EVALUATION OF NITROGEN FIXATION POTENTIALS OF SOME SOYBEAN GENOTYPES AND THEIR RESIDUE NITROGEN EFFECTS ON SUCCEEDING MAIZE CROP

BY

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SEPTEMBER, 2015

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DEPARTMENT OF CROP AND SOIL SCIENCES

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A 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. This is in partial fulfillment of the requirement for the award of Master of Philosophy Degree in Agronomy

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SEPTEMBER, 2015

DECLARATION

I hereby declare that this submission is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

……. **…….**

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Certified by

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(Head of Department)

DEDICATION

This Thesis is dedicated to my loving and supporting family who have always stood beside me and kept me in their thought and prayers throughout all my endeavours. I would also like to dedicate this work to my husband with whose financial support this work has become a success.

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ABSTRACT

An experiment was conducted at CSIR- Crops Research Institute of Ghana to determine the nitrogen fixation potentials of some soybean lines and varieties and subsequently, their residue nitrogen effects on a succeeding maize crop. The experiment was a randomized complete block design with four replications. The varieties were 'Anidaso', 'Nangbaar', 'Salentuya 1', 'Jengumah' and 'Quashie'. Soybean lines used were TGX 1990-5F, TGX 1987-62F, TGX 1989-20F, TGX 1904-2F and TGX 1990-8F. 'Obaatampa' maize variety was used as the reference crop. Data collected were plant height, number of primary branches, number of leaves, nodule count, nodule dry weight and effectiveness, number of pods per plant, number of seeds per pod, hundred seed weight and grain yield per hectare for soybean. Data collected on maize were plant height, stem girth, shoot dry weight, number of leaves, number of cobs per plant, number of seeds per cob, hundred seed weight and grain yield per hectare. The data were analyzed using ANOVA and means separated by LSD ($P< 0.05$) using GENSTAT. The results showed that all the soybean varieties and lines nodulated freely with the naturalized cowpea rhizobia. N fixation differed significantly ($p<0.05$) among the varieties and the Anidaso variety fixed the greatest amount of 59.1 kg N/ha. However, soybean grain yield was not the greatest in this variety. Again, the Anidaso variety left the largest amount of 14.3 kg/ha of N in its residue for succeeding crop. Maize grain yield results showed that applying the traditional fertilizer recommendation (100 kg N/ha of NPK and top dressing with ammonia fertilizer) was not significantly different from incorporating the soybean residue without any fertilizer application. The results indicate that farmers can reduce their cost of producing maize by incorporating soybean residue instead of fertilizer application.

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CHAPTER ONE

1.0 INTRODUCTION

Soybean (*Glycine max* (L) Merrill) is a species of legume native to East Asia, which is widely grown for its edible bean and several other uses. The world annual production as at 2010 was estimated at 261.6 million metric tonnes with the USA, Brazil and Argentina being the largest world producers (FAOSTAT, 2012). Nigeria is the leading producer of soybean in West Africa with 393,860 metric tonnes (FAOSTAT, 2012), and Ghana producing about 124,045 metric tonnes (MOFA, 2011).

Approximately, the protein content in soybean is 40% with oil content being 20%, ultimately making it the crop with the highest protein content and the largest gross output of vegetable oil among the cultivated crops in the world (FAOSTAT, 2009). In Ghana, it is ranked third in terms of production and utilization, after groundnut and cowpea among the grain legumes (MOFA, 2011). As a result of its nutritional value, there is an increasing demand for the crop for the production of soybean cake in the poultry industry and the oil industry as a raw material (MOFA and CSIR, 2005). It is also a source of essential amino acid and rich in minerals which improve the nutrient requirement of both humans and animals. Its oil and protein are of low cholesterol content as compared to those of animal sources such as meat, egg and milk, making it the edible oil of choice in Ghana (Adu-Dapaah *et al*., 2004).

Furthermore, soybean as a leguminous crop has the ability to fix atmospheric nitrogen for its own use and for use by succeeding crops in crop rotation, thereby precluding the need or cutting down the amount of nitrogen fertilizers applied to farmlands (Dadson and Noureldin, 2001). Cereal production in Ghana, especially maize is produced

predominantly by small holder resource poor farmers under rain-fed conditions (SARI, 1996), with low soil fertility and low application of external inputs being the two main reasons for low productivity. Continued research into soil fertility improvement by managing soil nutrients, especially nitrogen which is usually limiting is very essential. Much of the nitrogen in plants is used in chlorophyll molecules, which are essential for photosynthesis and further growth (Smil, 2000). Nitrogen gas (N_2), accounting for about 78% of all the gases in the atmosphere, is the largest constituent of the earth's atmosphere, yet this form of nitrogen is relatively inert and unusable by plants (Nancy and Porter, 2014). This form of nitrogen must be fixed or processed into useable forms for the plant. This fixation can be done either by chemical processing or by natural fixation involving bacterial conversion, which is either free living or symbiotic bacteria known as diazotrophs (Moir, 2011). Symbiotic nitrogen-fixing bacteria such as rhizobium usually live in the root nodules of legumes family known as fabaceae. Examples of species of this family include peas, alfalfa and soybeans. They do so by forming a mutualistic relationship with the plant; thus the rhizobium producing ammonium which is useable to plants in exchange for carbohydrates (Smil, 2000).

Approximately 30% of the total nitrogen fixed today is produced industrially using the Haber-Bosch process (Smith *et al*., 2004), which uses high temperatures and pressures to convert nitrogen gas and hydrogen source into ammonia, (Smil, 2000). The process by which nitrogen gas (N_2) is converted into ammonium (NH_4^+) is known as nitrogen fixation (Postgate, 1998), and it also occurs naturally in the air by means of lightning (Edwin, 1919). Industries today use the Haber-Bosch process to reduce atmospheric nitrogen into chemical fertilizers for use in conventional agriculture, which is a complex

process that requires large input of energy to proceed (Postgate, 1982). Consequences arising as a result of using this approach include using fossil fuels for the energy needed to produce this fertilizer, the resulting carbon dioxide emissions and pollution from burning these fuels, which have adverse effects on human health (Vitousek, 1997).

There has been a trend of decreasing concentration of minerals (such as iron, zinc, copper and magnesium) in many foods over the last 50 to 60 years as indicated by scientists, which have been attributed to intensive farming practices including the use of inorganic fertilizers (Thomas, 2007). Hubcap and Clemson (2012) have stated that over-fertilization of a nutrient can be as detrimental as under fertilization; thus the occurrence of fertilizer burns resulting in drying out of the leaves and damage or even death of the plant. Another report indicated that production of ammonium consumes about 5% of global natural gas consumption, which is somewhat under 2% of world energy production (IFA statistics, 2002).

A high contribution to climate change by carbon dioxide, methane and nitrous oxide has well been documented, which are produced during the manufacture of nitrogen fertilizer (Wood and Cowie, 2004). Increasing pest problems by increasing the birth rate, longevity and overall fitness of certain agricultural pests have also been associated with excessive application of nitrogen fertilizer (Jahn, 2004; Jahn *et al.*, 2001). There is also the issue of financial incentives to purchase and the capacity to acquire and use fertilizers in sub-Saharan Africa (Reardon *et al*., 1995; Reardon *et al*., 1999), which could be probably due to poverty status of most peasant farmers and insufficient education on fertilizer usage in this sub-region. Aside the nitrogen effects of rotating legumes with cereals, there are other beneficial effects which include, the breaking of cereal pest and disease cycles

(Francis and Clegg, 1990), soil structure improvement (Peoples and Craswell, 1992) and enhanced phosphorus availability through secretion of enzymes and acids in the legume rhizosphere (Schlecht *et al.,* 2006), and enhanced arbuscular mycorrhizal colonization (Harinikumar and Bagyaraj, 1988).

Since legumes are used in crop rotation systems in order to improve yield of succeeding crops, there are a number of questions; thus how much nitrogen they can fix and if the amount fixed into the soil is significant for agricultural production (Dadson and Noureldin, 2001). Boddey (1987) suggested that measuring the amount of nitrogen fixed into the soil is the most important thing one needs to do in other to answer the above questions.

It has been indicated by Stephen *et al.*, (2006) that maize is a cereal crop which is ranked first in the world in terms of its importance and seed production. In Ghana, it has been noted to be the most widely consumed staple food for the majority of the people which is an important source of carbohydrate, proteins, vitamins and minerals. It is being consumed in the forms of porridges, milled into flour for preparation of some local delicacies and grains boiled or roasted for eating. It therefore forms a very important component of our diets. Its production is however being challenged by low soil fertility problems, which has consequently led to decreased productivity. Soil fertility management programs, particularly application of fertilizers in tropical agriculture has the potential to dramatically increase production due to highly weathered soils and the limited reserves of nutrients (Stewart *et al.,* 2005). Nitrogen fertilizer rates of 90 kg ha to 150 kg ha as reported by (Akmal *et al*., 2010) has increased maize seed yield. Higher dry

matter production was also observed by Abbas *et al.* (2003) to be due to nitrogen fertilizer application.

However, issues of availability and affordability serve as a hindrance to peasant farmers in sub-Saharan Africa, as the removal of subsidies on fertilizer products tend to increase their costs. Considering the numerous challenges (cost, availability and environmental concerns) associated with fertilizer N application, there is the need for a replacement with an alternative source of N, such as biological nitrogen fixation (BNF), which is a more cost effective and sustainable source of N. If BNF is to replace N fertilizer, there is the need to evaluate the N fixation potentials of available legumes and accurate determination as to how much N the legumes will leave for subsequent cereal, especially maize production.

The main objective of this study was to determine the N-fixation potential of some selected improved soybean varieties and lines and the amount of residue N for profitable maize production. The specific objectives were to;

I. Determine the nitrogen fixing ability of selected improved soybean varieties.
II. Determine effect of nitrogen fixation on soybean growth and grain yield.
III. Determine the amount of nitrogen left in the crop residue for succeeding maize crop and to;
IV. Determine the sufficiency of the residue nitrogen for profitable maize production.

The above objectives were formulated based on the hypothesis that:

- Soybean variety will affect nitrogen fixation
- Nitrogen fixation will improve maize grain yield

CHAPTER TWO

2.0 LITERATURE RIVIEW

2.1 ORIGIN AND DISTRIBUTION OF SOYBEAN

Generally, scholars across the world agree that soybean originated from China. First the annual wild soybean (*Glycine soja*), the kindred ancestor of the current cultivated soybean (*Glycine max*), is found throughout China. *Glycine soja* distribution is limited to China, Japan, Korea and the Far East Russia in East Asia, but its distribution in China is the most extensive, its numbers the largest and its diversity of types the richest (Qui and Chang, 2010).

Fukuda (1933) thought that the origin of soybean is northeast China, based on the observations that semi-natural wild soybeans are extensively distributed in northeast China but not in other regions. He further stated that the extensive distribution of these wild soybeans in northeast China with only a few in other regions might well be influenced by differences in efforts to investigate and collect materials. Hymowitz (1970) also thought that the origin of soybean was the eastern part of northern China, which he referred to as winter wheat (*T. aestivum*), whereas Wang (1985) studied the origin of soybean by using ancient Chinese literature, inscriptions on bones and tortoise shells of the Shang dynasty based on which he also concluded that the earliest region for cultivating soybean was around the middle or downstream of the Yellow valley, which was seconded by Chang (1989) based on his study of the relationship between the origin of agriculture and the origin of soybean.

