KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI, GHANA

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CROP AND SOIL SCIENCES

GROWTH AND YIELD RESPONSE OF SOYBEAN VARIETIES TO INOCULATION AND NITROGEN LEVELS

BY

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BSc. Agriculture (Hons.)

NOVEMBER, 2016

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(BSc. AGRICULTURE)

A Thesis submitted to the Department of Crop and Soil Sciences, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology, Kumasi, in partial fulfilment of the requirements for the degree of

MASTER OF PHILISOPHY

IN

AGRONOMY

CERTIFICATION

I hereby declare that this submission is my own work towards the MPhil 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.

PRUDENCE OWUSU-MENSAH

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PROF. JOSEPH SARKODIE-ADDO (supervisor)

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ABSTRACT

Soybean, like any other legume can fix atmospheric N through symbiotic association with native rhizobia but, the amount of N_2 fixed is usually not enough due to existence of ineffective population of native rhizobia. A field experiment was conducted in 2015 at CSIR-CRI demonstration field to study the interactive effect of inoculation and nitrogen fertilization effect on the growth, nodulation and nitrogen fixation, grain yield and protein content, as well as remobilization of N using three soybean varieties. The experiment was a 3x6 factorial experiment laid out in a Randomized Complete Block Design with three replications. The factors were: soybean varieties (Anidaso, Quarshie and Salentuya 1) and the nitrogen sources: (control, 30 and 60 kg N/ha, inoculation alone, inoculation+30 kg N/ha and inoculation + 60 kg N/ha. The seeds were inoculated before sowing. All cultural practices were carried out when needed. The results showed that, inoculated plants of all three varieties established better than their corresponding uninoculated plants. Again, inoculation resulted in significantly greater nodule numbers, nodule dry weight and nitrogen fixation. Also growth, grain yield and protein content were all enhanced following seed inoculation. Application of fertilizer N at grain filling period increased seed yield, especially at the rate of 30 kg N/ha. Lastly, remobilization of N occurred in all treatments, but was greater in treatments with greater availability of N.

DEDICATION

This dissertation is dedicated with love and appreciation to my supervisor, Prof. Joseph Sarkodie-Addo.

ACKNOWLEDGMENT

Ebenezer, this is how far the Lord has brought me. First, I give thanks to God Almighty whose favour, direction and protection has kept me this far. My sincere appreciation goes to my supervisor Prof. Joseph Sarkodie-Addo, Prof. R. Akromah of Department of Crop and Soil Sciences, KNUST for their devotion and support throughout the period of this research work. My heartfelt appreciation also goes Dr. Stephen Amoah and Dr. J.Y. Asibuo of CSIR-CRI for their immense support and corrections toward this work.

I am grateful to Mr. Frank Adomako, Franklin Bosompem, Mr. Felix, Mr Bofah Henry, Nana Akuoko, Afia Gyaamah, Osofo Appiah, Mr Maxwell Lamptey, Bright and Wilson all of the legumes and oil seed division of CSIR-CRI, Fumesua for their invaluable contribution toward the completion of this work. I would also like to acknowledge the assistance received from Mr. Ulzen Jacob, Mr. Azumah Ayamah and Mr. Caleb Ocansey.

Special thanks to Mr. Godfred Afrifa Owusu, Mr. Stephen Arthur, Mr. Martin Tengan, Mr. Adu Boakye Daniel, Mr. Paul Marno and Snr. Muhamed all of the Department of Crop and Soil Sciences, KNUST for their time and assistance during the field and writing of this dissertation.

Finally, I wish to express my profound gratitude to my parents, Mr. George OwusuMensah and Madam Grace Linda Fordjour for their prayers, encouragement and support throughout my study and to my siblings, Anthony Owusu-Mensah, Eugene Oteng Boafo, Ophelia Agyenim-Boatemaah their support and inspiration. God bless you all.

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CHAPTER ONE

1.0 INTRODUCTION

Soybean **(***Glycine max* L) is an annual herbaceous plant in the *Fabaceae* (legume or bean family) (Tefera, 2011). It is an economically important leguminous crop on a worldwide scale and also the most important legume in China. Among the important food crops grown in every part of the continent is soybean. The crop can be cultivated in many places with low level of agricultural inputs (Gan *et al*., 2003; Dugde *et al.*, 2009). Soybean is an important protein source in the diet of humans and ration of animals; containing considerable amounts of all essential minerals, oils, vitamins and amino acids (Tefera, 2011).

The protein content of soybean is around 40% and the oil content is approximately 20% which is also 85% unsaturated and cholesterol-free and also the main source of vegetable oil worldwide. It is recorded as the legume with the highest protein content and vegetable oil among the other crops produced (IITA, 2009). Thirty percent of the oil produced worldwide is from soybean and it also serves as bio-fuel source (Graham and Vance, 2003).

Biological nitrogen fixation (BNF) occurs in many legumes due to symbiotic association with soil rhizobia. Soybeans like other legumes are also able to establish associations with other rhizobia specifically *Bradyrhizobium japonicuin* (Paulo *et al.*, 2009). It is widely known that inoculation of legumes with effective compatible rhizobia can enhance yields through biological nitrogen fixation and this accounts for a substitute and sustainable source of nitrogen for inorganic fertilizers. Introduction of rhizobia in the soil is a practice that has been used over decades in the absence of compatible rhizobia or when native rhizobia population is low or inefficient to fix nitrogen (Catroux *et al.*, 2001; Deaker *et al.*, 2004; Stephens and Rask, 2000).

Most farmers take advantage of legume-rhizobia associations by applying rhizobia inoculant to seeds or soils. Rhizobial inoculants are available in many formulations: granular inoculants are applied over the soil after sowing (Lupwayi *et al.*, 2006); liquid inoculants are mostly used in large areas, mainly with soybean, in South America (Paulo *et al.*, 2009). However, commercial inoculants are available as solid- in powder from peat or in granular form or as liquid formulation (Stephen and Rask, 2000). Inoculant use accounts for greater root biomass and an increase in nodulation, which increases plant vigour and yield. Even though the cost of inoculation is relatively low, nodule failure is very expensive, as without root nodulation with effective rhizobia, the plants will use soil nitrogen for their growth making the soil nitrogen deficient (Bowen and Hogg, 2010).

Soybean can fix between 50-80% of the nitrogen required (Solomon *et al*., 2012) but most soybean varieties cannot meet all the N required for growth and development of seeds only through fixation. *Bradyrhizobium japonicum* has been reported to be rarely available in Ghanaian soils because soybean does not originate from Ghana (Okogun and Sanginga, 2003). Moreover, in soils with no previous record of soybean production, bradyrhizobia populations are often not present, and therefore for successful nodule formation, it may require clearly identified bradyrhizobium species for N_2 fixation to be effective (Abaidoo *et al*., 2007). The success of inoculation however, does not only depend on the inoculant quality and proper inoculation practice but also on the achievement of efficient and effective BNF by considering factors that affect the performance of the rhizobia species such as climatic, edaphic, management factors and legume genotype (Giller, 2001; Sanginga *et al*.,1995; Giller and Wilson, 1991).

In soybean, nitrogen obtained from nodule and fertilizer is the most crucial element for ensuring good growth rate of the source (photosynthetic organs) and also ensuring growth of flower buds at vegetative stage. In effect, nitrogen translocation efficiency from vegetative to reproductive organs has influence on yield at pod filling stage (Nakamura *et al*., 2010). Reduction of N from the vegetative parts enhances leaf fall and thus limits the photosynthetic ability of the leaf canopy. This results in reduced yield by cutting short the seed filling period (Kumudini *et al.,* 2002).

Several studies have shown that nitrogen fertilizer applied during the reproductive stage (R1 to R5) is likely to increase the capacity and duration of inorganic N utilization periods. Supplying N to soybean plant during the peak of seed demand may supplement N existing resources, thus overcoming premature senescence and increase seed yield (Barker and Sawyer, 2005; Freeborn *et al.,* 2001).

1.1 Objectives

The main objective of this research was to determine the response of soybean to inoculation and different nitrogen fertilizer levels on the growth and yield of soybean.

The specific objectives of the study were to determine the;

1. response of soybean to inoculation and their effect on nodulation and nitrogen fixation,

2. effect of inoculation on growth, grain yield and crude protein content of soybean,
3. effect of nitrogen availability on nitrogen remobilization between R1 and R5 growth stages in soybean.

The above objectives were based on the hypothesis that;

1. inoculation of soybean will increase nodulation and nitrogen fixation,
2. inoculation will increase growth, grain yield and crude protein content of soybean,
3. nitrogen availability on nitrogen remobilization between R1 and R5 growth stages in soybean will positively be affected.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and history

Soybean (*Glycine max*) has protein and oil content of approximately 40% and 20% respectively. This crop has the highest protein content and vegetable oil among cultivated crops in the world. The total cultivated area of soybean in the world as at 2007 was 20.19 million, whiles total production was 220.5million (FA0, 2009).

Soybean is said to have originated and domesticated the eastern half of North China in the eleventh century B.C. or perhaps a bit earlier (Laswai *et al.*, 2005). Soybean was one of the five main crop plants of China along with rice, wheat, barley and millet. According to early authors, production was localized in China until after the ChineseJapanese war of 1894-95, when the Japanese began the commercial import of soybean oil cake to be used as fertilizer. Soybeans were shipped to Europe about 1908, and it gained world-wide attention and attraction. Awareness of soybean by Europeans was as early as 1712 through the writing of a German botanist. Seeds of soybeans are believed to have been sent from China by some missionaries as early as 1740 and planted in France (Lance and Garren, 2005).

