Response of Soybean to Rhzobial Inoculation and Nitrogen Management options in the Southern Guinea Savannah Zone of Ghana

BY

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(Bed Agriculture)

NOVEMBER, 2016

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

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SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CROP AND SOIL SCIENCES

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Thesis submitted to the Department of Crop and Soil Sciences, Faculty of Agriculture of the College of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, in partial fulfillment of the requirements for the award of

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in

Soil science

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DECLARATION

I hereby declare that this thesis is the result of my own work, except the references to other people's work which have been duly cited and acknowledged, and that it has not been presented in this university or elsewhere, either in whole or in part for the award of a degree.

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(HEAD OF DEPARTMENT) SIGNATURE DATE

DEDICATION

This Thesis is dedicated to my loving and supporting family, who have always supported me and kept me in their thought and prayers throughout all my endeavours, especially my mother COMFORT SARFO, and my late father BENJAMEN AHLIJAH.

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ABSTRACT

Field experiments were carried out in the Savelugu/Nanton and Yendi Municipalities in the northern region of Ghana in 2014 to investigate the effect of inoculation, rates and time of nitrogen fertilizer application on growth, yield, agronomic efficiency of mineral N and biological nitrogen fixation of soybean. The experiment was laid out in a splitsplit plot, arranged in a randomized complete block design with four replications at both locations. The main plot factor was inoculation (legume-fix inoculants), the sub-plot factor was nitrogen rates (0, 10, and 20 kg ha^{-1} N) in the form of sulfate of ammonia fertilizer (21 % N) and the sub-sub plot factor was time of nitrogen application (either starter N or late N application). Triple superphosphate (TSP) 46% P_2O_5 was applied as basal fertilizer to all treatment plots at the rate of 30 kg P ha^{-1}. Weed fallow was established at both locations as reference for the determination of N-fixation using nitrogen difference method (NDM).The crop parameters were evaluated on the basis of plant height, number of nodule and nodule dry weight at 50% flowering, number of nodule and nodule dry weight at R4 (full pod stage), dry matter yield at R4, pod number, pod weight, grain yield, mean hundred-grain weight, amount of N_2 fixed (BNF) and agronomic efficiency of mineral N fertilizer application in soybean production. The results showed that inoculation of soybean with rhizobia inoculants had no significant ($p > 0.05$) effect on all the response parameters measured at both locations. N rates at 20 kg ha^{-1}significantly ($p < 0.05$) increased dry weight of nodules at 50% flowering, hundredseed mean weight and grain yield by 29.88% at Puriya over the control. At Bunlong, the N rates only significantly ($p < 0.05$) increased dry weight of nodules at 50% flowering.

Starter N significantly increased nodule number at R4 stage by 19.8% and dry weight by 20% at Puriya but had no effect on other response parameters. At Bunglong however, starter N only increased the nodule number. Late application of N only increased nodule

dry weight at Bunglong. The interactions among the factors (inoculation, rate and time of N application) did not significantly influence biological nitrogen fixation and growth at both locations. The agronomic efficiency of the mineral N fertilizer applied was significant with the 20 and 10 kg N ha^{-1} rate increasing yield at Bunglong and Puriya respectively. It was therefore concluded that inoculation of soybean with rhizobia inoculant and nitrogen application did not increase growth and BNF. However, mineral N fertilizer application increased grain yield and agronomic efficiency in soybean production.

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CHAPTER ONE

I.0 INTRODUCTION

Economically, soybean (*Glycine max*) is an important leguminous crop worldwide (Plahar, 2006). It plays a very important function in the natural ecosystem and agriculture, where its ability to fix atmospheric N_2 in symbiosis with rhizobium makes it a very good colonizer of low-N environment (Graham and Vance, 2003). Biological Nitrogen fixation (BNF) abilities of legumes is an important method for sustainable crop-land management and is a very good source of providing N to plants under favourable atmospheric and environmental conditions (Hungria and Vargas, 2000; Chen *et al*., 2002). Mahamood *et al*. (2009) reported that soybean is a crop which has been proposed for the removal of the acute shortage of protein and oil worldwide.

Mpepereki *et al*.(2000) and Addo-Quaye *et al.*(1993) reported that the world's most widely used edible oil is Soybean oil, as it is having a natural taste and nearly undetectable odour and as it is low in cholesterol, which makes it the most chosen vegetable oil for domestic and industrial food processing.

Promotion of the nutritional and economic values of the crop is being done in Ghana by the Ministry of Food and Agriculture, and this has resulted in rapid expansion in production during the past decade (Sarkodie-Addo *et al*., 2006). In West Africa, soybean has become a major source of high quality and cheap protein for the poor and rural households (Abbey *et al*., 2001). Ghana's current soybean production is estimated at about 141,000 metric tons of soybean grain annually while total domestic need for cooking oil, seasoning and animal feed cake is estimated at nearly 182,000 metric tons per year (USAID, 2012).

In spite of the above benefits, soybean yield in Ghana, especially among the smallholder famers is very low. According to Lawson *et al*. (2008) the average yield for soybean

production in northern Ghana is estimated at about 1.5 t / ha on the farmers' field comparative to that of USA which is 4.6 t / ha and grain yield per unit area in Ghana is an average of 1.3 t / ha. This has been attributed to several factors including inaccessibility to certified seeds by farmers leading to poor germination, poor cultural practices and inherently low soil fertility (Lawson *et al*., 2008). Smallholder soybean farmers in northern Ghana hardly apply N fertilizer to soybean but rely on N_2 fixed by symbiotic processes alone for soybean production; however, forming nodules and fixing nitrogen, soybean needs specific rhizobia. In most soils, these rhizobia are not abundant, thus, necessitating the need to inoculate soybean seeds with the right rhizobium inoculant.

Currently, according to Mapfumo (2001) the contribution of biological nitrogen fixation on most of smallholder farms in Africa with N_2fixing legumes hardly exceeds 5 kg N / ha / year. Giller (2001) recorded more than 240 kg N / ha of amount N_2fixed in soybean in southern Africa on small holder farms with corresponding grain yields of more than 3.5 t / ha. This implies that the potential rates of soybean biological nitrogen fixation are not only limited by the effectiveness of the legume rhizobium association.

According to Wood *et al*. (1993), fertilizer N as starter N application is aimed at supplying soybean with readily available soil-N during seedling growth and has been revealed to increase soybean grain yield. N fertilizer is typically not applied to soybean because the crop is expected to supply its own N through symbiotic process; however, it is not all the time that the crop is able to supply its own N because of various adverse environmental effects. Although it has been reported that biological nitrogen fixation in legumes could be enhanced through rhizobial inoculation but Sosulski and Buchan (1978) reported that only rhizobial inoculation is not adequate for acquiring high yields of legumes due to poor nodulation and nitrogenase activity. These authors have suggested that legumes will need a high rate of plant N fertility to obtain high yields. The use of N fertilizer in soybean

production among smallholder farmers in Ghana is not a common practice but studies by Ahmed *et al*. (2014) on the impact of nitrogen fertilizer on pigeon pea in Sudan indicated that N application could improve grain yield and plant N concentration. Yet, there is dearth of information on the appropriate time of N fertilization and right amount of N fertilizer to be applied to soybean that could enhance growth, yield, agronomic efficiency of mineral N and BNF, hence, the need for this study.

Although, several studies on the response of soybean to inoculation have been conducted in Ghana, only few studies have looked at the response of soybean to inoculation in association with N fertilizer application either as starter or late N application. The study was therefore, conducted based on the hypothesis that Rhizobium inoculation and nitrogen fertilizer application increase BNF and yield of soybean.

The general objective of this study was to investigate the possibility of improving soybean productivity through the combined application of rhizobia inoculant and N fertilizer in the Guinea savannah zone of Ghana.

The specific objectives were:

i. To determine the effect of inoculation, mineral N rate and time of application on nodulation and BNF. ii. To examine the effect of inoculation, mineral N rate and time of application on growth and yield.

iii. To evaluate the agronomic efficiency of mineral N application in soybean production.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 World production

Soybean production is increasing rapidly all over the world as a result of the numerous benefits derived from the crop. Current world production of soybean is 220 million metric tons of grain per annum, of which the seven leading producers are the USA-thirty two percent, Brazil-twenty eight percent , Argentina-twenty one percent, China-seven percent, India-four percent, Paraguay-three percent, Canada-one percent and others-four percent (USDA, 2007). According to FAO data for 2005, the overall land area under soybean cultivation in the world was 95.2 million hectares per annum and the overall production was 212.6 million tons annually.

The main producing countries were United State of America (29 million hectares), Brazil (23 million hectares), and Argentina (14 million hectares) (IITA, 2009). Masuda and Goldsmith (2008) also gave the breakdown of world soybean production of 94 million hectares worldwide as follows: the U.S.A. accounted for over 30 million, Brazil for almost 22 million, Argentina for 15 million, China for 9.2 million, India for 8.2 million, Paraguay for 2.2 million and Canada for one million hectares, respectively.

Comparative to Sub-Saharan Africa, Masuda and Goldsmith (2008) revealed that an average of 1.16 million hectares of soybean with an average production of 1.26 million tons of grain in 2005 was grown. Nigeria (601, 000 hectares), South Africa (150, 000 hectares), Uganda (144, 000 hectares), Malawi (68, 000 hectares), and Zimbabwe (61, 000 hectares) were the largest African countries involved in the production of soybean.

2.2 Economic benefits of soybean

Soybean is more protein-rich than most vegetables in Africa (Dugje *et al*., 2009). Soybean has an average protein content of 40%. The seeds also possess about 20% oil on a dry matter basis, and this is 85% unsaturated and cholesterol-free. Borget (1992) has stated

that soybean contributes to the feeding of both humans and domestic animals. It has various nutritional and medicinal properties as well as industrial and commercial uses; and agronomic values such as soil conservation, green manure, compost and nitrogen fixation. Soybean can be cooked and eaten as a vegetable as well as processed into soy oil, soy milk, soy yoghurt, soy flour, and toffee (Rienke and Joke, 2005; MoFA and CSIR, 2005).

Rienke and Joke (2005) reported that soybean has high-quality protein and is a very good source of carbohydrates, oil, vitamins and minerals. Research has shown that the quantity of proteins in one kilogram of soybean is equivalent to the quantity of proteins in three kilograms of meat or 60 eggs or 10 litres of milk. Comparatively, the cost of buying one kilogram of soybean is much less than buying a similar quantity of meat or eggs (Ngeze, 1993). It can therefore, be an excellent substitute for meat in developing countries, where animal protein-rich foods which are often difficult to come by and expensive for poor families to afford.

Soybean oil is also rich and highly digestible, odourless and colourless, which does not coalesce easily. It is one of the most common vegetable cooking oils used in food processing industries, all over the world. It is also heavily used in industries, especially in the manufacture of paint, soap, typewriter ink, plastic products, glycerin and enamels (Rienke and Joke, 2005; Ngeze, 1993).

The cake obtained from soybean after oil extraction is also an important source of protein feed for livestock such as poultry, pig and fish. The expansion of soybean production has led to significant growth of the poultry, pig and fish farming (Abbey *et al.*, 2001; Ngeze, 1993; MoFA and CSIR, 2005). The haulms, after extraction of seed, also provide good feed for sheep and goats (Dugje *et al*., 2009). The high quality protein, low cholesterol oil and other nutritional values are beneficial in the treatment of nutritional diseases in children (MoFA and CSIR, 2005).