Literature related to soybean in past dynasties of China was collected by Guo (1993) who analyzed the arguments related to the origin of soybean and concluded that the

origin of cultivated soybean is northeast China, but the exact origin of soybean remain unknown and therefore thought that these arguments are not conclusive. Asia has the longest history of growing soybean, with China having the largest cultivated area of the crop. Japan, North and South Korea, Indonesia are some of the countries that cultivate soybean. In Japan, most soybean varieties are large-seed types and are used as vegetable soybean which is called 'edamame'. A 100 fresh seed weight is greater than 70g and the dry weight greater than 30g, whereas in South Korea, varieties are small seed types; thus 100 seed weight is less than 15g (Qui and Chang, 2010).

Reports by Hymouitz (1984) showed that soybean was cultivated in the USA as early as 1765, when Samuel Bowen, a sailor from the east India Company brought soybean from China to Savenna (Georgia). The USA twice sent scientists to China, Korea and Japan to collect soybean germplasm and several thousand accessions of soybean were collected from these countries, which have become the primary parents of soybean breeding in the USA.

In Africa, it was first introduced in 1857 (Shuttleff and Aoyagi, 2012) and later introduced by the Portuguese missionaries into Ghana in 1910, with major growing areas being, Bawku, Nakpanduri, Bimbilla and Karaga. The main problem facing farmers at that time was loss of seed viability during storage (Plahar, 2006).

2.2 PRODUCTION AND USES

The USA, Brazil and Argentina are ranked the first three leading producers of soybean in the world with the USA producing 72.86 million tonnes, Brazil 57.85 million tonnes and Argentina 47.48 million tonnes in 2007 (FAOSTAT, 2009). Africa has a small cultivated

area as compared to other countries, with Nigeria having the largest area, followed by South Africa, Uganda, Zimbabwe, Congo, Zambia and others. Also, there is a great potential for soybean development in Africa and therefore needs help and support from leading producing countries, not in terms of production alone but processing and utilization as well (Qui and Chang, 2010). In Ghana, the mean acreage under soybean cultivation is 3.4 acres per farmer (Plahar, 2006).

The consumption of soybean in China was about 44 million tonnes in 2006, but the country produced 15.50 million tonnes only, which led to the importation of 28 million tonnes of soybean from America. Currently, the industrial chain has developed with the economy of the country and products being changed from crude oil and bean meal to high-value-added products with the main products being bean curd, bean curd stick and bean curd cheese. In the USA, production has developed rapidly and in recent years, the cultivated areas of soybean in the USA have grown to more than 28 million hectares, which produces more than 80 million tonnes. In 2000, the production of soybean in Brazil and Argentina were 32.72 million tonnes and 20.21 million tonnes respectively and have recently reached more than 60 million tonnes and 40 million tonnes respectively (Qui and Chang, 2010).

Soybean is mainly processed to extract soybean oil while the seed which is rich in protein remains. Its oil can be used for the production of edible kitchen oil, salad oil and also printing ink and biodiesel through refining and deep processing. It is the main source of protein in livestock farming, which is normally used in the production of compound feed. It is high in proteins which are added to various foods in the processing industry. Wheat flour for instance is supplemented with a certain amount of soybean protein for the

production of bread. It can also be used to process protein fiber, which can be blended with cotton, wool or chemical fibers, with the resulting fabric being soft and of a high quality. Many soybean food products including both the fermented and non-fermented can be processed by using soybean as a raw material, and such foods include soybean milk, bean curd, soy paste, soybean cheese, soy sauce and others (Qui and Chang, 2010).

In Ghana, there is promotion of the crop by Ministry of Food and Agriculture due to its nutritional and economic values (Sarkodie-Addo *et al.,* 2006). It is used in processing soy meat, baby foods and dawadawa, which is used for seasoning stews and soups (Abbey *et al,* 2001). Several traditional delicacies such as gari, sauces, stew, soups, banku and kenkey are being fortified using soybean to improve their nutritional values (MOFA and CSIR, 2005).

2.3 IMPORTANCE OF NITROGEN TO CROP PRODUCTION

Nitrogen is one of the most important nutrients for crop growth, and also a critical limiting element for plant growth and production. This is so because it is a major component in chlorophyll, the most important pigment needed for photosynthesis as well as amino acids, the key building blocks of proteins (Wagner, 2011). Although it makes up 78% of the atmosphere, it is not directly available for use by plants, but is directly used in nitrogen fixation and industrial fertilizer manufacturing. It is used by plants in the form of nitrate or ammonium ions through the roots to produce protein (in the form of enzymes) and nucleic acids and readily transported from older to younger tissues, which account for the reason why a plant deficient in nitrogen will show yellowing in the older

leaves first , due to the destruction of chloroplasts and an absence of the green pigment chlorophyll (Charles Sturt University, 2014).

Cassman *et al.,* (2008) reported that nitrogen is often the most limiting factor in crop production and as a result, the application of fertilizer nitrogen results in higher biomass yields and protein yields, and concentration in plant tissue is commonly increased. They further stated that abundant supply in cereals decreases the relative proportion of lysine and threonine, thus reducing the biological value of the protein.

Other works by Tsai *et al.,* (1983) showed that protein concentration of corn grain increases with nitrogen supply due to preferential deposition of zein over other endosperm proteins, which correlated with the findings of Rendig and Broadbent (1979) that concentration of the protein fraction zein in corn grain was closely associated with the level of soil nitrogen, with each increment of nitrogen increasing the percentage of zein.

According to Rhykerd and Noller (1974), nitrogen is the most limiting factor for grassland productivity; hence its application profoundly affects grassland systems in many ways. Nitrogen fertilization, they noted at low rates increases forage yield with little effect on forage nitrogen. Increased water concentration and decreased soluble carbohydrates are commonly observed after nitrogen fertilization (Messman *et al*., 1991; Brink and Fairbrother, 1992).

Soybeans (*Glycine max* (L) Merrill) and groundnuts (*Arachis hypogaea* L) which are both nodulating legumes are still responsive under many conditions to increasing nitrogen levels in terms of yield, whereas the oil content appears to be less negatively impacted by nitrogen rates (Bishnoi and Dutt, 1980; Pawar *et al*.,1982; Abdel-Wahab *et*

al.,1988; Nagre *et al*.,1991). Meanwhile, several reports of decreasing oil concentration at higher nitrogen rates have been documented (Hassan *et al*., 1985; Jadhav *et al.*, 1994).

Inadequate supply of available nitrogen frequently results in plants that have slow growth, depressed protein levels, poor yield of low quality produce and inefficient water use (Mikkelson and Hartz, 2008) and further leads to greater disease susceptibility as compared to properly nourished plants. Excessive nitrogen can however be detrimental for crop growth and quality in addition to causing undesirable environmental impacts.

Typically, stunted and slower growth of plants characterizes insufficient nitrogen, and this also leads to the reduction of the amount of protein in the seed and plant. Also, the standability of crops as grain fill occurs tend to be affected by nitrogen deficiency, as a deficient plant will absorb nitrogen out of the leaves and stalk for grain fill and consequently weaken the stalk, causing standability problems (Nachurs, 2010). Soil nitrogen management will therefore be part of soil fertility programme which can lead to increased efficiency and profitability for the farmer.

2.4 SOURCES OF NITROGEN

Nitrogen is a key component in plant proteins and chlorophyll, thereby essential in photosynthesis. The need to know the various sources of nitrogen to crop production is therefore important as it will play a role in managing soil nitrogen for efficient and profitable productivity. Nitrogen fixation by legumes is one of the natural sources of nitrogen, thus some plants are able to manufacture their own nitrogen if they are colonized by rhizobium bacteria. This is typical of the plants from the legume family. This is as a result of the symbiotic association between the bacteria and the roots of the

legume plant, whereby the bacteria utilizes the sugars around the roots of the legume plant as an energy source and in turn fix nitrogen by converting nitrogen gas from the atmosphere into useable forms, such as ammonia for the plants use (Cassman *et al.,* 2008).

Mineralization of organic matter such as soil organic matter, cover crops and compost is essential to avoid deficiency of available nitrogen. Rates of mineralization depend on environmental factors such as temperature, soil moisture, the properties of the organic material, (such as C: N ratio, lignin content) and placement of the material (Mikkelson and Hartz, 2008).

Commercial fertilizers are also obtained from the atmospheric nitrogen pool which is about 78% of all the gases in the atmosphere. This is done through the Haber-Bosch processes, whereby nitrogen gas and hydrogen gas are combined to form ammonia (NH_3). This anhydrous ammonia is then used in the manufacture of other nitrogen fertilizers which can then supplement other nitrogen sources for crop nutrition (O'Leary *et al.,* 2002).

Poultry manure and other animal waste products were used as a source of supplemental nitrogen long before inorganic nitrogen fertilizer came into popular use. These, including composted plant residue continue to be used today as sources of nitrogen, especially by organic crop producers (Cassman *et al.,* 2008). Sodium nitrate is also mined from naturally occurring deposits in Chile and Peru, the location of the driest deserts on earth where nitrate salts accumulate over time, which is readily soluble when added to soil to meet the nitrogen demand during critical plant growth stages and not to meet the entire nutritional need of the crop (Mikkelson and Hartz, 2008).

2.5 CHALLENGES OF NITROGEN FERTILIZER APPLICATION

Progress in agricultural productivity in sub-Saharan Africa during the past several decades lags far behind than in other regions of the world and is well below that required to meet sub-Saharan Africa food security and poverty reduction goals, which is not surprising considering the less favorable agro-ecological conditions, lower investment in irrigation and much lower use of fertilizer (Kelly, 2006). While the expansion of cultivation area has slowed down considerably due to the increasing scarcity of uncultivated land, the population in sub-Saharan Africa still continues to grow rapidly which has led to the decline in food production per capital, with the likeliness of increasing food shortages in the near future if the trend continues (FAO, 2005). This according to the report makes it necessary for adoption of fertilizers, as rice and wheat yields in Asia began to grow dramatically which is partly due to the adoption of fertilizer-responsive rice and wheat varieties.

Fertilizer is any organic or inorganic material of natural or synthetic origin (other than liming materials) that is added to soil to supply one or more plant nutrients essential to the growth of plants (Wikipedia, 2014). By this, the additions of fertilizers to soils have the tendency to improve productivity, especially in sub-Saharan Africa where food insecurity is likely to hi,t as a result of our ever-increasing population and unfavorable farming conditions. Stewart *et al.* (2005) reported that 30-50% conservative estimates of crop yields are attributed to natural or synthetic fertilizer, with global market value likely to rise to more than US$185 billion until 2019 (Ceresan, 2013).

Significantly, inorganic fertilizer has supported global population growth with almost half the population on earth currently fed as a result of synthetic N fertilizer use (Sutton

et al., 2008). These fertilizer, especially inorganic nitrogen fertilizers, aside their contributions to reducing global hunger have been associated with several problems which if not taken care of , would pose danger to our environment and subsequently our health.

Firstly, nitrates in fertilizers can cause problems for our natural habitats and for human health if they are washed off soil into water courses or leached through soil into ground water (Debra, 2014). This is classified in the USA as non-source pollutants due to inability to quantify the amount entering water bodies and shallow aquifers (EPA, 2013). Nitrogen fertilizers can further be converted by soil bacteria into nitrous oxide, a greenhouse gas, not forgetting methane and carbon dioxide which are produced during the manufacturing of nitrogen fertilizers.