Soybean production developed rapidly in the 1950s in the USA and now USA is the largest soybean producing country world-wide, followed by Brazil, Argentina and China. These countries use various machineries for the commercial production and the commodity rate of soybean is relatively higher (Qiu and Chang 2010). In the late 1980s, soybean cultivation was identified in Africa, although little is known about the country to which it was first introduced but it was likely to have been cultivated earlier on the eastern coast of Africa since that was the region were the Chinese traded (Shurtleff and Aoyagi, 2007). In 1903, the next soybean cultivation in Africa was identified in South Africa at Cedara in Natal and Transvaal. Soybean was later Mauritius and Tanzania in

1907. In Ghana, the first soybean trial was in 1906 with poor results (Shurtleff and Aoyagi, 2009). However, in the late 1960s and early 1970s, research on soybean was at its optimum at CSIR-Crops Research Institute and the Agric Research Station of University of Ghana (Plahar, 2006).

2.2 Botany

Soybean (*Glycine max* L), a self-pollinating legume with 2n = 40 chromosomes. It has 30% carbohydrate, 20% oil and 40% protein and plays an important role in agriculture production world-wide. (Tefera, 2011; Flaskerud 2003). The origin is believed have been derived from *Glysine ussuriensis,* a legume of central China origin (Encyclopaedia Britannica, 2008). The soybean is bushy and erect annual plant which grows to a height up to 2m. The entire body is covered by short brown/white hairs. It has a taproot with deep main root branches extensively with its secondary roots which has spectacular nodulation (Joshi, 2015).

2.3 Morphological description

The soybean is an annual crop with an extensive taproot system, most of it in the soil. The taproots usually grow into the soil and adventitious root grow from the hypocotyls. The leaves are trifoliate and alternate with long petioles and small stipules and stipules; leaves are ovate and lanceolate with mucronate tip (Chaturvedi *et al.,* 2011). The flowers of soybean develop from auxillary bud along the main stem and branches. Inflorescence at each axil is a raceme consisting of 2-35 papilionaceous flowers (Smith, 1995). Flowers are usually self-pollinated but around 1% of cross pollination aided by insects does occur

(Chaturvedi *et al.,* 2011). Pods are short stalked and occur in groups of 13-15, 3-7cm long, hairy and light brown at maturity and slightly constricted between seeds (Chaturvedi *et al.*, 2011).

Three types of growth habits are identified with soybean cultivars: determinate, semideterminate and indeterminate. With the determinate, vegetative growth is nearly complete when the plant starts flowering whiles with the indeterminate, both vegetative and reproductive growth go on simultaneously. The semi-determinates have indeterminate stems that terminate vegetative growth unexpectedly after flowering periods (OECD, 2000). The stems are mostly concealed in a mass of hairs which are usually fine in nature. The function of the stem is to provide support to the flowers and leaves and also transport nutrients and water. Growth habits of soybean are erect and bushy and grow to height of 60-140cm depending on the planting date and variety type (Beldfield *et al.,* 2011).

Leaves, pods and stem are covered with fine brown or gray-like hairs. The leaves are trifoliate, having three- four different structures per leaf. Seeds vary in appearance, size, colour and shape, though they are mostly round or oval with a cream seed coat. Flowers have different colours ranging from white, purple and pink, small and usually pea-typed and 5-6mm long. Pod colour ranges from brown or black or yellowish. Fruits of soybean are hairy pods that develop in cluster of three to four seeds (Kumudini, 2010; Chaturvedi *et al.,* 2011; Rienke and Joke, 2005).

Growth and development of soybean have been marked out in two main stages: the vegetative growth and the reproductive growth. The vegetative growth is distinguished by completely unrolled leaf at the unifoliate node (V1), completely unrolled leaf at the first node above unifoliate node (V2), three nodes on main stem starting with the unifoliate node (V3) and the N nodes on the main stem starting with the unifoliate nodes (V4) (Nand *et al.,* 2010). The reproductive stage is also characterised by beginning of bloom (R1): one flower is at least present on the main stem, full bloom develops (R2): on any of the top two nodes, flowers can be identified, beginning of pod (R3): pods of 0.5cm in height are found on one of the top four nodes, pods develops fully (R4): pods of 1.9cm in height on one of the top four, seeding begins (R5): seeds of 0.3cm on one of the top four, seeds develops fully (R6): pods are fully filled with seeds on one of the top four, maturity begins (R7): one matured pod is found on the plant and complete maturity (R8): 95% pods change to matured pod colour (Pawel, 2015).

2.4 Climate and soil

2.4.1 Soil

Soybean does well on a wide range of soil but prefer well drained fertile loamy soils. This soil is very suitable for wide range of crops. The crop is intolerant to drought because of the nature of the roots. That is the shallow rooting system of soybean does not support absorption of water especially during the dry season. Therefore, soils with low water holding capacity and shallow soils are not suitable for soybean production (Beldfield *et al.,* 2011).

Soils with adequate water content are the best for soybean production and enhance germination of seeds and establishment of plant. Waterlog conditions cause harm to soybean between appearances of four-leaf stage. However, it becomes tolerant to waterlog after this stage.

Soybean does well on a wide range of soil pH and prefer soil pH of 4.5-8.5. Slightly acidic soils are however also suitable for soybean (Beldfield *et al.*, 2011). Maintaining optimum soil pH of 5.5-7.0 enhances nutrient availability, symbiotic nitrogen fixation and microbial breakdown of crop residue. Soil pH above 8.0 is suitable for soybean growth and even makes micronutrients such as zinc and iron to be deficient (Ferguson *et al.*, 2006). For high grain yield, loamy textured soils are required than clayey soils (Rienke and Joke, 2005).

2.4.2 Moisture supply

Soybean is a rain-fed crop and requires adequate water for growth and development since about 90% of the weight of plant is made of water operating in physiological and biochemical processes (Bilibio *et al*., 2011). However, it can grow to maturity with as low as 180mm of in-crop rain but 40-60% yield loss is expected compared with favourable conditions for growth. The required rainfall for soybean growth is 4601000mm depending on weather, crop management, duration of crop cycle, soil type and soil moisture stored. Below 180mm of rains, crop failure is likely to occur (Bilibio *et al*., 2011; Beldfield *et al.*, 2011). According to Rienke and Joke (2005), soybeans have two crucial periods for water requirement: from sowing to germination and time for growth of the bean in the pods. For a seed to germinate, it requires to take up 50% of its weight in water. However, at the germination stage much water causes severe damage than when little water is applied. Soil water saturation level should be between 50-85% to ensure growth. The need for water increases as the crop grow, the maximum requirement is during bean growth (7-8mm per day) and drops again. Excessive rainfall prior to flowering can retard growth and cause lodging whereas waterlogging also have a negative effect on growth and yield (Soybean production guidelines, 2010).

2.4.3 Photoperiod and temperature

Soybeans belong to the legume family which is adapted in the tropics, subtropics and the temperate regions (IITA, 2009). Soybean is greatly influenced by day-length. It is a short day crop which implies that, they flower when the night lengths exceed critical day-length or cannot flower under short nights. Different varieties respond differently to critical day-length it must obtain before plants start to flower (Beldfield *et al.,* 2011). Therefore, timing of development of flowers can be crucial to the adaption of the variety to a geographic location (Kumudini, 2010).

Soybean respond to different temperature ranges but optimum temperature range for growth of soybean is 20-30^{0}C. Temperature range of 35^{0}C and above are said to have a reduction effect on yields. Soil temperature optimum for germination and early seedling emergence is 25-30^{0}C (Beldfield *et al.,* 2011). In soybean, induction of flower is controlled mainly by temperature or temperature and photoperiod (Kumudini, 2010).

2.5 Soil available nitrogen

In soils with low mineral N, the amount of N that is fixed is usually high but this only become possible when there is adequate water and supporting nutrients that enhances plant growth (Unkovich *et al*., 2008). Several research have also shown that, soils with high level of nitrogen in the root area prevents formation, establishment of nodules and activity of nitrogenase (Abdel-Wahab *et al*., 1996; Peoples *et al*., 1995) as it requires minimum amount of energy for legumes to absorb soil N than biologically fixing N from the atmosphere (Cannell and Thornley, 2000). However, as soil mineral content N increases, N fixation is inhibited (Macduff *et al*., 1996). Gan *et al*. (2004) reported that, application of starter N enhances the establishment of nodules and fixation of N as compared to no mineral N application in some instances. Application of certain concentrations of starter N that enhances BNF differs with variety used and the conditions surrounding growth of crop but mostly smaller than 4m*M* for NH_4^+ and 2m*M* for NO_3^-. This according to Keyser and Li (1992) is due to the fact that symbiotic legume-rhizobium may not produce the required N during the early stages of growth needed to meet the N demand mineral N beneficial to help encourage early growth. N fertilizer applied at either vegetative or reproductive growth stage can increase crop biomass and pod by 16% and 44% respectively (Katulanda, 2011). Yinbo *et al*. (1997) reported that, legume response to N fertilizer application is highly dependent on application rates and time of application. N fertilizer application at R5 (pod filling) stage increases amount of plant N from fixation (Yinbo *et al*., 1997)

2.6 Why N application in soybean is necessary

Several contrasting statements have been published about response of legumes to nitrogen fertilizer. A report by Keyser and Li (1992) states that, addition of mineral N in the rhizosphere region resulted in increased nodule formation and suppressed functioning, resulting in lower amount of N fixed. Wood *et al*. (1993) stated that in the dry matter of soybean, only 25-60% of N originates from symbiotic fixation whiles the rest comes from soil N. The plant of soybean serves as a sink for N in the soil and use effectively the soil N regardless the source; therefore application of N fertilizers to soybean could be beneficial. A report by Panchali (2011) stated that, if the legumerhizobium association is not able to produce adequate nitrogen especially at the early growth stages to meet the amount of N demanded by plant due to such factors, then lower amount of mineral N application becomes required. The high N demand by legumes may need a high level of N in the soil to bring about yield maximization

(Sosulski *et al*., 1989). A confirmation by Katulanda (2011) also states that a 44% and 16% increase in crop and pod biomass respectively as to whether N application should be done at either the vegetative or reproductive growth stage. Osborne and Riedell (2006), Kucey *et al*. (1989) and Gan *et al*. (2003) were all in agreement to N fertilizer application to soybean at a particular growth stage to increase its production. Schmitt *et al*. (2001) stated that N fertilized soybean did not give higher oil content and grain yield. Panchali (2011) and Barker and Sawyer (2005) also stated that, N use in soybean at a particular growth may not be recommended. N use in soybean production cannot be left without consideration of factors such as environment, fertilizer type, time and rate of application among others should be considered before drawing conclusion on these controversies.