2.3 Environmental requirements

2.3.1 Soil

Soybean tolerate different soil conditions but does best on warm, moist, and well drained fertile loamy soils, that supply enough nutrients and good contact between the seed and the soil for rapid germination and growth (Hans *et al.*, 1997; Addo-Quaye *et al.*, 1993). Ngeze (1993) stated that, soybean does well in fertile sandy soils with pH between 5.5 and 7.0, and that the crop can tolerate acidic soils than other legumes but does not grow well in water logged, alkaline and saline soils.

Keeping the pH of soil between 5.5 and 7.0 enhances nutrients' availability such as nitrogen and phosphorus, the ability of microbes to breakdown crop residues and symbiotic nitrogen fixation (Ferguson *et al.*, 2006). Rienke and Joke (2005) reported high yields in loamy textured soil, and that if the seeds are able to germinate, they grow better in clayey soils.

2.3.2 Temperature and photoperiod

Plant breeders have argued that within the soybean species, there are varieties which react differently to photoperiod, and classified them as long day, short day and day neutral plants (Borget, 1992). Rienke and Joke (2005), described soybean as being typically a short day plant, physiologically adapted to temperate climatic conditions. However, some have been adapted to the hot, humid, tropical climate. In the tropics, the growth duration of adapted genotypes is commonly 90-110 days, and up to 140 days for the late maturing ones (Osafo, 1997). The relatively short growth duration is primarily due to sensitivity to the day length. This affects the extent of vegetative growth, flower induction, production of viable pollen, and length of flowering, pod filling and maturity characteristics (Norman *et al.*, 1995).

Most legumes require a temperature between 17.5 °C and 27.5 °C for development, optimum being 22 °C and the maximum about 40 °C. The seeds grow well between 15

°C and 40 °C temperatures, but the optimum is about 30 °C (Rienke and Joke, 2005). Addo-Quaye *et al.* (1993) have suggested the optimum temperature for growth as between 23-25°C.

2.3.3 Moisture requirements

Soybean requires optimum moisture for seeds to germinate and grow well. The optimum rainfall amount is between 350 and 750mm, well distributed throughout the growth cycle (Ngeze, 1993). Rienke and Joke (2005) and Addo-Quaye *et al.* (1993) described two periods as being critical for soybean moisture requirement; from sowing to germination and flowering to pod filling periods. During germination, the soil moisture should be between 50% and 85%, as the seed absorbs 50% of its weight of water before it can germinate. The amount of water needs increases, and peaks up at the vegetative stage, and then decreases from reproductive to maturity stage.

Huge differences in the quantity and the supply of water in the soil reduces yield of soybean. According to Bohnert *et al.* (1995), water in plants plays two main functions, these are (1) as an electron donor in the photosynthetic reaction processes and (2) as a medium in which plant nutrients are dissolved and transported. Troedson *et al*. (1985) reported that, soybean is quite susceptible to water stress, and usually respond to frequent watering by substantially increasing vegetative growth and yield. Direct influence of drought stress on the physiological development of soybean depends on its water use efficiency (Earl, 2002). In soybean management, the efficiency of water is a vital physiological feature which relates to the plants' capability to accommodate water stress. Passioura (1997) revealed that grain yield is a function of the quantity of water transpired, water use efficiency and harvest index.

Due to low photosynthetic rates and high evapotranspiration, soybean is less efficient in water use. Pandy *et al*. (1984) revealed that, leaf area, leaf area duration, crop growth rate and shoot dry matter are progressively decreased when drought stress is increased; hence, limits soybean yield. Sionit and Kramer (1977) reported that at flowering and early pod formation when drought stress occurs, it causes highest decrease of pod number and grains at harvest. Low soil moisture with high plant population may cause yield to decrease because of drought stress (Gary and Dale, 1997).

2.4 Fertilizer requirement

Soybean plant has a nutrient dense, high protein seed, and therefore, requires high amount of nutrients for its growth (Lamond and Wesley, 2001). Sarkodie-Addo *et al.* (2006) found out that legume can meet its nitrogen needs through symbiotic processes with N_2fixing bacteria of the species *Bradyrhizobium japonicum* from atmospheric nitrogen. Generally, the plant will not benefit from supplemental nitrogen fertilizer application, where there are indigenous populations of the appropriate *Bradyrhizobia* bacteria strains that cause effective nodulation of the roots and nitrogen fixation (Darryl *et al.*, 2004).

Gary and Dale (1997) have stated that, nitrogen fertilizer application prevent the benefit of *Rhizobia bacteria*, as the bacteria will not change the atmospheric nitrogen when soil nitrogen is readily available to the plant. However, where soybean have not been grown recently, inoculation of the seed with specific *Bradyrhizobia* strains is essential for effective nitrogen fixation (Darryl *et al.*, 2004). Malik *et al*. (2006) revealed that, soybean seed inoculation with *Rhizobium* in combination with phosphorus application at 90 kg per hectare, performed better in yield under irrigated conditions. Soybean can provide maximum seed yield with relatively low rates of available phosphorus in the soil. Also, since K levels are usually high in both surface soil and subsoil, most soils hardly require

potassium fertilizer for soybean production. Potassium fertilizer is not required if soil test shows more than 124ppm (Ferguson *et al.*, 2006).

Linderman and Glover (2003) have stated that, the basic nutrients N, P and K; N is supplied by the symbiotic bacteria in the nodules, while the others come from the soil, and will be taken into the plant as it takes up water.

2.5 Biological nitrogen fixation

Leguminous plants form vital symbiotic relationship with bacteria *rhizobium* and their families. Root nodule development can be divided into three stages; the stages are preinfection, infection and nodule organogenesis and nodule function and maintenance. The pre-infection of the legume plant begins by the root hairs releasing flavonoids, which cause rhizobia to be attracted by chemotaxis. Induction of nod-gene is needed for the release of *nod*-factorsto cause the bacteria to attach itself onto the root hairs. This is followed by the entry of the rhizobia to cause infection thread which creates a path for the rhizobia to move from the tip of the root hairs to the internal legume. Rolfe and Gresshoff (1988) reported that within the nodule, rhizobia differentiate into bacteriods, which fix N_2 into a plant usable form NH_4^+ using the enzyme nitrogenase. To execute N_2 fixation, bacteroids are required to get carbon and energy from the plant. The carbon and the energy are in the form of dicarboxylic acids. It is agreed that, the bacteroids give the host legume plant with ammonium or ammonia in return, which diffuses across the peribacteroid membrane and is integrated into amino acids in the plant cytosol of the nodular tissue. Most non-leguminous woody plants form a N_2-fixing symbiosis in root nodules with Actinomycetes of the genus *Frankia (*Clawson *et al.*, 1998) and also establish N_2-fixing symbiosis with trees (Nazaret *et al.*, 1991).

2.5.1 Factors influencing nitrogen fixation in legumes

Peoples *et al.* (1992) reported that forming effective N_2 fixing symbioses between legumes and their N_2 fixing bacteria is based on some environmental conditions, and this could be affected by farm management practices. There are many environmental factors affecting Biological Nitrogen Fixation. Some of these environmental factors include saline and sodic soils, extreme soil pH, low nutrient availability, mineral toxicity, extreme temperature, moisture stress, drought, water logging, inadequate photosynthates, and diseases can affect the development of the plant. Panchali (2011) reported that in environmental conditions like these, the most effective rhizobium strains cannot form effective association in nodulation and N fixation with the host plant.

The nodule functions can negatively be affected by moisture stress, as drought could decrease nodule weight and nitrogenase activity. According to Ramos *et al*. (2003) after exposure of the nodules to moisture stress for 10 days, the nodule cell wall begins to lose strength which results in senescence of bacteroids. Sousssi *et al*. (1998) and Kouas *et al.* (2010) reported that compilation of Na^+ decreases the growth of the plant, nodule formation, and symbiotic N fixation ability under sodic saline conditions. Similarly, Singleton and Bohlool (1984) reported that soil salinity affects the early interaction between the rhizobium and the host plant in nodule formation. They also observed a reduction in soybean nodulation due to the interference with the early stages of infection at salinity of 8 dS m^{-1}. Hungria and Franco (1993) identified that high temperatures decreases nitrogenase activities because of ineffective nodules formation. Extreme soil pH, either low or high can decrease the rhizobial colonization in the legume rhizosphere. van Jaarsveld (2002) reported that amount of N_2 fixed may be prevented by acidic soils. Nodulation and the amount of N_2 fixed are more intensely influenced than the plant growth under low soil pH conditions. Bordeleau and Prevost (1994) reported that highly alkaline

soils with pH greater than eight are high in sodium, chloride, bicarbonate and borate ions and these decrease the amount of N fixed.

Ronner and Franke (2012) reported that besides the environmental conditions, agricultural management factors affect percentage of N_2 obtained from the atmosphere. Some of the management factors including inoculation, phosphorus fertilizer application, and selection of genotype and plant population can influence the plant growth and development. According to Giller (2001) the purpose for inoculation is largely based upon the availability of compatible rhizobia in the soil and how effective they are. A legume which is promiscuous will rarely respond to inoculation, especially when the indigenous strains are available and can establish effective symbiotic relationship (e.g. cowpea or groundnut). Soybean is most commonly seen to respond to inoculation among grain legumes and most genotypes are highly specific and do not commonly form symbiotic relationship with the native population strains in Africa soils.

Also, some soybean varieties are better adapted to local environmental circumstances and are also more specific than others. In general, indeterminate and long duration genotypes are able to fix more N_2because they spend long time in terms of growth than short-duration and determinate genotypes. Roner and Franke (2012) reported that phosphorus fertilizer application in soybean production enhances nodulation processes and plant growth when phosphorus is low in the soil. According to Naab *et al*. (2009) and Makoi *et al*. (2009) densely populated plants either show negative response to the amount of N_2obtained from N_2 fixation because of the struggle for nutrients and water or show positive response to the amount of N obtained from N_2 fixation because of greater struggle for soil N.

2.5.2 Methods of estimating BNF

There are several methods of estimating symbiotic biological N fixation in legumes. In choosing a particular method, it depends on the system in question, the available resources and variety under study. Some of the methods are; total nitrogen difference (TND) method, the xylem sap analysis, the acetylene reduction assay (ARA), and the isotopic techniques. These techniques have their advantages and disadvantages, therefore, to acquire the exact estimation of amount of N_2 fixed in the field, the pitfalls of the techniques must be acknowledged to minimize their influence on the measurement of the symbiotic activities.

2.5.2.1 N-Difference Method

The N difference (ND) method is an alternative to the N balance technique and with this method the existing soil N rates under both the reference crop and the legume are considered. This method compares the total N of the N_2 fixing species with a neighboring non-N_2-fixing species. The difference between the two is assumed to be the amount of N_2 fixed. This method is based on some assumptions that: Inculcating the soil N changes over the growing season, the soil N component and the corresponding differences in the N uptake from the two crops can be determined, considered soil N changes and losses are the same between the crops.