The breakdown of the symbiotic relationships between plants roots and mycchorizal fungi have been associated with high levels of fertilizer (Caroll and Salt, 2004), which disrupts the numerous mycchorizal benefits that the plant gains from this association. These include; taking up deep seated nutrients especially phosphorus for the plants and water deep down the depletion zone, thereby reducing drought and making plants roots able to resist soil pathogens as a result of it serving as a cover for the root surface.

A report by New York Times (2008) showed that nitrogen-rich compounds found in fertilizer runoffs are the primary cause of serious oxygen depletion in many parts of the ocean, especially in coastal zones, which subsequently reduce the ability of these areas to sustain oceanic fauna. These same compounds according to a report by (soil.scijiunals.org, 2014) can cause soil acidification when added and may lead to decreases in nutrient availability which may be offset by liming.

Ogburn (2010) observed that the high temperatures and very high pressures needed to transform N_2 into NH_3 are energy intensive, with about 1% of the world's annual energy consumption being used to produce ammonia, most of which becomes nitrogen fertilizer; thus about 80million metric tonnes of annual global CO_2 emissions.

The need to subsidize fertilizer costs by government in sub-Saharan Africa (SSA) also poses a problem, as governments and donors in sub-Saharan Africa have strong resistance to using subsidies to speed up adoption of conservation practices, which have the potential to improve productivity and reduce poverty for the majority of SSA farmers, who are unlikely to gain access to land with controlled irrigation (Kelly, 2006). This same report suggested that high transportation costs at parts and poor road infrastructure contributes to high fertilizer costs. This deters farmers in SSA to purchase fertilizer, as a result of low income, poor access to credit and lack of marketing power.

Moreover, the biggest challenge facing extension services at present is the development of a strategy to inform farmers about available technologies and increase farmer's capacity to evaluate and adopt the most appropriate technology for their situation from available ones. These problems if properly dealt with would help improve the capacity of farmers in SSA to use fertilizer (Kelly, 2006).

Desai (2002) noted that the fertilizers economic potential in developing countries, which is determined by the prevailing fertilizer responses and prices is almost always much larger than actual use. This means, the outcome of the conversion of fertilizer's economic potential into farmers' effective demand and the fulfillment of this demand through fertilizer supply and distribution systems is consumption (Desai, 1988).

2.6 BIOLOGICAL NITROGEN FIXATION

According to Wagner (2011), nitrogen is a critical limiting element for plant growth and production. Other biomolecules such as ATP and nucleic acids also have nitrogen incorporated in them. This nitrogen, although abundant in the atmosphere is relatively inert. Plants can only utilize reduced forms of this element through; 1) the addition of ammonia and nitrate fertilizer, 2) the release of these compounds during organic matter decomposition, 3) the conversion of atmosphere nitrogen into compounds by natural processes such as lightning, and 4) biological nitrogen fixation (Vance, 2001).

Karanja *et al*. (2014) suggested that nitrogen as an essential element for plant growth and development is a key issue of agriculture, as most studies indicated that nitrogen fertilizers contributes to resolving the challenge the world is facing in feeding the human population. An enormous increase in the application of nitrogen fertilizer accompanied the green revolution, with a high heterogeneity of its distribution throughout the world; some areas subjected to pollution whereas others to depleted soil, decreased crop production and other consequences of inadequate supply. The same report further indicated that BNF is a key to sustain agriculture and reduce soil fertility decline, and therefore encouraged research on micro-organisms and plants able to fix nitrogen contributes largely to the production of bio-fertilizers. Thus it is important to ensure that BNF research and development will take into account the needs of farmers mainly in the developing countries.

BNF was discovered in 1901 (Beijerinck, 1901) and is carried out by a specialized group of prokaryotes. These organisms are able to utilize the enzyme nitrogenase to catalyze the conversion of atmospheric nitrogen to ammonia, which plants can easily assimilate to

produce the needed nitrogenous biomolecules. Such prokaryotes include aquatic organisms such as cyano-bacteria, free-living soil bacteria such as Azotobacteria, bacteria that form associative relationships with plants, such as Azospirillium, and most importantly, bacteria, such as *Rhizobium* and *Bradyrhizobium*, which form symbioses with legumes and other plants (Postgate, 1982).

BNF occurs when atmospheric nitrogen is converted into ammonia by an enzyme called nitrogenase (Postgate,1998) and the microbial genes required for nitrogen fixation are widely distributed in adverse environments (Gaby and Backley, 2011). The reduction of atmospheric nitrogen is a complex process that requires a large input of energy to proceed (Postgate, 1982), as the nitrogen molecule is composed of two nitrogen atoms joined by a triple covalent bond; thus making the molecule highly inert and non-reactive. Microorganisms that fix nitrogen require 16 moles of adenosine triphosphate (ATP) to reduce each mole of nitrogen (Hubbell and Kidder, 2009) and they obtain this energy by oxidizing organic molecules. Non-photosynthetic free-living microorganisms must obtain these molecules from other organisms, while photosynthetic micro-organisms such as cyanobacteria use sugars produced by photosynthesis, with associative and symbiotic N-fixing micro-organisms obtaining theirs from their host plant's rhizospheres (National Research Council, 1994; Hubbell and Kidder, 2009).

Micro-organisms fix nitrogen symbiotically by partnering with a host plant. The plant provides sugars from photosynthesis that are utilized by the N-fixing microorganisms as a carbon source and the microbe in turn provides fixed nitrogen to the host plant for its growth. This whole process begins when the bacteria colonize the host plants root system by being attracted to flavonoids released by the host legume's roots. The bacteria then

begin to attach themselves to epidermal cells called root hairs, and the host legume in turn senses chemicals produced by the rhizobia called nod factors, that cause the colonized root hairs to curl and form the shepherd's crook. The rhizobia then penetrate the root hairs and form a tabular structure called an infection thread, and once the bacteria reach the root itself, they stimulate cortical cell divisions that lead to the formation of a nodule. As the nodule begins to form, the bacteria become surrounded by a plant derived membrane and are released inside plant cells forming the nodule. The bacteria subsequently lose their cell wall and undergo a profound change in cell morphology to form large irregularly shaped branching cells called bacteroids and then they are entirely dependent on the host plant for their energy needs and in return fix nitrogen for the plant (Wagner, 2011).

BNF for more than 100 years have commanded the attention of scientists concerned with plant mineral nutrition and has been exploited extensively in agriculture (Burris, 1994; Dixon and Wheeler, 1986). Its importance as a primary source of nitrogen for agriculture has, however, diminished in recent decades as increasing amounts of fertilizer nitrogen have been used for the production of food and cash crops (Peoples *et al.,* 1995).

International emphasis on environmentally sustainable development with the use of renewable resources is however likely to focus attention on the potential role of BNF in supplying nitrogen for agriculture (Dixon and Wheeler, 1986; Peoples *et al*., 1995). Moreover, the expanded interest in ecology has drawn attention to the fact that BNF is ecologically benign and its greater exploitation can reduce the use of fossil fuels and can be helpful in restoration of misused lands to productivity (Burris, 1994; Sprent and Sprent, 1990).

2.7 FACTORS AFFECTING BIOLOGICAL NITROGEN FIXATION

As indicated by Mohammadi *et al*. (2012), BNF is an efficient source of fixed nitrogen that plays an important role in land remediation, with interest being focused on the symbiotic systems of leguminous plants and rhizobia, because their associations have the greatest quantitative impact on the nitrogen cycle. Estimated values of BNF for various legume crops and pasture species are often impressive, usually falling in the range of 200-300 kg N ha^{-1} per year, which is the basis for the significance of rhizobium and legume symbioses as a major contributor to BNF. N-fixation, along with photosynthesis as the energy supplier is the basis of the soil environment under a constant state of change and as such, can be relatively stressful for both macro and micro-organisms, with fluctuations in pH, nutrient availability, temperature and water status among other factors being greatly influencing growth, survival and metabolic activity of N-fixing bacteria and plant, and their ability to enter into symbiotic interactions (Werner and Newton, 2005).

While some stress factors simultaneously affect both symbiotic partners, others may differently influence each partner to a seemingly different degree by different mechanisms and therefore it is important to understand how the micro and macro-symbionts interact at cellular and molecular levels in order to properly discuss how these factors influence symbiotic N-fixation (Mohammadi *et al.,* 2012). The flavonoid gene inducers for instance are specific for a particular legume-rhizobium interaction and their productivity is influenced by environmental variables like plant fertility, pH and nod factors (Schmidt *et al*., 1994), with the specific sensitivity of the symbiotic N-fixation dependent legumes to salinity being well documented for initiation, development and function of nodules (Saadallah *et al.,* 2001).

According to Paul and Clark (1996), N-fixation depends on photosynthesis to provide ATP for energy and carbon compounds as electron donors, which consequently make both duration and rate of N-fixation dependent on both past and current conditions that influence cyanobacteria carbon balances, such as moisture, temperature, light intensity and supply of assimilates. Moreover, cyanobacteria are physiologically active only when wet, consequently making all N-fixing activities in soil cyanobacteria cynolichens ultimately controlled by moisture (Kershaw, 1985; Nash, 1996). Availability of liquid water ultimately determines the extent of N-fixation, as it requires the products of photosynthesis, with levels needed to initiate and optimize N-fixation varying widely with species, habitats and pre-collection conditions (Belnap, 2001). Nitrogen accumulation values for soil-crust cyanobacteria range from a water content of 6% dry weight to total water immersion (Jones,1977; Kershaw, 1985; Belnap *et al*., 1999), with cyanolichens inducing collema species requiring a water content of at least 80% dry weight for initiation of net carbon fixation activity (Lange *et al*, 1998).

A report by Mohammadi *et al*. (2012), showed that soil nutrient status has a tremendous influence of the symbiosis, as well as independent growth and survival of both partners, and therefore fixation tends to decrease with legume age, mainly because of the concomitant increase in soil N. A negative exponential relationship was observed between N fertilizer rate and N-fixation when N was applied to 0 to 20cm of the top soil, with differences being attributed to a variation in N supply derived from indigenous sources, such as irrigation, atmospheric deposition, net soil N mineralization and possibly other factors affecting growth and N fixation (for example, soil pH, and drought) (Ledgard and Steele, 1992).

They also indicated that phosphorus stimulated growth and increased mineral N uptake from solution without affecting the amount of N derived from the atmosphere under non-limiting N conditions. Calcium might in some cases, offset the deleterious influence of low pH on root growth and ion uptake and increase nod-gene induction and expression (Richardson *et al*., 1988) and its deficiency with or without the confounding influence of pH affecting attachment of rhizobia to root hairs and nodulation and nodule development (Alva *et al*., 1990).

