachs *et al*. (2010) reported that, inoculation of different legumes with rhizobia

isolated from one host species, invariably only a few of such cross-inoculated strains can nodulate the new host, and only a lesser number efficiently and effectively fix nitrogen on the new host. This trend proposes that several ineffective rhizobia that have been recognised may be very effective if inoculated on a more compatible host. In agricultural settings mismatches of host-symbiont might be widespread where plants, bacteria, and even soils are transported among sites (Sachs *et al*., 2010). On the other hand, although the definition of rhizobia is dependent on their infection ability on legumes. Lineages of multiple rhizobia have been studied, which revealed that much difference within these groups is represented by strains that don't exhibit any ability to infect legumes (Sachs *et al*., 2010). The N_2-fixing symbiosis on N fixation is characterized by legume x rhizobium specificity. Some host varieties are superior to others in their ability to fix N_2, and in turn, some rhizobia strains have similar superior capability. Sachs *et al*. (2010) and Friesen (2012) reported that, one hypothesis presented in several studies is that the appearance of rhizobial exploitation is the result of mismatched host-symbiont pairs. Furthermore, a basic concept of the rhizobia–legume relationship or interaction is the effectiveness of the symbiosis, thus the amount of N_2 fixed by rhizobia and made available to the plant. Moreover, for successful N fixation, legumes allocate about 15 % of their assimilates to the rhizobium strain to supply them energy. Differences in effectiveness of symbiotic interactions can be wide (Sprent, 2007). Due to this potential difference, Howieson *et al.* (2005) defined four classifications of symbiotic interaction: no symbiotic interaction thus plants do not nodulate, an ineffective or parasitic interaction; where nodules formed do not fix N_2; partially effective symbiosis where 20 – 75 % of the plant biomass produced is achieved by nitrogen–fed control and an effective symbiosis, where nodulated plants produce more than 75 % of the plant biomass

achieved by a nitrogen–fed control. A better understanding of the factors that guide specificity and effectiveness in the rhizobia–legume symbiosis will be needed if sustainable increases in productivity are required in agricultural systems in order to cope with the increasing population in the world, higher N fertilizer prices and other pressure (Howieson *et al*., 2008). Perret *et al*. (2000) reported that specificity between symbiotic partners reduce the formation of ineffective, non–fixing nodules by the host legumes. Rhizobia vary in their response to different signal molecules produced by legumes, thus some have narrow host range and develop nodules with a restricted number of legumes. On the other hand, legumes may also be host to a specific kind of symbiont or form symbiosis with varied range of rhizobia. Increase in N fixation can be enhanced by the specificity of the relationship. Giller (2001) reported that grain legumes can yield up to 300 $kgha^{-1}yr^{-1}$ whilst some other tree legumes fix as much as 600 $kgha^{-1}yr^{-1}$ when well matched with their symbionts.

2.7 Factors affecting rhizobia inoculation and BNF

Efficient nitrogen fixation depends on rhizobia strain, host plant, environmental and soil conditions and their interaction. Application of foreign superior rhizobia strains into the soil does not give an assurance of a higher BNF hence increase in yield (Lupwayi *et al*., 2000). On the contrary, absence of all other factors that affect nitrogen fixation, a rhizobium strain introduced in the soil should be able to compete with the native or indigenous rhizobia in nodule formation. The effectiveness and efficacy of an introduced strain is decreased by several of these factors. These factors have the capacity to influence the symbiotic relationship that exist between legumes and rhizobia. It limits the ability of the rhizobia to develop nodules with optimum nitrogen fixing ability (Slattery and Pearce, 2001). Hence successful inoculation relies on a number of biotic and abiotic factors (Chianu *et al.*, 2009). The most important abiotic

factors include drought, salinity, waterlogging, temperature, soil acidity, inadequate mineral nutrition and mineral toxicities (Abdel-Latef and Ahmed, 2015) and biotic factors such as competition of ineffective indigenous rhizobia, insect pest and diseases (Serrage and Adu-Gyamfi, 2004; Sofy *et al*., 2014). These factors can interpose persistence of rhizobia in the soil, the infection process, nodule development and nodule functioning and symbiotic nitrogen fixation (Mohamed *et al*., 2014). Therefore, it is not likely that a persistent and competitive strain of rhizobia can express its full capabilities for nitrogen fixation in the presence of these factors that limit, reduce or minimize the vigour of the host plant (Abd-Alla *et al*., 2014).

2.7.1 Nitrogen availability

Soils with low mineral nitrogen (N) usually have high amount of nitrogen from fixation but sufficient water and other nutrients that have the capability to support plant growth (Unkovich *et al*., 2008). Increase or maximization in the levels of soil mineral N in the rhizosphere suppressed nodule formation and functioning (Saito *et al.*, 2014). Generally, increase in nodulation should maximize or enhance the amount of N_2–fixed but this principle could be limited by a number of environmental factors. For example, the amount of N produced by the legume – rhizobium symbiosis during the early stages of growth may not be sufficient to meet the N requirement of the legume and as such small application of chemical N is necessary to enhance early growth (Saito *et al.*, 2014). Application of chemical N at either flowering or vegetative stage can potentially increase or enhance pod number and biomass of the crop by 44 % and 16 %, respectively (Katulanda, 2011). There are a number or various contradictory reports on legume response to application of nitrogen. When soil nitrate is low there is a higher possibility of getting positive response to inoculation and the legume has a high potential for growth and in the same manner N_2-fixation can potentially be hindered

by high soil nitrates (Saito *et al.*, 2014). Legumes response to application of nitrogen depends on the rate and time of application (Amba *et al*., 2013).

2.7.2 Phosphorus availability

Soils that are deficient in phosphorus (P) reduce or limit the formation of nodules and also affect the persistence of rhizobia in soils (Giller, 2001). Phosphorus play an important role for legumes as P is needed in large quantities in N_2 fixation. The high amount of P needed by legumes is consistent with the participation of P in plant growth and the high rate of energy needed for symbiotic nitrogen fixation and assimilation of N in nodules (Nkaa, 2014). Phosphorus is a key component of ATP which is required to supply energy for the rhizobia for N_2 -fixation; about 16 molecules of ATP which is similar to 10 kg of carbohydrate is needed by the rhizobia for every kilogram of nitrogen fixed (Mmbaga, 2014). Soil with low P may tend to reduce yield by affecting fixation of nitrogen in nodules and as a result causing deficiency of N in plant shoot (Nkaa, 2014). Significant amount of phosphorus is fixed in soil with high or low pH and unavailable to plant (Chen, 2006). Legumes need about 30 kg P ha^{-1} for optimal N_2–fixation and growth (Yakubu *et al.,* 2010). Moreover, applying P-solubilizing bacteria (FAO, 2006) tend to decrease the pH of the soil resulting in dissolution of bound forms of phosphate hence making P available to plant (Chen, 2006). Thus, an ideal option for maximizing available P concentration to plant is the use of P solubilizing bacteria (PSB) as applied chemical P is usually adsorbed by soil particles leading to a reduction in the concentration or levels of available P to plant and decreasing fixation of N. PSB can be introduced into the soil by applying to seeds just as inoculants before planting (Chen, 2006).

2.7.3 Other constraints affecting nodulation

Superior rhizobium strains may be present in the soil but their full potential may not be fully attained if there are nutritional and environmental limitations as these nutritional and environmental factors determine the amount of N that can be fixed. Nutrient deficiencies limit legume–rhizobium symbiosis that lead to N_2-fixation and in turn adversely affect yield (Mabrouk and Belhadj, 2012). In order to obtain efficient symbiosis between the host and rhizobium to achieve optimum growth through nitrogen fixation, there must be sufficient supply of all the nutrients essential for rhizobium and host legume growth (Mmbaga, 2014). Aluminium and manganese produce toxic effects which have adverse effect on plants, reduce the persistence of rhizobia and limits fixation of N (Giller, 2001). Soils with low pH tend to have high concentrations of aluminium and most legumes will not nodulate under such conditions (Mubarik and Sunatmo, 2014). On the other hand, calcium application is known to regulate aluminium and manganese toxicity and increase the persistence of rhizobia and enhance the ability of the rhizobia to infect legumes (Giller, 2001). Meristematic activities in both the legume and the nodule are enhanced by boron but their deficiency causes nodules dysfunction (Weisany *et al*., 2013). However, sulphur do not have any direct effect on nodulation (Giller, 2001) but its deficiency will lead to lower protein yield as it forms part of many amino acids (Weisany *et al*., 2013). Cobalt, zinc and chloride have no effect on nodulation but are needed by the host legume for growth (Weisany *et al.*, 2013). Nodulation and nitrogenase activity have been affected or influenced soil moisture (Ramos *et al.*, 2003). Biological nitrogen fixation is very sensitive to moisture stress. Soil rhizobia number decreases with drought resulting in a decrease in nitrogen fixation rate (Niste *et al.*, 2013).

2.7.3.1 Native or indigenous rhizobia populations

Adamovich and Klasens, (2001) reported that, significant nitrogen fixation process also depends on the existence and persistence of Rhizobia in soils and their efficiency. Response to inoculation by plants is highly influenced by the quality of the indigenous rhizobia (Date, 2000). Unkovich *et al*. (2008) reported that the absence of sufficient numbers of efficient and effective rhizobia in the soil is one of the most common factors limiting a legume's ability to fix Nitrogen. Symbiotically effective indigenous rhizobia in the soil will have competitive advantage over rhizobia strains introduced in the soil because of its large population and has already adapted to the conditions of the area. Ampomah *et al*. (2008) revealed that, rhizobia that are symbiotically compatible may or may not be present in the soil depending on the cropping history and the type of crop grown in that area. A strong competition present by indigenous rhizobia to the establishment of an introduced rhizobia strain, most often leads to inoculant failure. Castro *et al*. (1999) reported that after studying nodulation of peanuts in the presence of native rhizobia and introduced strains realised that indigenous rhizobia are more competitive. This situation can be resolved by applying high rates of the rhizobia strain to be introduced. Significant qualities of inoculants must be applied to legumes, to enhance the competitive advantage of the introduced strains overcome the competition presented by the native rhizobia (Geetha and Sanket2013). Boahen (2008) reported that inoculation can lead to the establishment of large rhizobia population in the rhizosphere and improved nodulation. Literature suggests that agronomists must factor in natural selection on both symbionts and their crop plants to optimize crop production. Rhizobium strain in an inoculum, must be able to survive in soil under the different field conditions when applied in an agricultural setting (Santos *et al.*, 1999). This means that the inoculum strains must be able to outcompete

the native rhizobia for nodulation, efficiently escape from senescent nodules and survive in the soil in order to infect the next season of cultivated hosts. Inoculation with highly effective N-fixing rhizobia strain requires establishment and survival in the soil environment. A persistent and effective rhizobia strain has several advantages, and is preferable to the repeated inoculation in the subsequent season. To be established in the field, introduced rhizobia strain must cohabit with predators and competitors, and maintain itself during period of low nutrient availability. In the course of introducing new rhizobia strains it may create competition barrier. However, Thies *et al*. (1991) reported that, competition from indigenous rhizobia is not necessarily the major determining factor for lack of response to inoculation; rather the availability of sufficient soil population to meet the N_2 fixation requirements of the host is the primary reason for failure of crops to respond to inoculation.

2.7.3.2Biological Agents

Leaves defoliation caused by pest and diseases impair photosynthesis. Hence there is the reduction in symbiotic effectiveness between rhizobium and host legume as the supply of nutrients (carbohydrate) which serve as energy for the rhizobia is reduced and this can adversely influence fixation of nitrogen (Giller, 2001). The ability of the host legume to persist under these stress conditions is very important for the symbiotic relationship (Zahran, 1999). Plants and weeds compete for growth resources which affect photosynthesis hence decreasing the nutritional supply to rhizobia which influence the amount of N_2 fixed.

2.8 Why the need to inoculate

Inoculation becomes very necessary where compatible rhizobia are absent, when native rhizobia are ineffective in fixing atmospheric nitrogen, when a legume is introduced in an area for the very first time and where there is small population of

compatible rhizobia to enhance nodule formation (Herridge *et al*., 2002). Inoculation trials are generally set up with three treatments consisting of: non–inoculated treatment receiving no fertilizer, non-inoculated plants supplied with N fertilizer and an inoculated treatment (Date, 2000). The inoculation is done to establish the maximum rhizobia number that could be applied to significantly increase yields and as well as to provide an opportunity to assess the competitive ability and effectiveness of the introduced superior strains; the un-inoculated treatment measures the impact or influence of soil N and gives a clue about the presence or absence of indigenous rhizobia and their symbiotic effectiveness and lastly the N fertilized treatment measures the plants maximum potential yield when N is not limiting (Date, 2000). Inoculants are sometimes applied as insurance against crop failure Deaker *et al.* (2006) as there is more problems associated with not inoculating at all than over inoculation (Herridge *et al.*, 2002).