Alternative proposition is that root N between the reference and the N_2-fixing species is the same and once it is not effective and applicable to effectively uproot roots from field crops, the ratios between the root N and shoot N are considered to be the same between the crops. According to McCauley (2011) this proposition is very hard to prove in field settings. Root N, encompassing losses of root N to the soil may represent a large pool of amount of N_2-fixed that is not considered in the measurement of N fixation. The N difference technique is complex when dealing with intercropped legumes, for its competition could influence the capability of the non-legume fixing reference crops and

the legume crops to access soil N (Giller, 2001). Danso *et al.* (1992) reported that the N difference method is very good for sandy or low-N soils, since increase in soil N also heightens the error in Biological Nitrogen Fixation measure. The advantage of using this method is that it is simple and low cost method when facilities for dry matter determination and total N analysis are available.

2.5.2.2 Xylem sap analysis

Biological Nitrogen Fixation products are translocated to the parts of the plant via the xylem and the nitrogen assimilated from the soil is either conveyed to shoots as NO_3 or changed to amides before they are conveyed. Giller and Wilson (1991) reported that most legumes convey many of their N fixed in the form of ureides. Herridge *et al*. (1996) revealed that the amounts of N in the xylem sap as ureides are directly proportional to the proportion of N fixed.

According to Ngome (2006) BNF is closely connected to the amount of amide N in the xylem sap in legumes which never convey fixed N as ureides. Herridge *et al*. (1996) reported that xylem sap analysis has some limitations. Nonetheless, this procedure is reliable and it is deemed to be in line with ^{15}N isotopic procedure.

2.5.2.3Acetylene reduction assay (ARA)

Ngome (2006) reported that this method was coined from observations made in 1980s that the nitrogenase helped to increase the reduction of acetylene (C_2H_2) to ethylene (C_2H_4). Danso *et al*. (1992) also reported that Acetylene reduction assay method accounts for most estimates of BNF in legumes since that time (1980). According to Danso *et al.* (1992) incubating whole plants, decapitated roots or excised nodules in an environment containing acetylene, the quantity of ethylene produced is sometimes changed into total N_2 fixed by multiplying it by a conversion factor of three. These days, Acetylene reduction assay is hugely limited to quantitative studies because of the following: (i) the conversion factor of

three does not count in most situations and huge mistakes are likely to evolve, (ii) it needs interpolation between single and short-term measurements to get time-integrated measurements and (iii) because it is very challenging to get all nodules in the field for detailed assessment of Biological Nitrogen Fixation (Ngome, 2006). The Acetylene reduction assay is questionable because the product ethylene can conceal the performance of the nitrogenase by 50% after 30 minutes; however, it is the technique widely used (Olga-Cristina and Cornella, 2009).

2.5.2.4 ^{15}N Isotope and Natural abundance Methods

According to Chalk (2000) the above stated method could be more advantageous than N balance method as they give a yield-independent measure of N fixation. To estimate the amount of N fixed using this method depends on the naturally-occurring ^{15}N abundance in the atmosphere. Atmospheric ^{15}N is uniform globally; however, transformation of N processes that preferentially go for or against ^{15}N; biological material and soil tend to have ^{15}N concentrations which differ from that of atmosphere (McCauley, 2011). The microbes discriminating against the heavier ^{15}N isotope in favor of the lighter ^{14}N isotope causes most soils to become enriched in ^{15}N over time. Hauggaard-Nielsen *et al*. (2010) reported that the extent of soil ^{15}N enrichment in a field can differ massively and is affected by biochemical and physical conditions. The addition of a known amount of plant available ^{15}N to the system, usually with ^{15}N-labeled fertilizer, and adding a non-Nfixing reference crop, the Isotope Dilution technique can partially adjust for this variability. The natural abundance method is used to quantify N fixed by calculating the difference between the legume and the reference plant, thus, getting N from both the soil and the atmosphere, after accounting for isotopic fractionation between N and ^{15}N in the aboveground shoot of the legume. The first proposition of the Natural Abundance method is that reference plant and the legume are getting similar pools of soil N. The proposition demands that both plants

should be grown closer to each other and again both plants should have similar rooting morphology and stature. According to McCauley (2011) another proposition is that there is either no discrimination or similar discrimination between ^{14}N and ^{15}N in the plants' uptake and metabolism of N.

2.6 Effects of nitrogen application on BNF, growth and yield

Soybean supplies part of its N needs through symbiotic N_2 fixation like most annual legumes, when the plants are inoculated. Sosulski and Buchan (1978) have revealed that rhizobial inoculation alone is not adequate to get high yields of legumes due to poor nodulation and nitrogenase activity. According to Sosulski and Buchan (1978) to achieve maximum yield, legumes may need high rate of plant N fertility. Kucey and Hynes (1989) reported that there may be the presence of native rhizobia population for legumes in tropical soils, however, these native populations may not be effective to bring about N_2 fixation under semi-arid environments.

The application of N-Fertilizer to soybean is dependent upon some principles of possible soil-N requirement during the growth of the soybean. Periods in the growth of the soybean in which soil N is very much important include: (i) in the period of seedling growth before formation of nodules (Harper, 1974; Hatfield *et al*., 1974) and (ii) in the period of highest N needs such as reproductive growth stage three to four(pod fill stage) (Diebert *et al*., 1979).

According to Touchton and Rickerl (1986) during the growth of seedling, the application of N fertilizer as starter-N is aimed at giving the soybean crop with readily available soilN and it has revealed to improve soybean yields. However, Beard and Hoover (1971) reported that fertilizer-N application as starter N may reduce nodulation and N fixation in soybean. Diebert *et al*. (1979) observed a 26 to 48%decrease in N fixation when fertilizer-

N was applied in excess of 7.2 kg ha^{-1}at planting, but application of 22 kg ha^{-1} N was required to decrease the amount of N_2 fixed if N application were delayed. According to Beard and Hoover (1971) there is a decrease in nodulation when more than 22.5 kg N/acre is applied as starter N, but when 45 kg N/acre is applied at reproductive stages it would not affect number of nodules.

According to Herman (1982) and Happer (1971, 1974)the period at which soybean requires more N is during the reproductive growth stages, and it is greatly marked by peak N fixation. Harper (1974) identified that fixed-$N_2$and soil-N were necessary for higher grain yield of soybean and that soybean plants at reproductive growth stage two is capable to respond to N fertilizer. According to Brevedan *et aI*. (1977) and Deibert *et al*. (1979) studies have revealed that most N used by soybean at reproductive growth stages three and four was provided by the soil. According to Brevedan *et aI*. (1978); Gascho (1991) and Oplinger (1991) the addition of N-fertilizer during the reproductive growth stages have benefited the growth of soybean. Oplinger (1991) identified yield increases with N fertilization at reproductive growth stage three whiles Brevedan *et al*. (1978) identified grain yield increases when N was applied at reproductive growth stages one and two.

Anne-Sophie *et al*.(2002) reported that mineral N in the soil inhibited symbiotic nitrogen fixation, nonetheless, it was relative to start of nodulation and N_2 fixation at early vegetative growth only if the concentration is low. High concentrations of mineral N inhibits the effects of mineral N on nodulation and N_2 fixation of soybean (>5 mM), but the effects are far lesser when the concentrations are low. However, N fertilizer application affects nodulation of bean plants and therefore, the usually-appropriatesuggested rates of 40-60 kg N ha^{-1} suppress N_2 fixation (Graham, 1981; Ruschel *et al.*,

1979). Although, there are a few reports on positive effects of low nitrate concentrations on N_2 fixation in legume species such as soybean (Streeter, 1982;Gremaud and Harper,

1989;Gulden and Vessey 1997); an attempt to increase yield of soybean with the application of N fertilizer, two main periods of application of N were identified. First is the application of N at R1 to R5 growth stages.

According to Fehr and Caviness (1977) N_2-fixing capabilities starts to diminish after the reproductive growth stage five which meets the period of highest N needs (Shibles, 1998). Nonetheless, trials with N application at R1 to R6 stages have brought out results which were inconsistent. Gascho (1993) and Wesley *et al*. (1998) reported that soybean with high yield potential, irrigated soybean was significantly increased when nitrogen was applied at R3 to R4 stages. However, Freeborn *et al*. (2001); Schmitt *et al*. (2001) and Gutiérrez-Boem *et al*. (2004) revealed that other rain-fed studies showed

insignificant effect on grain yield with N fertilization at R3 and R4 growth stages.

The effect of N application at R3 on soybean in Lowa was examined and it revealed that soybean grain yield was not positively influenced by application of N fertilizer. Nonetheless, plant dry matter (DM) and plant N concentration were positively influenced (Barkar and Sawyer, 2005). Starter N fertilizer application at flowering or at V1 and V2 increased crop biomass and the pod yield by 16 and 44 % respectively. According to Panchali (2011) the amount of plant N concentration gotten from the symbiotic relationship is highest if N application is done at the reproductive growth stage four (pod filling stage) where demand for N is greater. Osborne and Riedell (2006) identified that the application of urea ($(NH_2)_2$ CO_3) or ammonium nitrate (NH_4NO_3) at planting at the rates of 8, 16, and 24 kg ha^{-1} positively influenced the plant biomass and plant N as compared to when no N was applied. Again, no increase was observed either in seed protein or oil content when grain yield of soybean was positively influenced by 16 % at the rate of 16 kg ha^{-1}N compared to the control. Ahlam *et al*. (2005) conducted an

experiment on the impact of nitrogen fertilization and rhizobium inoculation on pigeon pea in Sudan and found out that there was an increase in seed yield.

Gomaa (1989) Abdel-Ati *et al*. (1996) and Awad (1998) revealed that only using biofertilizers, excluding rates of mineral fertilizers from the appropriate suggested rates was less effective than the appropriate suggested rates of mineral fertilizers according to soil
fertility.

The findings of Wesley *et al*. (1998) had inconclusive results when they researched on the impact of the application of fertilizers-N on soybean qualities. According to Soresen and Penas (1978) various factors can affect the N_2intake and plant response to N fertilizer. The authors identified that conditions such as soil pH, moisture and temperature negatively affected plant response to application of N fertilizer. Hardy *et al*. (1971) reported that if plants were under normal temperature and humidity conditions, N fixation would begin 14 days after planting. Therefore, it would be of handy for the initial growth of plant if small amount of N fertilizer was included at planting.
Bergersen (1985) reported that the use of N fertilizer before planting would be of handy and could end up in production of nodules nine days after germination. The research carried out in Lowa showed that foliar N use at vegetative growth stage five would increase N absorption and plant yield. According to Haq and Mallarino (2000) the improvement of yield because of N fertilizer application seen in areas where the climatic conditions or soil type has reduced the soil moisture; low moisture would reduce early growth and nutrients availability.

According to Sij *et al*. (1979) the application of N fertilizer as starter N would increase the initial vegetative growth. Sij *et al*. (1979) again reported that the application of N fertilizer

as starter N had no effect on plant height and its yield. Terman (1977) confirmed that N distributed uniformly would result in 20 % increase in vegetative growth; however, plant yield was not affected. According to Starling *et al*. (2000) the use of mineral N at planting would increase plant growth and yield. According to Flannery (1986) and Wesley *et al*. (1998) N fertilizer must be applied at flowering stage in areas with high yield until the R3 stage; in this condition the response of plant to the use of N fertilizer would be positive.