2.7 Inoculation

At the first introduction of legumes into the soils, nodules formed on root may fail to develop as results of lower population of effective and compactible rhizobium in soil. Thus, addition of rhizobium of appropriate strain is very important where legumes have not been sown or there are no native rhizobia populations (Ledgard and Steele, 1992). Inoculation is aimed at coating legume seeds with adequate rhizobia of appropriate strain to result in rapid and effective nodulation of that particular legume on the field (Sinha, 1997). Inoculant formulations are in various forms which are highly dependent on the method of application. These include liquid or frozen concentrates, inoculated granules, porous gypsum granule and natural peat granules. The most commonly used type is the peat- based inoculant which is applied directly to seeds or in liquid form (Sham *et al*., 2005).

Peat inoculant is prepared by addition of rhizobia broth culture to sedge peat. Adequate moisture is incorporated to ensure multiplication and growth of rhizobia (Ledgard and Steele, 1992; FAO, 1984). Rhizobia population > 1000/g soil is required for optimising nodulation and fixation of N; thus the main aim of inoculation is to increase the desirable strain rhizobia strain number in the rhizosphere (Lupwayi *et al*., 2000), therefore increasing BNF and yield of grain. At other times too, inoculation is applied as a security against failure of crops (Deaker *et al*., 2004) as less production related problems such as producing crops which are N deficient is avoided. In legumes, inoculation with a desirable rhizobium was found to be the most useful agronomic practice in yield maximization (Gudni and Graig, 2003). However, the poor competition with indigenous strains, unfavourable climatic conditions and other related stresses affect their number and viability (Batilan and Johnson, 1995). Per estimate, legumes can fix 200 kg N/ha/year under favourable condition (Giller, 2001).

Legume-N benefit can only be accomplished in the existence of effective and efficient rhizobial strains which can be introduced or native. Inoculation of seeds before sowing is very important and can lead to large rhizobial population establishment in the rhizospere which enhances nodulation and N fixation. Yields of soybean were very low and production of the crop faced a lot of challenges. Most important among them was shortage or absence of effective bacteria for soybean nodulation (Khidir, 1997). For better yields, it has been reported that, the crop should be inoculated with the right rhizobium strain (Hardson and Atkins, 2003).

Inoculation of soybean has been found to be an alternative for nitrogen fertilization. Rhizobium inoculation has also been found to show a great potential as using fertilizer and it is relatively cheaper and enhances plant growth and quality of seeds. Although, addition of N fertilizer in small quantity to the soil improves nodulation, nodule development and fixation of N is drastically reduce when high amount of N concentrations are added to the plants (Ahmed, 2013). In an experiment, Fenin et al. (2002) concluded that, only 6% of native rhizobia population in Ghanaian soils are effective with 68% and 26% been moderately and ineffective respectively which there encourages inoculation. In other field trial in Yamgambi Congo, it was observed that inoculated soybeans increased yields by 80-300% (Shurtleff and Aoyagi, 2009).

2.8 Nodulation, biological nitrogen fixation (BNF) and factors affecting BNF

Soybeans like other legume live in a symbiotic association with soil-dwelling bacteria that help to fix nitrogen through the root nodules. *Rhizobium*-legume relationship is of importance to natural as well as agricultural systems. With soils of limited nitrogen levels, legume roots are infected by nitrogen-fixing bacteria of Rhizobiaceae family, succeed in the formation of root nodules, which serve as habitat and also provide adequate supply of food for bacterial symbiont which also provide anaerobic conditions (such as low oxygen) for the fixation nitrogen (Eckardt, 2006).

Development of the root nodule can be grouped into three (3) main stages of preinfection, nodule initiation and differentiation. At initial stage which is the pre-infection stage, flavonoids which are released by the legume root hairs as chemotaxis for rhizobial symbionts and also trigger the nod genes expression (Eckardt, 2006). The expression of the gene produces nod factor which activate series of sequential events including curling of root hair around the rhizobia invading, the invasion of the rhizobia into the plants by the infection thread and the beginning of cell division of the cortex of the root that indicate the nodule formation at the earliest stage. With the presence flavonoids in the layer of soil immediately adjacent plant roots where microbial activities are highest, rhizobia colonize and multiply (Hopkins and Hurner, 2004).

Soybean plants live in symbiotic relationship with nitrogen fixing bacteria (rhizobia) called the *Bradyrhizobium japonicum* (Sarkodie-Addo *et al.*, 2006). The mutual relationship between rhizobium bacteria on the root nodules and the soybean plant is considered the most desirable systems of nitrogen fixation. The soybean bradyrhizobium for example can fix 57.94 kg/ha of nitrogen when the soybean plant is inoculated (Shurtleff and Aoyagi, 2009). *Rhizobium* bacteria which are hosted in the roots of certain legume plants provide carbohydrate for growth of bacteria while the bacteria fix N_2 in the atmosphere into NH_4^+, which is changed into plants usable amino acids. Rhizobia are able to utilize nitrate in the soil and changing it to gaseous oxide of N by the process of denitrification. This process occurs in various rhizobium and bradyrhizobuim strains (Ledgard and Steele, 1992; Russelle, 2008).

The amount of fixed N is greatly influenced by both the rhizobium and the characteristics of the host plant. Improved strains of the rhizobium are normally selected in improved BNF in certain special conditions but the strains selected should be capable to establish well in the field and it is dependent on factors including their ability and effectiveness (Ledgard and Steele, 1992). Inoculation has been found to be an important technology for improving productivity of crop and the fertility of soil (Keyser and Li, 1992). Seeds inoculated with rhizobium have been found to increase total N and yield of grains in soybean cultivars.

N requirements of soybean are met in a complicated manner as the crop is able to utilise both soil N and N from the atmosphere (Mrkovack *et al*., 2008). Nitrogen fixation capacity at the early growth stages is low but increase at a faster rate at later stage. At the R3 and R4 stages, N fixation reaches maximum and begin to decline at R5 which coincide with the time of peak of N demand. R5 stage is characterised by high level of demand of photosynthates from nodules and pods, make easier the energyzation rate of the thylokiod membrane and promote photosynthesis (Diaz *et al*., 2009).

N fixation process is affected by the physiological states of the host plant. Other environmental conditions negatively affect the growth and activity of the plants that fix nitrogen. Factors such as nutrient deficiency, temperature extremes, salinity, plant disease, mineral toxicity, unfavourable pH, inadequate photosynthesis, grazing and inadequate or excess soil moisture act as limiting factors on host legume vigor (Zahran, 1999).

Salinity effect on legumes can be critical because of their low tolerance to salinity and the high response of the symbiotic nitrogen fixation to stress. Rhizobial infection of root hair and development of nodules are very responsive to saline conditions (Rao *et al*., 2002). Colonization of roots by rhizobia is not affected by saline condition but rather new nodules development, growth and efficiency of fully grown nodules are affected (Rao *et al*., 2002).

Acidic soils have adverse effect on growth, survival and fixation of nitrogen by microorganisms while *rhizobium*-legume relationship is also affected (Bordeleau and Prevost, 1994). Rhizobial colonization in the rhizosphere can be drastically reduced by soil pH extremity (van Jaarsveld *et al*., 2002). Highly acidic soils (pH < 4) are characterised by extremely lower levels of calcium, phosphorous accompanied with manganese and aluminium toxicity, which have impact on both the rhizobia and host plant. In soils with low pH, nitrogen fixation and nodulation are undesirably affected (Bordeleau and Prevost, 1994).

Temperature changes affect nitrogen fixation and nodulation as the ability of rhizobium strains to compete in native environment is highly affected (Bordeleau and Prevost, 1994). High temperature is one of the limiting factors affecting fixation of nitrogen in the tropics and sub-tropical region. Activity of nitrogenase is extremely reduced due to ineffective nodule formation under high temperature (Hungria and Franco, 1993).

Moisture stress has effect on the morphological changes of rhizobia strains. Some strains have been reported to show irregular forms under low water levels. Reduction in nodulation and infection in legumes are as a result of moisture stress (Zahran, 1999). Nodulation is affected by both drought and soil moisture. Number, weight and size of nodules are affected by drought. The amount of N fixed is greatly dependent on soil moisture availability and is decreased with water stress (Guriqbal, 2010). Carbon shortage, oxygen limitation and nitrogen metabolism regulation are the three main factors related to the effects of drought on BNF (Ladrera *et al*., 2007). Sinclair *et al*. (2007) also stated that N_2 fixation with dryness of soil causes reduction in yield as a result of insufficient N for production of protein, which is a very crucial product of seed.

Rasmo *et al.* (2003) reported that bacteriods senescence resulted after exposing common bean to ten (10) day moisture stress due to degradation of the cell wall of nodule. Again, conditions such as drought also adversely affect weight of nodule and activity of nitrogenase.

2.9 Rate of nitrogen application on soybean yield

Wood *et al*. (1993) reported that the architecture of soybean plant that supports grain production is highly affected by early season growth. Starter N applied at R1 was found to increase yield. Yields of dry matter recorded at R1 stage were 26 and 30% greater than the zero-N control. Also, according to Valinejad *et al*. (2013), starter N application at R1, R3 and V2-V4 were significantly greater than control (zero-N applied). Application of N early in growth season results in poor formation of nodule and yield increase above soybean with no N applied at a later stage (Elbelhar and Anderson, 2000). However, Gan *et al*. (2003) recorded that, application of starter N at 25kg/ha resulted in the reduction yield, total N accumulated and total amount of N_2 fixed in all three genotypes used. Nitrogen applied at the R3 to R6 has shown positive impact on soybean growth. Application of N was found to have increased grain yield at flowering stage and at early seed pod fill (Wood *et al*., 1993).