2.9 Ways towards improving N_2-fixation

Methods directed towards improving BNF rely on the combined effect of legume genotypes, the rhizobium strain, the environment and the management of the factors mentioned above (Nana and Alemneh, 2015). The genetic potential of plants in fixing nitrogen may be enhanced by breeding for improved cultivars of legumes; which can lead to 10 % increase in nitrogen fixed comparative to the existing cultivars (Hirel *et al*., 2011). Good legume growth is required for symbiosis as it provides rhizobia with nutrients (Keyser and Li, 1992). Mechanisms directed towards screening for superior rhizobia strains or selection of rhizobia must factor in the desirable qualities described above (section 2.4). Practices that control or regulate rhizobia population, reduce the inhibitory influence of soil nitrate and biomass of legumes can alter the inputs of N fixed substantially (Peoples *et al*., 1995). One of the effective ways of enhancing N

fixation is by intercropping cereals with legumes in soils rich in nitrogen as it is assumed that the cereal will establish effective rooting system than the legume thus making use of the nitrogen in the soil before the legume become well established (Bedoussac *et al*., 2007). Lupwayi (2000) suggested that carrier for rhizobia and methods of inoculation should be reviewed under extensive conditions so that site or country specific recommendations can be made rather than generalized recommendation. The environment is the most difficult factor to change and hence efforts must be aimed towards maximizing systems that best fit a particular condition and also using strains and legumes that have wide adaptions to different climatic conditions (Giller and Cadisch, 1995). Climatic, edaphic and nutritional issues are part of the environmental factors (Peoples *et al*., 1995). Educating people about handling, benefits, availability and use of rhizobia inoculant brings about a significant effect or influence on improving BNF in developing countries (Keyser and Li, 1992). Without requisite skills and proper management by the researcher or farmer the above interventions will not hold to yield the needed results or outcome.

2.10 Characteristics of rhizobia needed to ensure effective symbiosis

Competition presented by native rhizobia could be overcome by selecting rhizobia strain that are extremely efficient and effective with the legume it forms symbiotic relation with (Ampomah *et al*., 2008). The following attributes are identified as the best qualities needed by rhizobia for nitrogen fixation: ability to compete with the native rhizobia, persist in the soil, survive in seed pellets, fix nitrogen under varying environments conditions, adapt to adverse environmental conditions and lastly ability to multiply in broth and persist in inoculant carries.

2.11 Maintenance of soil fertility through BNF

Nutrient losses from the soil results from crop removal, leaching and erosion (Stoorvogel, 1993). Although these losses are inevitable or unavoidable, it could be controlled through the application of bio-fertilizers, organic and inorganic fertilizers (Giller and Cadisch, 1995) or in combinations. Losses of nutrients through erosion can be minimized or reduced by legumes in farming systems as some legumes form canopy, which minimize the impact of rain drops on the soil (Giller and Cadisch, 1995). Legumes contribute to soil fertility maintenance through N_2–fixation as it enriches the soil with N through decomposition of litter (Giller and Cadisch, 1995). Moradi *et al.* (2014) revealed that the amount of N contributed by groundnut to the growth of maize in intercropping system 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. Yakubu *et al.* (2010) reported groundnut–rhizobium symbiosis can result to N fixation of about 65 – 335 kg ha^{-1} $year^{-1}$, although the amount of N fixed by symbiotic system may differ according the method used to measure N_2-fixation (Sellstedt *et al*., 1993). Plants remains after harvesting, if not transferred from the field can contribute to increasing soil fertility.

2.12 Quantifying biologically fixed nitrogen

The easiest method for estimating biologically fixed N is the N balance method where an N- fixing crop and non-N fixing (Anglade *et al*., 2015) crop are grown adjacent to each other and the difference in N in the plant tissue at harvest between the two crops is assumed to be the quantity of N fixed biologically. Even though this technique is not expensive and simple to use in the field, it has revealed to be very inaccurate and undependable in largely over– or underestimates the influence of soil N in the system (Mc Cauley, 2011). The methodologies for the quantification of N fixed falls into three

broad techniques. The first assess N_2 fixation as the net increase in total N of a plant–soil system (N balance method). The second focuses at separating plant N into two fractions one absorbed from the soil and the other obtained from the N_2 fixation (N difference, ^{15}N isotope dilution, ^{15}N natural abundance and ureide methods). The last group measures nitrogenase activity, the enzyme in charge for fixation of nitrogen (acetylene reduction and hydrogen evolution methods). Therefore, methodologies such as N balance method, N difference method, acetylene reduction method and ^{15}N methods could be used for estimating N fixed.

2.12.1 Nitrogen difference method

The N difference method has been recommended as an option to the N balance method where levels of soil available N under both crops are taken into account. Respective difference in N uptake from the two crops can be estimated by adding the soil N component, soil N transformations over the growing season, assuming equality between the crops in terms of soil N transformations and losses (Unkovich *et al*., 2008). Another assumption of the N difference technique is that N uptake pattern and the root N between the two crops are similar. These assumptions are difficult to be confirmed in the field setting because N in root, including losses of root N to the soil, may represent a large pool of fixed N that is overlooked in N fixation estimation (McCauley, 2011). Since it is not practical to efficiently or effectively harvest roots from field plants, shoot N and root N ratios could be assumed to be similar between the crops. This technique is complicated especially when intercropped legumes are involved because intercrop competition may negatively influence the legume and non-legume reference crop's ability to access soil N (Giller, 2001). The difference between total N uptake (recovery)in legume and the total N uptake of an adjacent non-N_2 fixing reference species, such as a grasses, cereals or some other non-legume is calculated in

this method (Unkovich *et al.*, 2008). When available soil N is low, this method is considered reliable (Ashworth et al., 2015), though significant differences may only be seen in total N uptake when BNF levels is above 20 kg N ha^{-1} (Zuberer, 2005, Ashworth *et al*., 2015).

2.12.2 Acetylene reduction assay technique

This method is the most widely used method in estimating N_2 fixation in a symbiotic system (Unkovich and Baldock, 2008). It is based on the principle that nitrogenase which convertsN_2 to NH_3, also has the capacity to convert acetylene (C_2H_2) to ethylene (C_2H_4). This technique measures the rate of acetylene conversion to ethylene by the nitrogenase enzyme; the amount of nitrogen derived from the atmosphere is estimated by using the amount of ethylene produced by multiplying it by a conversion factor or ratio (Danso, 1995). The acetylene reduction assay can be used to detect nitrogenase activity since it is highly sensitive (Unkovich *et al*., 2008). It is also relatively inexpensive and simple (Danso, 1995). The limitation of this technique is the fact that measurement only reflects activity of nitrogenase for the period of the assay. There are variations in the enzyme's diurnal and seasonal activities and as such several measurements are needed for the correct estimations of the N_2 fixed (Unkovich *et al*., 2008). The validity of the acetylene reduction assay technique is questionable because of the use of conversion ratio (Danso, 1995). There is also auto–inhibition acetylene conversion to ethylene (Danso, 1995). Moreover, acetylene is hazardous to man as it can explode (Unkovich *et al.*, 2008).

2.12.3 ^{15}N Methods

This method consists of the ^{15}N enrichment methods and ^{15}N natural abundance. This approach provides an accurate estimation of N Fixed biologically but it is expensive and needs specialised equipment and skills (Danso, 1995). It is generally based on the

principle that the concentration of ^{15}N in the atmosphere is different from that of soil N available for plant use and therefore the difference in the analyses of ^{15}N of the N_2-fixing plant and the non-fixing plant is considered as the amount of N fixed.

2.13 Quality of an inoculant

Quality control of an inoculant is defined as a series of activities put up to maintain the value of the inoculant during and after production to certify that the inoculant contains adequate and viable number of rhizobia to colonise the rhizosphere for plentiful nodulation (Beck *et al*., 1994). The inoculant carrier has an impact on its quality but the most vital concerns are the rhizobia numbers and age; the inoculant should contain adequate number of rhizobia with few dying over time (Herridge *et al*., 2002). There are numerous types of carriers but the peat is more appropriate because of the protection it offers to the rhizobia combined with its capability to nurture the organism (Herridge *et al*., 2002). Peats, be it sterile or non-sterile are commonly used but the first one is ideal because it holds 100-fold more rhizobia and also produces superior inoculant products than the latter but the cost of sterilization is high (Lupwayi *et al*., 2000; Herridge *et al*., 2002). Day (1991) reported cases of positive response and negative response by plants due to the use of sterile carrier inoculant and non-sterile carrier inoculant, respectively. This is due to the fact that non–sterile carrier inoculants contain low numbers of workable rhizobia or enormous number of contaminants (Lupwayi *et al*. 2000). Inoculants with workable rhizobia are similar to inoculants with contaminants or dead rhizobia on appearance hence one cannot differentiate between quality and non-quality inoculants by mere observation; in view of this some producers are unenthusiastic to institute checks for quality control (Thompson, 1991). This causes farmers therefore to lose interest in inoculant because of limited response to inoculation (Lupwayi *et al*. 2000). For this reason, there should be hard-headed actions

to check the quality of inoculant to guarantee that high quality inoculants are sold to farmers. Manufacture of quality inoculants without any real laid down measures to check and keep its quality is likewise worse as producing low quality inoculant. Principles for regulating and retaining inoculant quality may vary among countries (Herridge *et al*., 2002). However, it serves the same purpose of safeguarding the production and upkeep of high quality inoculants for farmers. Countries like Canada and France have working principles which have resulted to the production of high quality inoculants for farmers (Herridge *et al*., 2002; Lupwayi *et al*., 2000). For example, between 1974 and 1998 the number of workable rhizobia in inoculants sampled from the Canadian market improved from 15 % to 95 % as an outcome of effective quality control systems (Lupwayi *et al*., 2000).

Lupwayi *et al*. (2000) emphasised the characteristics of quality inoculants as follows: inoculants should have less contaminant with no effect on its effectiveness, should have large cell numbers of superior rhizobia strain, adequate shelf, formulation that is effective, proper packaging, life be easy to apply and have clear labelling with instructions for use.

The quality of inoculants can be assessed using any of the following methods outlined by Lupwayi *et al.* (2000): plate counts of viable cells, most probable number (MPN) or plant infection, microscopic examination, immunological techniques, immuno-spot blot and colony-lift, indirect fluorescent antibody identification of rhizobia in broth and syringe filter enzyme immunoassay, immunoblot test for rhizobia identity.

2.14 Persistence and performance of rhizobium strains

Persistence is the ability of a rhizobium strain to survive and perfume efficiently and effectively in the soil over time. Alexander (1986) reported that in order for introduced

rhizobia to become established in the field, they must cohabit with predators and competitors and sustain themselves when soil available nutrient are low. The survival of inoculated strain in the field can also be influenced by changing conditions in the environment and management practices. Knowledge of the variations happening in the populations of rhizobia and the issues influencing such variations may result in the selection of adapted inoculant strain and to improved use of inoculants (Ojo and Fagade, 2002). Ojo and Fagade (2002), observed increased population sizes of rhizobia for *Leucaena leucocephala* from 360 cells g^{-1} to 8.5 x 10^4 cells g^{-1} of soil after ten years of fallow. Ranga Rao *et al.* (1981) reported that yields of soybean were sustained in a field without further inoculant or fertilizer nitrogen input after *Bradyrhizobium japonicum* persisted in the soil for 2-year fallow period. Moreover, Moawad (2005) found that common bean and two rhizobium inoculant strains (Ph 163 and CE3) for common bean persisted in clay and silt loam soils, respectively for a whole year after the first inoculation. Zahran (1999) reported that, the only safe and cost effective approach for growing groundnut for commercial purposes is still through biological nitrogen fixation by groundnut-rhizobia symbiotic systems. This will need insuring efficient rhizobia strains in soil where the plant is grown in rotation with other plants (Zahran, 1999).