Again, Brevedan (1987) identified that the application of N fertilizer from early R1 stage to the end of this stage in a greenhouse experiment resulted in a yield increase of 28% to 33 %. More so, Wesley *et al*. (1999) revealed that N application at the early R3 stage resulted in yield increase in 4 trials in an on farm experiments whiles it did not have any effect on the concentration of oil and proteins in the grains. Nonetheless, other studies conducted in the south of the United States revealed that the application of mineral N before planting would increase the grain protein, weight and yield of soybean; but had no effect on soybean oil concentration (Ham *et al*., 1975). Schmitt *et al*. (2001) revealed that the use of fertilizer N did not positively affect grain yield and oil concentration; its effect on protein was also limited. Other research carried out by Bly *et al*. (1998) Riedell *et al*. (1997) and Woodard *et al*. (1998) to examine the effect of the timing of the use of fertilizer-Non yield revealed that the use of mineral N as starter N application positively influenced the yield; however, the use of fertilizer-Nat the middle of pod-filling stage could not significantly affect yield. Pikul *et al*. (2001) identified that using low rate of N as starter N fertilizer application lower than 15 Kg N ha^{-1}, significantly affected grain yield compared to no N fertilizer application at planting. Because the reasons were not satisfying enough for increased grain yield, researchers decided to conduct other researches on this issue.

Schmitt *et al*. (2001) carried out an experiment to examine the effects of the source of N, time and mode of N fertilizer use on soybean grain yield, plant growth, protein, and oil. The authors reported that the use of fertilizer-N had no positive influence on the soybean grain yield or the oil content. Barker and Sawyer (2005) reported that soybean oil, grain yield, protein, and fiber content were not positively influenced with the fertilizer–N application at the rates of 45 and 90 kg ha^{-1} at early reproductive stage. Gan *et al*. (2003) identified that early application of nitrogen at V2 and R1 growth stages at a rate of 25 kg ha^{-1} increased the soybean plant dry matter and the N concentration. On the contrary, Panchali (2011) reported that the plant total biomass, N concentration and the grain yield could not be improved when N was applied at the seed filling stages (R3 / R5). Gan *et al*. (2003) again reported that application of N at R1 and R3 stages dramatically decreased the soybean nodulation, whereas at vegetative growth stage one there was a positive influence, which increased the soybean nodulation.

2.7 The need to inoculate soybean

Herridge *et al*. (2008) reported that soybean plants, which yields are high, need more nitrogen and it is estimated that Biological Nitrogen Fixation can cover 60 to 70 % of the nitrogen needs of the plant. Salvagiotti *et al.* (2008) reported that the requirement of nitrogen by soybean plants is supplied by Biological Nitrogen Fixation and it is estimated at about an average of 50 – 60 %. In areas where soils lack appropriate rhizobia, the response of soybean to inoculation is strong when the rhizobia are introduced into a new environment (van Kessel and Hartley, 2000). Soils with insufficient provision of inorganic N presumptively have a yield advantage to crops which have been inoculated in such soils. However, van Kessel and Hartley (2000) reported that the response of yield to inoculation was hugely unstable and influenced by innate field differences, and by variability in environmental and edaphic factors.

Nonetheless, according to Lindstrom (2010) unless soybean is grown on soils which lack effective indigenous strains of *Bradyrhizobium japonicum* for at least five or more years, the soils would usually lack effective indigenous strains of *Bradyrhizobium japonicum* and introduced strains may disappear completely without it been repeated over time, though rhizobial strains are more suitable to new environments. Hiltbold *et al*. (1985) correlated the number of *Bradyrhizobium japonicum* in Lowa experimental fields with soybeans planted at the locations during the last thirteen years and finalized that in areas where legume crops were going to be planted for the first time on the land, inoculation of seeds with the necessary strains of bacteria before sowing was vital.

According to Thies *et al*. (1991) the response of legume to inoculation is hugely based upon the number of indigenous rhizobia which are existing in the soil before, the management practice and the availability of soil nitrogen. The legume response to inoculation remains extremely site specific and it is dependent upon conditions widely above the competitiveness and effectiveness of the strain(s) applied and host cultivar(s) seeded.

Choudhry (2012) observed greater differences in strain effectiveness in various trials though N_2 fixation in grain legumes was much focused on choosing best rhizobial strains. Even, whether the soils' nitrogen is enough to coincide with the nitrogen requirement of the crop, the most effective rhizobia-host plant symbiosis will fix little N_2. When the need for nitrogen by the host is increased by management practices and enough nutrient availability, even, a less effective rhizobia-host plant symbiosis may well fix more nitrogen. Mengel (1994) reported that the activities of the nitrogenase are flexible processes that adjust to the N_2need of the host plant. The quantity of N_2 fixed becomes extremely dependent on the need of nitrogen by the host plant than on the innate abilities of the rhizobia to fix N_2. According to Choudhry (2012) increasing the quantity of fixed

N_2 by grain legumes as compared to attempting to increase the effectiveness of the rhizobia-host plant symbiosis, management practices that increase N_2 need will be an effective means.

Several researches have shown significant increase of rhizobia inoculation on plant biomass, nodulation and grain yield. According to Dorivar *et al.* (2009) the use of rhizobia inoculants positively influenced soybean grain yield by an average of 130 kg ha^1 and that N accumulation, plant biomass and grain N were also increased in soybean with the use of inoculants on soybean seed. Again, a study conducted on three groundnut genotypes in a sandy loamy soil identified that rhizobia inoculation positively influenced the quantity of N_2 fixed by 46 % over the control which were not inoculated.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site

The studies were conducted on-farm at Puriya in the Yendi Municipality and Bunglong in the Savelugu/Nanton Municipality both in the Northern Region of Ghana from July to December,

2014. The study sites fall within the Guinea Savannah agro-ecological zone with a unimodal rainfall of 1,100 mm per annum, which gradually builds up from May, June to October and declines towards November to April.

Puriya lies 202m above sea level on latitude N09° 24'56.2" and longitude W000° 12'21.3". Bunlong also lies 189m above sea level on latitude N09° 35' 58.8" and longitude W000° 48' 02.7". The soil in the area is predominantly Fluvic luvisols classified locally as the Tingoli series and the vegetation type is a tree Savannah (FAO, 2008).

3.2 Preparation of the land

The land at both locations were slashed with cutlass, ploughed and harrowed with tractor. The land was then leveled and the plots laid out using tape measure, garden line and pegs.

3.3 Experimental design and treatments

The experimental design was a split-split plot arranged in randomized complete block design with four replications. The interventions tested were inoculated and un-inoculated as the main plot factor, rates of N fertilizer (0 kg/ha, 10kg/ha, 20kg/ha) as the sub-plot factor and time of nitrogen application (at first trifoliate (V1) and early pod, R3) stages as the sub-sub plot factor. The size of the plot was 25 m^2(10 x 2.5 m) at a planting distance of 50x 10cm and spacing between plots and blocks were 50 cm and 1m, respectively. To assess Biological Nitrogen Fixation in this study, weed fallow was established on each field; whereby on each field, six plots each with dimension 4m^2 was demarcated and the three fertilizer rates applied as starter N and late N. Rates of fertilizer; thus, 0kg, 10kg and 20kg N/ha of ammonium sulfate fertilizer were applied to the first three plots at V1 stage and the same quantity applied at the R4 stage on each of the fields. A 30 kg/ha phosphorus was applied as triple super phosphate (46% $P_2 O_5$) to all soybean plots.

3.4 Seed inoculation

The inoculant (LEGUMEFIX) contained 10^{10} cells g^{-1} of *Bradyrhizobium japonicum* strain USDA 532C together with a polymer sticker allowing dry inoculation

(www.legumetechnology.co.uk). The soybean seeds were moistened with water and mixed with the inoculant at the rate of 5g of the inoculant per one kilogram of the soybean seeds and air-dried for 20 minutes before planting.

3.5 Agronomic practices

Planting of the soybean seeds were done in July 28th and 29th, 2014 at Puriya and Bunglong respectively.

Weeding was done 4 and 8 weeks after emergence (WAE) with hoe.

The TSP was applied uniformly to all plots at a rate of 30 kg P/ha as basal fertilizer to all treatment plots at two weeks after planting (WAP) and a split application of ammonium sulfate was done at 2 WAP and at early pod stage (R3).

3.6 Data collection

3.6.1 Soil sampling

Initial soil samplings were done at each site or location prior to ploughing. Per field, 9-10 cores (0-20cm) depth were taken in a "W" shape design and mixed and sub-sample taken. The soil samples were air-dried, ground and sieved using 2 mm mesh, before the soils were analyzed for physicochemical properties using standard laboratory procedures. The hydrometer method (Anderson and Ingram, 1993) was used to analyse the particle size analysis. Soil pH was measured by using a pH meter in the supernatant suspension of 1: 2.5 soils and water mixture. Walkley and Black method (Walkley and Black, 1934) was used to measure soil organic carbon. The Kjeldahl method was used to measure total nitrogen of the soil. Bray 1 method (Bray and Kurtz, 1945) was used to determine available phosphorus. Cation Exchange Capacity was measured by leaching the soil with neutral 1 N ammonium acetate (FAO, 2008). Table 1 shows the soil characteristics of the experimental fields.

3.7 Laboratory analysis of soil and plant samples

3.7.1 Particle size analysis

The hydrometer method was used to determine the soil texture. Air-dried soil sample weighing 51g was put in a measuring cylinder and 50 ml of calgon (sodium hexamethaphosphate) was added. The suspension was then shaken and allowed to stand. Corrected hydrometer readings at 40 seconds and 5 hours were taken.

Calculation:

$$\% \text{ sand} = 100 - \left[\left(\frac{A}{W}\right) \times 100\right]$$

$$\% \text{ clay} = 100 \times \left(\frac{B}{W}\right)$$

$$\% \text{ silt} = (\% \text{ sand} + \% \text{ clay})$$

Where:

$A = \text{corrected hydrometer reading at 40 seconds}$

$B = \text{corrected hydrometer reading at 5 hours}$

$W = \text{weight of dry soil}$

The textural class was then determined from the textural triangle.

3.7.2 Soil pH

Soil pH in water (1: 2.5 soil: water ratio) was determined using the pH meter. A soil sample of mass 20 g was weighed into hundred milliliters bottle to which water of volume fifty milliliters was added. The suspension was frequently stirred for 30 minutes. The pH meter was calibrated with buffer solutions of pH 4.0 and 7.0. The glass electrode was immersed into the upper part of the suspension and this gave the pH readings.

3.7.3 Soil organic carbon

Modified Walkley and Black's Wet oxidation method as outlined by Nelson and Sommers (1982) was used to measure soil organic carbon. Two grammes of a soil sample were

weighed into 500 ml conical flask. One reference sample and a blank were included. 10 ml of 1.0 N (0.1667 M) potassium dichromate and concentrated H_2SO_4acid of volume twenty milliliters were added to the sample and the blank flasks. The content of the flasks were mixed in a circular pattern and it was allowed to stand for thirty minutes on a fume cupboard. A 10 ml of concentrated orthophosphoricacid (H_3PO_4) and 200 ml of distilled water were added after thirty minutes and allowed to cool down.

Diphenylamine indicator (1 ml) was then added and titrated with 1.0 M ferrous solution.

$$\% C = \frac{N \times (Vbl - Vs) \times 0.003 \times 1.33 \times 100}{g}$$

Where:

N = Normality of $FeSO_4$ solution

V_{bl} = ml of $FeSO_4$ used for blank titration

Vs = ml of $FeSO_4$ used for sample titration

g = mass of soil taken in grammes

0.003 = milli-equivalent weight of C in grammes (12/4000).