A field study conducted at the Islamic Azad University of Tabriz, Iran in 2011 on the effect of nitrogen fertilization of soybean showed that, at seed filling stage, nitrogen demand is very high and that was found to have accounted for leaf fall and remobilization of nitrogen. Addition of 15 kg N/ha was found to have caused a small delay of two (2) days in in fall whereas with full dose, effective vegetative growth was recorded (Golparvar *et al*., 2012). Other studies conducted by Flavio *et al*. (2004) using two nitrogen rates (100 and 50 kg N/ha) applied at R3 and R5 stages showed an increased soil nitrate availability during seed filling stage following N fertilization. However, no yield responses were observed when irrigated soybean was treated with N fertilization at R3 or R5, even at high yield site of 5,000kg/ha (Freeborn *et al*., 2001).

Reports from Wesley *et al*. (1999) stated that yield of soybean increased to nearly 12% when nitrogen was applied at R3 growth stage under irrigation. Hatfield and Follett (2008) also reported that increasing nitrogen levels increase yield but oil content is negatively affected with rates of nitrogen levels.

Addition of nitrogen at the R4 growth stage produced a minimal increase of seed protein from 3.72-37.6% (Schmitt *et al*., 2001). At a rate of 25 kg N/ha, N applied early as top dressing was found to have promoted the total biomass of soybean plant and the N accumulation at the seed filling stage (R5) which increased yield (Gan *et al*., 2003).

2.10 Nitrogen application and remobilization on yield of soybean

The integral part of all protein is nitrogen and is one of the main macronutrient elements required for plant growth and photosynthesis. Nitrogen is available in most soils with other adequate essential elements in quantities for plant growth (Pro soils, 2013). In agricultural crop production, available and usable N is the most limiting factor for high yields (Agren *et al*., 2012). N is limiting because large amounts are taken with crop through harvest and it is easily lost through leaching, runoff or erosion and gaseous exchange. Also, large amount of N is removed because, the grain alone has a high protein content of 40% and the protein contains about 16% N (DuPont, 2014; Salvagiotti *et al*., 2008).

High yielding newly released cultivars have been found to exhibit higher production of dry matter and N remobilised at seed filling than old cultivars (Shiraiwa and Hashikawa, 1995). Topdressing with N fertilizer could be a sure way to satisfy the high N demand especially at seed filling stage. Topdressing has been found to increase the seed yield and assimilation of N (Nishioka and Okumura, 2008). Gan *et al*. (2003) in an experiment on the effect of nitrogen fertilizer top-dressing at various reproductive stages concluded that, due to the high requirement level of soybean, N fixation only cannot cater for the amount of N required to maximise yield. Yield increase by N is as a result of its influenced on a number of agronomic and quality parameters. Nitrogen application significantly influenced grain yield due to the role it plays in chlorophyll and amino acid synthesis (Oz, 2008). Mastrodomenica and Purcell (2011) in an experiment stated that part of N is translocated from vegetative tissue across different maturity groups.

Remobilization of phloem –mobile nutrient can lead to rapid reduction on the N concentration in the vegetative shoot that leaf fall is induced and plant behave as _selfdestructing‘ system (Marschner and Marschner, 2012). Bebeley (2013) showed that remobilization of N only occurred in soybean during the grain filling stage. Again, at R5-

R7 growth stage, N is remobilized from leaves and other plant parts for seed production (Egli, 2010). Nitrogen supplied from fertilizer N during the R1 and R5 stages enhance the duration of mineral N to the utilization periods, while ensuring continual N fixation (Barker and Sawyer, 2005). Mengel *et al*. (2006) reported high significant response to highest N rate (80 kg N/ha) applied on yield and yield parameter. Gan *et al*. (2003) also reported that application of N at 50 kg N/ha significantly increased N accumulation at both yield at V2 or R1 growth stages. Zhao *et al*. (2014) also stated that, leaf N content increases during R1-R5 stages which increases pod number and seed number and declines, therefore, N fertilizer applied can increase seed filling period which subsequently increase yield.7 h

2.11 Inoculation and nitrogen effect on soybean growth and yield

Soybeans have shown different responses to inoculation and nitrogen application at different levels. Mrkovacki *et al*. (2008) indicated that, greatest N content, mass and length was associated with 60 kg N/ha while, higher nodule number, N content and mass were produced with 30 kg N/ha. However, application of 90 kg N/ha reduced nodulation and subsequently reduced inoculated yield since higher N application inhibits infection thread formation which creates a path for the rhizobia to travel from the root hair tip to the internal legume to initiate nodule formation (Eckardt, 2006). In other experiment, it was established that, inoculation alone was inadequate to supply the nitrogen need of the crop at 30 kg N/ha but 60 kg N/ha alone resulted in higher yield. Dry matter, bean yield and weight, number of pods, N uptake and N content of seed were higher at higher N application combined with inoculant application. However, seed oil content dropped with inoculant and nitrogen application (Ahmed, 2013).

Diep *et al*. (2002) conducted a similar experiment on effect of nitrogen fertilization and rhizobial inoculation on vegetable soybean and concluded that, pods per plant and green pod yield were highest at 25 kg N/ha but at 100 kg N/ha the lowest pod number and pod yield was observed. However, at 50 kg N/ha yield of was slightly reduced. Zhou *et al*. (2006) conducted a study on the effect of rhizobial inoculation and N fertilization on photosynthesis in soybean. It was observed that at R5 growth stage, plant biomass, leaf area and chlorophyll content were found to have improved. Also, stomatal conductance and maximum photochemical efficiency were markedly improved. Plant growth and photosynthesis after rhizobial inoculation were also improved.

Again, Ahmed (2013) conducted a field at the demonstration farm of the Faculty of Agriculture on the interactive effective of nitrogen fertilization and inoculation on soybean yield with 40 and 80 kg ha^{-1} urea and one strain of rhizobium. The results obtained showed that number of nodule varied with nitrogen fertilizer levels. Also, as rate of nitrogen increases, nodule number and nodule dry weight decreased. Seed yield increased with nitrogen levels and inoculation application as compared inoculated non fertilized control plants by 83-89%. Namva *et al*. (2013) also stated that *rhizobium* showed an increase protein content than that of control but with no effect on oil content in seed.

A similar experiment stated that nodule number per plant, nodule rating, nodule volume per plant and nodule dry weight was influenced by the *Bradyrhizobium japonicum* strains. Dry matter production and nitrogen uptake were highly significant at midflowering stage.

Seed yield, number of seeds per pod, thousand seed weight, and number of pods per plant, total nitrogen uptake and above-ground dry biomass were significantly affected by inoculation with *Bradyrhizobium* strains alone. Varieties however, had no effect on yield and yield components (Solomon *et al*., 2012).

Another study conducted on the effect of liming, Bradyrhizobium inoculation and nitrogen fertilizer application on nodulation and growth response showed that, the combined effect of liming and bradyrhizobium inoculation increased significantly and increased nodule volume and weight compared with un-limed and non-inoculated treatment application. However, application of nitrogen did not have any significant influence on nodulation parameters of the crop (Bekere *et al*., 2013).

2.12 Inoculation and nitrogen effect on crude protein content of soybean growth and yield

Inoculation has been shown to increase yield and protein content. An increase in protein content of inoculated soybean was reported by Egamaberdiyeva *et al*. (2004) and Elsheikh *et al*. (2008). Morshed *et al*. (2008) reported a 22% increase in protein content in soybean inoculated with rhizobium. This increase could have been a result that, fixing of N_2 was efficient with the inoculation where adequate N_2 was fixed and translocated to the seeds. Moreover there is enhanced symbiotic association with rhizobium for better growth and yield (Saed and Elsheikh, 1995). Fertilizer N applied at 50 kg N/ha was reported to increase protein content slightly in fenugreek but no significant difference was recorded. This could be because the N fertilizer was applied at sowing and the effect was however not extended to pod filling stage. Gaydou and Arrivets (1983) also obtained similar results with soybean where N fertilizer did affect protein content significantly. Babiker *et al*. (1995) reported significant increase in protein content of faba bean.

2.13 Importance and uses of soybean

Soybean is a pulse as well as oilseed crop with high yield potential. The seed contains 40% protein and 20% edible oil. The protein content is 5% rich in lysine which is not present in many cereals (Joshi, 2015). The crop can be grown successfully in many states of Nigeria with low levels of agricultural inputs (Dudge *et al*., 2009).

Soybean is the most nutrient rich, easily digestible and the cheapest source of oil of the bean family. It is consumed as soybean milk, tofu: a curd that resembles cheese, soya kebab and soy sauce in East Asia (Encyclopaedia Britannica, 2008). It is among the few crop plant that provides a complete protein due to the fact that it contains all eight (8) essential amino acids for human health (Soytech, 2015). Soybeans with other legumes have been suggested to reduce cancer risks and low cholesterol in serum. It has also proven as an alternative to a therapy for hormone replacement for postmenopausal in women (Graham and Vance, 2003).

Industrial uses soybeans are numerous. Crushed soybean can be grouped in two components; oil (lecithin inclusive) and protein (meal or flour). Soybean can be processed into flour which can be used soy candy, doughnut milk, meat pie and pancake. The oil can be refined to be used by industries to produce anti-corrosive agents, soaps, shampoo and detergents, core oil, lubricants, diesel fuel, hydraulic fluids, disinfectants, fungicides, herbicides, cosmetics, waterproof cements and metal casting agents (Adu-Dapaah *et al*., 2004: Graham and Vance, 2003). Other industrial uses of soybean include paints, in dressing or water proofing for textiles and paint removers (Shurtleff and Aoyagi, 2009). Soybeans like other legumes live in symbiotic association with nitrogen fixing bacteria to fix atmospheric nitrogen. Soybeans specifically form this symbiotic association with *Bradyrhizobium japonicum* and *Sinorhizobium* species. This attribute together with its oil, protein and wide environmental adaptation ensures its continuity to be an important food crop world-wide (Keyser and Li, 1992).