2.15 Summary of literature review

Legume rhizobia symbiosis resulting in the conversion of atmospheric nitrogen to ammonia for plants use is restricted by numerous factors. Among such factors are the density and effectiveness of indigenous rhizobia which this has been known to hinder significant response to rhizobia inoculation as in many cases they are able to out compete foreign rhizobium strains for nodule occupancy. High levels of soil mineral

N has been reported to hinder nodulation and N_2-fixation. Moreover, nutritional and environmental factors like pH, temperature, moisture, phosphorus and light also affects the symbiotic relationship between legumes and rhizobia. The indigenous rhizobia are usually low in numbers or ineffective and are therefore not able to fix adequate nitrogen to meet the nitrogen requirement of plants. A conventional method to increase biological nitrogen fixation is to study the combined effect of legume genotypes, rhizobium strains and the environment under different management systems. Varieties of promiscuous legume were introduced to nodulate freely with the indigenous rhizobia because of the insufficiency of rhizobia inoculants. The indigenous rhizobia are often low in numbers or ineffective and are therefore not able to fix adequate nitrogen with the promiscuous legumes to meet the nitrogen requirement of plants. This may require the introduction of introduce foreign rhizobium strains to promote symbiosis. Unfortunately, the success of this relies on the ability of the introduced strains to establish and out compete the resident rhizobia for such symbiosis to occur which usually is not the case rather the vice versa. Competitiveness of an inoculant strain is vital for successful nodulation and N fixation as well as the selection of rhizobium strain for inoculant preparation. High application of inoculant strain can sometimes help overcome poor persistence and competitiveness of the introduced strain. On the contrary, such quantities are hard to obtain for practical legume inoculant. Hence is vital to find highly effective strains that are persistent and competitive in situ. Although inoculation with effective strains can help increase legume performance, there is no doubt that specificity exists between rhizobium strain and legume variety and their compatibility is a gateway to successful nodulation and enhanced N fixation. Moreover, different legume species or cultivar of a legume species respond differently to different rhizobium strains under different

environmental and soil conditions. Therefore, it is prudent to identify the compatibility between rhizobium strains and legume species or varieties of different legume species towards sustainable productive groundnut-based cropping systems.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site and characteristics

The laboratory work was carried out at the Department of Crop and Soil Sciences at the Kwame Nkrumah University of Science and Technology, Kumasi.

The field trials were conducted at three different locations in northern Ghana from July to November 2015, with one location in each region; Cheshegu in the Tolon district (Northern region) (latitude N09°27'18.2" to longitude W000°57' 22.4"), Binduri in the Binduri district (Upper East region) (latitude N10°56'57.6" to longitude W000°18' 52.0") and Tanina in the Nadowli district (Upper West region) (latitude N09°53'13.0" to longitudeW002°27' 48.5"), located in the Guinea and Sudan savannah agro ecological zones. The study sites have a unimodal rainfall distribution with an annual rainfall ranging between 1000 and 1200 mm. The rainfall duration lasts for5 – 6 months commencing from April or early May and reaches its peak in August or early September. Periods of drought span for 6 months beginning from mid-November to April and average temperatures range between 26 and 30 °C with slight variation throughout the year. The fields had no known recent history of rhizobia introductions and had not been grown with any groundnut variety. The field at Cheshegu and Binduri were previously grown with maize while that at Tanina had not been grown with any crop.

3.2 Field experimentation

3.2.1 Source of planting materials

Groundnut variety 1(Sumnut 22) and 2 (Chinese) were obtained from the International Institute of Tropical Agriculture (IITA) Tamale station and SARI, respectively while inoculants used were obtained from Crop and Soil Sciences' microbiology laboratory at KNUST.

Groundnut variety 1(Sumnut 22) is a late maturing variety (120 days' maturity) and takes 55 to 60 days to attain 50% flowering growth and variety 2 (Chinese) is a medium maturing variety (95 days' maturity) stage and takes 40 to 45 days to attain 50 % flowering (Etwise *et al*., 2013).

3.2.2 Land preparation

The field was ploughed and harrowed with a disc plough and harrow respectively at all sites and each plots measured 4 m x 4 m.

3.2.3 Inoculant preparation

The peat-based inoculant was prepared in the soil microbiology laboratory of Kwame Nkrumah University of Science and Technology. The isolates were cultured on yeast mannitol agar (YMA) incubated at 28 °C. The cultured isolates were then looped unto yeast extract mannitol broth and placed in an orbital incubator at a temperature of 28 °C at 125 rpm until it became turbid. Peat imported from IITA, Nigeria, was bagged (50 g peat/bag) and gamma radiated at Ghana Atomic Energy Commission (GAEC). Using a 20 ml sterile syringe with 18-gauge needle, 50 ml of the *Bradyrhizobium spp.* broth cultures were withdrawn from the broth and transferred into a 50 g peat aseptically under the laminar flow cabinet as described by Somasegaran and Hoben, (2012). The bags were then aseptically sealed, labelled accordingly and gently massaged until the inoculum was evenly absorbed by the peat. The freshly prepared

inoculants were then incubated 28 °C for two weeks to cure (Somasegaran and Hoben, 2012).

3.2.4 Inoculant application and Planting

The seeds were treated under shade with inoculum of respective isolate @ 5 g kg^{-1} of seeds just before sowing. A sticker (Gum Arabic) was used in this study at a ratio of 1.5 g to 15 ml clean lukewarm water before adding the inoculant. Seed sowing was done at a spacing of 60 x 20 cm at each location. Three seeds were planted per hill and thinned to two after two weeks to maintain optimum plant population.

3.2.5 Treatments and experimental design

The experiments consisted of eighteen treatments combinations of two groundnut varieties and nine nitrogen sources designated as follows:

Test crop: Groundnut

Variety 1: Samnut 22

Variety 2: Chinese

Nitrogen sources:

The treatments were as follows: five indigenous rhizobium isolates (52b1, 53e, 91a, 9d and 9g); two commercial inoculants (strain BR3267 and Biofix); positive N control (+N) (only N; 100kgNha^{-1}); and negative control (-N) (without inoculant and N). All the treatments received basal application of 30 kg P per hectare from triple superphosphate (TSP). The urea was applied in splits; 20 kg N at two weeks after planting and 80 kg N at 50% flowering growth stage. Fertilizer application was done using the band placement technique to ensure fertilizer use efficiency and as well to minimize growth of weeds. The experiment was a two factorial experiment arranged in a Randomized Complete Block Design (RCBD) with 3 replications.

3.3 Data collected

3.3.1 Nodulation

At mid flowering, ten consecutive plants were carefully dug out from the row immediately adjacent to the border rows of each plot. The shoots were cut at about 5 cm above the root. The roots of the plants were put in rubber bags together with detached nodules collected from the soil. The roots were washed under running tap water in a 1 mm mesh sieve to remove adhered soil. The nodules were detached gently and counted. Counted nodules were dried in an oven for 48 hours at 60 °C and weighed to obtained nodule dry weight.

3.3.2 Shoot dry weight

Shoots of 10 plants separated from the root during nodule sampling at mid flowering were dried in an oven at 60 °C for 72 h. The weights of the dried shoots were recorded to obtain shoot dry weight.

3.3.3 Number of pods per plant and grain yield

Groundnut were harvested at physiological maturity of 95 DAP and 120 DAP for Chinese and Samnut 22, respectively from a 4 m x 4 m measured area to determine pod number and grain yield. Ten plant were randomly selected after harvest and their pods detached and counted. The pods were then air dried and threshed to obtain the grain. The grains obtained were dried in an oven for 72 h at 60 °C and weights of the dried grains recorded. The weights of the dried grains were then used to evaluate the grain yield per hectare as reported by Okogun *et al*. (2005).

3.3.4 Measurement of N_2 fixation

The amount of nitrogen fixed biologically was determined using the Total N Difference (TND) technique using guinea grass (*Panicum maximum*) as reference plant (Ashworth *et al*., 2015). The total amount of nitrogen in shoot at mid flowering

and reference plant were assessed and the amount of N_2 fixed calculated using the modified equations of Mary *et al*. (1995). In order to account for the nitrogen that remained in the roots and/or was lost in the soil, the calculated value was then multiplied by a factor of 1.4 (Unkovich *et al.*, 2008).

$$\text{Total N in plant} = \frac{\text{shoot dry weight} \times \% \text{ N in shoots}}{100}$$

Amount of N fixed = Total N in legume – Total N in reference crop/plant

3.4 Laboratory analyses

3.4.1 Soil sampling and sample preparation

Soil samples were taken using the W design (Peters and Laboski, 2013) from each of the experimental fields with a soil auger to a depth of 0-20cm. Five soil core samples were sampled from each plot, mixed thoroughly and composite samples sampled into transparent polythene bags and kept in the refrigerator at 4 °C before laboratory analysis.

3.4.2 Determination of soil physical characteristics

3.4.2.1 Distribution of particle size

This was determined using the hydrometer method described by Anderson and Ingram (1993). A 51-g of air dried soil sample was weighed into a 1L screw lid shaking bottle and100ml distilled water was added and swirled thoroughly to obtain a uniform mixture. A 20 ml part of 30% H_2O_2 was added, followed by the addition of 50 ml of 5% sodium hexametaphosphate (dispersing agent). Drops of amyl alcohol was added and swirled gently. A mechanical shaker was used to shake the mixture for 2 h and the content transferred into a 1L sedimentation cylinder and the first hydrometer and temperature reading recorded after 40 seconds and the first temperature reading was also taken with a thermometer. The sedimentation cylinder was then allowed to stand undisturbed for 3 h and the second hydrometer and temperature readings recorded.

Calculation

% Sand = 100 – [H1 + 0.2 (T1 - 20) – 2] × 2

% Clay = [H1 + 0.2 (T2 - 20) – 2] × 2

% Silt = 100 – (% Sand + % Clay)

where

H1 = 1st hydrometer reading at 40 seconds

T1 = 1st temperature reading at 40 seconds

T2 = Temperature reading at 3 hours

H2 = 2nd hydrometer reading at 3 hours

-2 = Salt correction to be added to hydrometer reading

0.2 (T - 20) = Temperature correction to be added to hydrometer reading.

3.4.3 Determination of soil chemical properties

3.4.3.1 Determination of soil pH

The pH of the soil was determined with the use of Eutech 510 pH meter in a 1:2.5 soil to distilled water ratio. A 10 g air-dried soil was weighed into a 100 ml beaker and a 25 ml distilled water was added, stirred thoroughly for 20 minutes with a stirring rod and the soil water suspension allowed to stand for 15 minutes. pH meter was calibrated with buffer solutions of pH 7.0 and 4.0. before taking readings with the electrode in the supernatant solution (Hazelton and Murphy, 2007).

3.4.3.2 Determination soil organic carbon

The Walkley and Black modified procedure as described by Nelson and Somers (1982) was used to determine the organic carbon. The organic matter underwent a wet combustion process with a mixture of potassium dichromate and sulphuric acid. The excess dichromate after the process was titrated against ferrous sulphate. A 1-g of soil was weighed into a 500-ml Erlenmeyer flask. A blank was included in each batch of

analysis. Ten millilitres of a 0.166 M (1.0 N) potassium dichromate solution was added to the soil and the blank and20 ml of concentrated sulphuric acid was carefully added, swirled and allowed to stand for 30 minutes on an asbestos sheet followed by addition ofa250 ml distilled water and 10 ml concentrated orthophosphoric acid and allowed to cool. A 1 ml of diphenylamine indicator was added and titrated with 1.0M ferrous sulphate solution.

Calculation:

$$\% \text{ Organic C} = \frac{\mathrm{M} \times 0.39 \times \mathrm{mcf}\,(\mathrm{V1}-\mathrm{V2})}{\mathrm{g}}$$

where:

M = molarity of ferrous sulphate solution

V1 = mL ferrous sulphate solution required for blank titration

V2 = mL ferrous sulphate solution required for sample titration

g = weight of air –dry sample in gram

mcf = moisture correction factor (100 + % moisture) / 100

0.39 = 3 x 0.001 x 100 % x 1.33 (3 = equivalent weight of C)

1.3 = a compensation factor for the incomplete combustion of organic matte

3.4.3.3 Determination of total nitrogen

This was determined using the Kjeldahl method involving digestion and distillation method as described by Bremner and Mulvancy (1982). A blank was included in every batch of analysis to compensate for traces of nitrogen in reagents and water used. 10 ml of distilled water was added to 10 g of soil in a Kjeldahl digestion flask. A 5 ml mixture of selenium and concentrated sulphuric acid were added after 30 minutes, mixed judiciously and digested for 3 hours till a colourless solution was obtained. Fifty millilitres of distilled water were used to dilute the digest and allowed to cool. The digest was made to 100 ml with distilled water and mixed well. A 20 ml of 40% NaOH

solution was added to 10 ml aliquot of the digest transferred to the reaction chamber followed by distillation. The distillate was collected over 4% boric acid using bromocresol green as an indicator. The distillate was titrated with 0.02 N HCl solution.

Calculation:

14g of N in one equivalent weight of NH_3

$$\text{Weight of N in the soil} = \frac{14 \times (A - B) \times N}{1000}$$

where:

A= volume of standard HCl used in the sample titration

B = volume of standard HCl used in the blank titration

N = Normality of standard HCl

Mass of soil sample used, considering the dilution and the aliquot taken for distillation

$$= \frac{10g \times 10}{100}$$

Thus, the percentage of nitrogen in the soil sample is,

$$\% \text{ Total N} = \frac{14 \times (A - B) \times N \times 100}{1000 \times 1}$$

Note:

when N = 0.1 and B = 0

Total N = A x 0.14 %.