1.33 = correction factor used to change the Wet combustion C value to the true C value since the Wet combustion method is about 75 % efficient in estimating C value (i.e. 100/75 = 1.33).

3.7.4. Soil total nitrogen

The Kjeldahl digestion method was used to determine soil total nitrogen. 0.5 g soil sample was weighed into kjeldahl digestion flask.5 ml of distilled water was then added. Selenium mixture and concentrated sulphuric acid (5 ml) were added and mixed carefully after 30 minutes. The sample was then digested for 3 hours until a clear digest was obtained. A 50 ml of distilled water was then used to dilute the digest and mixed well and allowed to cool. The volume of the solution was made to 100 ml with distilled water and mixed well. The mixture was heated strongly to digest the soil to a permanent clear green colour. A solution

of 25 ml aliquot was then transferred to a Tecator distillation flask and 20 ml of 40 % NaOH solution was added followed by distillation. The distillate was collected in 2.0 % boric acid and by using bromocresol green as indicator it was titrated with 0.02 N HCl. A titration and blank distillation were also conducted to cater for the traces of nitrogen in the chemicals used and the water used.

The % N in the sample was expressed as:

$$\% \, N = \frac{N \times (a - b) \times 1.4 \times mcf}{w}$$

Where:

N = concentration of HCl used in titration

a = ml HCl used in sample titration b = ml HCl used in blank titration w = weight of air-dried soil sample

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

$1.4 = 14 \times 0.001 \times 100\ \%$ (14 = atomic weight of N)

3.7.5 Available phosphorus

Bray's No. 1 extracting solution (0.03 M NH_4F and 0.025 M HCl) as described by Bray and Kurts (1945) was used to extract available phosphorus. A 35 ml of extracting solution of Bray's No. 1 was added to a 5 g soil sample which was weighed into a shaking bottle (50 ml). The mixture was then shaken for 10 minutes and filtered through a whatman No. 42 filter paper. 10 ml of the colouring reagent (ammonium molybdate and tartarate solution) was added to an aliquot of 5 ml of the extract which was pipetted into a test tube and the solution uniformly mixed. A blank was included and treated the same way as the sample. The solution was then allowed to stand undisturbed for ten minutes for the development of the blue colouration. The absorbance values were recorded at 660 nm wavelength on a spectrophotometer. A standard series of 0, 1, 2, 3, 4, and 5mgP / l were prepared from 20 mg / l phosphorus stock solution.

$$P\ (mg/kg\ soil) = \frac{(a - b) \times 35 \times 15 \times mcf}{w}$$

Where:

a = mg/l P in sample extract

b = mg/l P in blank mcf = moisture correcting factor 35 = ml extracting solution

15 = ml final sample solution

W =sample weight in grammes

Table 3.1: Selected physical and chemical initial soil properties of the study area.

Property	**Value**	
	Puriya	**Bunglong**
Sand (%)	70	72
Silt (%)	22	11
Clay (%)	8	16
Texture	Sandy Loam	Sandy Loam
pH(H_2O,1:2.5)	5.40	5.30
Organic matter (%)	1.72	0.57
Total N (%)	0.07	0.02
NO_3^-N (mg/kg)	0.23	0.93
Available P (mg/kg)	3.43	3.11
Exchangeable Cations (cmol (+) / kg)		
Ca	3.47	3.20
Mg	2.67	0.81
K	0.19	0.09
Na	0.10	0.06
Exchangeable acidity (cmol (+) / kg)	0.60	0.65
Effective cation exchange capacity (cmol (+) / kg)	6.83	4.61

3.7.6 Determination of plant total nitrogen

The Kjeldahl digestion method was used to measure total N in the plant. Two grammes ofthe plant material was weighed into a 500 ml Kjeldahl digestion flask and one spatula of catalyst (copper sulphate + sodium sulphate +selenium powder mixture) followed by the addition of concentrated H_2SO_4 acid of volume 20 ml. Heat was strongly applied to the mixture to digest the plant material to a permanent green colour. The digest was allowed to cool and transferred to the 100 ml volumetric flask and distilled water was added to make up to the mark. A volume of the digest, 10 ml aliquot was transferred into a Tecator distillation flask and 20 ml of 40 % NaOH solution added. Steam from the Foss Tecator apparatus was allowed to flow into the flask. The ammonium distilled was then collected into a 250 ml flask which contains 15 ml of 4 % boric acid with mixed indicator of bromocresol green and methyl red. 0.1 N HCL solution was then titrated with the distillate. A blank digestion, distillation and titration were done to check traces of nitrogen in the reagent and the water used.

The expression for total N in the sample;

$$\%N = \frac{N\,(a-b)\,\times\,1.4\,\times\,N\times V}{s\,\times\,t}$$

Where;

N= normality of the Hydrochloric acid a = ml of the Hydrochloric acid used in sample titration b = ml of the Hydrochloric acid used in blank titration

1.4 = 14 x 10^{-3} x 100 % (14 = atomic weight of N) V = total volume of digest s = mass of oven dry plant sample taken for digestion in grammes (2.0 g) t = volume of aliquot taken for distillation (10.0 ml)

3.8 Measurement of Crop Parameters

3.8.1Height of plant

At both locations, five plants were randomly selected and tagged for height determinations which were taken at 50% flowering, and the average for each plot was calculated.

3.8.2 Number and dry weight of nodules

At 50% flowering and full pod stages, sampling area of 1.5m^2 from three rows of 1m length was established for nodulation assessment leaving 50 cm from the border lines from each plot at both locations. Ten plants were randomly selected from the established sampling area and gently dug out with hand trowel, washed through a fine sieve with water to take away soil particles and organic debris. The average nodules per plant were calculated after the number of nodules on each plant had been determined. The nodules' fresh weight were determined, oven dried at 60°C for 48 hours and nodule dry weight recorded.

3.8.3 Shoot dry weight determination

At full pod stage at both locations, sampling area of 1.5 m^2 from three rows of 1 m length was established leaving 50cm from the border lines from each plot for determination of dry matter. All the plants were cut at the ground level from each of the plots and weighed and sub-samples of 100g were taken, air dried for three days and followed by oven drying at 60°C for 48 hours and dry weight recorded.

3.8.4 Determination of yield and yield components at harvest

At both locations, a net plot size of 6m x 1.5m (9m^2) from three rows of 6 m length was established leaving 50 cm from the border lines for yield measurement; all plants were counted and recorded. Per treatment plot, ten plants were randomly selected and pod load was determined. The pods which were taken from the ten plants were put together with the pods from the respective net plot and threshed with the pods harvested in the harvest area of each treatment plot; the grains were weighed on an electronic balance. These were then

extrapolated to obtain total grain yield per hectare. The grains were oven-dried at 60°Cfor 48 hours to constant weight and hundred seeds from each treatment were randomly selected and weighed. This was repeated three times and the mean100-grain weight determined.

3.8.5 Determination of amount of N_2fixed

Total Nitrogen Difference (TND) technique was used to determine BNF. The determination of the amount of N_2 fixed was carried out by comparing total nitrogen of the legume with that of the reference plant.

BNF = N uptake by legume – N uptake by reference crop

Where Total N in plants = (Dry matter weight kg ha^{-1}× % N in plants)

$$\%\text{N from } N_2 - \text{fixation} = \frac{(\text{N}_{\text{legume}} - \text{N}_{\text{reference}})}{\text{N}_{\text{legume}}} \times 100$$

3.8.6 Estimation of agronomic efficiency

The agronomic efficiency of N was determined by comparing the grain yields in the treatment where fertilizer N was applied with that where fertilizer N was not applied. The results were then divided by the respective amount of fertilizer N applied.

Calculation:

$$\text{N-AE} = \frac{Y_F - Y_C}{F_{appl}}$$

Where:

N-AE[kg(kg N)$^{-1}$] = agronomic efficiency of nitrogen

Y_F [kg ha^{-1}] = grain yield where fertilizer N was applied

Y_C [kg ha^{-1}] = grain yield where N fertilizer was not applied

F_{appl} [kg N ha^{-1}] = N fertilizer amount applied

3.9Analysis of data

All data were subjected to ANOVA (analysis of variance) using the Genstat statistical package (12th edition). Least significant difference was used to separate means at ($P < 0.05$). Correlation analysis between yield and yield component was also carried out.

CHAPTER FOUR

4.0 RESULTS

4.1 Plant height

Table 4.1 shows the results of the effect of inoculation, rates of N and time of N application on plant height at 50% flowering. Plant height was not significantly ($P > 0.05$) affected by inoculation, nitrogen rates and time of nitrogen application at the two locations. Similarly, the interactions among inoculation, nitrogen rates and time of nitrogen application did not significantly ($P > 0.05$) affect plant height (Table 4.1).

Table 4.1: Plant height of soybean as affected by inoculation, mineral N rate and time of application at 50% flowering.

Treatment	Plant height (cm)	
	Bunglong	**Puriya**
Inoculation (I) Un-inoculated	42.00	44.74
Inoculated	42.00	49.92
Pr (I)	NS	NS
LSD (0.05)	10.73	8.98
Nitrogen rates (kg/ha) (N)		
0	41.10	46.55
10	41.90	46.61
20	43.90	48.74
Pr (N)	NS	NS
LSD (0.05)	4.72	5.34
Time of N application (T)		
Starter N	41.90	47.85
Late N	42.10	46.81
Pr (T)	NS	NS
LSD (0.05)	2.68	2.98
Interactions Pr (I x N)	0.51	0.72
Pr (I x T)	0.69	0.62
Pr (N x T)	0.21	0.13
Pr (I x N x T)	0.64	0.95
C V (%)	10.50	10.40

4.2 Nodules number and Nodule dry weight at 50% flowering

The results of the effect of inoculation, rates of nitrogen and time of application on number of nodule and nodule dry weight at 50 % flowering are presented in Table 4.2. There were no significant ($P > 0.05$) effects of inoculation on number of nodule and dry weight of nodule at both locations. Again, there was no significant ($P > 0.05$) effect of nitrogen rates on number of nodules at both locations. However, nodule dry weights at both locations were significantly ($P < 0.05$) influenced by nitrogen rates. Nitrogen application at rate of 10 and 20 (kg Nha^{-1}) significantly ($P < 0.05$) increased nodule dry weight by 74.19 % and 77.42 % respectively over the control both at Puriya and Bunglong.

Time of nitrogen application also had no significant ($P > 0.05$) effect on number of nodules at any of the two locations. However, nodule dry weight was significantly ($P < 0.05$) influenced by time of application at Bunglong but not at Puriya. At Bunglong, the effect of late application of Non dry weight of nodules was significantly higher ($P < 0.05$) than nitrogen applied as starter N. The interactions among inoculation, nitrogen rates and time of nitrogen application did not significantly ($P > 0.05$) affect number of nodules and dry weight of nodules at any of the locations (Table 4.2).

Table 4.2: Effect of inoculation and N management on nodulation at 50 % flowering.