Low soil pH is generally accepted as an indicator of conditions under which some other soil properties may limit crop growth rather than as a primary cause of poor growth and in addition to the direct effects of soil acidity factors on plants, growth of legumes may be reduced indirectly through inhibition of nodulation and nitrogen fixation. Rhizobia may have different tolerances to soil acidity factors than the host plant in this regard (Mohammadi *et al.,* 2012). It was further stated by Graham and Vance (2000) that more than 1.5g ha^{-1} of acid soils limit agriculture production worldwide and as much as 25% of the earth's croplands are impacted by problems associated with soil acidity. A nearly 10^{-3} decrease in the number of *S. meliloti* in soils with a pH less than 6 compared to those with a pH greater than 7 were also reported by Brockwell *et al*. (1991). Temperature was noted to have a marked influence on survival and persistence of rhizobia. As indicated by Werner and Newton (2005), cowpea rhizobial strains from the hot dry Sahel savanna of West Africa grow at 37^{o}C, and more than 90% of the strains isolated from this region grew well to 40^{0}C. Soil temperature also greatly influences competition for nodulation (Triplett and Sadowsky, 1992) and this effect may in part be due to a temperature induced delay in nodulation or the restriction of nodules to the sub-surface region. Relatively high

root temperature has also been shown to influence infection, N-fixing ability and legume growth (Hungria and Franco, 1993) and has a strong influence on specific strain and cultivar interactions (Arayankoon *et al*., 1990).

2.8 MEASUREMENT OF BNF

To ensure proper management and fully realize the benefits of the legume-rhizobium symbioses, it is necessary to be able to quantify the amount of nitrogen fixed and having measured the effectiveness of atmospheric N-fixation, the macro or micro-symbionts as well as agronomic factors can be manipulated with the objective to improve BNF. A suitable method to quantify N-fixation is therefore necessary in any program aiming at increasing N-fixation (Hardarson and Danso, 1993).

Herridge *et al*. (2008) observed that plant associated N-fixation currently contributes 50-70 million tonnes annually to the global agricultural N budget, and therefore increasing or just maintaining that level of input requires a substantial investment in fundamental research to optimize the various N-fixing systems and have them applied. They further stated that undertaking experiments to identify treatment effects on neither N-fixation, nor on-farm surveys to determine activity at a regional or country level is impossible, unless the process can be accurately and reliably quantified. Furthermore, studies have demonstrated measureable inputs of fixed N with tropical grasses such as sugarcane in the order of 10-65 kg N/ha per year, but there are few conclusive data to indicate that agronomically significant amounts of N are fixed by bacteria associated with non-legumes in temperate agriculture (Boddey *et al*., 1995).

Identification and understanding of the principal factors regulating N-fixation or managing N-fixation for the benefit of the environment or agricultural productivity is not possible, unless it can be reliably measured (Herridge *et al.*, 2008) and therefore the application of current methodologies for measuring plant-associated N-fixation in different situations is necessary. They further showed that no single accurate way of measuring N-fixation is available, since all current methodologies have limitations, and therefore measuring the exact amount of N-fixed continues to be a challenge.

There are many techniques available for measuring legume BNF in the field and in controlled environments (Goh *et al.*, 1978; Sheehy *et al.*, 1991; Herridge *et al.*, 2008; Carlsson and Huss-Danell, 2008). These methods are able to reveal the response of N-fixation to varying factors in real situations, but they are limited to the conditions prevailing at the time of measurement and cannot be used to predict N-fixation (Liu *et al.*, 2010). Among these are the N difference method, the ^{15}N isotope method and the acetylene reduction method.

The N difference method refers to the difference between the total N yield of the nodulated (N-fixing) plant and that of a non-nodulated (Non-fixing) plant preferably of the same species. The accuracy of the estimates in this method depends upon the structural and functional similarities of the two root systems; thus the two crops must have the same growth cycle, rooting habit and root system etc. in order to ensure that they take up the same amount of nutrient from the soil which is one of the principles behind this method. The other principle is that the amount of N they take up is the available soil N (Martensson and Ljungren, 1984).

The acetylene reduction method, the N-fixing system is placed within an atmosphere enriched with 10% acetylene. A sample of the system is removed after a short incubation time (1-2hrs) and the ethylene resulting from the reduction of acetylene by the nitrogenase is analyzed (Witty and Minchin, 1988).

The ^{15}N enrichment method is based on the comparison of non-fixing and nitrogen-fixing plants grown in soil to which ^{15}N has been added as labeled urea, nitrate or ammonia. The nitrogen fixing plants obtain N from two sources; air and soil and thus have a lower content in isotope ^{15}N than non-nitrogen fixing plants which absorb only labeled soil N. The percentage of the plant nitrogen derived from N-fixation is calculated from the ^{15}N atom percent excess in non N-fixing and N-fixing plants respectively (Boddey *et al*., 1995).

Adjei-Nsiah *et al.* (2008), evaluated productivity, yield and N-fixation in different cowpea varieties and their subsequent residual N effects on a succeeding maize crop. Five cowpea varieties were evaluated for grain yield, N-fixation, biomass production and contribution to productivity of subsequent maize grown crop rotation. The ^{15}N natural abundance technique was used to estimate N-fixation which ranged between 61%-77% among varieties and the resulting amount of N fixed in above-ground biomass ranging between 32 and 67 kg N ha^{-1}.

Sarkodie-Addo *et al*. (2006), also evaluated the N-fixing potentials of medium-maturing soybean lines. The N difference method was used in determining the amount of N fixed, and results showed that all the lines nodulated freely with the naturalized rhizobia in the soil and residue N varied among soybean. The amount of N fixed ranged from 70.6 to 100.5 kg/ha, which was positively correlated with total seed yield. The line that produced

the greatest amount of N and nodule dry weight was as well negatively correlated with nodule numbers. This was in accordance to reports by Giller (2001), who observed that the ability to form nodules is not enough to obtain an effective nitrogen fixation symbiosis.

Other works by Dayathilake *et al*. (2001) who evaluated the N-fixation potential of cowpea and mungbean lines and its effects on succeeding maize crop using the ^{15}N methodology, indicated that cowpea varieties derived 45-70% of their N requirements from BNF. Their results further showed that cowpea lines contributed positively towards N yield of the succeeding maize crop, while the residual effect of preceding mungbean lines was not promising and the BNF capability varied among genotypes.

The measured amounts of N fixed by symbiotic systems may however differ according to the method used to study N fixation (Sellstedt *et al*., 1993). The proportion of N derived from fixation varies subsequently from 0 to as high as 97% (Keysher and Li, 1992), with an average estimate in soybean to be 75 kg N ha-1, using average commercial yields and assuming that 50% of the N was derived from fixation (LaRue and Patterson, 1981).

2.9 FUTURE OF BNF

According to Zahran (1999), the history of BNF shows that interest generally has focused on the symbiotic systems of leguminous plants and rhizobia, because these associations have the greatest quantitative impact on the nitrogen cycle; thus a tremendous potential for contribution of fixed nitrogen to soil ecosystems exists among the legumes (Brockwell *et al*., 1995; Peoples *et al*., 1995; Tate, 1995). It has also been observed that, out of 700 genera and about 13,000 species of legumes, only a portion of about 20%

(Sprent and Sprent, 1990) have the ability to fix N, with estimates showing that the rhizobial symbiosis with the somewhat greater than 100 agriculturally important legumes contributes to nearly half of the annual quantity of BNF entering soil ecosystems (Tate, 1995).

People *et al*. (1995) observed that atmospheric N fixed symbiotically by the association between rhizobium species and legumes represents a renewable source of N for agriculture, with estimated values for various legume and pasture species commonly falling in the range of 200-300 kg N ha-^{1}. Yield increases of crops planted in rotation with legumes are often equivalent to those expected from application of 30-80 kg ha-^{1} of fertilizer, with inputs of fixed N for alfalfa, red clover, pea, soybean and cowpea estimated to be about 65-335 kg N ha-^{1} per year (Tate, 1995).

Brockwell *et al*. (1995) reported that inputs into terrestrial ecosystems of BNF from symbiotic relationship between legumes and their rhizobia amount to as least 70 million tonnes of N per year and therefore calls for the augmentation of this enormous quantity as the world's population increases and natural resources that supply fertilizer N diminish. This according to Zahran (1999) could be achieved through the development of superior legume varieties, improvements in agronomic practices and increased efficiency of the N-fixing process itself by better management of the symbiotic relationship between plants and bacteria.

Keysher and Li (1992) noted that soybean like other nodulated legumes, utilizes two sources of N for its growth; soil mineral N and atmospheric N, and has also been characterized as being rather non-responsive to the application of fertilizer N (Mengel *et al*., 1987; Scott and Aldrich, 1983) and the N requirement is the highest among

agronomic crops (Sinclair and De Wit, 1975). Herridge and Bergerson (1988) had stated that increasing the amounts of N fixed in soybean, and the portion of total plant N derived from fixation, may only be achieved with tremendous yield increases, whereas Patterson and LaRue, (1983) and George *et al.*, (1988) concluded that late maturing cultivars fix more N and yield more than earlier types due to longer reproductive phase, when rates of N fixation and seed biomass accumulation are high.

Research strategies aimed at improving BNF in legumes in general and soybean in particular has been recognized frequently (Russell *et al.* 1989; Scott and Aldrich, 1983), with soil microbiologists having extensive experience in the selection of rhizobia and bradyrhizobia for symbiotic effectiveness with legumes (Brockwell *et al.*, 1982; Burton, 1980; Date,1976). In addition to selecting highly effective strains for a given legume genotype, other important attributes were listed by Brockwell *et al.* (1982); such as competitive ability, N-fixing ability over a range of environmental conditions, nodulation and N-fixation in the presence of soil N, ability to multiply to survive in inoculant and to migrate from initial site of inoculation.

It has also been indicated by Keyser and Li (1992), that selection for improved N-fixation by the host plant include the ability to nodulate and fix N in the presence of high soil-N levels, development of soybeans with the ability to restrict nodulation by selected indigenous populations and still nodulate with effective inoculant strains and the development of soybeans that nodulate promiscuously with indigenous *Bradyrhizobium* species. Selection and breeding of soybeans for a lack of dependence on *B. japonicum* has been carried out by workers at the International Institute of Tropical Agriculture in Nigeria; thus as a result of the difficulty in production and distribution of inoculant in

parts of Africa, soybean lines which are promiscuous in their nodulation have been sought.

A report by Hardarson *et al.* (1989) showed that profuse nodulation occurred throughout the root system and nodules formed in the bottom part where roots were younger and contributed large amounts of fixed N to the soybean during seed formation when inoculant was distributed throughout the soil. Burton (1980) then reviewed the need for improved inoculant-delivery systems, but the delivery of large numbers of bradyrhizobia is a challenge, with the best systems being the soil-applied granular and seed bed sprayed inoculants to date. Other management variables that increase yield should also increase the amount of N fixed and this was emphasized by Eaglesham (1989) that N is not always the primary limiting factor and in its absence, there will not be a response to inoculation and therefore, other factors which limit soybean yield will also limit inoculation and N response.

Findings by Cassman *et al*. (1981) showed that field-grown soybean has a higher P requirements when it is dependent on BNF for its N supply as compared to mineral N dependency. This shows that phosphorus is also a common limiting nutrient in many soils, and its management is important for attaining high yields of soybean (Keysher and Li., 1992) and this was evident in a study where soybean dependent on BNF but not supplied with P attained only 28% of the maximum yield obtained at optimum P levels.