3.4.3.4 Determination of available phosphorus

The readily acid – soluble forms of phosphorus were extracted with Bray No. 1 solution as described by Olsen and Sommers (1982). A 5-g soil sample was weighed into 100 ml extraction bottle and 35 ml of Bray 1 solution (0.03 M NH_4F and 0.025 M HCl**)** was added. The mixture was shaken for 10 minutes in a reciprocal shaker and filtered through Whatman No. 42 filter paper. A 5-ml of the aliquot of the filtrate was pipetted into 25 ml flask and 10 ml colouring reagent (ammonium paramolybdate) was

added, followed by addition of a pinch of ascorbic acid. After mixing well, the mixture was allowed to stand for 15 minutes to develop a blue colour. A standard series of 0, 1.2, 2.4, 3.6, 4.8, and 6.0 mg P L^{-1} was prepared by pipetting respectively 0, 10, 20, 30, 40 and 50 ml of 12.0 mg P L^{-1} in 100 ml volumetric flask and made to volume with distilled water. The available phosphorus was then extrapolated from the standard curve.

Calculation:

$$P\ (mg\ kg^{-1}) = \frac{(a - b) \times 35 \times 15 \times mcf}{g}$$

Where:

a = mg P l^{-1} in the sample extract

b = mg Pl^{-1} in the blank

g = sample weight in grams

mcf = moisture correction factor

35 = volume of extraction solution

15 = final volume of the sample solute

3.4.3.5 Extraction of exchangeable cations

The exchangeable cations (calcium, magnesium, potassium and sodium) in the soil were determined in 1.0 N ammonium acetate (NH_4OAc) extract (Black, 1965). A 10-g sample was weighed and transferred into an extraction bottle and 100 ml of 1.0 N ammonium acetate solution was added. The extraction bottle with its content was shaken for 1 hour after which the supernatant solution was filtered through a Whatman No. 42 filter paper. Hydrogen plus aluminium were determined in 1.0 *M* KCl extract as described by Page *et al.* (1982).

3.4.3.5.1 Determination of exchangeable calcium and magnesium

This was determined by transferring 10 ml of the soil extract (described in section 3.2.3.5) into a conical flask and 5 ml of ammonium-chloride-ammonium hydroxide

buffer solution was added followed by 1 ml of triethanolamine. Few drops of potassium cyanide and Eriochrome Black T solution were then added. For the determination of calcium alone, 10 ml of the soil extract was transferred into a conical flask and 10 ml of potassium hydroxide solution was added followed by 1 ml of 30 % triethanolamine. Three drops of potassium cyanide solution and a few crystals of cal-red indicator were then added. The mixture was titrated with 0.02 M EDTA solution from red to a blue end point. Exchangeable magnesium was calculated by subtracting the value of calcium alone from calcium + magnesium value.

Calculation:

$$\text{Ca + Mg } (\text{cmol}^{(+)}\ \text{kg}^{-1}) = \frac{0.02 \times (V1 - V2) \times 1000}{g}$$

Where:

V1 = ml of 0.01 *M* EDTA used in the sample titration

V2 = ml of 0.01 *M* EDTA used in the blank titration

G = weight in grams of air–dried soil extraction

0.02= concentration of EDTA used

3.4.3.5.2 Determination of exchangeable potassium and sodium

Exchangeable potassium and sodium in the soil extract (described in section 3.2.3.5) was determined by flame photometry (Sparks *et al*., 2001). A standard series of potassium and sodium were prepared by diluting 1000 mg/L of both potassium and sodium to 100 mg/L. This was done by taking 25 mg portion of each solution into a 250 ml volumetric flask and the volume was made up to the mark. Portions of 0, 5, 10, 15 and 20 ml of the 100 ml standard solution were put into 200-ml volumetric flasks, respectively followed by an addition of 100 ml of 1.0 M NH_4OAC solution to each

flask and made up to the volume with distilled water. The standard series obtained for potassium and sodium were 0, 2.5. 5.0, 7.5, 10 mg/L. Sodium and potassium were measured in the soil extract at the wavelengths of 589 nm and 766.5 nm, respectively.

Calculations:

Exchangeable K ($cmol^{(+)}\ kg^{-1}$ soil) = $\frac{(A-B)\times 250 \times mcf}{(10 \times 39.1 \times g)}$

Exchangeable Na ($cmol^{(+)}\ kg^{-1}$ soil) = $\frac{(A - B) \times 250 \times mcf}{(10\times 23 \times g)}$

Where:

A = mgl^{-1} K or Na in the diluted sample

B = mgl^{-1} K or Na in the blank sample

g = air–dried sample weight of soil in grams

mcf = moisture correction factor

3.5 Plant tissue analysis

The shoots of the plants were milled in a stainless steel mill and nitrogen content determined using the procedure described in section 3.4.3.3.

3.6 Enumeration of rhizobia population

Enumeration of indigenous rhizobia population was done by the most probable number plant infection technique (Vincent, 1970; Somasegaran and Hoben, 2012). Soil samples were taken from a depth of 0–20 cm, bulked and a composite sample was taken and used for the MPN count. Seeds of groundnut variety, Chinese was used as a trap host for the estimation of the indigenous rhizobia population. The groundnut seeds were surface-sterilized with 95% ethanol and 3% (v/v) hydrogen peroxide (H2O2) solution. The seeds were successively rinsed in sterilized distilled water and then incubated at 30^{o}C on moistened filter paper in petri dishes. Their emergence was observed after 2 day and healthy well-grown seedlings with similar sizes and radicles were transferred aseptically into growth pouches containing 65 ml of nitrogen - free

mineral nutrient solution (Broughton and Dilworth, 1971). After 5–7 days, the pouches were sorted for uniformity and reorganized in quadruplicates prior to inoculation of plants. 20 g of the composite soil sample was diluted in 80 ml of distilled water (5^{-1}). This was mixed thoroughly on a vortex mixer. A series of soil dilution from 5^{-2} to 5^{-6} was made by taking 5 ml of diluents into 20 ml sterilized distilled water and mixing was done on a vortex mixer. One millilitre (1 ml) of each dilution was used to inoculate the groundnut (Chinese) seedlings. Plants were watered as and when required. The set up was monitored for 28 days after which scoring was done for the presence or absence of nodules. Population estimates were assigned using the MPNES software (Woomer *et al.,* 1990).

3.7 Performance of the locally selected rhizobia isolates after eight months of introduction

This study was conducted under a greenhouse condition at the Department of Horticulture, Faculty of Agriculture, KNUST, Kumasi.After harvesting plants, plots were left fallow from time of harvest (November 2015) to the beginning of the following farming season (June 2016) to pursue the persistence of the rhizobium isolates in the soil. Soils were randomly collected from each plot at a depth of 15 cm with an auger at all three locations and each set thoroughly mixed to make a composite sampleand used to assess the persistence and effectiveness of the selected rhizobia strains. Enumeration of rhizobia population was done in growth pouches using the Most Probable Number procedure (Somasegaran and Hoben, 2012). Field capacity of the soils was determined using the cylinder method (Somasegaran and Hoben, 2012). Pots with holes drilled beneath and lined with tissue were filled with 3 kg of composite sample. The pots were planted with the same groundnut cultivars used in the field experiment. No additional inoculation was performed. In order to evaluate total N fixed by the legume, maize (*Zea miaze*) was included as reference crop. The

experiment was done using completely randomized design (CRD) replicated three times. A basal dose of P at 30 kg ha^{-1} and 20 kg N ha^{-1} from triple super phosphate and urea, respectively were applied two weeks after planting. At 8 weeks from planting, measurements of nodule number and nodule dry weight were made to determine nodulation success; plant dry weight was determined as an indication of growth response, and N_2 fixed was determined as an indication of nitrogen fixation efficiency and by extension the persistence of introduced rhizobia and sustained effectiveness.

3.8 Statistical analysis

Data from the field and greenhouse experiments were subjected to analysis of variance (ANOVA) using SISVAR statistical software version 5.1.3(Ferreira, 2008). Significant differences were assessed at 5% (P = 0.05) and 1% (P = 0.01) level of significance for field and greenhouse work, respectively. Separation of means was done using Scott-Knott test tool.

CHAPTER FOUR

4.0 RESULTS

4.1 Physico-chemical properties and the most probable number count

The physico-chemical properties of the study sites are as shown below in Table 4.1. The soil texture at the three study sites were loamy sand at Binduri and Tanina and sandy loam at Cheshegu. The pH levels were medium acidic across location ranging from 6.10 – 6.38. The organic carbon (OC) levels at all the study sites were very low; 0.42 % at Cheshegu, 0.96 % at Binduri and 0.62 % at Tanina. Total N at all the study sites were very low ranging from 0.022 - 0.030 %. Results recorded on available phosphorus (P) ranged from low to moderate (17.61 – 20.54 mg kg^{-1}). In general, the fertility status of the soil at

the study sites was very low. The estimated population sizes of the indigenous rhizobia at the study sites were 26.3cells g^{-1} soil, 11.2 cells g^{-1} soil and 32.8 cells g^{-1} soil for Cheshagu, Binduri and Tanina, respectively.

Table 4.1 Soil physico - chemical characteristics and MPN count at the study sites

Soil parameters	Cheshegu	Binduri	Tanina
Total N	0.024	0.022	0.030
Available P ($mgkg^{-1}$)	17.61	17.61	20.54
Exch. K ($cmolkg^{-1}$)	0.03	0.016	0.015
% Organic C	0.42	0.96	0.62
pH (1:2.5)(H_2O)	6.10	6.24	6.38
Exch. Ca ($cmol^{(+)}kg^{-1}$)	3.64	2.54	2.6
Exch. Mg ($cmol^{(+)}kg^{-1}$)	0.22	0.28	0.18
Exch. Na ($cmol^{(+)}kg^{-1}$)	0.39	0.28	0.35
% Sand	56.4	78.60	84.56
% Clay	5.88	14.56	5.88
% Silt	37.72	6.84	9.56
Texture	Sandy loam	Loamy sand	Loamy sand
MPN (Rhizobia cell g^{-1} soil)	26.3	11.2	32.8

4.2 Rainfall pattern at the study sites

The daily accumulated and number of rains received at the study sites are highlighted in Figures 1, 2 and 3. Generally, the rainfall pattern at Cheshegu was much better than Binduri and Tanina. There were short durations of drought between 10 and 20 days after planting at Binduri and Tanina with a daily average rainfall of 3.75 and 3.96 mm, respectively during the plant flowering stage (Figures 2 and 3) whereas at Cheshegu, the recorded rainfall averagely was 8.22 mm during the same growth stage (Figure 1). During podding, daily average rainfall recorded at Binduri (5.95 mm) and Tanina (5.35 mm) were relatively low compared to that of Cheshegu (7.65 mm)

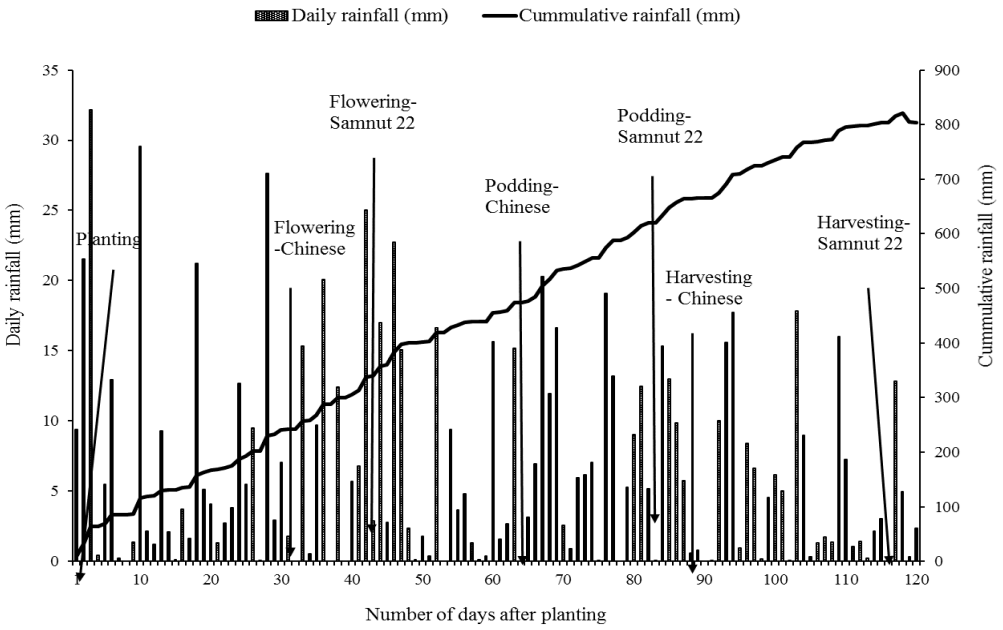

Fig 1. Rainfall status at Cheshegu from planting to harvesting of groundnut varieties