Soybean is used in the formulation of poultry meal in Nigeria. The crop has the potential to increase farmers‘ income especially when grown as cash crop, improve soil fertility through nitrogen fixation and control *striga*: an endemic parasitic weed that reduces yield and quality of agricultural produce (Dudge *et al*., 2009). It is a source of protein in livestock feed world-wide. Percentage of crude protein produced per ton is 440-480kg which of high quality and highly digestible (FAO, 2009; Dudge *et al*., 2009). Soybean can be cooked and eaten as a vegetable. Vegetable soybean is harvested during the late reproductive stage (after R6 and before R7) when pods are still green and development of seed fill is 80-90% (MoFA and CSIR, 2005; Diep *et al*., 2002). It contains more protein than fish and beef that have about 18% of protein content. Products from soybeans are also cholesterol free and high in fiber, vitamin B1 and B2, calcium and phosphorous and that explains its high demand for health reasons (Greenberg and Huartung, 1998). Haulms obtained after extraction of seed serve as source of feed for cattle, sheep and goat (Dudge *et al*., 2009). It can be harvested and sown as shelled beans or fresh or frozen pods (Diep *et al*., 2002).

The crop can help contribute to poverty and malnutrition reduction especially in expecting mothers and children (Myaka *et al*., 2005; IITA, 2009). Soybeans production can serve as source of employment, income, improve standard of living and quality of animal products and improve human and animal health (Malema, 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of experimental site

The study was carried out at Council for Scientific and Industrial Research –Crops Research Institute (CSIR-CRI), Fumesua- Kumasi (06º41‘N, 01º28‘W). The site is located within the semi- deciduous rain forest zone and experiences a bi- modal rainfall. The major growing season starts from April through to June and minor season also stretches from September through to December with an annual rainfall of 1500 mm. Soil in Fumesua is characterised as Ferric acrisols (FAO/UNESCO, 1990).

3.1.1 Land preparation

The land was ploughed and disc-harrowed. Plots measured 2.5 m x 3 m each and 1 alley between plots, 1 m and 2 m between replicates was also constructed.

3.2 Enumeration of population of soil rhizobia

Population of rhizobia in the study area was estimated using the Most Probable Number (MPN) (Vincent, 1970). Viable seeds were sterilized with hydrogen peroxide and alcohol (Somasegaran and Hoben, 1994). The seeds were initially germinated in petri dishes which contained moist cotton wool which was also sterilized. The seeds were incubated between temperature range of 20 ºC and 30 ºC. Seeds were later transferred to plastic growth pouches which contained Dilworth and Broughton plant nutrient solution (Broughton and Dilworth, 1971) using forceps. The growth pouches were neatly put on a wooden rack which was stored in the greenhouse. The number of rhizobia was estimated using the formula $MPN = \frac{mxd}{v}$

Where; m= most likely number in column for number of dilution d= lowest dilution with series v= aliquot used for inoculation (1 ml)

3.3 Inoculation

Soybean seeds (var Anidaso -V_1, Quarshie -V_2 and Salentuya I -V_3) were obtained from the Council for Scientific and Research-Crops Research institute (CSIR-CRI) with maturity period between 90 and 110 days. Inoculation was done at the Soil Microbiology Laboratory at KNUST. Soybean seeds (var Anidaso -V_1, Quarshie -V_2 and Salentuya I -V_3) were inoculated with a *Bradyrhizobium japonicum* contained in a peat based inoculant at a rate of 3.5 g per 100 g of seed using the slurry method (Woomer *et al*., 1994).

3.4 Planting

Planting was done on in October, 2015. Seeds were planted at 3 seeds per hill at 5 cm within row and 60 cm between rows and later thinned to two seeds per hill after two weeks of planting. The uninoculated seeds were planted first to avoid contamination of the inoculated seeds. Refilling was done after one week of sowing.

3.5 Experimental design and treatments

The experiment was a 3x6 factorial trial with treatments arranged in a Randomized Complete Block Design (RCBD) with three (3) replications. The first factor was three soybean varieties (Anidaso, Quarshie and Salentuya 1). All three varieties had maturity period of 90-110 days. The second factor was the N sources (Control, 30 kg N/ha, 60 kg N/ha, inoculation, inoculation+30 kg N/ha and inoculation+60 kg N/ha. The 30 and 60 kg N/ha were applied between R5-R6 growth stages at the beginning of seeding and pod filling respectively). The N fertilizer was applied as sulphate of ammonia by burying the fertilizer in 5 cm dug trenches beside the plants.

3.6 Data collection

3.6.1. Seedling emergence

This was determined at ten days after planting by counting the emerged plants in the three innermost rows. This was expressed as percentage of number of plants to seeds sown.

3.6.2 Plant height

Plant height was measured from five random plants using a metre rule. This was done by measuring the plant from the ground level to the tip of the stem. This was done at 25, 45 and 65 days after planting and means calculated for each treatment.

3.6.3 Canopy spread

Canopy spread was measured from five random plants using a metre rule. This was done by measuring the widest points and the means calculated for each treatment. This was done at 25, 45 and 65 days after planting.

3.6.4 Number of leaves

Number of leaves of soybean varieties was counted at 25, 45 and 65 days after planting and means calculated for each treatment.

3.6.5 Number of branches

Number of branches from the above plants was counted and the means calculated for each plot.

3.6.6 Nodule count and effectiveness

Five plants selected randomly from each treatment were dug with a spade. The plants were washed with clean water to detach the roots from soil. The nodules were removed from the roots and counted. The nodules were cut open with the aid of a knife. Effectiveness was determined with the help of a hand lens. Reddish or pink nodules were declared effective. Nodules were sampled once at flowering.

3.6.7 Nodule dry weight

The nodules were oven dried for 48 hours at a temperature of 70 ºC. The nodule dry weights were then determined using a weighing scale.

3.6.8 Number of pods per plant

Five middle rows plants were selected from each plot and all the pods were detached manually and counted. The average pod number was calculated.

3.6.9 Pod length

Five random pods from each treatment were picked and length taken with a measuring rule. Averages were calculated**.**

3.6.10 Number of seeds per pod

Seed numbers per pod were determined by randomly selecting ten pods per plot and threshing them. Seed were counted, and mean number was calculated for each plot.

3.6.11 Seed width

This was obtained by randomly selecting ten seeds from each plot, and measuring their width with calipers, and their means were calculated.

3.6.12 100 Seed weight

This parameter was measured by counting 100 seeds from each plot and oven drying, and their weights were measured.

3.6.13 Grain yield

Plants from an area from each plot were harvested, sun dried, threshed and winnowed. The grains were oven dried at a temperature of 60ºC for 72 hours. The dry weights of the grains were taken.

3.6.14 Harvest index

The harvest index (H.I.) of the crop was obtained using the equation as:

$$Harvest\ index\ (H.I.) = \frac{Economic\ yield}{Biological\ yield}$$

Where;

Economic yield= grain yield

Biological yield= biomass yield

3.6.15 Haulm weight

The haulm of the all the five tagged plants from each plot were dried and weighed and averages were calculated.

3.6.16 Total N fixation

This was determined by using the N- difference method as described by Bell and Nutman (1972) with maize as the reference crop. This method relies on Kjeldahl's N determination which is calculated as:

N_2 fixed = Total N (fc) – Total N (nfc)

$$\%Nda = \frac{\text{Total N (fc)} - \text{Total N (nfc)}}{\text{Total N (fc)}} \times 100$$

Where; fc = fixing

crop nfc = non

fixing crop

%Nda =proportion of N derived from the atmosphere

3.6.17 N Remobilization between R1 and R5

Sampling was done at the beginning of bloom (R1) and full pod filling stage (R5) at two weeks interval. Plants from each plot were randomly selected and divided into stem and leaves. The plants were oven dried at 80 ºC for 48 hours. Using the Kjeldahl's method, N concentration for both parts were determined. The difference in N concentration of the vegetative tissue of R1 - N concentration of the vegetative tissue of R5 was calculated as remobilized N.

3.6.18 Seed and residue N

The plant residues as well as the seeds were milled in a miller, after which nitrogen concentration were determined using Kjeldahl's N determination method comprising of digestion and distillation.

3.6.19 Crude protein content

This was achieved by multiplying the values obtained from seed N by a constant (6.25%) which is the amino acid percentage in soybean.

3.7 Cultural practices

3.7.1 Irrigation

Water was supplied to the plants from the early vegetative growth stage through to the pod filling stage after the rains had stopped.

3.7.2 Thinning

This was done at 20 days after planting when soil had enough moisture with seedling establishment. Seeds were thinned to two plants per hill.

3.7.3 Weeding

This was done manually with hoes at second and sixth week after planting to control weeds.

3.7.4 Pest management

Incidence of leaf miners and pod sucking bugs were visually identified on the experimental field. Both insects were controlled with Cypermetrin + Dimethoate 10 EC at 100ml in 15 L of water using a knapsack.

3.7.5 Harvesting

Harvesting was done at physiological maturity when the pods had turned brown.

3.8 Statistical analysis

All data obtained was analysed with Genstat 12th edition. The Least Significance Difference (LSD) was used to determine treatment differences at 5% probability. Count data were log transformed before analyses were run.

CHAPTER FOUR

4.0 RESULTS

4.1 Crop establishment

The results for crop establishment are presented in Figure 4.1. The results showed that among the inoculated seeds, Salentuya 1 resulted in greatest crop establishment to Quarshie and Anidaso with inoculated seeds of Anidaso resulting in the least crop established. Also, among the uninoculated seeds, Quarshie resulted in the greatest crops established whiles Anidaso resulted in least crops established.