Treatment	Nodule number (no/plant)		Nodule dry weight (g)	
	Bunglong	**Puriya**	**Bunglong**	**Puriya**
Inoculation rates (%) Un-inoculated	9.56	7.11	0.48	0.46
Inoculated	8.21	10.34	0.46	0.48
Pr (I)	NS	NS	NS	NS
LSD (0.05)	2.11	6.20	0.03	0.06
Nitrogen rates (kg/ha) 0	9.74	7.38	0.31	0.31
10	8.33	8.90	0.54	0.54
20	8.60	9.87	0.55	0.55
Pr (N)	NS	NS	< 0.001	< 0.001
LSD (0.05)	2.03	2.18	0.03	0.03
Time of N application Starter N	8.75	8.45	0.45	0.47
Late N	9.03	8.99	0.48	0.47
Pr (T)	NS	NS	0.03	NS
LSD (0.05)	3.81	1.69	0.02	0.02
Interactions Pr (I x N)	0.14	0.61	0.24	0.70
Pr (I x T)	0.65	0.92	0.10	0.60
Pr (N x T)	0.88	0.93	0.88	0.13
Pr (I x N x T)	0.95	0.97	0.22	0.75
C V (%)	31.10	32.00	7.50	5.80

4.3 Nodule number and nodule weight at full pod stage

The results of number of nodules and dry weight of nodules at R4 (full pod) stage are presented in Table 4.3. There were no significant (P > 0.05) effects of inoculation rates and nitrogen rates on number of nodules and dry weight of nodules at both locations.

Table 4.3 Effect of inoculation and N management on nodulation at full pod stage.

Treatment	Nodule number (no/plant)		Nodule wt (g/plant)	
	Bunglong	**Puriya**	**Bunglong**	**Puriya**

Inoculation rates (%)				
Un-inoculated	4.53	4.65	0.09	0.04
Inoculated	8.85	8.90	0.12	0.06
Pr (I)	NS	NS	NS	NS
LSD (0.05)	10.07	9.70	0.04	0.06
Nitrogen rates (kg/ha)				
0	6.11	6.30	0.09	0.05
10	7.68	7.68	0.09	0.06
20	6.34	6.34	0.13	0.05
Pr (N)	NS	NS	NS	NS
LSD (0.05)	1.99	1.95	0.07	0.02
Time of N application				
Starter N	7.30	7.39	0.11	0.06
Late N	6.12	6.17	0.10	0.05
Pr (T)	0.01	0.003	NS	0.03
LSD (0.05)	0.77	0.73	0.03	0.01
interactions				
Pr (I x N)	0.12	0.13	0.86	0.09
Pr (I x T)	0.32	0.58	0.47	0.59
Pr (N x T)	0.03	0.02	NS	NS
Pr (I x N x T)	1.00	0.70	0.66	0.004
C V (%)	19.00	17.70	53.20	29.10

However, time of nitrogen application significantly ($P < 0.05$) affected both number of nodules and dry weight of nodules at Puriya.

At Bunlong, the time of N fertilizer application significantly ($P < 0.05$) increased nodule number but had no effect on nodule dry weight. Application of starter N resulted insignificantly ($P < 0.05$) greater nodule number compared with late application of N at both locations. However, at Puriya, time of N application did not influence nodule dry

weight. The interactions between inoculation and nitrogen rates and inoculation and time of nitrogen application did not significantly affect ($P > 0.05$) number of nodules and dry weight of nodules at both locations. However, the interaction between nitrogen rates and time of nitrogen application significantly ($P < 0.05$) affected nodule number at both locations but did not have any significant ($P > 0.05$) effect on nodule dry weight at both locations. The interactions among inoculation rates, nitrogen rates and time of nitrogen application did not significantly ($P > 0.05$) affect number of nodules and dry weight of nodules at Bunlong. The interactions, however significantly ($P < 0.05$) influenced nodule dry weight but not nodule number at Puriya (Table 4.3).

4.4 Shoot biomass at full pod stage.

The results of the effect of inoculation and rate and time of N application on dry matter yield at full podding are presented in Table 4.4. Inoculation rates, nitrogen rates and time of nitrogen application had no significant effects ($P > 0.05$) on dry matter yield of soybean at both locations. The interactions also did not significantly ($P > 0.05$) affect dry matter yield at both locations (Table 4.4).

Table 4.4: Dry matter yield of soybean as affected by inoculation, nitrogen rate and time of nitrogen application on two fields at full pod stage (R^1).

	Dry Matter yield (kg/ha) Treatment	
	Bunlong	**Puriya**

[1] .5 Pod number and pod weight

Table 4.5 shows that inoculation, N application rate and time of application did not significantly ($P > 0.05$) affect number of pods per plant and pod weight per plant at the two locations. The interactions between nitrogen rates and time of application; and

Inoculation (%)		
Un-inoculated	4070.0	2510.6
Inoculated	4120.0	2980.0
Pr (I)	NS	NS
LSD (0.05)	2409.0	932.3
Nitrogen rates (kg/ha)		
0	3300.0	2400.2
10	4400.0	2700.9
20	4580.0	3130.2
Pr (N)	NS	NS
LSD (0.05)	1217.0	622.6
Time of N application		
Starter N	4130.0	2880.1
Late N	4070.0	2601.4
Pr (T)	NS	NS
LSD (0.05)	398.0	307.9
Interactions		
Pr (I x N)	0.84	0.48
Pr (I x T)	0.07	0.97
Pr (N x T)	0.95	0.13
Pr (I x N x T)	0.36	0.21
C V (%)	16.0	18.5

interactions among inoculation rates, nitrogen rates and time of nitrogen application did not significantly ($P > 0.05$) affect number of pod and pod weight of soybean at Puriya and Bunglong. However, the interaction between inoculation rates and nitrogen rates significantly ($P < 0.05$) affected pod weight but had no significant ($P > 0.05$) effect on number of pod at Bunlong. More so, the interaction between inoculation rates and time of

nitrogen application significantly ($P < 0.05$) affected number of pod but did not significantly ($P > 0.05$) affect weight of pod at puriya (Table 4.5).

Table 4.5: Effect of inoculation and N management on pod number and pod weight.

	Pod number (No /plant)		Pod weight (g/plant) Treatment	
	Bunglong	**Puriya**	**Bunglong**	**Puriya**
Inoculation rates (%)				
Un-inoculated	59.10	70.80	17.67	20.10
Inoculated	63.40	67.10	19.56	17.80
Pr (I)	NS	NS	NS	NS
LSD (0.05)	13.33	14.88	5.47	5.99
Nitrogen rates (kg/ha)				
0	59.70	64.30	18.50	16.40
10	62.70	72.10	18.50	22.20
20	61.40	70.50	18.84	18.30
Pr (N)	NS	NS	NS	NS
LSD (0.05)	11.12	9.30	3.30	5.45
Time of N application				
Starter N	61.60	68.80	18.62	19.60
Late N	61.00	69.20	18.60	18.20
Pr(T)	NS	NS	NS	NS
LSD (0.05)	10.11	7.46	3.00	5.19
Interactions Pr				
(I x N)	0.12	0.74	0.02	0.23
Pr (I x T)	0.77	0.05	0.94	0.82
Pr (N x T)	0.94	0.12	0.97	0.32
Pr (I x N x T)	1.00	0.96	0.92	0.35
C V (%)	27.20	17.80	26.50	45.20

4.6 Grain yield and hundred seed weight

Table 4.5 shows the results of the effect of inoculation and rate and time of N application on grain yield and mean grain weight at the two locations. At both locations, the inoculated plots yielded higher than the un-inoculated plots, although the differences were not significant.

Table 4.6: Grain yield and hundred-grain weight of soybean as affected by inoculation rate, nitrogen rate and time of nitrogen application on two fields.

Treatment	Grain yield (kg/ha)		100-seed weight(g)	
	Bunglong	**Puriya**	**Bunglong**	**Puriya**
Inoculation (%) Un-inoculated	2182	2015	10.70	10.01
Inoculated	2669	2284	10.50	9.88
Pr (I)	NS	NS	NS	NS
LSD (0.05)	647.50	446.50	0.96	0.72
Nitrogen rates (kg/ha)				
0	2471	1854	10.70	9.55
10	2247	2186	10.40	10.02
20	2558	2408	10.70	10.28
Pr (N)	NS	0.003	NS	<0.001
LSD (0.05)	340.70	280.30	0.60	0.26
Time of N application				
Starter N	2417	2132	10.50	9.75
Late N	2433	2167	10.40	10.15
Pr (T)	NS	NS	NS	<0.001
LSD (0.05)	162.10	177.90	0.37	0.18
Interactions				
Pr (I x N)	0.01	0.81	0.15	0.41
Pr (I x T)	0.17	0.51	0.83	0.36
Pr (N x T)	0.45	0.26	0.10	0.002
Pr (I x N x T)	0.43	0.95	0.36	0.78
C V (%)	11.00	13.60	5.70	3.00

At both locations, the plots that received the highest application rate (20 kg N /ha) also gave the highest grain yield, although it was only significantly different from the other rates at Puriya but not at Buglong. At Puriya, nitrogen application rates of 10 and 20 kg / ha N positively ($P< 0.05$) influenced grain yield by 17.91% and 29.88 % respectively over

the control. The mean hundred grain weight was not significantly influenced by inoculation at both locations. Nitrogen application rate, however, significantly influenced mean grain weights at Puriya with the 20kg N/ha rate giving the highest mean weight. Again nitrogen application of 10 and 20 kg / ha N significantly ($P < 0.05$) increased hundred seed weight by 4.92 % and 7.64 % respectively over the control. Time of N application also significantly ($P<0.05$) influenced mean grain weight at Puriya with the late application

	Nitrogen Fixed (kg/ha)	

giving the highest weight. Interaction between inoculation and nitrogen application rate significantly affected ($P < 0.05$) grain yield at Bunglong but not at Puriya. Interaction between Nitrogen rates and time of nitrogen application did not significantly affect ($P > 0.05$) grain yield and hundred grain weight at Bunlong. It however, significantly ($P > 0.05$) affected mean grain weight at Puriya. The interaction of inoculation and time of application and inoculation rates, nitrogen rates and time of nitrogen application did not significantly ($P > 0.05$) affect grain yield and hundred grain weights at both locations (Table 4.6).

4.7 N fixed

Table 4.7 shows the results of the effect of inoculation, rates of nitrogen and time of nitrogen application on nitrogen fixation in soybean at both locations. Inoculation, nitrogen rates and time of application had no significant ($P > 0.05$) effect on the amount of nitrogen fixed in soybean, although at both locations, the inoculated plots fixed higher amount of N than the un-inoculated plots. The interactions also had no significant effect on nitrogen fixed at both locations (Table 4.7).

Table 4.7: Nitrogen fixation of soybean as affected by inoculation, nitrogen rate and time of nitrogen application on two fields.

Treatment	Bunlong	Puriya
Inoculation rates (%)		
Un-inoculated	49.90	27.14
Inoculated	50.83	35.95
Pr (I)	NS	NS
LSD (0.05)	45.82	17.71
Nitrogen rates (kg/ha)		
0	42.56	30.24
10	53.96	37.92
20	54.57	26.47
Pr (N)	NS	NS
LSD (0.05)	23.13	11.83
Time of N application		
Starter N	52.82	33.16
Late N	47.91	29.93
Pr (T)	NS	NS
LSD (0.05)	7.56	5.85
Interactions		
Pr (I x N)	0.84	0.47
Pr (I x T)	0.07	0.97
Pr (N x T)	0.55	0.21
Pr (I x N x T)	0.36	0.22
C V (%)	24.80	30.60

4.8 Correlation matrix

The results of the correlation matrix for grain yield, biomass yield, number of pod per plant, number of nodule per plant at R4, dry weight of nodule per plant at R4, nodule number per plant at flowering and amount of nitrogen fixed at both locations are presented in Table 4.8.