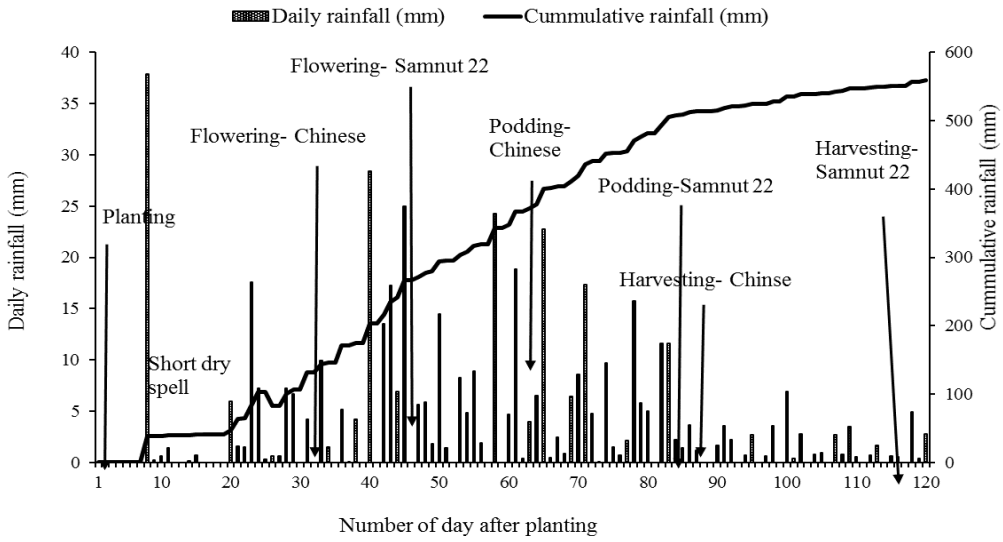

Fig 2. Rainfall status at Binduri from planting to harvesting of groundnut varieties

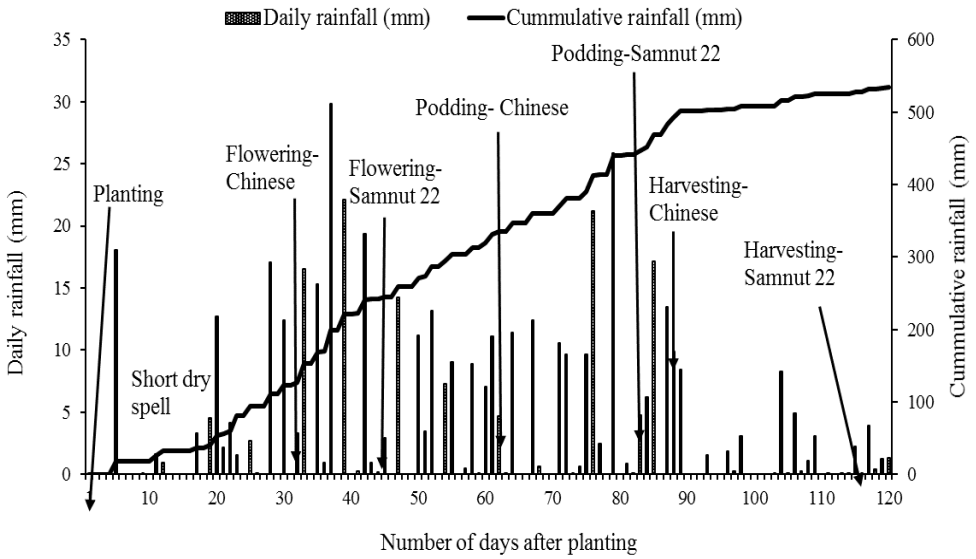

Fig 3. Rainfall status at Tanina from planting to harvesting of groundnut varieties

4.3 Effect of groundnut varieties and rhizobia interaction on nodulation, N fixed, growth and yield of groundnut.

The performance of the groundnut varieties in response to the rhizobia isolates inoculation was mostly higher at Cheshegu than at Binduri and Tanina. Generally, the responses of Samnut 22 to inoculation with the isolates were significantly ($P < 0.05$) greater than those of Chinese across the different locations (Tables 4.2, 4.3, 4.4, 4.5, 4.6 and 4.7).

4.3.1. Nodulation of groundnut varieties in response to rhizobia inoculation.

Groundnut varieties interacted differently and significantly ($P \leq 0.05$) to *rhizobium* isolates on nodulation at all study sites (Table 4.2 and 4.3).

At Cheshegu, Samnut 22 produced the highest nodules (135) plant^{-1} with isolate 53e and represented 34, 29and 47 % more nodules produced plant^{-1}than those of Biofix, BR3267 inoculant and un-inoculated (-N) controls, respectively. The maximum nodule dry weight for Samnut 22 was produced by isolate 53e inoculation (0.49 g plant^{-1}). This represented an increase of 48, 75 and 133 % over those of Biofix, BR

3267 inoculant and the un-inoculated (-N) control, respectively. The groundnut variety Chinese responded to inoculation with isolate 9g to record the highest nodule number (121) plant^{-1}. This was similar to the performance of isolate 53e (119 nodules plant^{-1}) and represented increased nodule number of 16, 20 and 53% over those of Biofix, BR3267 and un-inoculated (-N) control, respectively. Chinese responded to isolate 9g inoculation to produce the highest nodule dry weight (0.38 g plant^{-1}). This was comparable to that of Chinese inoculated with isolate 53e (0.34 g plant^{-1}), respectively, and represented an increased nodule dry weight of 41, 55 and 111 % over those of Biofix, BR 3267 inoculant and un-inoculated (-N) control, respectively.

At Binduri, the inoculation of Samnut 22 with isolate 9d produced the highest number (144) of nodules plant^{-1} and this was 37, 21and 33 % higher than the nodules produced from the interactions with Biofix, BR3267 and un-inoculated controls (-N). Samnut 22 interacted with isolate 9d to produce the highest nodule dry weight of 0.47 g plant^{-1} while Samnut 22 inoculated with BR3267 recorded the lowest dry weight of 0.18 g plant^{-1}. The percentage increase due to isolate 9d inoculation was 57 and 135 % relative to Biofix and the negative control (-N), respectively. For Chinese, inoculation with isolate 9d and Biofix separately, produced statistically similar nodule numbers which were significantly higher than those of BR 3267 inoculated and the un-inoculated control treatments. Inoculation of Chinese with isolate 9d produced nodule dry weight comparable to the nodule dry weight produced by inoculating with Biofix and increased nodule dry weight by more than three-folds compared to that of BR3267 inoculant; and about three-fold compared to the negative control treatment.

At Tanina, Samnut 22 produced the highest nodule plant^{-1} in response to inoculation with isolate 53e which represented increases of 26, 17 and 27 % over those of Biofix, BR3267 inoculated and the un-inoculated and (-N) controls treatments. The highest

nodule dry weight for Samnut 22 was recorded by isolate 53e inoculation (0.30 g plant^{-1}) while the absolute control for Samnut 22 produced the lowest nodule dry weight (0.11 g plant^{-1}). Inoculation with isolate 53e increased nodule dry weight by 131, 69 and 173 % over those of Biofix, and BR3267 inoculated and the un-inoculated (-N) control treatments, respectively. The inoculation of Chinese with isolate 9gproduced the highest numbers (95) of nodules plant^{-1}. This was at par with the response to strain BR3267 inoculation (92 nodules plant^{-1}) and represented an increase in nodule numbers by 7 and 11 % over those of Biofix and un-inoculated controls treatments, respectively. Nodule dry weight obtained from inoculating Chinese with isolate 9g was comparable to the nodule dry weight recorded with BR3267 inoculation and these were significantly ($P < 0.001$) higher than that of the negative control treatment.

Table 4.2 Response of nodule number of groundnut varieties to rhizobia inoculation

	Cheshegu		Binduri		Tanina	
Nitrogen source	Samnut 22	Chinese	Samnut 22	Chinese	Samnut 22	Chinese
	Nodule number plant^{-1}					
52b1	106 Ab	103 Ab	127 Ab	89 Bc	84 Ac	88 Ab
53e	135 Aa	119 Ba	128 Ab	91 Bc	108 Aa	85 Bb
91a	111 Ab	100 Bb	117 Ac	97 Bb	86 Ac	87 Ab
9d	99 Ab	97 Ab	144 Aa	105 Ba	87 Ac	85 Ab
9g	94 Bb	121 Aa	128 Ab	93 Bc	91 Ab	95 Aa
BIOFIX	101 Ab	104 Ab	105 Ad	106 Aa	86 Ac	89 Ab
BR3267	105 Ab	101 Bb	119 Ac	88 Bc	92 Ab	92 Aa
N+	81 Ad	79 Ac	94 Ae	80 Bd	87 Ac	86 Ab
N-	92 Ac	88 Ac	108 Ad	82 Bd	85 Ac	86 Ab
F Pr. (V×N.S)	< 0.001		< 0.001		< 0.001	
CV (%)	6.4		5.0		3.4	

Means with different lowercase letters vertically (compare isolates) and uppercase letters horizontally (compare variety) indicate significant difference ($P < 0.05$) between treatments by the Scott Knott test

Table 4.3. Response of nodule dry weight of groundnut varieties to rhizobia inoculation

	Cheshegu		Binduri		Tanina	
Nitrogen source	Samnut 22	Chinese	Samnut 22	Chinese	Samnut 22	Chinese
	Nodule dry weight (g plant^{-1})					
52b1	0.36 Ab	0.26 Bb	0.43 Ab	0.14 Bc	0.14 Ad	0.11 Ac
53e	0.49 Aa	0.34 Ba	0.23 Ad	0.14 Bc	0.30 Aa	0.12 Bc
91a	0.33 Ab	0.24 Bb	0.21 Ad	0.10 Bc	0.12 Ad	0.12 Ac
9d	0.23 Ac	0.22 Ab	0.47 Aa	0.38 Ba	0.20 Ab	0.10 Bc
9g	0.19 Bd	0.38 Aa	0.24 Ad	0.23 Ab	0.16 Ac	0.18 Aa
BIOFIX	0.33 Ab	0.27 Bb	0.30 Bc	0.35 Aa	0.13 Ad	0.15 Ab
BR3267	0.28 Ac	0.24 Ab	0.18 Ae	0.11 Bc	0.18 Ac	0.20 Aa
N+	0.12 Ae	0.15 Bd	0.19 Ad	0.06 Bd	0.12 Ad	0.08 Ab
N-	0.21 Ad	0.18 Bc	0.20 Ad	0.13 Bc	0.11 Bd	0.12 Ab
F Pr. (V×N.S)	< 0.001		< 0.001		< 0.001	
CV (%)	9.5		8.5		12.7	

Means with different lowercase letters vertically (compare isolates) and uppercase letters horizontally (compare variety) indicate significant difference ($P < 0.05$) between treatments by the Scott Knott test

4.3.2. Shoot dry matter production of groundnut varieties in response to rhizobia inoculation.

Shoot dry weights of the groundnut varieties at mid flowering were significantly ($P \leq 0.05$) increased by rhizobia isolates inoculation (Table 4.4). At Cheshegu, inoculation of Samnut 22 with isolate 53e produced similar shoot dry weight obtained from the positive control (+N) control treatment and these were significantly higher than dry matter produced by the un-inoculated (-N) and the plants inoculated with the reference strains. The highest shoot dry weight (1206.6 kg ha^{-1}) recorded for Chinese was obtained with the isolate 9g inoculation while the un-inoculated treatment recorded the lowest shoot dry weight of 531.8 kg ha^{-1}. Shoot dry matter produced by inoculation with isolate 9g was at par with plants that received urea but significantly, higher than that of the -N control treatment and those inoculated with BR 3267 and Biofix.

At Binduri, inoculation of Samunt 22 with isolate 9d produced the highest shoot dry weight (886.5 kg ha^{-1}) and this was similar to that of urea treatment (847.2 kg ha^{-1}) but significantly higher than that of negative control (-N) and those inoculated with BR3267 and Biofix. For Chinese, the inoculation with isolate 9d recorded the highest shoot dry weight of 884.9 kg ha^{-1} and the un-inoculated (-N) treatment recorded the lowest shoot dry weight (497.4 kg ha^{-1}). Isolate 9d inoculation produced comparable shoot dry weight with the urea fertilised treatment and increased shoot dry weight by 17, 39 and 78 %, compared to those of Biofix, BR 3267 inoculant and the un-inoculated (-N) control treatments, respectively.

At Tanina, the isolate- 53e and Samnut 22 interaction recorded the highest shoot dry weight (1072.4 kg ha^{-1}) and this was at par with the response to the positive (+N) control (1025.5 kg ha^{-1}) but significantly higher than the response obtained from the un-inoculated (-N) control and the Biofix and BR3267 inoculated treatments. The

response to isolates 9d inoculation was 8.6 % higher than the un-inoculated (-N) control. Chinese response to strain BR3267 inoculation produced the highest shoot dry weight of 976.4 kg ha^{-1}. This was comparable to isolate 9g inoculation (962.7 kg ha^{-1}) and the un-inoculated (+N) control (974.0 kg ha^{-1}) but significantly higher than absolute control (837 kg ha^{-1}).