Soybean genotype well adapted to a given site is probably one of the best and simplest strategies for improving BNF, through yield improvement which of course assumes that the soybean is well nodulated with effective bradyrhizobia (Keysher and Li, 1992) and this was verified by a data from an international Soybean Variety Experiment (ISVEX)

that, the varieties may differ in their yield performance in a given environment and even at different sites in the same environmental zone (Jackobs *et al.,* 1985; Judy and Whigham, 1978). BNF therefore aims at sustaining the environment, as biologically-fixed nitrogen could be directly 'absorbed' by plants and keep the environment almost 'untouched' (Cheng, 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 EXPERIMENTAL SITE

The experiment was conducted at the CSIR-Crops Research Institute (CRI) at Fumesua in 2014. Soybean was planted in the major season, between June and September and then followed by maize in the minor season; between September and December (2014). CRI lies in the semi-deciduous forest zone in the Ashanti region of Ghana, which is characterized by sandy loam soils with latitude 6º43'N and longitude 1º31'W (Parkes *et al*., 2012).

3.2 EXPERIMENT ONE: DETERMINATION OF THE N-FIXING POTENTIALS OF SELECTED IMPROVED SOYBEAN VARIETIES AND LINES

3.2.1 LAND PREPARATION

The land was slashed, ploughed and harrowed just before the major season rains. Lining and pegging was done according to plant spacing; 75cm x 5cm for soybean. Plot length was 4m with 4 rows on each plot. This gave a plot size of 22.5m^2. The distance between blocks was 1.5 m and that within plots was 1 m. A plot of 'Obaatampa' maize variety was planted at 75 cm x 20 cm as a reference crop in each replication.

3.2.2 EXPERIMENTAL DESIGN

Five soybean varieties, five soybean lines and one maize variety were the treatments which were arranged in Randomized Complete Block Design and replicated four times, giving a total of 44 plots. A total land area of 59 m x 13.5 m was used.

3.2.3 INITIAL SOIL ANALYSIS

Soil samples were taken randomly in 4 areas on the field for laboratory analysis for the following;

3.2.3.1 Soil total Nitrogen

This was done using the macro Kjeldahl method. A 10 g soil sample was digested with a mixture of 10 g copper sulphate, 1.0 g selenium and 100 g potassium sulphate. The digestion was done using 30 ml of concentrated sulphuric acid which was then distilled with 10 ml of 4% boric acid, 15 ml of 40% sodium hydroxide and 4 drops of indicator. Ammonium sulphate solution was then used to titrate the mixture and the titre value was used to obtain the percentage nitrogen in the soil.

3.2.3.2 Available phosphorus

The Bray-1 extraction method was used to determine soil available phosphorus. Dilute acid fluoride was used for the extraction**.**

3.2.3.3 Potassium

The ammonium acetate extraction method was used. This was done by adding 100 ml of ammonium acetate to 10 g of the soil sample in a bottle, shaken for about 90 minutes and then filtered. A flame photometer was used for the reading.

3.2.3.4 Organic carbon

The Walkley and Black method was used to determine the percentage organic carbon of the soil. This was done by adding 20 ml of concentrated sulphuric acid and 20 ml of distilled water to 20 g of the soil sample in a conical flask. It was digested, titrated and values recorded.

3.2.3.5 Organic matter

The Van Bemmelen Factor (1.724) was multiplied by the % organic carbon to determine the % organic matter.

3.2.3.6 Soil pH

An electrocalomel electrode pH meter was used to measure pH in 1:2:5 soils to water suspension.

3.2.4 TREATMENTS

These 5 varieties; Nangbaar, Anidaso, Jenguma, Quashie and Salentuya 1 and five lines; TGX 1990-5F, TGX 1987-62F, TGX 1989-20F, TGX 1904-2F AND TGX 1990-8F were planted. Maize variety obaatampa was used as the reference crop. All seeds were obtained from the legumes section of the Crops Research Institute.

3.2.5 PLANTING

Planting was done on the 30th of May, 2014 at two seeds per hill for soybean (75cm x 5cm) and maize (75cm x 20cm) which was later thinned to one seed per hill.

3.2.6 CULTURAL PRACTICES

The weedicide with 260 g glyphosate at a rate of 300 ml per knapsack was sprayed as pre-emergence herbicide to control weeds on the field. This was done a day after planting.

Refilling was done on all plots on the 9th June, 2014. This was done due to poor seed germination and seedling emergence.

Thinning was done when plants were well established, 22 days after planting to one plant per hill on the 23rd June, 2014.

Weed was controlled manually by the use of a hoe; thus on the 30th June and 21st July, 2014.

Pests were controlled by spraying with Power which contains 25 g lambda cyhalothrine as the active ingredient at a rate of 50 ml per knapsack on the 18th June and 15th July 2014.

The insecticide with 480 g chlorpyrifos-ethyl at a rate of 100 ml per knapsack was again sprayed to control post flowering insects of soybean. This was done on the 2nd August, 2014.

3.2.7 DATA COLLECTION

3.2.7.1 Days to emergence

This was done when about 50% of seedlings have emerged; by counting the seeds in the two central rows and the date recorded.

3.2.7.2 Plant stand

The number of plants in the two central rows of each plot was counted and recorded.

3.2.8 GROWTH DATA SAMPLING AND NODULE COUNT

Five plants were sampled consecutively from the two central rows of each plot at 25, 50 and 75 days after emergence for the following parameters:

3.2.8.1 Plant height

This was done by the use of a measuring rule to measure plants from the ground level to the tip of the stem and the mean calculated and recorded for each treatment.

3.2.8.2 Number of leaves per plant

Each of the five plants was taken and the number of leaves of the five plants were then counted and the mean recorded for each treatment.

3.2.8.3 Number of branches per plant

The number of branches on each plant was counted and means recorded accordingly.

3.2.8.4 Stem girth

The thickness of the stem of each of the 5 plants was measured using a caliper and their means recorded.

3.2.8.5 Dry matter

The 5 sampled plants were put in labeled envelopes, taken to the laboratory and oven dried at 60^0C for 72 hours, it was weighed and the mean recorded. This was done using a weighing balance.

3.2.8.6 Nodule number

The 5 sampled plants which were carefully dug out from the ground had their nodules counted. This was done by inserting their roots in a bucket of water so as to wash off soil particles and make nodules clearly seen. All nodules were detached from the roots counted and placed in a plastic container with a lid. They were counted and their means calculated and recorded for each treatment.

3.2.8.7 Nodule effectiveness

Nodules were taken to the laboratory and the effectiveness was determined. This was done by the use of a small knife to cut open the nodules and their effectiveness recorded. Those with reddish or pinkish color were considered effective, while those that are colorless or with a greenish coloration were considered non-effective. The percentage effectiveness was recorded accordingly.

3.2.8.8 Nodule dry weight

Nodules were oven dried at 60^0C for 24 hours and then weighed using the sensitive balance. The mean was calculated and recorded.

3.2.8.9 Days to 50% flowering

The number of days from planting to the time that 50% of the plants from the two middle rows of each plant have flowered was recorded. This was done by visual examination.

3.2.8.10 Days to maturity

The number of days from planting to the time that about 50% of pods had turned brown and 75% of leaves had shedded was recorded.

3.2.8.11 Plant stand at harvest

This was done at harvesting, by counting the number of plants in the two central rows of each plot and their numbers recorded.

3.2.9 HARVESTING

This was done at maturity by harvesting all plants from the two central rows of each plot. Plants were labeled and tied around each bunch of plants. Also, 5 plants were harvested from the lateral rows of each treatment and labeled accordingly. All harvested plants were taken from the field to a conducive place where the pods were detached from the plants. All seeds were threshed from the pods and the residue including the haulm was returned on the field on their individual plots. This was done with the exception of the 5 plants harvested from the border rows.

3.2.10 YIELD ANALYSIS

3.2.10.1 Harvest index

The 5 plants harvested from the lateral rows with their pods attached to them were sun-dried, for some days and then weighed. The total biomass weight was recorded. The pods were then detached from the plants and the seeds threshed. The total seed weight was recorded. This was then divided by the total biomass weight to get the harvest index expressed in percentage.

3.2.10.2 Number of pods per plant

All pods detached from the 5 plants were counted and their means recorded for each plot. Thus mean number of pods per plant=number of pods/number of plants

3.2.10.3 Number of seeds per pod

All seeds threshed from the pods of the 5 plants were counted. The number of seeds was divided by the number of pods to get the number of seeds per pod. Thus mean number of seeds per pod=number of seeds/number of pods

3.2.10.4 Hundred seed weight

One hundred seeds were counted from the total seeds of the 5 plants and then weighed. The weight was recorded accordingly.

3.2.10.5 Total grain yield

All threshed seeds from the two central rows of each plot was weighed to get the mean seed weight. This represents total grain yield in grams per m^2, which was converted to kilogram per hectare.

3.2.11 N-FIXATION

The macro Kjeidahl's method was used to determine the amount of nitrogen in seeds and residues. Seeds and residues of both soybean and maize were oven dried at 80^0C for 48 hours and then ground into powder forms. They were analyzed separately by digestion with 30 ml of concentrated sulphuric acid. Distillation was done using 40% caustic soda solution with 10 ml aliquot to distil off ammonia gas over boric acid. Titration was done with 0.1M HCl with the help of an indicator until colour changed from greyish green to pink. N content of seeds and residues were recorded separately for each plot. Total N content of both soybean and maize was obtained by adding the seed N content to the residue N. The amount on N fixed by the difference method was obtained for each plot by subtracting the maize total N to soybean total N.

3.3 EXPERIMENT TWO: DETERMINATION OF RESIDUE N FROM SOYBEAN AVAILABLE FOR SUCCEEDING MAIZE CROP

Maize was planted in the minor season on the same field, which was sown to the soybean variety. Soybean residue was returned to their respective plots. The variety used was the 'Abontem' which matures in about 85 days. It was sown at a distance of 75 cm x 20 cm. pre-emergence herbicide Round up was used to spray the field a day after planting.

3.3.1 CULTURAL PRACTICES

Thinning was done at about 15 days after planting on the 30th September, 2014. Plant stand was left at one plant per hill.

Weeding was done 3 weeks after planting by the use of a hoe. This was on the 8th October, 2014.

On the 14th November, 2014, an incidence of the smut disease of maize was recorded which led to the destruction and burning of all affected cobs.

The chemical containing 25 g lambda cyhalothrin at a rate of 50 ml per knapsack was used to spray the crops from the 20th September, 2014 until plants had tasseled. This was done at 14 days intervals to control insect pests.

The weedicide Gramozone at a rate of 150 ml of which contains 276 g paraquate chloride as active ingredient and 200 ml Atrazine which contains 500 g Atrazine were mixed and used to spray weeds at tasselling.

Fertilizer NPK 15-15-15 was applied at a rate of 100 kg per ha to plants at 2 weeks after planting on the 26th September, 2014 to plots that were previously cropped with maize in the major season. There was top-dressing with sulphate of ammonia 2 weeks afterwards on the 10th October, 2014.

3.3.2 DATA COLLECTION

3.3.2.1 Days to emergence

The number of days from planting to the time seedlings had emerged was recorded for each plot and the means recorded.

3.3.2.2 Plant stand

The number of plants from the two central rows of each plot was counted and recorded. This was done at 2 weeks after planting.

3.3.3 GROWTH ANALYSIS

Five plants were randomly sampled from the middle row of each plot at 3 sampling times: 20, 40 and 60 days after planting for the following parameters:

3.3.3.1 Plant height

This was done by using a measuring rule to from the ground level to the tip of the stem and the mean recorded.