Table 4.8: Correlation coefficient of some selected parameters

† Numbers against the parameters in columns correspond with variables in rows. * =Significant at P=0.05; ** =Significant at P=0.01; *** =Significant at P<0.001

@R4: at full pod stage; @flwg: at flowering stage; mt: matter; plt: plant.

The results showed highly significant positive correlation between grain yield and number of pods per plant (r = 0.47, P< 0.001). There was also highly significant positive correlation between dry matter yield and amount of N fixed (r=0.97, P < 0.001). The correlation between dry matter yield and nodule dry weight per plant at R4 was also highly significant positive (r=0.02, P < 0.05) and significant positive correlation between number of nodules per plant at R4and dry weight of nodule per plant at R4 (r=0.32,P < 0.05) at Bunglong. Similarly, at Puriya, grain yield was significantly and positively correlated with pod

	Parameters †	2	3	5	6	7
				BUNGLONG		
1.	Grain yield	0.02	0.47***	0.09	-0.1	0.08	0.01
2.	Dry mt yield		0.02	-0.31	0.02*	-0.06	0.97***
3.	Numb of pod/plt			-0.03	-0.15	0.32	0.01
4.	Nod numb/plt @R5				0.32*	-0.29	-0.34
5.	Nod dry wt/plt @R5					-0.1	-0.02
6.	Nod numb/plt @flwg						-0.03
7.	N fixed						
				PURIYA			
1.	Grain yield	0.15	0.35*	0.19	0.17	0.42**	0.02
2.	Dry mt yield		0.12	-0.04	-0.19	-0.07	0.96***
3.	Numb of pod/plt			-0.3	-0.28	0.36***	0.06
4.	Nod numb/plt @R5				0.85***	0.64***	-0.06
5.	Nod dry wt/plt @R5					0.5***	-0.21
6.	Nod numb/plt @flwg						-0.13
7.	N fixed						

number per plant(r=0.35,P < 0.05). Nodule number at R4 also highly, positively and highly significantly correlated with dry weight of nodules at R4 (r=0.85,P < 0.001). Nodule number per plant at flowering was also high, positive and highly significantly correlated with grain yield(r=0.42, P < 0.001), pod number per plant(r=0.36, P < 0.001), nodule dry weight per plant at R4 (r=0.64, P < 0.001), and nodule number per plant at R4 (r=0.50, P < 0.001).

4.9 Agronomic efficiency of mineral N fertilizer

Table 4.9 shows the results of agronomic efficiency of mineral N fertilizer application in soybean at Bunglong and Puriya. Inoculation positively (P < 0.05) influenced agronomic efficiency of N at Bunglong. The un-inoculated significantly (P < 0.05) increased agronomic efficiency by 200% over the inoculated. However, at Puriya, there was no significant (P > 0.05) effect of inoculation on agronomic efficiency of mineral N in soybean.

The rates of nitrogen applied significantly (P < 0.05) affected agronomic efficiency of mineral N in soybean at both locations. At Bunglong, the nitrogen application at the rate of 20 kg N/ha significantly (P < 0.05) increased agronomic efficiency of mineral N fertilizer application in soybean by 228.6% over the 10 kg N/ha. However, at Puriya, the nitrogen application at the rate of 10 kg N/ha significantly (P < 0.05) increased agronomic efficiency of mineral N fertilizer application and the increase was by a percentage of 35.7.

Time of N application significantly (P < 0.05) affected agronomic efficiency of mineral N fertilizer application at Bunglong with the application of the starter N resulting in significantly (P < 0.05) higher agronomic efficiency of mineral N fertilizer application compared to the late N. However, at Puriya, time of N application had no significant (P < 0.05) effect on agronomic efficiency of mineral N application in soybean. There was also

significant (P < 0.05) combined effect of the inoculation and rate of N application on agronomic efficiency of mineral N in soybean at Bunglong.

Table 4.9: Agronomic efficiency of mineral N fertilizer Agronomic efficiency of mineral N (kg kg^{-1} N/ha) Treatment

	Bunlong	**Puriya**
Inoculation (%) Un-inoculated	15.00	19.00
Inoculated	5.00	25.00
Pr (I)	0.04	NS
LSD (0.05)	9.50	29.50
Nitrogen rates (kg/ha) 0	0.00	0.00
10	7.00	38.00
20	23.00	28.00
Pr (N)	<0.001	0.01
LSD (0.05)	6.60	23.10
Time of N application Starter N	14.00	21.00
Late N	6.00	23.00
Pr (T)	0.03	NS
LSD (0.05)	7.10	15.60
Pr (I x N)	0.03	0.83
Pr (I x T)	0.51	0.53
Pr (N x T)	0.28	0.38
Pr (I x N x T)	0.18	0.75
C V (%)	116.60	118.30

Table 4.10 Combined effects of inoculation and nitrogen rate at Bunglong

	N rates (kg/ha)		
Inoculation			
	0	10	20
un-inoculated	0	14	32
Inoculated	0	0	14
Pr (Ix N)	0.03		
LSD	10.1		

CV	60.4

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effect of inoculation, nitrogen rates and time of nitrogen application on plant height.

At Puriya and Bunglong, inoculation rates, nitrogen rates and time of nitrogen application had no significant influence on plant height of soybean (Table 4.1). The nonsignificant effect of inoculation on soybean height could probably be due to the competitiveness of the indigenous rhizobia in the soils at both locations which outcompeted the inoculated rhizobium isolate, even though the native rhizobia population in the experimental fields was not assessed. The present findings of this study on the effect of inoculation on soybean corroborates the findings of Rudresh *et al*. (2005) who made similar observation but contradicted the findings of Amani (2007) and Caliskan *et al*. (2007) who reported that plant height increases with application of inoculants together with nitrogen fertilizer. This non significant effect of nitrogen rates on plant height in this study could also be attributed to lower rates of nitrogen used. The present study contradicts the findings of Ali *et al*. (2013) who recorded significant effect of nitrogen application on plant height of chickpea with the application of 100 kg urea / ha and that of Hassan (1981) that increasing mineral nitrogen increased the height of soybean plants.

The insignificant effect of time of N application on height of soybean could probably be due to the prolong drought experienced at V1 and R4 stages. The results of time of application of nitrogen on soybean is in line with the observation made by Wood *et al*. (1993) who researched on nitrogen fertilizer application/timing in Alabama and recorded non-significant effect on plant height.

5.2 Effect of inoculation rates, nitrogen rates and time of nitrogen application on nodulation.

Inoculation rates and nitrogen rates had no significant influence on number of nodules and weight of nodule of soybean at Puriya and Bunglong (Table 4.3). The lack of response of

nodulation to inoculation could be attributed to the setting in of nodule senescence at the reproductive growth stage of soybean (Ramos *et al*. 2003), which increases to a peak at the R5 growth stage. Conversely, the adverse effect of the inoculation rate on the crop could also be attributed to moisture stress and drought conditions which led to the reduction in nodule weight. This phenomenon agrees with the findings of Ramos *et al*. (2003) that the nodule cell wall begins to lose form which leads to nodule senescence of bacteriods after the exposure of the nodules to moisture stress for 10 days. The higher figures recorded in the non-inoculated treatment comparatively to the inoculated plots at Bunlong could probably be due to active indigenous nodulating bacteria in the experimental soil as reported by Bekere and Hailemariam (2012). However, in this study, we did not assess the initial rhizobia populations in the soil. At Puriya, the observation that inoculation had no effect on nodulation could also be due to moisture stress which was experienced at the vegetative growth stage three. The findings of this study corroborate the findings of Ladrera *et al*.

(2007) that drought stress negatively affects nodulation.

Nitrogen application rates did not significantly affect nodule number per plant at Puriya and Bunglong. The non-significant effect of nitrogen application rates on nodule number are in agreement with the findings of Cassman *et al*. (1980) and Seneviratne *et al*. (2000) that the presence or absence of nitrogen in the soil does not significantly affect nodulation of the crop. However, it significantly affected nodule dry weight at both locations probably due to the secretion of growth hormones by rhizobia and thereby improving root growth (Ahemad and Khan 2012) and uptake of nutrients. This can increase the plant chances to get soil nutrients and increasing the availability of P through solubilizing unavailable P (Zaidi *et al*. 2009).

Although, time of N application had no significant effect on dry weight of nodules at Bunlong, starter N significantly increased nodule number at both locations and also significantly affected nodule dry weight at Puriya. The greatest nodule number observed under starter N application could probably be due to lower concentration of nitrogen applied as observed by Anne-Sophie *et al*. (2002) who reported that high concentrations of mineral N inhibits the effects of mineral N on nodulation and N_2 fixation of soybean (>5 mM), but the effects are far lesser at lower concentrations. The results of this study corroborated the results of Gan *et al*. (2003) who reported that application of mineral N at vegetative growth stage one had a positive effect, which increased the soybean nodulation whereas the application of mineral N at early bloom and early pod filling stages hugely decreased the soybean nodulation.

Among the interactions, nitrogen rate and time of application significantly affected nodule number at Puriya and Bunglong.

5.3 Effect of inoculation and N management option on dry matter yield of soybean at R4 stage.

Inoculation, nitrogen application rate and time of nitrogen application did not positively influence dry matter yield at the two locations (Table 4.4). The recorded insignificant inoculation influence on dry matter yield was possibly due to its non-promotive effect on stem growth in length and width and the inability of the inoculation to significantly influence nodulation. Other results including Groppa *et al*. (1998); Shamsuddin and Ang (1999) and Bekere *et al*.(2012) have shown no significant effect of rhizobium inoculation on dry matter yield in soybean. On the contrary, Solomon *et al*. (2012) reported that the effect of inoculation of rhizobium strains significantly influenced dry matter production at mid-flowering. The insignificant influence of the nitrogen rates could probably be due to the inhibitory effect of N application on nodulation and N_2 fixation. The insignificant

influence of time of nitrogen application on dry matter yield observed in this study contradicts the findings of Gan *et al*. (2003)that early application of nitrogen at a rate of 25 kg / ha at V2 and V3 growth stages promoted the soybean plant total biomass. However, this present result confirms the findings of Panchali (2011) that N applied at the reproductive growth stages three to five could not positively influenced the plant total biomass, N concentration and the grain yield.

5.4 Effect of inoculation and N management option on yield and yield components at harvest.

The results of this investigation revealed that inoculation did not significantly affect pod number and pod weight of soybean. Several studies (Abdel-Fattah *et al*. (2011) and Bhuiyan *et al*. (2008) have however; shown that inoculating mung bean and soybean with *Bradhyrizobium* significantly increased pod number and pod weight. The insignificant effect of inoculation on pod number and pod weight observed in this study could probably be due to its non-promotive effect on growth and dry matter accumulation.