Table 4.4. Response of shoot dry weight of groundnut varieties to rhizobia inoculation

	Cheshegu		Binduri		Tanina	
Nitrogen source	Samnut 22	Chinese	Samnut 22	Chinese	Samnut 22	Chinese
	Shoot dry weight (kgha^{-1})					
52b1	1214.1Ab	763.7Bc	824.5Aa	524.4Bd	705.2Ae	649.7Ac
53e	1375.3Aa	1186.8Ba	822.6Aa	518.4Bd	1072.4Aa	695.8Bc
91a	1070.4Ac	697.8Bc	730.5Ab	532.1Bd	762.9Ae	560.9Bd
9d	1050.3Ac	741.6Bc	886.5Aa	884.9Aa	991.4Ab	588.2Bd
9g	932.7Bd	1206.6Aa	795.7Aa	729.7Ab	838.2Bd	962.7Aa
BIOFIX	967.7Ad	716.8Bc	728.3Ab	746.0Ab	878.8Ac	692.2Ac
BR3267	1186.3Ab	847.6Bb	718.0Ab	635.5Ac	976.6Ab	976.4Aa
N+	1307.0Aa	1124.8Ba	847.2Aa	820.7Aa	1025.5Aa	974.0Aa
N-	1025.3Ac	531.8Ad	650.3Ab	497.4Bd	912.1Ac	837.0Ab
F Pr. (V×N.S)	< 0.001		< 0.001		< 0.001	
CV (%)	4.9		7		6.2	

Means with different lowercase letters vertically (compare isolates) and uppercase letters horizontally (compare variety) indicate significant difference ($P < 0.05$) between treatments by the Scott Knott test.

4.3.3. Biological nitrogen fixed by groundnut varieties in response to rhizobia inoculation.

The response of the groundnut varieties to inoculation was varied and significant ($P \leq 0.05$) with variety and rhizobia isolates interaction across the different locations (Table 4.5). At Cheshegu, the isolate 53e Samnut 22 symbiosis recorded the highest N_2 fixed of 49.63 kg ha^{-1} and the 9g-inoculated Samnut 22 treatment recorded the lowest N_2 fixed of 31.13 kg ha^{-1} (Table 4.5). The inoculation with isolate 53e increased the amount of N fixed by 52, 20 and 19 % respectively over those of Biofix, BR 3267 inoculant and un-inoculated (-N) control. Inoculation of Chinese with isolate 9g and isolate 53e separately, obtained comparable amounts of N fixed of 46.78 kg ha^{-1} and

44.98 kg ha^{-1}, respectively which were significantly ($P < 0.05$) higher than those recorded with no inoculation (-N) (21.49 kg ha^{-1}), Biofix (26.5 kg ha^{-1}) and BR 3267inoculant (29.99 kg ha^{-1}).

At Binduri, isolate 9d-inoculated Samnut 22 produced the highest amount of N_2 fixed of 37.78 kg ha^{-1} and the BR 3267-inoculated Samnut 22 recorded the lowest amount of N fixed of 27.28 kg ha^{-1} (Table 4.5). Inoculation with isolate 9d increased N_2 fixed by 22, 28 and 59 % over those of the absolute control (-N), Biofix and BR 3267 inoculant. For Chinese, inoculation with isolate 9d produced a significantly ($P < 0.05$) higher amount of N_2 fixed (36.81 kg ha^{-1}) than all the other treatments. Percentage increases due to isolate 9d inoculation were 26, 36 and 105 %, respectively over those of Biofix, strain BR 3267 and un-inoculated –N controls treatments.

Samnut 22 responded to isolate 53e inoculation to produce the maximum N_2 fixed of 42.61 kg ha^{-1} at Tanina (Table 4.5). This significantly increased N_2 fixed over those of Biofix, strain BR 3267 and un-inoculated (-N) controls treatments by 62, 22 and 39 %, respectively. Chinese responded to strain BR 3267 and isolate 9g inoculations to produce a significantly ($P < 0.05$) higher amount of N_2 fixed than those of the Biofix-inoculated and the un-inoculated (-N) control treatments.

Table 4.5. Response of N_2 fixed of groundnut varieties to rhizobia inoculation.

	Cheshagu		Binduri		Tanina	
Nitrogen source	Samnut 22	Chinese	Samnut 22	Chinese	Samnut 22	Chinese
	N fixed (kg ha^{-1})					
52b1	42.23Ab	26.48Bc	34.48Aa	24.48Bc	23.80Ad	20.89Ac
53e	49.63Aa	44.20Ba	36.28Aa	24.93Bc	42.61Aa	21.82Bc
91a	37.76Ac	25.92Bc	30.86Ab	27.42Ac	26.45Ac	17.45Bd
9d	40.25Ab	29.85Bb	37.78Aa	36.81Aa	33.62Ab	17.16Bd
9g	31.13Bd	46.78Aa	35.84Aa	31.89Bb	27.66Bc	33.26Aa
BIOFIX	36.05Ac	26.50Bc	30.67Ab	31.70Ab	27.49Ac	22.85Ac
BR3267	43.00Ab	29.99Bb	27.28Ac	29.79Ab	35.56Ab	34.48Aa
N+	41.02Ab	32.98Bb	29.67Ab	31.96Ab	33.83Ab	29.89Bb
N-	31.93Ad	21.49Bd	31.83Ab	23.70Bc	31.51Ab	30.78Ab
F Pr. (V×N.S)	< 0.001		< 0.001		< 0.001	
CV (%)	5.8		7.9		7.4	

Means with different lowercase letters vertically (compare isolates) and uppercase letters horizontally (compare variety) indicate significant difference ($P < 0.05$) between treatments by the Scott Knott test

4.3.4. Pod number of groundnut varieties in response to rhizobia inoculation.

The responses of the groundnut varieties were different and significant ($P \leq 0.05$) in pod formation to the interaction with the rhizobia inoculant (Table 4.6.). At Cheshegu, the response of Samnut 22 to isolate 53e inoculation produced the highest number (41) of pods plant^{-1}. This was similar to that produced by the un-inoculated (+N) control (39 pods plant^{-1}) and represented an increase of 86, 32 and 33 % over those of Biofix, strain BR3267 and un-inoculated (-N) control treatments, respectively. For Chinese, inoculation with isolate 9g produced the maximum number (55) of pods plant $^{-1}$. This was comparable to those produced by plants inoculated withisolate53e (51 pods plant^{-1}) and the un-inoculated (+N) control (44 pods plant^{-1}). These values represented increases of 209, 72 and 68 % over responses to Biofix (23 pods plant^{-1}), strain BR3267 (22 pods plant^{-1}) and un-inoculated (-N) control (18 pods plant^{-1}), respectively.

At Binduri, Samnut 22 inoculated with isolate 9d recorded the highest number (37) of pods plant $^{-1}$. This was similar to the responses to isolates 9g (32 pods plant^{-1}), 53e (33 pods plant^{-1}), 52b1 (31 pods plant^{-1}) inoculations and the un-inoculated (+N) control

(30 pods plant^{-1}) interactions. Percentage increases due to isolate 9dinoculation were 48, 95 and 85 % relative to those of Biofix, strain BR3267 and un-inoculated (-N) control combinations, respectively. Isolate 9d-inoculated Chinese and the urea fertilised Chinese treatments produced comparable number of pods plant $^{-1}$ (33 and 32, respectively) which were significantly higher than those produced in the Biofix, BR3267 inoculant and the un-inoculated (-N) control treatment by 44, 50 and 83 %, respectively.

Samnut 22 responded to isolate 53e inoculation to produce the highest number of (33) pods plant^{-1} at Tanina. This was at par with those produced by the 9d (28 pods plant^{-1}) and un-inoculated (+N) control (30 pods plant^{-1}) treatments and represented an increase of 36, 31 and 46 % over those of Biofix, BR3267and un-inoculated (-N) controls treatments, respectively. Inoculation of Chinese with strain BR3267 produced the highest number (27) of pods plant^{-1}. This was at par with number of pods plant^{-1} produced by inoculation with isolates 52b1 (22 pods plant^{-1}), 53e (23 pods plant^{-1}), 91a (25 pods plant^{-1}), 9g (23 pods plant^{-1}) inoculated treatments and the 28 pods plant^{-1} of the un-inoculated +N control interactions.

Table 4.6. Pod formation in groundnut varieties in response to rhizobia inoculation

	Cheshagu		Binduri		Tanina	
Nitrogen source	Samnut 22	Chinese	Samnut 22	Chinese	Samnut 22	Chinese
	Pods plant^{-1}					
52b1	24 Ac	24 Ad	31 Aa	20 Bb	24 Ab	22 Aa
53e	41 Ba	51 Aa	33 Aa	19 Bb	34 Aa	23 Ba
91a	25 Ab	27 Ad	23 Ab	21 Ab	19 Bd	25 Aa
9d	27 Bb	32 Ac	37 Aa	33 Aa	28 Aa	20 Bb
9g	21 Bc	55 Aa	32 Aa	24 Bb	22 Ac	23 Aa
BIOFIX	22 Ac	25 Ad	25 Ab	23 Ab	24 Ab	19 Bb
BR3267	31 Ab	32 Ac	19 Ac	22 Ab	23 Bb	27 Aa
N+	39 Ba	44 Ab	30 Aa	32 Aa	30 Aa	23 Ba
N-	31 Ab	33 Ac	20 Ab	18 Ab	21 Ac	20 Ab
F Pr. (V×N.S)	< 0.001		0.003		0.003	
CV (%)	6.2		12		12.8	

Means with different lowercase letters vertically (compare isolates) and uppercase letters horizontally (compare variety) indicate significant difference ($P < 0.05$) between treatments by the Scott Knott test

4.3.5 Grain yield of groundnut varieties in response to rhizobia inoculation.

Grain yield of groundnut varieties was differently and significantly ($P \leq 0.05$) influenced by inoculation with rhizobia isolates (Table 4.7). At Cheshegu, Samnut 22 responded to isolate 53e inoculation to produce the highest grain yield of 1923.2 kg ha^{-1} which was comparable to yield (1862 kg ha^{-1}) recorded in the positive control (+N) treatment. Inoculation with isolate 53e increased grain yield by 21, 25 and 29 % over those of the Biofix, strain BR 3267 inoculated and un-inoculated (-N) control treatments, respectively. For Chinese, inoculation with isolate 9g gave the highest grain yield of 1702.4 kg ha^{-1} and this was comparable to the yield (1688.8 kg ha^{-1}) produced by the urea fertilised plants and plants inoculated with isolate 53e. Inoculation with isolate 9g increased grain yield by 31, 27 and 24 % over those of Biofix, BR3267 inoculated and the un-inoculated (-N) control treatments, respectively.

At Binduri, inoculation with isolate 9d recorded the highest grain yield of 1359 kg ha^{-1} for Samnut 22. This was comparable to yields produced by inoculation with isolates 52b1 (1277.1 kg ha^{-1}), 53e (1293.0 kg ha^{-1}), 9g (1289.7 kg ha^{-1}) and the un-inoculated (+N) control (1255.1 kg ha^{-1}). These increases due to isolate 9d inoculation were 14.2, 22.1 and 20.9 % over those of Biofix, BR3267 inoculated and un-inoculated (-N) control treatments, respectively. Inoculation of the Chinese variety with isolate-9d produced the highest grain yield of 1197.6 kg ha^{-1}. This yield was comparable to that of un-inoculated (+N) control (1078.7 kg ha^{-1}) and it represented an increase of 34, 40 and 36 % over those of the Biofix, strain BR3267 inoculated and the un-inoculated (-N) control treatments, respectively.

At Tanina, the isolate 53e-inoculated Samnut 22 treatment produced the highest grain yield of 1189.9 kg ha^{-1} and this was statistically similar to the yield (1123.3 kg ha^{-1}) recorded by the positive (+N) control treatment. Inoculation with isolate 53e increased grain yield by 35.9, 51.4 and 43.7 % over those of Biofix, BR3267 inoculated and the un-inoculated (-N) control treatments, respectively. For Chinese, BR3267-inoculated treatment produced the highest grain yield of 871.9 kg ha^{-1} and this was comparable to the yields of the +N treatment (816.6 kg ha^{-1}) and the isolates 53e (825.1 kg ha^{-1}), 91a (805.4 kg ha^{-1}) and 9g-inoculated (830.6 kg ha^{-1}) treatments. Inoculation with isolates 53e, 91a and 9g were significantly ($P < 0.05$) higher than yields recorded in the absolute control (-N) and that of the Biofix inoculant.

Table 4.7. Response of grain yield of groundnut varieties to rhizobia inoculation.