3.3.3.2 Number of leaves per plant

All leaves on each of the 5 sampled plants were counted and their means recorded.

3.3.3.3 Stem girth

The thickness of the stems of each of the 5 plants was measured using a vernier caliper and the means recorded.

3.3.4 REPRODUCTIVE DATA

3.3.4.1 Days to tasselling

The number of days from planting to the time that about 50% of the plants had tassels was recorded.

3.3.4.2 Days to maturing

This was recorded when about 85% of cobs had turned brown and silks had begun to fall off the cobs.

3.3.5 HARVESTING

This was done at maturity. All cobs from the two central rows of each plot were harvested into sacks and labeled. Five plants were again harvested from the lateral rows.

3.3.6 YIELD ANALYSIS

3.3.6.1 Harvest index

The 5 plants were dried including their pods and their total weight recorded. This gave the total biomass weight. All seeds were threshed from the cobs and then weighed to get the total seed weight of the 5 plants. The total seed weight was divided by the total biomass weight to get the harvest index.

3.3.6.2 Number of cobs per plant

All cobs from the 5 plants were detached and then counted. This was then divided by the number of plants and their means recorded.

3.3.6.3 Number of seeds per cob

All seeds were detached from the cobs of the 5 plants and then counted. The number of seeds was then divided by the number of cobs to get the number of seeds per cob.

3.3.6.4 Hundred seed weight

One hundred seeds were counted from all the seeds from the 5 plants weighed and then recorded.

3.3.6.5 Total grain yield

All cobs harvested from the two central rows were shelled and the seeds weighed to get the mean seed weight. This represents the grain yield in grams per meter square to be converted to kilogram per hectare.

3.3.7 DATA ANALYSIS

All data was analyzed using the Analysis of Variance (ANOVA) and the MSTAT-C statistical package. Treatment differences were compared using the Least Significant Difference (LSD) procedure at 5% level of probability.

CHAPTER FOUR

4.0 RESULTS

4.1 RESULTS OF EXPERIMENT ONE

4.1.1 PLANT HEIGHT

The effect of variety on plant height are presented in Table 4.1. Soybean variety affected plant height significantly ($p< 0.05$) at all sampling times. At 25DAE, TGX 1990-8F produced the tallest plants (24.53cm) while Quashie produced the shortest plants. Plant height of TGX 1990-8F was statistically similar to those of Nangbaar, TGX 1904-2F and TGX 1987-62F, but significantly higher than all other treatment means. At 50DAE, TGX 1987-62F produced the tallest plants which effect was significantly higher than all treatment means, with the exception of TGX 1990-8F. At 75DAE, TGX 1987-62F again produced the tallest plants, which effect was significantly higher than those of Anidaso, Quashie, TGX1990-5F and TGX1989-20F, but statistically similar to all other treatment means. Quashie produced the least plant height at all sampling times. The treatment effect of the Quashie variety was significantly lower than all other varietal effects except those of Jengumah, TGX 1990-5F and TGX 1989-20F. Treatment effect of line TGX 1990-8F was also significantly higher than that of line TGX 1989-20F. All other varietal effects were similar.

Table 4.1 Height of soybean varieties at three sampling periods

	Plant Height (cm)		
Variety	25 DAE	50 DAE	75 DAE
Nangbaar	21.7	37.1	46.9
Anidaso	20.2	35.3	43.1
Jengumah	19.5	38.7	51.1
Quashie	15.9	32.8	39.0
Salentuya 1	20.4	38.0	50.5
TGX 1990- 5F	18.8	35.6	42.8
TGX 1987-62F	21.9	46.2	57.5
TGX 1989-20F	16.6	32.6	42.2
TGX 1904-2F	20.9	38.9	51.4
TGX 1990-8F	24.5	41.9	51.7
LSD (5%)	3.8	8.9	12.5
CV (%)	9.4	3.4	5.0

4.1.2 NUMBER OF LEAVES PER PLANT

Table 4.2 shows varietal effect on number of leaves of the soybean varieties. At 25 DAE and 50 DAE, variety did not significantly ($p > 0.05$) affect leaf production. However, at 75 DAE, treatment effect of TGX 1989-20F variety was significantly lower than that of Quashie. All other treatment differences were not significant.

Table 4.2 Number of leaves of soybean plants at three sampling periods

	Number of leaves		
Variety	25 DAE	50 DAE	75 DAE
Nangbaar	14.3	22.8	31.2
Anidaso	14.6	21.3	32.0
Jengumah	13.7	23.9	33.1
Quashie	12.1	22.9	35.4
Salentuya 1	12.4	25.2	33.9
TGX 1990-5F	14.2	20.4	25.6
TGX 1987-62F	13.9	24.8	30.3
TGX 1989-20F	12.2	22.6	23.3
TGX 1904-2F	12.2	18.3	27.9
TGX 1990-8F	12.3	25.6	28.3
LSD (5%)	NS	NS	11.6
CV (%)	7.3	5.5	7.9

4.1.3 NUMBER OF BRANCHES

Table 4.3 shows varietal effects on the number of branches per plant. At 50 DAE, Jengumah produced the greatest number of branches, which was significantly ($P< 0.05$) higher than that of TGX 1990-5F which produced the least. All other treatment differences were statistically similar. Treatment effects of TGX 1990-5F, TGX 1989-20F and TGX 1990-8F were significantly lower than that of Jengumah which produced the greatest branches at 75 DAE. Treatment effects of Anidaso and Salentuya 1, which were statistically similar to that of Jengumah were also significantly higher than those of TGX 1990-5F and TGX 1989-20. All other varietal differences were not significant ($p> 0.05$). Varietal effects of Salentuya 1 was also significantly higher than that of TGX 1990-5F line.

Table 4.3 Number of branches of soybean varieties at two sampling periods

	Number of branches	
Variety	50 DAE	75 DAE
Nangbaar	5.67	6.43
Anidaso	6.05	7.00
Jengumah	6.75	7.40
Quashie	5.60	6.50
Salentuya 1	6.45	7.25
TGX 1990-5F	4.00	5.15
TGX 1987-62F	5.05	6.03
TGX 1989-20F	4.45	5.40
TGX 1904-2F	5.90	6.65
TGX 1990-8F	5.00	5.45
LSD (5%)	2.42	1.60
CV (%)	24.3	20.7

4.1.4 STEM GIRTH

Varietal effect on stem girth is presented in Table 4.4. There were no significant differences (P> 0.05) among treatments at 25 DAE. At 50 DAE, TGX 1987-62F produced the greatest stem girth, which was significantly higher than those of Quashie and TGX 1904-2F. All other treatment differences were not significant at 5% level of probability. At 75 DAE, treatment effects of Quashie and TGX 1989-20F were significantly lower than that of TGX 1987-62F which produced the greatest stem girth (0.750). The treatment effect of Nangbaar variety was also significantly higher ($p< 0.05$) than that of variety TGX 1989-20F. All other varietal differences were not significant at 5% level of probability.

Table 4.4 Stem girth of soybean varieties at three sampling periods

	Stem girth (cm)		
Variety	25 DAE	50 DAE	75 DAE
Nangbaar	0.185	0.465	0.705
Anidaso	0.185	0.440	0.690
Jengumah	0.175	0.410	0.630
Quashie	0.180	0.350	0.545
Salentuya 1	0.205	0.470	0.690
TGX 1990-5F	0.220	0.405	0.635
TGX 1987-62F	0.175	0.570	0.750
TGX 1989-20F	0.190	0.405	0.510
TGX 1904-2F	0.180	0.395	0.665
TGX 1990-8F	0.205	0.475	0.635
LSD (5%)	NS	0.152	0.195
CV (%)	8.8	7.1	4.6

4.1.5 NUMBER OF NODULES

Table 4.5 shows the results of nodule number at 3 sampling times. Soybean variety did not significantly ($P > 0.05$) affect nodule production at both 25 DAE and 50 DAE. At 75 DAE however, TGX 1904-2F produced the greatest number of nodules (21.05) which was significantly higher ($p < 0.05$) than that of TGX 1990-5F only. All other treatment differences were not significant at 5% level of probability.

Table 4.5 Nodule number of soybean varieties at three sampling periods

	Number of nodules		
Variety	25 DAE	50 DAE	75 DAE
Nangbaar	6.30	12.25	13.50
Anidaso	6.40	12.50	17.25
Jengumah	4.85	11.40	13.00
Quashie	4.15	16.40	18.00
Salentuya 1	6.20	16.75	18.55
TGX 1990-5F	4.60	13.60	12.65
TGX 1987-62F	3.10	13.30	16.70
TGX 1989-20F	2.97	10.35	15.75
TGX 1904-2F	6.20	10.60	21.05
TGX 1990-8F	5.30	10.85	17.95
LSD (5%)	NS	NS	8.10
CV (%)	39.1	19.0	11.5

4.1.6. NODULE EFFECTIVENESS

Table 4.6 shows varietal effects on percentage nodule effectiveness of the soybean plants. At 25 DAE, TGX 1989-20F had the greatest percentage nodule effectiveness and was statistically similar to all other varietal effects, except those of TGX 1987-62F and Quashie variety. Percentage nodule effectiveness of Anidaso and Nangbaar varieties were also significantly higher than that of TGX 1987-62F, which recorded the least. At 50 DAE, TGX 1989-20F recorded the highest percentage nodule effectiveness which was statistically higher than those of varieties TGX 1987-62F and TGX 1904-2F only. Varietal effects of TGX 1987-62F were significantly lower than all other varietal means except those of Anidaso and Jengumah varieties. Treatment effect of TGX 1904-2F was

also significantly lower than those of the Quashie and TGX 1989-20F varieties only. All other varietal differences were not significant. The Jengumah variety had the greatest nodule effectiveness at 75 DAE, but this was significantly higher than the effects of TGX 1987-62F, Salentuya 1 and TGX 1990-8F only. Line TGX 1987-62F recorded the least nodule effectiveness, which was significantly lower than those of Quashie and Nangbaar varieties. All other treatment effects were not significant.

Table 4.6 Percentage effectiveness of nodules of soybean varieties at three sampling periods

	Percentage effectiveness		
Variety	25 DAE	50 DAE	75 DAE
Nangbaar	39.5	76.2	51.2
Anidaso	48.2	71.2	47.5
Jengumah	23.9	73.8	58.8
Quashie	20.2	82.5	50.0
Salentuya 1	34.6	78.8	40.0
TGX 1990-5F	23.6	76.2	41.2
TGX 1987-62F	9.0	57.5	31.2
TGX 1989-20F	51.7	83.8	42.5
TGX 1904-2F	27.7	62.5	46.2
TGX 1990-8F	28.3	63.8	37.5
LSD (5%)	30.6	18.7	18.4
CV (%)	50.8	10.2	6.6

4.1.7 NODULE DRY WEIGHT

Table 4.7 shows the varietal effects on nodule dry weight. There were significant differences ($P< 0.05$) among varieties in nodule dry weight on all 3 occasions. Anidaso

produced the greatest nodule weight at 25 DAE, which was significantly higher than those of Jengumah, Quashie, TGX 1990-5F, TGX1987-62F and TGX 1989-20F. Quashie produced the least nodule weight. At 50 DAE, nodule weight of Nangbaar was significantly higher than those of Jengumah, TGX 1987-62F and TGX 1904-2F. All other treatment differences were statistically similar. At 75 DAE, variety TGX 1904-2F recorded the greatest nodule dry weight but this was significantly higher than those of TGX 1990-5F, Jengumah and Quashie varieties only. Treatment effect of TGX 1990-5F which was the lowest was significantly lower than those of TGX 1989-20F, TGX 1904-2F and TGX 1990-8F only. All other varietal differences were not significant.