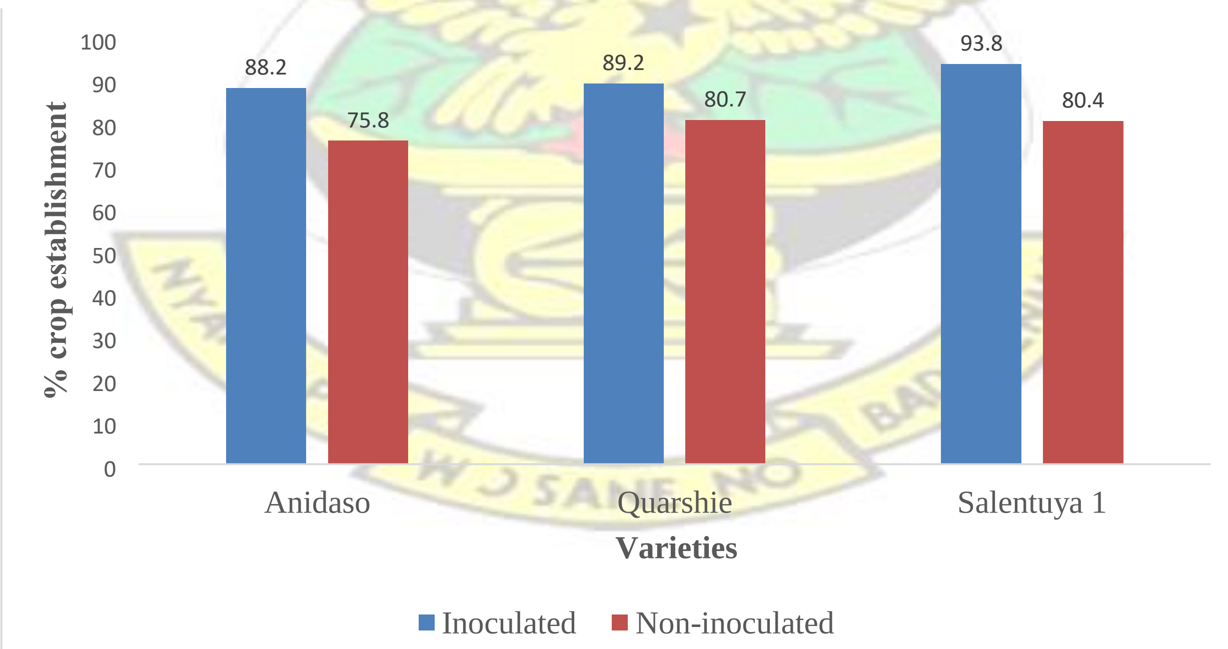

Figure 4.1 Effect of inoculation regime on crop establishment of soybean.

4.2 Plant height

The results of plant height recorded at 25, 45 and 65 DAP are presented in Table 4.1. Varietal effect was significant (P < 0.05) only at 25 DAP, with height of Anidaso plants being significantly higher than in the other varieties. Nitrogen treatment affected plant height on all sampling days. On each sampling date, the inoculated treatment effect was the greater, and this was significantly higher than all other treatment effects, except the inoculation + 30 kg N/ha treatment at 25 and 45 DAP, and also inoculation + 60 kg N/ha at 65 DAP. The control treatment effect was the lowest at 25 DAP, the 30 kg N/ha treatment effect was the lowest at 45 DAP and lastly, the 60 kg N/ha effect was the least at 65 DAP (Table 4.1).

4.3 Canopy spread

The results for canopy spread recorded for 25, 45 and 65 DAP are shown in Table 4.1. Varietal effect showed significant (P<0.05) difference at 25 and 45 DAP only. On both days, the treatment effect of Anidaso was significantly higher than that of the Quarshie variety only. Treatment effects of Quarshie and Salentuya 1 were similar. Varietal differences at 65 DAP was not significant. Canopy spread was significantly affected (P<0.05) by N treatments on all sampling days. At 25 DAP, inoculation + 60 kg N/ha treatment effect resulted in greatest canopy spread, and this was significantly higher than all other treatment effects except inoculation + 30 kg N/ha treatment. Inoculation alone was also significantly higher than sole N and the control treatment. At 45 and 65 DAP, inoculation + 30 kg N/ha treatment was greater, and this was significantly higher than all other treatments, except inoculation alone and inoculation + 60 kg N/ha treatment effects. On both sampling days, sole inoculation was significantly higher than the sole N and control treatment (Table 4.1).

Table 4.1 Effect of variety, inoculation and N fertilizer application on plant height and canopy spread of soybean

Treatments	Plant height (cm)			Canopy spread (cm)		
	25 DAP	**45 DAP**	**65 DAP**	**25 DAP**	**45 DAP**	**65 DAP**
VARIETIES						
Anidaso	16.91	37.06	47.71	20.15	45.79	48.93
Quarshie	15.36	35.86	47.44	18.16	43.07	49.62
Salentuya 1	15.84	36.49	47.26	19.06	44.61	49.78
LSD (5%)	0.63	NS	NS	1.28	2.69	NS
N SOURCES						
Control	14.02	31.17	42.52	14.00	36.37	43.96
30 kg N/ha	14.04	30.90	41.56	14.01	35.41	42.90
60 kg N/ha	14.46	31.04	39.91	15.02	37.29	41.17
Inoculation (Ino)	18.28	43.36	53.53	22.66	52.39	56.36
Ino+30 kg N/ha	18.11	41.6	54.82	24.48	54.17	57.59
Ino+60 kg N/ha	17.31	40.76	52.49	24.57	51.33	54.67
LSD (5%)	0.90	2.22	3.02	1.82	3.80	3.20
CV (%)	5.8	6.4	6.6	9.9	8.9	6.8

4.4 Number of leaves

The results for number of leaves are presented in Table 4.2. There were significant (P< 0.05) differences among the varieties at 25 and 65 DAP. At 25 DAP, treatment effect of Anidaso variety was significantly higher than that of Quarshie variety only. At 65 DAP, the Salentuya 1 varietal effect was significantly higher than that of Anidaso variety only. Leaf production was significantly (P< 0.05) affected by N treatment on all sampling days. At 25 DAP, the inoculated + 60 kg N/ha treatment effect was the greatest, and this was significantly (P< 0.05) higher than all other treatment effects, except those of inoculation only and inoculation + 30 kg N/ha treatments. The sole inoculation was significantly higher than sole N and control treatments. At 45 DAP, inoculation only was significantly higher than all other treatments, except combined inoculation and N fertilizer treatment. At 65 DAP, the inoculation + 60 kg N/ha treatment effect was significantly higher than all other treatment effects, except the sole inoculation.

4.5 Number of branches

Soybean variety did not significantly (P> 0.05) affect branch production on all sampling days (Table 4.2). Nitrogen treatment, however, had significant (P< 0.05) effect on branch production on all sampling days. At 25 DAP, inoculation and its combinations supported similar branch production, but either effect was significantly (P< 0.05) higher than sole N and control treatment effects. At 45 DAP, inoculation only was significantly (P< 0.05) higher than all other treatment effects, except inoculation + 60 kg N/ha treatment. At 65 DAP, the control and 30 kg N/ha treatment effects were similar, and either effect was significantly lower than all other treatment effects.

Table 4.2 Effect of variety, inoculation and N fertilizer application on number of leaves and branches of soybean

Treatments	Number of leaves			Number of branches		
	25 DAP	45 DAP	65 DAP	25 DAP	45 DAP	65 DAP
VARIETIES						
Anidaso	20.15	11.00	16.33	6.22	10.61	17.78
Quarshie	18.16	11.17	16.67	5.56	11.06	18.78
Salentuya 1	19.06	10.94	17.61	6.00	10.28	18.67
LSD (5%)	1.28	NS	1.06	NS	NS	NS
N SOURCES						
Control	14.00	8.89	13.67	4.11	8.56	14.33
30 kg N/ha	14.01	8.33	14.22	4.44	7.44	14.89
60 kg N/ha	15.02	8.78	17.67	4.44	8.22	19.22
Inoculation (Ino)	22.66	13.89	18.78	7.56	14.11	20.89
Ino+30 kg N/ha	24.48	12.78	17.67	7.56	12.44	19.44
Ino+60 kg N/ha	24.57	13.56	19.33	7.44	13.11	21.67
LSD (5%)	1.82	1.17	1.50	1.06	1.37	2.75
CV (%)	5.5	6.0	4.6	8.4	6.8	7.8

4.6 Number of pods

The results for pod number per plant are presented in Table 4.3. There were significant differences among varieties as the Salentuya 1 variety produced significantly greater number of pods than Anidaso variety only. Inoculation significantly ($P< 0.05$) increased number of pods per plant. Inoculation alone and inoculation + 30 kg N/ha treatment effects were similar, and either effect was significantly higher than all other treatment effects. Among the other treatments, the 60 kg N/ha treatment effect was the lowest, but this was significantly ($P< 0.05$) lower than sole 30 kg N/ha. All other treatment differences were not significant ($P> 0.05$).

4.7 Pod weight

The results of pod weight per plant are presented on table 4.3. Varietal effect significantly ($P<0.05$) affected pod weight, with the Salentuya 1 varietal effect being significantly higher than that of Anidaso. Nitrogen treatment significantly affected pod weight ($P< 0.05$). Inoculation alone and inoculation + 30 kg N/ha supported similar effects, and either effect was significantly higher than all other treatment effects. The 30 kg N/ha treatment effect was also greater than that of the 60 kg N/ha treatment. All other treatment effects were statistically similar.

4.8 Pod length

The results for pod length are presented in Table 4.3. Varietal differences were not significant ($P>0.05$). Inoculation significantly ($P< 0.05$) affected length of pod. The 30 kg N/ha, inoculation + 30 kg N/ha and inoculation only treatment effects were similar, but either effect was significantly higher than all the other treatment effects. The control was significantly higher than 60 kg N/ha and the inoculation + 60 kg N/ha treatments.