	Cheshegu		Binduri		Tanina	
Nitrogen source	Samnut 22	Chinese	Samnut 22	Chinese	Samnut 22	Chinese
	Grain yield (kg ha^{-1})					
52b1	1355.2Ac	1269.1Ab	1277.1Aa	911.0Bb	793.9Ac	755.6Ab
53e	1923.2Aa	1598.1Ba	1293.0Aa	890.1Bb	1183.9Aa	825.1Ba
91a	1466.5Ab	1388.7Ab	972.7Ac	821.8Bb	755.7Ac	805.4Aa
9d	1523.1Ab	1262.2Bb	1359.0Aa	1197.6Ba	976.7Ab	744.9Bb
9g	1379.9Bc	1702.4Aa	1289.7Aa	987.2Bb	756.0Ac	830.6Aa
BIOFIX	1533.1Ab	1304.3Bb	1190.5Ab	891.5Bb	871.3Ac	769.0Ab
BR3267	1596.4Ab	1340.1Bb	1112.6Ab	856.8Bb	781.9Ac	871.9Aa
N+	1862.1Aa	1688.8Aa	1255.1Aa	1078.7Ba	1123.3Aa	816.6Ba
N-	1292.2Ac	1177.6Ac	1124.4Ab	882.9Bb	824.1Ac	705.1Ab
F Pr. (V×N.S)	0.006		0.009		0.004	
CV (%)	6.3		7.4		6.7	

Means with different lowercase letters vertically (compare isolates) and uppercase letters horizontally (compare variety) indicate significant difference ($P < 0.05$) between treatments by the Scott Knott test.

4.4 Relationships between measured N_2 fixing traits.

The correlation among N fixing traits is presented in Table 4.8. Nitrogen fixed significantly ($P \leq 0.05$) correlated positively with nodule number and nodule dry weight. However, the highest correlation was observed between number of nodules and nodule dry weight at Cheshegu ($r = 0.94$) and Tanina ($r = 0.98$) while at Binduri, this was between N_2 fixed and nodule number ($r = 0.80$).

Table 4.8 Correlation coefficients of relationships between N_2 fixed and N fixing parameters

Parameter	N_2 fixed kg ha^{-1}	Nodule number	Nodule dry weight (g $plant^{-1}$)
Cheshegu			
N_2 fixed (kg ha^{-1})	-		
Nodule number	0.46*	-	
Nodule dry weight (g $plant^{-1}$)	0.45*	0.94*	-
Binduri			
N_2 fixed(kg ha^{-1})	-		
Nodule number	0.80*	-	
Nodule dry weight (g $plant^{-1}$)	0.50*	0.68*	-
Tanina			
N_2 fixed(kg ha^{-1})	-		
Nodule number	0.67*	-	
Nodule dry weight (g $plant^{-1}$)	0.65*	0.98*	-

*correlation is significant at $P < 0.05$

4.5 Greenhouse study

4.5.1 Persistence of rhizobia isolates after eight (8) months fallow.

The initial estimated population sizes of the indigenous rhizobia in soils of Cheshegu, Binduri and Tanina sites were 26.3, 11.2 and 32.8 cells g^{-1} soil at field planting and increased to 96.9, 107.9 and 65cells g^{-1} soil, respectively after 8 month of fallow period. However, the rhizobium isolates were not able to significantly ($P \geq 0.05$) increase the rhizobia population over the un-inoculated plots at all the study locations (Table 4.9).

Table 4.9 Most probable number counts of rhizobia population after 8 months of groundnut inoculation with elite rhizobia isolates

Cheshegu		
Rhizobium isolates	MPN (cellg-1 soil)	Confident interval (P = 0.05)
53e	202.6	70.3-534.0
52b1	96.9	33.6-279.3
9d	107.9	37.4-311.0
9g	131.8	45.7-379.9
91a	131.8	45.7-379.9
BR3267	96.9	33.6-279.3
BIOFIX	107.9	37.4-311.0
[a]Control plots	26.3	9.1-75.7
Eight months after planting	96.9	33.6-534.0
Binduri		
53e	283.4	98.3-817.1
52b1	218.4	75.8-629.7
9d	283.4	98.3-817.1
9g	40.4	14-116.5
91a	81.3	25.3-234.3
BR3262	218.4	75.8-629.7
BIOFIX	57.1	19.8-164.7
[a]Control plots	11.2	3.9-32.6
Eight months after planting	107.9	37.4-311.0
Tanina		
53e	131.8	45.7-379.9
52b1	96.9	33.6-279.3
9d	131.8	45.7-379.9
9g	96.9	33.6-279.3
91a	96.9	33.6-279.3
BR3262	96.9	33.6-279.3
BIOFIX	55.2	19.2-159.3
[a]Control plots	32.8	11.4-94.5
Eight month after planting	65	22.6-187.4

[a;,]Counts prior to introduction of rhizobia strains through groundnut inoculation

4.5.2 Residual effect of rhizobia isolates on two groundnut varieties.

The groundnut varieties responded variedly and significantly ($P \leq 0.01$) to the persisting bradyrhizobium isolates after 8 months in the soils from all the study sites (Tables 4.9 and 4.10). Soils from 53e inoculated Samnut 22 treatment increased nodule number pot^{-1} by 94.4, 25 and 138.6 % and nodule dry weight by 268.9, 133.8 and 374.1 % over those of Biofix, BR3267 and un-inoculated (-N) control treatments in Cheshegu soils (Table 4.9). Although, Samnut 22 responded in soils previously inoculated with isolates 53e, 91a, 9d and 9g to increase N fixed and shoot dry weight over those of BR3267 and un-inoculated (-N) control treatments, shoot dry weight produced were comparable to those produced from previous Biofix and nitrogen fertilized plants treatment soils. Similar results of nodule number and nodule dry weight increases were recorded for previous Samnut 22-isolate 53e treatment in Binduri soils. Furthermore, the response of Samnut 22 in soils previously inoculated with isolates 53e and 9d also led to an increased N fixed and shoot dry weight over those of Biofix, strain BR3267 inoculated treatments and the un-inoculated (-N) control treatments. Shoot dry weight produced was comparable to those from previously nitrogen fertilized treatments. Soils from Tanina which previously received Samnut 22-9g inoculant treatment gave the highest nodule number and nodule dry weight relative to those of Biofix, BR3267-inoculated and the un-inoculated (-N) control treatments. Moreover, Samnut 22 responses in soils previously inoculated with isolates 53e, 9g and 91a increased N fixed relative to those of Biofix, BR3267-inoculated and the un-inoculated (-N) control treatments. However, responses in soils previously inoculated with all the isolates increased shoot dry weight relative to those of BR3267-inoculated and the un-inoculated (-N) control treatments (Table 4.10).

Table 4.10 Response of Samnut22 groundnut to persisting rhizobia isolates.

Nitrogen source	Nodule number per pot	Nodule dry weight ($mgpot^{-1}$)	N_2 fixed ($gpot^{-1}$)	shoot dry weight ($gpot^{-1}$)
		Cheshegu		
52b1	101 d	120.0 d	22.9 b	13.33 b
53e	210 a	553.3 a	36.3 a	19.87 a
91a	95 d	140.0 d	33.3 a	17.59 a
9d	115 c	413.3 b	34.6 a	17.77 a
9g	96 d	113.3 d	34.7 a	17.38 a
BIOFIX	108 c	150.0 d	36.2 a	17.17 a
BR3267	168 b	236.7 c	20.5 b	11.91 b
N+	55 e	53.3 e	27.0 b	22.76 a
N-	88 d	116.7 d	22.5 b	13.16 b
CV (%)	6.5	8.6	11.4	7.8
		Binduri		
52b1	113 g	146.7 d	29.88 b	20.4 b
53e	304 a	343.3 a	35.85 a	25.6 a
91a	150 e	190.0 c	30.23 b	18.8 b
9d	229 b	226.7 b	34.00 a	25.2 a
9g	131 f	160.0 d	28.33b	15.6 b
BIOFIX	123 g	150.0 d	30.57 b	19.6 b
BR3267	180 c	220.0 b	29.90 b	19.1 b
N+	62 h	76.7 e	32.46 a	25.3 a
N-	167 d	196.7 c	23.70 c	14.1 b
CV (%)	2.6	5.3	10.2	9.7
		Tanina		
52b1	198 e	210.0 b	25.69 b	18.2 a
53e	211 d	200.0 b	34.19 a	22.5 a
91a	231 c	186.7 b	30.57 a	18.8 a
9d	255 b	206.7 b	27.65 b	18.7 a
9g	316 a	300.0 a	38.03 a	19.2 a
BIOFIX	170 f	146.7 d	27.47 b	18.4 a
BR3267	204 d	190.0 b	21.86 b	15.5 b
N+	68 g	86.7 e	34.93 a	21.9 a
N-	206 d	180.0 c	21.13 b	15.4 b
CV (%)	2.5	5.8	8.6	9.6

Means within a column with the same alphabet are not significantly different ($P > 0.01$) between treatments by the Scott Knott test.

In general, the performance of Chinese in response to the persisting rhizobia isolates were lower than that of Samnut 22 (Table 4.10). In Cheshegu soils, the response of Chinese in soils from un-inoculated (-N) control and isolate 91a plots recorded the highest number of nodules. A similar trend was observed for nodule dry weight except that soils previously inoculated with isolate 9g produced the second highest nodule dry weight. However, the responses of Chinese to the persisting rhizobia isolates led to no significant difference in N fixed and shoot dry weight. In Binduri soils, Chinese response in soils previously inoculated with isolates 53e and 9d resulted in 2-fold increase in nodule number and a double increase in nodule dry weight relative to the un-inoculated (-N) control. A similar trend of non-significant response was observed in shoot biomass for Chinese in Binduri soils. However, Chinese isolate 9d treatment recorded a significant increase in N fixed relative to strain BR3267 and the un-inoculated (-N) control. In Tanina soils, Chinese responded in soils from the un-inoculated (-N) control and isolate 9d plots to record the highest nodule number. The nodule dry weight followed the same trend (Table 4.11). Nonetheless, Chinese responded in soils previously inoculated with all the rhizobia isolates to increase N fixed and shoot dry weight over those of BR3267-inoculated and the un-inoculated (-N) control treatment controls.

Table 4.11 Response of Chinese groundnut to persisting rhizobia isolates

Nitrogen source	Nodule number per pot	Nodule dry weight ($mgpot^{-1}$)	N_2 fixed ($gpot^{-1}$)	shoot dry weight ($gpot^{-1}$)
Cheshagu				
52b1	101 d	123.3 d	25.74 a	13.17 a
53e	141 c	116.7 d	22.95 a	13.41 a
91a	178 b	270.0 a	22.51 a	14.79 a
9d	110 d	130.0 d	19.85 a	12.17 a
9g	156 c	240.0 b	21.78 a	13.41 a
BIOFIX	154 c	196.7 c	19.30 a	12.47 a
BR3267	192 a	293.3 a	24.85 a	14.26 a
N+	103 d	93.3 e	19.86 a	15.32 a
N-	177 b	206.7 c	19.56 a	11.67 a
CV (%)	6.5	8.6	11.4	7.8
Binduri				
52b1	117 e	136.7 c	17.73 b	11.94 a
53e	205 b	216.7 a	18.47 b	13.09 a
91a	199 b	180.0 b	14.30 b	11.29 a
9d	215 a	210.0 a	27.36 a	16.29 a
9g	153 c	140.0 c	19.28 b	12.93 a
BIOFIX	130 d	160.0 b	23.27 a	14.14 a
BR3267	115 e	130.0 c	15.72 b	14.35 a
N+	55 g	63.3 e	19.92 b	16.05 a
N-	95 f	110.0 d	14.65 b	11.80 a
%CV	2.1	5.3	10.2	9.7
Tanina				
52b1	145 d	180.0 b	36.21 a	18.71 a
53e	213 b	170.0 b	28.00 b	15.63 a
91a	149 d	146.7 c	35.86 a	17.38 a
9d	262 a	160.0 c	34.33 a	16.65 a
9g	197 c	200.0 a	31.30 a	13.44 b
BIOFIX	131 e	140.0 c	23.41 c	16.34 a
BR3267	125 e	170.0 b	27.74 b	13.85 b
N+	63 f	86.7 d	20.37 d	16.80 a
N-	252 a	186.7 a	23.25 c	12.86 b
CV(%)	2.5	5.8	8.6	9.6

Means within a column with the same alphabet are not significantly different ($P > 0.01$) between treatments by the Scott Knott test.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Selected physico-chemical properties and MPN of the experimental sites.