Table 4.7 Nodule dry weight of soybean varieties at three sampling periods

	Nodule dry weight (g)		
Variety	25 DAE	50 DAE	75 DAE
Nangbaar	0.033	0.143	0.131
Anidaso	0.050	0.129	0.147
Jengumah	0.013	0.047	0.091
Quashie	0.003	0.090	0.091
Salentuya 1	0.023	0.113	0.186
TGX 1990-5F	0.015	0.078	0.084
TGX 1987-62F	0.013	0.069	0.148
TGX 1989-20F	0.005	0.065	0.201
TGX 1904-2F	0.023	0.073	0.216
TGX 1990-8F	0.028	0.129	0.207
LSD (5%)	0.033	0.073	0.110
CV (%)	42.7	26.5	16.0

4.1.8 PLANT DRY MATTER

Results of dry matter yield are shown in Table 4.8. At 25 DAE, results indicated that TGX 1990-5F produced the greatest dry matter which was significantly higher than those of Quashie and TGX 1989-20F. All other treatment means were not significant at 5% probability level. Quashie produced the least dry matter at 50 DAE, which was significantly lower than the effect of TGX 1990-8F only. The rest of the treatment means were statistically similar. Varietal differences at 75 DAE were not significant ($p > 0.05$).

Table 4.8 Plant dry matter of soybean varieties at three sampling periods

	Dry matter (g)		
Variety	25 DAE	50 DAE	75 DAE
Nangbaar	2.74	9.53	31.3
Anidaso	2.80	9.72	27.5
Jengumah	2.93	8.71	27.1
Quashie	2.08	6.58	23.1
Salentuya 1	3.10	9.75	32.6
TGX 1990-5F	3.53	10.02	26.7
TGX 1987-62F	2.44	11.61	34.9
TGX 1989-20F	2.16	8.35	21.6
TGX 1904-2F	3.12	8.25	29.9
TGX 1990-8F	3.14	14.67	44.8
LSD (5%)	1.29	7.12	NS
CV (%)	13.6	15.1	8.9

4.1.9 PLANT STAND, DAYS TO EMERGENCE AND DAYS TO 50% FLOWERING

Table 4.9 shows the results of plant stand, days to emergence and days to flowering as affected by variety. Soybean variety did not affect plant stand significantly ($p > 0.05$). Days to emergence differences were only significant between TGX 1904-2F and Anidaso varieties. Other treatment differences were statistically similar. Days to flowering were significantly affected by varieties. Those of Nangbaar, Anidaso and TGX 1987-62F were statistically similar and significantly higher than that of TGX 1990-8F. Varietal differences between TGX 1904-2F and TGX 1990-8F was also significant. All other treatment differences were not significant.

Table 4.9 Plant stand, days to emergence and days to 50% flowering of soybean varieties

Variety	Plant stand	Days to 50% emergence	Days to 50% flowering
Nangbaar	94.8	5.50	47.50
Anidaso	95.2	4.25	47.75
Jengumah	92.8	5.75	44.75
Quashie	95.0	5.25	44.75
Salentuya 1	91.0	5.25	45.00
TGX 1990-5F	69.5	5.75	44.75
TGX 1987-62F	89.8	5.25	48.25
TGX 1989-20F	89.2	5.00	45.00
TGX 1904-2F	75.5	6.50	46.50
TGX 1990-8F	83.8	5.25	44.50
LSD (5%)	NS	1.77	1.20
CV (%)	13.7	28.2	0.6

4.1.10. DAYS TO 50% MATURITY AND PLANT STAND AT HARVEST

Table 4.10 shows results of days to maturity and plant stand at harvest. Quashie variety recorded the longest days to maturity (98.00), which was significantly higher than those of all soybean lines except Nangbaar, Anidaso, Jengumah and Salentuya 1 varieties. The effects of TGX 1990-5F and TGX 1990-8F were significantly lower than all other treatment effects, except those of TGX 1987-62F and TGX 1989-2F lines. Plant stand at harvest was lowest in the TGX 1990-5F line, and this was significantly lower than all treatment effects, except TGX 1904-2F line. Quashie plots recorded the greatest number of plants at harvest.

Table 4.10 Days to 50% maturity and plant stand at harvest of soybean varieties

Variety	Days to 50% maturity	Plant stand at harvest
Nangbaar	96.00	78.8
Anidaso	94.00	77.2
Jengumah	94.25	79.5
Quashie	98.00	81.0
Salentuya 1	95.25	69.8
TGX 1990-5F	81.00	41.8
TGX 1987-62F	84.00	76.8
TGX 1989-20F	85.50	71.0
TGX 1904-2F	92.25	61.5
TGX 1990-8F	81.00	69.5
LSD (5%)	4.62	22.7
CV (%)	0.7	14.6

4.1.11 NUMBER OF PODS, NUMBER OF SEEDS AND ONE HUNDRED SEED WEIGHT

Table 4.11 shows results of mean number of pods per plant, number of seeds per pod and one hundred seed weight as affected by variety. Mean number of pods per plant ranged between 63.2 and 38.7 from TGX 1990-5F and Nangbaar respectively. There were no significant ($P<0.05$) differences among treatments except between these two. Mean number of seeds of TGX 1987-62F and TGX 1989-20F were statistically similar and significantly higher than those of Jengumah and Quashie. Varietal effects on 100 seed weight were significantly different. TGX 1990-8F, which recorded the greatest seed weight (18.12), was statistically similar to that of TGX 1990-5F (17.12), but statistically higher than all other treatment means, except that differences between TGX 1990-5F and TGX 1989-20F was not significant.

Table 4.11 Number of pods per plant, number of seeds per pod and one hundred seed weight of soybean varieties

Variety	No of pods per plant	No of seeds per pod	100 seed weight (g)
Nangbaar	38.7	1.5	11.38
Anidaso	50.6	1.5	10.75
Jengumah	59.1	1.2	13.00
Quashie	47.3	1.9	10.00
Salentuya 1	53.6	1.7	9.75
TGX 1990-5F	63.2	1.5	17.12
TGX 1987-62F	57.6	2.0	11.12
TGX 1989-20F	44.0	1.9	15.12
TGX 1904-2F	48.5	1.8	11.12
TGX 1990-8F	55.8	1.5	18.12
LSD (5%)	24.5	0.5	2.47
CV (%)	16.4	11.3	3.3

4.1.12. TOTAL GRAIN YIELD AND HARVEST INDEX

Results of total seed weight and harvest index of soybean varieties are shown in Table 4.12. Variety significantly ($p< 0.05$) affected grain yield, with those of Anidaso, Quashie, Nangbaar, TGX 1904-2F and Jengumah being significantly lower than that of TGX 1990-8F, which recorded the greatest yield of 839 kg/ha. Other treatment differences were not significant. Harvest index of all soybean varieties were significantly higher than that of Nangbaar, which recorded the least (0.193), with the exception of Anidaso. The greatest was recorded by TGX 1990-5F (0.453), and this was significantly higher than all other treatment means, except those of TGX 1987-62F, TGX 1989-20F and TGX 1990-8F.

Table 4.12 Total grain yield and harvest index of soybean varieties

Variety	Total grain yield (kg/ha)	Harvest index
Nangbaar	604	0.193
Anidaso	620	0.248
Jengumah	695	0.300
Quashie	617	0.270
Salentuya 1	719	0.290
TGX 1990-5F	731	0.453
TGX 1987-62F	740	0.405
TGX 1989-20F	784	0.450
TGX 1904-2F	678	0.320
TGX 1990-8F	839	0.425
LSD (5%)	129	0.074
CV (%)	6.7	3.7

4.1.13. Seed N, residue N, total N and fixed N

Tablel 4.13 shows varietal effects on seed N, residue N, total N and fixed N. Results showed that Anidaso directed the largest amount of nitrogen into the seed; whiles line TGX 1904-2F directed the least among all soybean cultivars. Apart from Nangbaar and Salentuya 1, treatment effect of Anidaso variety was significantly higher than all other treatment effects. The treatment effect of TGX 1904-2F was also lower than those of Anidaso, Nangbaar and Salentuya 1 varieties. Other treatment differences were not significant. The Anidaso variety retained the greatest amount of N in the residue (1.43g), which was significantly higher than those of Nangbaar, TGX 1990-5F and TGX 1987-62F lines. Soybean line TGX 1990-5F directed the least amount of N in the residue, but this was similar to all other treatment means, except those of Anidaso, Jengumah and Quashie varieties. Total plant N as greatest in the Anidaso variety and this was significantly higher than all other treatment effects, except those of Nangbaar, Quashie and Salentuya 1 varieties. All other treatment effects were similar. Anidaso variety fixed the greatest amount of N while TGX 1990-5F fixed the lowest. Treatment effect of the Anidaso variety was significantly higher than all other treatment effects, except those of Nangbaar, Quashie and Salentuya 1 varieties. Treatment effect of TGX 1990-5F was also lower than those of Nangbaar, Anidaso, Salentuya1 and Quashie varieties. All other treatment effects were similar.

Table 4.13 Residue N, seed N, total N and fixed N of soybean varieties

Variety	Seed N (kg/ha)	Residue N (kg/ha)	Total plant N (kg/ha)	Fixed N (kg/ha)
Nangbaar	63.5	9.2	72.6	53.6
Anidaso	63.8	14.3	78.1	59.1
Jengumah	58.0	13.2	71.2	52.2
Quashie	58.0	14.0	71.9	52.9
Salentuya 1	61.6	10.3	71.9	52.9
TGX 1990-5F	56.9	7.7	64.6	45.5
TGX 1987-62F	57.3	8.1	65.4	46.3
TGX 1989-20F	57.3	10.3	67.5	48.5
TGX 1904-2F	55.8	9.9	65.7	46.7
TGX 1990-8F	59.8	10.3	70.1	51.0
LSD (5%)	4.8	4.9	6.9	6.9
CV (%)	1.5	1.1	1.4	1.8

4.2 RERSULTS OF EXPERIMENT TWO

4.2.1. DAYS TO EMERGENCE, PLANT STAND AND DAYS TO 50% TASSELING

Table 4.14 shows the results of residue N effect on days to emergence, plant stand at 21 days and days to 50% tasseling of maize plants. Treatment effects on days to emergence were not significant ($p>0.05$). Plant stand at 21 days was significantly affected by residue N. The greatest plant stand was recorded by maize following Jengumah residue (35.8) which was significantly higher than the fertilizer- applied treatment. Treatment of TGX 1990-5F residue was also greater than the fertilizer applied treatment effect. Other treatment differences were not significant. The fertilizer applied treatment took the longest days to 50% tasseling, which was significantly higher than all other treatment

effects. Treatment effects of TGX 1987-62F and TGX 1989-2F were significantly lower than those of Salentuya 1, Anidaso, Jengumah, Quashie and the fertilizer applied treatments.