The insignificant influence of the nitrogen rates on pod weight could be due to lower rates of N applied. This result confirms the findings of Abdel-Fattah *et al*. (2011) who observed a decrease in green pod dry weight per plant when the rate of 20 kg mineral N/fed was applied comparative to higher levels.

Time of nitrogen application did not also positively influence dry weight and number of pods per plant. This phenomenon, according to Konlan *et al*. (2013) could be attributed to the adverse effects of the relatively low rainfall received and water stress at the pod filling stage.

None of the interactions positively influenced pod number and weight at Bunglong and Puriya except the combination of inoculation and nitrogen application at Bunglong. This

probably could be due to lower rate of nitrogen applied in combination with higher inoculants. The findings of this result corroborate with that of Abdel-Fattah*et al*. (2011). Soybean grain yield and hundred seed mean weight was not affected by inoculation at both locations. The lack of effect of rhizobia inoculation on yield and yield component in this present work could be due to the non-promotive effect of inoculation on nodulation. This present study is in agreement with Barsum and Abd-El-Gawad (1990) who found that the weight of seeds per pod was not significantly affected by inoculation with *Bradyrhizobium japonicum*. Similarly, Elsheikh and Ibrahim (1999) noticed non significant influence in the thousand mean grain weight with inoculation of guar by *Rhizobium*.

The rates of nitrogen applied could not positively affect the grain yield and mean 100 grain weight at Bunlong. This probably could be due to the lower organic matter content at Bunglong which was 0.57% compared to 1.72% at Puriya (Table 3.1). This result confirms the finding of Dorivar *et al*. (2009) that grain quality parameters, including mean 100 grain weight, protein, and oil concentration were not positively influenced with either inoculation or N fertilization. However, the effect of N application was positive on grain yield and 100 seed weight at Puriya. Al- Ithawi *et al*. (1980) and Touchton and Rickerl (1986) reported that nitrogen fertilizer positively affected growth and yield of soybean. The work of Wood *et al*. (1993) also confirms it, that they observed positive grain yield response to N fertilization at five locations out of seven.

Time of nitrogen application could not influence grain yield and 100 seed weight at Bunlong. Gascho (1993) and Wesley*et al*. (1998) reported that soybean with high yield potential, irrigated soybean was significantly increased when nitrogen was applied at R3 to R4 stages. However, studies by Freeborn *et al*. (2001); Schmitt *et al*. (2001) and

Gutiérrez-Boem *et al*. (2004) showed no significant effect on grain yield from the application of fertilizer N at R3 and R4 growth stages.

The effect of the application of N fertilizer to soybean at R3 in Lowa was examined and it revealed that soybean grain yield was not positively influenced by application of N fertilizer; however, plant dry matter was increased (Barkar and Sawyer, 2005).

Oplinger and Bundy (1998) and Randall and Schmitt (1998) also reported that soybean yield increases have been inconsistent with N application at early vegetative growth. Yield responses which have been measured differed with sites, the type of soil, inorganic soil N level, genotype of soybean, disease presence and growing season. Oplinger and Bundy (1998) and Randall and Schmitt (1998) concluded that soybean yield response has not been consistent with N application.

The result of this present study contradicts the findings of Wood *et al.* (1993) that N applied at R4 is the most reliable application time for increasing grain yield. Wesley *et al*. (1999) reported that on farm trials revealed that the application of N at the reproductive growth stage three (R3) resulted in yield increase in four trials. The nonsignificant effect of time of N application on yield in this present study could be associated with the insignificant number of pods per plant. This confirms the findings of Abdel-Fattah *et al*. (2011) that greater number of pods per plant and number of seeds per pod were significantly associated with high seed yield.

Among all the interactions, inoculation and nitrogen application positively influenced grain yield only at Bunlong, this probably is because of the lower total soil N at Bunglong compared to that of Puriya. The results of the present study that inoculation and N application positively influenced grain yield corroborated the findings of AbdelFattah *et al*. (2011) that inoculation of plants with *Bradyrhizobium japonicum* with the application of varied rates of mineral N fertilizer affected the reproductive growth and vegetative

parameters and grain yield per plant. Again, nitrogen application and time of application positively influenced hundred seed mean weight only at Puriya.

5.5 Effect of inoculation rates, nitrogen rates and time of nitrogen application on nitrogen fixation of soybean on two fields.

The results of the present investigation showed that inoculation, nitrogen rates and time of nitrogen application did not significantly affect the amount of nitrogen fixed by soybean at both locations (Table 4.7).The inability of inoculation to positively influence the nitrogen fixed could probably be attributed to its non-promotive effect on nodule number (Table 4.2). This present study contradicts the findings of Dorivar *et al*. (2009) that greater plant N concentration and plant total N were found with the inoculated soybean compared with the non-inoculated soybean. Regarding the nitrogen rates, its non significant effect could be attributed to its depression of nodulation because of N fertilization which is attributed to inhibition of the formation of infection threads (Dadson and Acquaah, 1984; Agha *et al*., 2004) or suppression of nitrogen fixation by nodules (Linderman and Glover, 2003) and this finding is in line with that of Dorivar *et al*. (2009) that fertilizer N application had no effect on plant N concentration or total plant N. However, Barker and Sawyer (2005) and Tewari *et al*. (2007) have reported an increase in plant N with fertilizer N application in their studies.

The non-significant effect of time of nitrogen application could probably be attributed to the negative effect that nitrogen fertilizer had on nodulation and its subsequent reduction in fixation of nitrogen. The finding of this present work is in line with the work of Beard and Hoover, (1971) that fertilizer-N application as starter N decreases nodulation and N fixation of soybean. However, it contradicts that of Diebert *et al*. (1979) who reported an increase in the amount of N fixed when the application of fertilizer-N was done in excess

of 18 kg N/acre as starter N, but application of 54 kg N/acre was required to decrease the amount of N fixed if N application was delayed. None of the interactions had positive effect on N fixed.

5.6 Correlation analysis of grain yield, growth and nodulation parameters. From the correlation analysis, it is observed that, only number of pod per plant had significant positive correlation to grain yield per hectare of soybean at both locations. The results showed a positive linear relationship between grain yield and number of pods per plant across rates of nitrogen fertilizer and it time of application. This is an indication that, the higher the number of pods per plant, the greater the grain yields. Phakamas *et al.* (2008) reported similar observation that, number of pods per plant was positively correlated with seed yield in peanut varieties. The results also confirm the report of Baligar and Jones (1997) that, legume seed yield is a function of number of pods per plant.

5.7 The effect of inoculation and N management options on agronomic efficiency of mineral N fertilizer application on two fields.

The results of this investigation revealed that inoculation had significant effect on agronomic efficiency at Bunglong but not at Puriya (Table 5.7).The observation made in this present study at Bunglong that inoculation significantly affected agronomic efficiency could be attributed to the low total N in the soil and non-significant effect of inoculation on mineral N fertilization observed at Puriya could be due to relatively higher total N in the soil as compared to that at Bunglong. The findings of this present study corroborate that of Vanlauwe *et al*. (2011) that higher soil N probably is the reason for non-responsiveness of common bean to inorganic N application**,** irrespective of rate of inoculation.

Nitrogen application rate significantly affected agronomic efficiency in soybean at both locations. High fixer legume, for instance, soybean, needs less inorganic N than the less fixer legume (Giller, 2001).The significant response of the applied N rate on yield of soybean measured at both locations could be attributed to the low mineral N fertilizer application. This present study corroborates the findings of Agaw *et al.* (2015) that the rates of N beyond 20 kg N ha^{-1} applied revealed non-significant effect on agronomic efficiency of N in common bean.

Time of N application only had significant effect on agronomic efficiency of mineral N application at Bunglong but had no significant effect at Puriya. The significant effect at Bunglong and the non-significant effect at Puriya of time of N fertilizer application on agronomic efficiency in soybean could be due to the different inherent soil fertility statuses that prevailed at both locations. The present study corroborate the findings of Jarrell and Beverly (1981) that plants grown at the lowest nutrient concentrations will inevitably have the highest utilization quotient because of dilution effects. Similarly, Minotta and Pinzauti (1996) reported the highest nitrogen use efficiency of peach at low fertile soil when compared with those obtained from higher fertile soil.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

From the results of this study, the following conclusions could be made:

1. Inoculation, rate and time of mineral N application had no effect on nodulation and BNF in soybean at the two locations.
2. Inoculation did not affect growth and grain yield of soybean at both locations. The mineral N rate (0, 10 and 20 kg N/ha) and time of application did not significantly affect growth at both locations. However, the mineral N fertilizer application increased grain yield of soybean at Puriya but not at Bunglong; with the 20 kg N/ha having the higher yield increase. It could be concluded that applying 20 kg N/ha could increase yield in soybean production.
3. Agronomic efficiency of mineral N fertilizer application in soybean production was significant; obtaining optimum yield from the N fertilization at the rate of 20 kg N/ha at Bunglong and 10 kg N/ha at Puriya. It could therefore be concluded that the application of mineral N fertilizer at the rate of 10 or 20 kg N/ha could be efficient in soybean production in similar agro-ecological zones in Ghana.

6.2 Recommendation

In relation to the inconsistencies in the results at both locations, it is recommended that further trials involving local genotypes or varieties of soybean may be conducted to get comprehensive data, improving the understanding and to come up with sounder conclusion since measured yield response varies with soybean variety. Furthermore, because of the erratic nature of rainfall these days due to changing climate, it is further recommended that

such trials or experiment should be irrigated since moisture stress affects soybean productivity.

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APPENDICES

Appendix 1. Rating of soil chemical properties

Soil nutrient (mineral) content	Rating
Organic Matter (%)	
< 1.5	Low
1.6 - 3.0	Moderate
> 3.0	High
Nitrogen (%)	
<0.1	Low
0.1 - 0.2	Moderate
> 0.2	High
Phosphorus, P (mg kg^{-1}) - Bray's	
No. 1	
<10	Low
10 - 20	Moderate
> 20	High
Calcium, Ca (cmol $_{(+)}$ kg^{-1}) Mg	
< 5	Low
10 - 20	Moderate
> 10	High
Exchangeable Potassium (cmol $_{(+)}$ kg^{-1})	
< 0.2	Low
0.2 - 0.4	Moderate
> 0.4	High

From Crop and Soil Research Institute (CSIR)

Appendix2. Correlation coefficient of some selected parameters

	Parameters †	2	3	4	5	6	7
				BUNGLONG			
1.	Grain yield	0.02	0.47***	0.09	-0.1	0.08	0.01
2.	Dry mt yield		0.02	-0.31	0.02*	-0.06	0.97***
3.	Numb of pod/plt			-0.03	-0.15	0.32	0.01
4.	Nod numb/plt@R5				0.32*	-0.29	-0.34
5.	Nod dry wt/plt@R5					-0.1	-0.02
6.	Nod numb/plt@flwg						-0.03
7.	N fixed						
				PURIYA			
1.	Grain yield	0.15	0.35*	0.19	0.17	0.42**	0.02
2.	Dry mt yield		0.12	-0.04	-0.19	-0.07	0.96***
3.	Numb of pod/plt			-0.3	-0.28	0.36***	0.06
4.	Nod numb/plt@R5				0.85***	0.64***	-0.06
5.	Nod dry wt/plt@R5					0.5***	-0.21
6.	Nod numb/plt@flwg						-0.13
7.	N fixed						