4.9 Number of seeds per pod

The results of number of seeds per pod are shown in Table 4.3. Varietal effect was not significant ($P>0.05$). Sole application of 30 kg N/ha, inoculation alone, and inoculation + 30 kg N/ha produced similar effects, and each effect was significantly higher than all other treatment effects. The control treatment was significantly higher than 60 kg N/ha treatment effect.

4.10 One hundred seed weight

The results for hundred seed weight are presented Table 4.3. Varietal effect was significant ($P<0.05$) as weight of Anidaso was significantly lower than those of Quarshie and Salentuya 1. Nitrogen treatments significantly affected mean seed weight. The inoculation + 30 kg /ha N treatment effect was significantly higher than all other treatment effects. Inoculation alone was also greater than that of the control and inoculation + 60 kg N/ha treatments. Sole N application treatment effects were also greater than the control and inoculation + 60 kg N/ha treatment effects.

Table 4.3 Effect of variety, inoculation and N fertilizer application on pod number, pod weight, pod length, number of seed per pod and hundred seed weight of soybean

Treatments	Pod number per plant	Pod weight (g)	Pod length (cm)	Number of seeds per pod	Hundred seed weight (g)
VARIETIES					
Anidaso	103.60	103.60	3.27	2.89	13.67
Quarshie	108.70	119.50	3.27	2.72	14.42
Salentuya 1	126.50	126.70	3.31	2.83	14.87
LSD (5%)	15.30	22.35	0.18	0.41	0.74
N SOURCES					
Control	96.00	95.70	3.38	2.67	12.07
30 kg N/ha	100.20	110.6	3.66	3.33	14.77
60 kg N/ha	75.90	77.60	2.58	2.11	14.31
Inoculation (Ino)	155.80	175.6	3.79	3.22	15.04
Ino+30 kg N/ha	158.70	158.10	3.89	3.33	18.36
Ino+60 kg N/ha	91.00	82.20	2.41	2.22	11.37
LSD (5%)	21.64	31.61	0.26	0.58	1.04
CV (%)	11.2	28.4	8.2	9.3	7.8

4.11 Seed N

The results for seed N are presented in Table 4.4. Varieties did not show significance (P>0.05) on seed N. Inoculation alone and inoculation + 30 kg N/ha produced significantly (P<0.05) greater seed N than all the other treatments. Among the other treatments, the 60 kg N/ha treatment produced the lowest seed N than all the other treatment effect.

4.12 Residue N

The results for residue N are presented in Table 4.4. Varieties did not show significance (P>0.05) on residue N but N treatment did. Inoculation alone, Inoculation + 30 kg N/ha, Inoculation + 60 kg N/ha produced and 60 kg N/ha produced significantly greater (P<0.05) residue N than all the other treatment effects. The 30 kg N/ha treatment produced higher residue N than control but was not significant. Among the other treatments, the control treatment produced the least residue N than all the other treatment.

4.13 Total N

The results for total N are presented on Table 4. Total N was significantly greater (P<0.05) in Quarshie than Anidaso and Salentuya 1. Inoculation alone, inoculation + 30 kg N/ha produced significantly greater (P<0.05) N than all other treatment effects. The control treatment and 60 kg N/ha produced similar total N but was not significant. Among the other treatments, the control treatment produced the lowest total N than all the other treatment effects.

Table 4.4 Effect of variety, inoculation and N fertilizer application on seed N, residue N and total N of soybean

Treatments	Seed N (kg/ha)	Residue N (kg/ha)	Total N (kg/ha)
VARIETIES			
Anidaso	41.70	10.05	51.75
Quarshie	42.80	9.65	52.45
Salentuya 1	40.70	10.94	51.64
LSD (5%)	NS	NS	NS
N SOURCES			
Control	43.10	6.41	49.51
30 kg N/ha	48.90	7.74	56.64
60 kg N/ha	40.60	9.76	49.94
Inoculation (Ino)	74.90	9.34	84.24
Ino+30 kg N/ha	72.50	7.56	80.06
Ino+60 kg N/ha	50.00	8.48	58.48
LSD (5%)	11.02	1.93	12.27
CV (%)	27.0	23.2	23.4

4.14 Number of nodules

The results of number of nodules are presented in Table 4.5. Varietal effect was not significant (P>0.05) on nodule number but N treatment was. Inoculation alone and inoculation + 30 kg N/ha produced significantly (P<0.05) greater nodules than all other treatments. The 30 kg N/ha treatment effect was also higher than the other treatments, all of which produced statistically similar number of nodules.

4.15 Nodule dry weight

Nodule dry weight was significantly higher (P<0.05) in the Anidaso than in Quarshie and Salentuya 1 (Table 4.5). Inoculation alone and inoculation + 30 kg N/ha treatment effects were significantly higher than all other treatment effects. The effect of 30 kg N/ha treatment was also greater than the other treatments.

4.16 Crude protein

Crude protein content was significantly higher (P<0.05) in the Salentuya 1 than in Quarshie and Anidaso (Table 4.5). Inoculation alone and inoculation + 30 kg N/ha, 30 kg N/ha, 60 kg N/ha treatments produced higher crude protein content than the control and inoculation + 60 kg N/ha treatment effects. Among the other treatments, the control produced the lowest crude protein content than all the other treatment effect.

Table 4.5 Effect of variety, inoculation and N fertilizer application on nodule number, nodule dry weight, total grain and remobilized N of soybean

Treatments	Number of nodule/plant	Nodule dry weight (g)	Crude protein (%)
VARIETIES			
Anidaso	47.28	0.53	30.23
Quarshie	45.83	0.48	30.19
Salentuya 1	46.72	0.49	33.13
LSD (5%)	NS	0.03	NS
N SOURCES			
Control	22.29	0.38	33.42
30 kg N/ha	51.00	0.53	35.50
60 kg N/ha	62.65	0.36	36.70
Inoculation (Ino)	67.33	0.70	39.80
Ino+30 kg N/ha	32.67	0.71	38.79
Ino+60 kg N/ha	32.67	0.33	32.67
LSD (5%)	4.33	0.04	6.28
CV (%)	9.7	8.4	18.33

4.17 Total grain yield

Varietal differences for grain yield were not significant (P> 0.05) (Table 4.6). Inoculation alone and inoculation + 30 kg N/ha treatment produced significantly greater grain yield than all other treatments. The 30 kg N/ha treatment effect was also greater than the other treatment effects. Additionally, 60 kg N/ha treatment effect was also greater than those of the control and inoculation + 60 kg N/ha treatment.

4.18 Remobilized N

The results for remobilized N presented in Table 4.6 showed that, there were no significant (P>0.05) differences among the varieties. The greater amount of remobilized N was in the inoculation + 30 kg N/ha treatment and this was significantly higher than in all treatments, except sole inoculation. Similar amount of N was remobilized in inoculation alone and inoculation + 30 kg N/ha treatments. The least amount of N remobilized was in the inoculation + 60 kg N/ha treatment, but this was similar to the amount from 60 kg N/ha treatment.

Table 4.6 Effect of variety, inoculation and N fertilizer application on total grain yield and remobilized N of soybean

Treatments	Total grain yield (kg/ha)	Remobilized N (kg/ha)
VARIETIES		
Anidaso	869.00	24.41
Quarshie	843.00	25.18
Salentuya 1	850.00	24.18
LSD (5%)	NS	NS
N SOURCES		
Control	432.00	23.36
30 kg N/ha	1021.00	27.06
60 kg N/ha	584.00	19.09
Inoculation (Ino)	1356.00	29.64
Ino+30 kg N/ha	1276.00	31.51
Ino+60 kg N/ha	444.00	17.90
LSD (5%)	118.10	2.20
CV (%)	14.4	9.3

CHAPTER FIVE

5.0 DISCUSSION

5.1 Response of soybean growth to N

Growth was measured as crop establishment, plant height, canopy spread leaf and branch production. Crop establishment is characterized by ability of seedling to germinate and survive after treatment application. The results obtained showed that inoculated seeds of Anidaso and Salentuya 1 established better than their corresponding uninoculated seeds. Ahmad and Mohammad (2007) reported that rhizobium inoculation resulted in better growth but the growth could be greatly affected by environmental and genetic factors such as soil temperature and moisture content. Gunarto (2000) also reported that the increase in crop establishment could also be due to the enhancement of the productivity of the soil as a result of the activities of the bacteria and nutrients availability. The result of Lampttey *et al*. (2014) is in agreement to the results obtained from this study.

The soybean varieties responded differently in plant height when inoculated with the bacteria *Bradyrhizobium japonicum*. A review by Hungry and Bohrer (2000) indicated significant differences among different cultivars of soybeans inoculated with *Bradyrhizobium japonicm* with respect to plant height. Differences in height of plants could also be as a result of genetic effect (Magani and Kuchinda, 2009). Inoculation of seeds before planting has been found to help promote soybean growth and development (Wafaa *et al*., 2002). Wu and Wu (1996) stated that inoculation with *Rhizobium japonicum* in soybean resulted in an increased height of crop especially at seedling, production of flowers through to fruiting stages. Amin *et al.* (2008) observed better performance of inoculated soybean in terms of height than the uninoculated control. Bekere *et al.* (2012), Malik *et al.* (2006), Hernanadez and Cuevas, (2003) and Lampttey *et al.* (2014) reported similar findings in their studies. Similar observations have been made in inoculated lentils (Hoque and Haq, 1994) and in mungbean (Harahap 1994). Inoculation or application of N both makes nitrogen available for plant height. Indeed, N has been described as the most limiting nutrient for plants to increase in height (Salisbury and Ross, 1992; Gardner *et al.*, 1985).