The soil fertility status at Cheshegu, Binduri and Tanina were generally low (Table 4.1). This is in agreement with findings of Buri *et al*. (2009), that soils in the Savannah zones of Ghana are predominantly low with low organic matter, nitrogen and available P levels. Since these values (C, N and P) are low, there is a potential for yield increment when they are in sufficient quantities. The levels of pH at all locations were medium acidic as reported by Landon (2014) and as such conducive for rhizobium growth and survival. This is in line with Drew *et al*., (2012), who reported that rhizobia can grow and survive under a pH range of 6.0 – 7.5. The results of the MPN determination showed that the soil of Binduri had relatively lower indigenous rhizobia population than those of Tanina and Cheshegu (Table 4.1). In principle, the presence of small numbers (> 50 rhizobia cells g^{-1} of soil) of native rhizobia is noted not to interfere with positive inoculation responses according to Slattery and Pearce (2001).

5.2. Response of nodulation of groundnut varieties to rhizobia inoculation

The results of the study showed that nodulation of groundnut varieties was significantly increased by inoculation with the local rhizobium isolates at all the study locations. This nodulation response may have been influenced by the low numbers of indigenous rhizobia at the study sites. According to Slattery and Pearce (2001), a native rhizobia population size of less than 50 rhizobia cells g $soil^{-1}$ can provide benefits to seed inoculation with introduced rhizobia. The observation therefore could suggest that introduced rhizobium isolates had a better competitive advantage over the native rhizobia due to their high numbers in soils. Moreover, the inoculation of the rhizobium isolates might have also increased the number of rhizobia within the

rhizosphere and hence more nodules plant^{-1} were produced. Similar significant responses of groundnut to inoculation has been reported by Yakubu *et al*. (2010) and Ashraf *et al.* (2006). Sajid *et al*. (2010) also reported significant responses on nodulation of three groundnut varieties (KS-1, BARD-1 and Chakori) following rhizobium inoculation. The difference in nodulation recorded by the isolates at the study locations could be attributed to the amount of rainfall that was received in the first 20 days. For instance, at Binduri and Tanina there were short dry spells with relatively low rainfall compared to Cheshegu (Fig 1, 2 and 3). Nodulation is known to be affected by moisture stress (Hungria and Varga, 2000; Bordeleau and Prevost, 1994). Abdulmalik *et al.* (2015), reported that different rhizobium strains respond differently to different legume species or cultivar of a legume species under different environmental and soil conditions. Moawad *et al*. (2005) observed different nodulation responses on nodulation of two beans cultivars with two elite rhizobium strains (CE 3 and Ph. 163 strains) in clay and silt soils, respectively.

5.3 Responses of shoot dry weight and total N of groundnut varieties to rhizobia inoculation

The results of this study showed that groundnut varieties responded significantly to inoculation of the isolates to increase N_2 fixed and shoot dry weight over the un-inoculated (-N) control and the reference strains. This result is in line with the observations of Yakubu *et al.* (2010) and Ashraf *et al.* (2006) who reported increases in N_2 fixed and shoot dry weight of groundnut following inoculation at Rawalpindi under rain fed condition. The study also showed that the groundnut varieties responded significantly to the mineral N fertilizer application across locations. This shows that N was limiting in the soils at the study sites and demand for fixed N was not being met by the native rhizobia population which was also low (less than 50 rhizobia cells g^{-1}

soil). Mathenge *et al*. (2016) reported that the potential of legumes to fix dinitrogen is likely to be high when the mineral N of the soil is low compared to conditions of soils with richer mineral N. Therefore, the low nitrogen levels recorded at these sites might have provided the opportunity for the increased N_2 fixed. This result also indicates that the isolates were compatible with the groundnut varieties and the symbioses could lead to more fixation of atmospheric nitrogen by the macrosymbionts. Perret *et al.* (2000) reported that the specificity between symbiotic partners minimizes the formation of non-fixing nodules by the host plant to enhance N_2 fixation. According to O'Hara (2001), P is an important element in rhizobium nutrition and symbiosis and therefore basal application of phosphorus could have improved the efficiency of the introduced rhizobium strains by supplying increased amounts of Adenosine triphosphate (ATP) needed for the nitrogen fixation process.

The increased N_2 fixed, in the groundnut varieties by the test isolates, probably increased rate of photosynthesis to produce large amount of carbohydrate to be allocated to the host vegetative parts, hence the observed more vigorous vegetative growth. The ability of Samnut 22 to respond better to test isolates in shoot dry weight and N_2 fixed than Chinese, generally, indicates that Samnut 22 was likely to be more compatible to the test isolates than Chinese or possibly attributed to the genetic variability existing between the groundnut varieties. This is in relation to the observation of Ashraf et al. (2006), who reported different levels of N fixed and shoot dry weights in two groundnut genotypes (ICG-4993 and ICG-7326) in response to three rhizobia inoculant (TAL-1371, TAL-1000 and NC-92.).

5.4 Response of pod formation and grain yield of groundnut varieties to rhizobia inoculation

Grain yield varied significantly with variety and rhizobium isolate across locations. Test isolates performed similarly to nitrogen fertilised plants in respect of pod formation and grain yield. This confirms that these isolates are symbiotically effective in supplying sufficient nitrogen through BNF to have increased the grain yield of the groundnut. The increased N_2 fixed probably resulted in the development of more leaves and shoots which enabled the plant to produce and sink more photosynthates to the lower parts and thus more pods plant^{-1}and grain yield were produced. Yakubu *et al.* (2010) and Ashraf *et al.* (2006) reported a positive response of pod number and grain yield of groundnut following inoculation and related this to the presence of effective strains in the inoculant. The result is also in agreement with the observation of Sajid *et al.* (2010) who reported significant increase in pod number and yield of groundnut following inoculation over the control. The positive responses due to inoculation with the local isolates and nitrogen fertilization at all the study locations also confirm that nitrogen was limiting in the study soils and that there was the need for inoculation. Comparative to Cheshegu, the low yields of groundnut recorded at Binduri and Tanina could be attributed to the low amounts of rainfall recorded at flowering stage of the plant which might have affected plant growth as well as the symbiotic processes at these locations (Fig 1, 2 and 3). Dry spell affects the amount of photosynthate produced hence the nutrition of the symbiotic organism which in turn affects the general growth of the plant especially podding and seeding. Ulzen *et al.* (2016) reported low yields of cowpea (ranged from 649 – 758 kg ha^{-1}) following inoculation and nitrogen fertilizer application at Nyagli in Upper West region as compared to yields obtained at Nyankpala (ranged from 828 – 1278 kg ha^{-1}) in

Northern region and related it to the short dry spell experienced at Nyali during the flowering stages of the crops.

5.5 Relationships between N fixed biologically and N fixing parameters of groundnut

The observation of significant positive correlation between N fixed biologically and N fixing traits (nodule number and nodule dry weight) across all the study locations implies that indirect selection of suitable varieties via these traits could result in improvement of N_2-fixed. The results suggested that the rate of N fixed kept pace with the rate of nodule number and nodule dry weight. It can therefore be inferred that N fixed is largely dependent on the effect of nodule number and nodule dry weight. Hence, groundnut varieties and bradyrhizobium isolates that promote high nodulation will have positive influence on N fixed in groundnut production. Rosario *et al*. (1997) reported similar observation that, N fixed, the proportion of the nitrogen in the plant contributed by fixation, was highly significantly correlated with nodulation.

5.6 Persistence of introduced rhizobia isolates through inoculation of groundnut

These isolates increased the rhizobia population sizes over that of the un-inoculated soils. This could be ascribed to higher nodulation in the previously inoculated treatments plots leading to release of large numbers of rhizobia into the rhizosphere through nodule senescence during the crop cycle. Moreover, it is also possible that the availability of sufficient organic carbon from decaying organic matter from plant litter and root exudates from non-legume host plants in the bulk soil (non-specific rhizosphere effect) could have provided the isolates enough carbohydrate for sustained energy production. These factors supported their survival and possibly the maintenance of their effectiveness. This is in agreement with Drew *et al.* (2012) who reported that it is not unusual to measure more than 100 to 1000 rhizobia cells per gram

in the top 10 centimetres of soil at the end of summer if there is availability of sufficient decaying organic matter and root exudates for rhizobia utilization.

However, the wide range of confidence interval recorded for the isolates, could possibly be attributed to dilution errors/contamination, microbial antagonism and non-random distribution or clumping of organisms (particularly with gum-producing organisms such as rhizobia), which led to ununiformed nodulation pattern between and within replications of the various dilution levels (where less positives were detected at a higher dilution level than at a lower level of dilution between replication or positive detected in replication and not replication 1 with dilution levels) of the soils containing these isolates when inoculated on groundnut in a growth pouch during the estimation of rhizobia population using the MPN count technique. This might have led to the creation of wide variation/error between and within dilution levels, hence the wide confidence interval. Similar observation had been reported by Scott and Porter (1986) who observed wide variation with the use of plant infection technique in estimating rhizobia population in inoculants containing either *Rhizobium meliloti or R. trijblii*.

5.7 Residual effect of introduced rhizobia isolates on groundnut

The groundnut varieties nodulated profusely and significantly in response to the rhizobia populations even after 8 months of fallow. This was ascribed to the increase in rhizobia numbers by the isolates above the thresh hold (100 cell g of soil) needed to cause prompt nodulation with maintained higher degree of infectivity. This is in line with Drew *et al.* (2012) who reported that prompt nodulation can occur when rhizobia population in the soil is well above the thresh hold needed (100 cell g of soil). The similar response pattern of groundnut varieties in shoot dry weight in soils previously inoculated with the isolates and the nitrogen-fertilized plants, confirmed that these isolates were able to maintain and sustain their symbiotic effectiveness to supply

enough N for their host plants through BNF to increase shoot dry weight. This could be attributed to the increased population sizes of the isolates over the native rhizobia in the un-inoculated (-N) control soils over time. Samnut 22 appeared to be more consistent in performing better in soils previously inoculated with isolate 53e than the other isolates in soils from all the study sites. The results also showed that, Samnut 22 was more compatible in performance with the persisting isolates on nodulation, N fixed and shoot dry weight than Chinese across location as previously observed in the field experiment. The results of this study are in line with the reports of other researchers, who found that soybean and lentil rhizobium strains were able to survive and persist in clay soil for three consecutive seasons after the first inoculation (Moawad *et al.*, 2005). Furthermore, Moawad *et al*. (2005) found that two common bean varieties (BRONCO and GIZA 6) responded differently and significantly on nodulation, N fixed and shoot dry weight in clay and silt loam soils previously inoculated with two rhizobium strains (Ph 163 and CE3), respectively after the first inoculation.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Data obtained from the field study, in general, showed that inoculation with isolates 53e and 9g consistently gave higher grain yield at all study locations. Based on the data from Cheshegu, superior isolate for Samnut 22 was 53e and that for Chinese was 9g. Additionally, data obtained at Binduri, also showed that superior isolates for Samnut 22 were 52b1, 53e, 9d and 9g and that for Chinese was 9d. Furthermore, Tanina's data indicates that superior isolate for Samnut 22 was 53e and that for Chinese were 53e, 91a and 9g.

Data obtained from the greenhouse, in general, showed that local rhizobium isolates with high residual effect were 53e and 9d for all locations. Based on data from cheshegu soils, isolates 53e, 91a, 9d and 9g had high residual effect. In Binduri soils only isolate 53e and 9d had high residual effect while in Tanina soils all the isolates had high residual effect after eight (8) months of fallow.

6.2 Recommendation

Based on the field study it can be recommended that, these rhizobium isolates (53e and 9g), (52b1, 53e, 9d and 9g) and (53e, 91a) have the potential to be used as peat based inoculants for smallholder groundnut farmers to enhance groundnut production at Cheshegu, Binduri and Tanina, respectively.

The residual effect study should be repeated to establish the number of seasons these isolates can survive to increase the rhizobia population in the soils of these sites with maintained effectiveness to enhance groundnut production.

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APPENDICES

Appendix 1. Components of Yeast Mannitol Media

Chemicals	**Yeast Mannitol Agar**	**Yeast Mannitol Broth**
	Quantity Measured g/L	
K_2HPO_4	0.5	0.5
$MgSO_4.7H_2O$	0.2	0.2
NaCl	0.1	0.1
Yeast extract	0.5	0.5
Mannitol	10	10
Agar	15	-
Distilled water	1000 L	1000 L

(Vincent, 1970)

Appendix 2. Broughton and Dilworth N-free Plant Nutrient Solution

Stock Solutions	Element	Form	g/L
1	Ca	$CaCl_2 \cdot 2H_2O$	294.1
2	P	KH_2PO_4	136.1
3	Fe	Fe-citrate	6.7
	Mg	$MgSo_4 \cdot 7H_2O$	123.3
	K	K_2SO_4	87.0
	Mn	$MnSO_4 \cdot H_2O$	0.338
4	B	H_3BO_3	0.247
	Zn	$ZnSO_4 \cdot 7H_2O$	0.288
	Cu	$CuSO_4 \cdot 5H_2O$	0.100
	Co	$CoSO_4 \cdot 7H_2O$	0.056
	Mo	$Na_2MoO_2 \cdot 2H_2O$	0.048