Canopy spread was significantly affected by inoculation across all sampling periods. Plants generally have the potential to attain certain height and canopy width but the size of the canopy is usually dependent on factors such as variety, crop spacing and nutrients available. Stefanescu and Palanciuc (2000) reported that canopy spread being greatest with inoculation was as a result of increased fixation of N and improved nutrition in soybean which usually causes greater and vigorous vegetative growth. The increase in canopy spread in the inoculated plants in this experiment could be as a result of greater N availability for improved growth in shoot of such plants (Ahmed *et al*. 2006). This result is in agreement with the observation of Malik *et al*. (2006) who indicated that inoculation of soybean seeds before planting enhanced the production of growth factors such as larger leaves production. Lampttey *et al*. (2014) and Osei *et al.* (2014) also reported a significant increase in canopy spread with inoculation at different sampling times.

Number of leaves and branches were influenced positively with inoculation. Several researchers have reported on the importance of inoculation on number of leaves and branches. Erman *et al.* (2009) reported that inoculation of seeds before planting increased the number of leaves, leaf area and branches. Similar reports have been made in other studies including Tairo *et al.* (2013), Zhang *et al* (.2002), Okereke *et al.* (2001) and Hayat *et al.* (2004). Moreover, addition of N fertilizer to inoculated seeds has been found to give the best number of branches and leaves. Stefanescu and Palanciuc (2000) observed that addition 30 kg N/ha to inoculated soybean increased the vegetative growth and yield. Ahmed (2013) reported that, application of a small quantity of N fertilizer (40 kg N/ha) in addition with rhizobial inoculation resulted in a greater number of branches and leaf number in the Sudan. A report by Mrkovacki *et al*. (2008) also indicated that the greatest number of branches and leaves could be obtained when 60 kg N/ha is applied to inoculated soybean.

5.2 Response of soybean grain yield and yield components

Number of pods was greatly affected by inoculation as well as N application. Rhizobium inoculation as well the small addition of N fertilizer has been found to have an influence on pod number. Kazemi *et al.* (2006) reported that rhizobium inoculation resulted in 11% increase in pod number the over control. In another experiment on inoculation of bean cultivars with different bacteria strains, a significant difference was obtained in pod number per plant. This highest pod number per plant was obtained with Maurada inoculated with the bacteria Semia 481. Increased pod number in inoculated plants may also be due to increased number of leaves which influence the production and translocation for greater pod production.

In another experiment, inoculation with *Bradyrhizobium japonicum* increased the number of pods per plant, seed per plant as well as seed weight (Dahmardeh *et al*., 2010; Morad *et al.*, 2013). Addition of a small quantity of nitrogen to inoculated soybean has been found to significantly increase yield parameters. Inoculation and application of fertilizer rates up to 30 kg N/ha showed a significant increase in number of pods per plant (Rashid *et al*., 2000; Hoque and Haq 1994). However, addition of 60 kg N/ha significantly depressed the positive impact of inoculation on number of pods and seed yield. This is in agreement with the findings of Ahmed (2013), Yagmur and Kaydan (2011) who stated that high nitrogen fertilizer doses following inoculation negatively affected the number and effectiveness of rhizobium, which affected nitrogen fixation as well as yield.

In the present study, seed number per pod was significantly influenced by seed inoculation, as has been reported by Solomon *et al.* (2012) and Egamberdiyeva *et al.* (2004). Anjum *et al*. (2006) reported a significant increase in seed number per pod in mungbean when inoculated with *Rhizobium* spp. Azarpour *et al.* (2012) and Karasu *et al*. (2011) in similar experiments reported significance in the number of seeds per pod in inoculated soybean when N fertilizer was applied. However, addition of 60 kg N/ha to inoculated seeds did not show any significant increase in seed number per pod as reported by Ahmed (2013) and Karahan and Şehirali (1999).

Results of this study further indicated that, different varieties of soybean respond differently when inoculated with *Rhizobium* spp in terms of weight of 100 seeds. A review by Hungry and Bohrer (2000) indicated significance difference among different cultivars of soybeans inoculated with *Bradyrhizobium japonicum* with respect to 100 seed weight. Several researchers have reported significance difference in 100 seed weight. Khan *et al*. (2003) in a similar experiment in chickpea indicated that inoculation increased 100 seed weight. Mohamed (2000) also reported significance difference in 100 seed weight for faba bean when inoculated. Osman *et al.* (2010) also reported significance difference in the weight of 100 seed when faba bean was inoculated with *Bradyrhizobium japonicum.* From the experiment, sole application of 30 and 60 kg N/ha applied at pod filling showed a significance difference in 100 seed weight in the varieties. Karasu *et al.* (2011) reported that application of 30 kg N/ha and 60 kg N/ha resulted in significance increase in 100 seed weight. Barker and Sawyer (2005) observed that addition of small doses of N to inoculated seeds increased 100 seed weight.

Inoculation significantly increased total grain yield in the study. The increase in yield in soybean varieties following inoculation might be as a result of soil N availability and adequate soil moisture. This agrees with the findings of Argaw (2014) who stated that genotype and seed inoculation significantly increased seed yield over control. Shrivastava *et al.* (2000) in an experiment stated an average of 11% increase in yield with seed inoculation with *B. japonicum* over control. Lampttey *et al*. (2014) in a similar experiment reported that, seed inoculation significantly increased yield due to increased nodulation and nodule dry weight and stated a 49% increase in pod number per plant and 38% in weight of pod number per plant following inoculation.

5.3 Variety, N and crude protein content of soybean

Inoculation increased crude protein content for all three varieties over their control. This finding confirms that of Elskeikh *et al.* (2000) who reported significant increase in crude protein following seed inoculation. Lucas-Garcia *et al.* (2004) and Rugheim and Abdelgani (2009) reported significance increase in crude protein content following seed inoculation. Morshed *et al*. (2008) reported a 22% increase in protein content in soybean inoculated with rhizobium. This increase could have been as a result that, fixing of N_2 was efficient with the inoculation where adequate N_2 was fixed and translocated to the seeds. Moreover, there is enhanced symbiotic association with rhizobium for better growth, and protein content yield (Saed and Elsheikh, 1995).

5.4 Nodulation and nitrogen fixation

According to Hansen (1994), the maximum number nodules obtained are known to be different within and between legume species but the present results showed no varietal differences. However, nodule count was significantly increased by inoculation. This is in agreement with Agraw (2014) who stated that rhizobial inoculation significantly increased nodule number and dry weight over control. Melchiorre *et al*. (2011) and Jonas *et al*. (2011) in similar experiments stated that response of soybean to inoculation is greatly affected by the number of effective rhizobia in the soil. Several researchers have found inoculation to have a positive correlation with nodulation and nitrogen fixation. Okereke *et al.* (2004) and Tahir *et al*. (2009) also reported significant responses to inoculation and higher nodule count compared with the control. The experiment also showed that few numbers of nodules were observed on the soybean plants that were not inoculated. This agreed with Bekere and Hailemariam (2012) that naturalized rhizobia are not very effective nodulators. Ulzen (2013), Okogun *et al.* (2005) and Chemining‘wa *et al.* (2007), however, reported no significant increase in nodulation and nodule dry weight following inoculation. A report by Singleton and Tavarez (1986) showed that, for a legume to respond positively to inoculation, the native rhizobia population should be higher than $1x10^2$ rhizobial cells g^{-1} soil.

5.5 Variety and treatment effect on N remobilization

The results indicated N remobilization in all the varieties, although differences were not significant. Nitrogen remobilization in soybean is a well-known phenomenon, which is as a result of its high protein content (about 40%). Because of its high protein content in the seed, which cannot be supplied by current photosynthesis, the plant tends to remobilize stored N in leaves and stems to the fill seeds (Bebeley, 2013). In this study, the amount of N remobilized was greatest in the treatments with greater total N content. This is an indication that insufficient quantities of N were available to be remobilized.

CHAPER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This experiment showed that seed inoculation resulted in significantly greater nodule number, nodule weight and nitrogen fixation in the varieties. Vegetative growth in terms of plant height, canopy spread, number of leaves and branches as well as grain yield and protein content were all enhanced following seed inoculation. Furthermore, application of fertilizer N at grain filling period increased seed yield, especially at the rate of 30 kg N/ha and lastly, remobilization of N occurred in all treatments, but was greater in treatments with greater availability of N.

6.2 Recommendations

i. Combined applications of inoculation and N-fertilizer (30 kg N/ha) seemed to be an appropriate management practice for increasing the productivity of soybean.

ii. The experiment should be repeated in different agro-ecological zones at different cropping season to ascertain the findings of this study.

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APPENDIX

Analysis of variance

Variate: EFF

Source of variation d.f. s.s. m.s. v.r. F pr. nitrogen 5 11359.70 2271.94 99.26 <.001 variety 2 53.93 26.96 1.18 0.319 nitrogen.variety 10 258.96 25.90 1.13 0.367

Residual	36	824.00	22.89
Total	53	12496.59	

Message: the following units have large residuals.

units 8	-9.33	s.e. 3.91

Tables of means

Variate: EFF

Grand mean 44.37

nitrogen	1	2	3	4	5	6
	27.56	48.67	34.33	60.56	64.56	30.56

variety	1	2	3
	45.56	43.11	44.44

nitrogen variety	1	2	3
1	26.67	27.67	28.33
2	50.00	46.33	49.67
3	37.67	29.67	35.67
4	66.33	59.00	56.33
5	63.33	65.67	64.67
6	29.33	30.33	32.00

Standard errors of differences of means

Table	nitrogen	variety	nitrogen variety
rep.	9	18	3
d.f.	36	36	36
s.e.d.	2.255	1.595	3.906

Least significant differences of means (5% level)

Table	nitrogen	variety	nitrogen variety
rep.	9	18	3
d.f.	36	36	36
l.s.d.	4.574	3.234	7.922

Stratum standard errors and coefficients of variation

Variate: EFF

d.f.	s.e.	cv%
36	4.784	10.8

```
276   "General Analysis of Variance."
277   BLOCK "No Blocking"
278   TREATMENTS nitrogen*variety
279   COVARIATE "No Covariate"
280   ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7;
PCONTRASTS=7; FPROB=yes;\
281   PSE=diff,lsd; LSDLEVEL=5] Hsw_g
```