Table 4.14 Residue N effects on days to emergence, plant stand at 21 days and days to 50% tasseling of maize

Residue	Days to emergence	plant stand at 21 days	Days to 50% tasseling
Nangbaar	7	30.5	51.0
Anidaso	7	29.5	52.0
Jengumah	7	35.8	51.8
Quashie	7	28.2	51.8
Salentuya 1	7	30.5	51.8
TGX 1990-5F	7	35.0	51.3
TGX 1987-62F	7	31.2	51.0
TGX 1989-20F	7	27.8	51.0
TGX 1904-2F	7	27.2	51.3
TGX 1990- 8F	7	26.0	51.5
Fertilizer	7	20.8	52.8
LSD (5%)	-	10.8	0.6
CV (%)	0.0	22.5	0.3

4.2.2 PLANT HEIGHT

Results of residue N effect on maize plant height are represented in Table 4.15. Effect of residue N on plant height at 20 DAP was significant ($p< 0.05$), with that of Nangbaar recording the greatest effect and this was significantly higher than those of Quashie, TGX 1990-5F, TGX 1987-62F, and TGX 1989-20F residue plots. All other treatment differences were not significant. At 40 DAP, maize following incorporation of Jengumah

residue recorded the greatest effect, while that of Nangbaar recorded the least effect. There were significant differences among some other treatment means. Treatment effect Jengumah plots was higher than those of Nangbaar, TGX 1990-5F, TGX 1987-62F, TGX 1989-2F and the fertilizer treatments. At 60 DAP, treatment differences were not significant ($p > 0.05$).

Table 4.15 Residue N effect on maize plant height at three sampling periods

	Plant height (cm)		
Residue	20 DAP	40 DAP	60 DAP
Nangbaar	23.5	57.4	165.2
Anidaso	23.2	69.5	169.2
Jengumah	20.9	79.7	165.5
Quashie	19.5	63.3	156.2
Salentuya 1	21.4	75.8	170.8
TGX 1990-5F	18.7	62.0	159.5
TGX 1987-62F	18.8	62.0	158.2
TGX 1989-20F	18.9	61.8	153.0
TGX 1904-2F	21.1	75.4	170.5
TGX 1990-8F	21.8	76.0	161.5
Fertilizer	20.0	58.4	149.0
LSD (5%)	3.9	16.8	NS
CV (%)	11.2	2.2	3.0

4.2.3. STEM GIRTH

Table 4.16 shows the results of residue N effect on maize stem girth. Residue N effect on maize stem girth was significant ($p < 0.05$) at 20 DAP only between Anidaso residue plots and those of fertilizer applied and TGX 1990-5F plots. All other treatment differences

were statistically similar. At 40 DAP treatment differences were not significant. At 60 DAP, treatment effect of the fertilizer applied plots was the highest, but this was significantly higher than that of the TGX 1989-20F residue plots only. All other treatment effects were similar.

Table 4.16 Residue N effect on maize stem girth at three sampling periods

	Stem girth (cm)		
Residue	20 DAP	40 DAP	60 DAP
Nangbaar	4.93	11.30	14.65
Anidaso	5.08	13.00	16.52
Jengumah/	4.25	13.47	15.75
Quashie	3.93	11.45	13.77
Salentuya 1	4.15	11.82	14.32
TGX 1990-5F	3.68	11.95	13.87
TGX 1987-62F	4.08	11.70	14.55
TGX 1989-20F	4.78	11.87	12.72
TGX 1904-2F	3.93	12.27	15.25
TGX 1990-8F	4.90	12.55	14.25
Fertilizer	3.80	12.97	16.62
LSD (5%)	1.27	NS	3.19
CV (%)	4.9	4.0	2.2

4.2.4 NUMBER OF LEAVES

Table 4.17 shows the results of residue N effect on maize number of leaves. Anidaso residue plots recorded the greatest effect which was significantly ($P< 0.05$) higher than those of TGX 1990-5F, TGX 1987-62F and fertilizer applied plots at 20 DAP. Treatment effects of TGX 1987-62F and the fertilizer applied plots were also significantly lower

than the Nangbaar plots. All other treatment differences were not significant at 5% level of probability. At 40 DAP, number of leaves from the TGX 1989-20F was the lowest, and this was significantly lower than those of Salentuya 1 and Jegumah residue incorporated plots. Other treatment differences were not significant ($p > 0.05$). At 60 DAP, the Salentuya 1 residue plots recorded the greatest number of leaves, but this was significantly higher than that of TGX 1989-20F residue plots only. Treatment effect of the Jengumah and Nangbaar residue plots were also significantly higher than that of TGX 1989-20F residue plots. All other treatment differences were not significant ($p > 0.05$)

Table 4.17 Residue N effect on number of leaves of maize plants at three sampling periods

	Number of leaves		
Residue	20 DAP	40 DAP	60 DAP
Nangbaar	6.75	8.50	9.95
Anidaso	6.85	8.70	9.70
Jengum	6.40	9.00	10.05
Quashie/	6.33	7.90	9.55
Salentuya 1	6.15	9.35	10.20
TGX 1990-5F	5.85	7.90	9.35
TGX 1987-62F	5.65	8.25	9.35
TGX 1989-20F	6.05	7.80	8.60
TGX 1904-2F	6.00	8.00	9.40
TGX 1990-8F	6.35	8.70	9.30
Fertilizer	5.55	8.60	9.45
LSD (5%)	0.92	1.05	1.15
CV (%)	2.5	1.7	1.9

4.2.5 MAIZE DRYMATTER

Table 4.18 shows the results of residue N effect on dry matter yield. Residue N affected dry matter production at all sampling times significantly ($p< 0.05$). TGX 1990-8F and Nangbaar residue treatments produced the greatest dry matter at 20 DAP but this effect was significantly higher than those of Quashie and fertilizer applied treatments only. Other treatment differences were not significant. Sampling at 40 DAP showed that the greatest dry matter yield was obtained from the TGX 1990-8F residue plots, which effect was significantly higher ($p< 0.05$) than those of Nangbaar, Anidaso, Quashie, TGX 1989-20F and TGX 1990-5F residue plots only. Furthermore, the fertilizer applied treatment effect was also significantly higher than those of TGX 1989-20F, TGX 1990-5F, Quashie, Anidaso and Nangbaar residue plots. At 60 DAP, the fertilizer applied treatment effect was significantly higher than all other treatment effects. All other treatment differences were not significant.

Table 4.18 Residue N effect on maize dry matter at three sampling periods

	Dry matter yield (g)		
Residue	20 DAP	40 DAP	60 DAP
Nangbaar	0.8	17.9	54.8
Anidaso	0.7	21.5	61.9
Jengumah	0.7	33.1	68.4
Quashie	0.6	20.4	46.9
Salentuya 1	0.8	29.8	66.4
TGX 1990-5F	0.7	20.2	57.3
TGX 1987-62F	0.6	25.7	50.5
TGX 1989-20F	0.7	20.4	51.2
TGX 1904-2F	0.8	24.7	57.8
TGX 1990-8F	0.8	39.4	69.0
Fertilizer	0.6	38.5	100.5
LSD (5%)	0.2	17.0	29.8
CV (%)	11.2	13.5	17.4

4.2.6 DAYS TO 50% MATURITY, PLANT STAND AT HARVEST AND HARVEST INDEX OF MAIZE

Table 4.19 shows the results of residue N effect on days to maturity, plant stand at harvest and harvest index of maize. Days to maturity was not significantly affected by residue N significantly ($p>0.05$). Plants stand at harvest was similar in Jengumah and TGX 1990-5F residue incorporated plots, and both effects were significantly higher than that of TGX 1990-8F treatment only. Maize harvest index was not affected by residue incorporation.

Table 4.19 Residue N effect on days to 50% maturity, plant stand at harvest and harvest index of maize

Residue	Days to 50% maturity	Plant stand at harvest	Harvest index
Nangbaar	81	30.5	0.34
Anidaso	81	29.5	0.31
Jengumah	81	35.8	0.30
Quashie	81	28.2	0.28
Salentuya 1	81	30.5	0.38
TGX 1990-5F	81	35.0	0.35
TGX 1987-62F	81	31.2	0.34
TGX 1989-20F	81	27.8	0.36
TGX 1904-2F	81	27.2	0.32
TGX 1990-8F	81	26.0	0.34
Fertilizer	81	20.8	0.28
LSD (5%)	NS	10.8	NS
CV (%)	0.0	22.8	11.1

4.2.7 NUMBER OF COBS PER PLANT, NUMBER OF SEEDS PER COB, 100 SEED WEIGHT AND TOTAL GRAIN YEILD

Table 4.20 shows the results of residue N effect on number of cobs per plant, number of seeds per cob and hundred seed weight. Residue incorporation did not significantly ($p > 0.05$) affect number of cobs per plant. Number of seeds per cob were significantly affected by residue N, with Nangbaar residue plots producing the greatest (437), followed by fertilizer applied maize plots (432). Both were statistically similar and significantly higher than those of Anidaso, Jengumah, TGX 1987-62, TGX 1989-20F and Quashie residue incorporated treatments. All other treatment differences were not significant. One hundred seed weight was greatest in the Jengumah residue plots and this was

significantly higher than all other treatment effects, except those of salentuya 1 and TGX 1989-20F residue treatments only. All other treatment differences were not significant. Maize seed yield was not significantly ($p > 0.05$) affected by residue N.

Table 4.20 Residue N effect on number of cobs per plant, number of seeds per cob, 100 seed weight and total grain yield

Residue	Number of cobs per plant	Number of seeds per cob	100 seed weight (g)	Grain yield (tons/ha)
Nangbaar	1.05	437	22.38	0.9
Anidaso	1.10	295	21.50	0.9
Jengumah	1.00	291	25.75	1.1
Quashie	1.00	278	21.88	0.9
Salentuy1	1.05	378	23.75	1.1
TGX 190-5F	1.00	337	21.88	1.0
TGX 1987-62F	1.05	294	21.00	0.8
TGX 1989-20F	1.00	308	23.38	0.8
TGX 1904-2F	1.00	430	22.62	1.1
TGX 1990-8F	1.00	362	22.00	1.1
Fertilizer	1.00	432	22.38	1.2
LSD (5%)	NS	107.2	2.97	NS
CV (%)	1.7	3.2	3.7	18.9

CHAPTER FIVE

5.0 DISCUSSION

5.1 VARIETAL EFFECTS ON NODULATION AND N-FIXATION

All the soybean varieties nodulated freely with the naturalized rhizobia in the soil. The cowpea miscellany, *Bradyrhizobium sp*, has been reported to be a compatible rhizobia with a host of tropical legumes including soybean, cowpea, groundnuts and Bambara groundnuts (Sarkodie-Addo, 1991; Giller, 2001; Berchie, 2010; Vuodzie, 2012; Konlan *et al.*, 2013). These rhizobia species are native of tropical soils, well adapted and highly competitive against introduced rhizobia in inoculants. Though they are not generally very efficient in N-fixation among these legumes, they are found to occupy greater portion of nodules; this renders tropical legume species unable to support greater N-fixation as the temperate species (Sarkodie-Addo *et al.*, 2006).

Nodulation varied among the varieties (Table 4.5). The Salentuya 1 variety supported the largest amount, 41.50 nodules whilst line TGX 1989-20F supported the least number of nodules over the three sampling periods. The differences were probably due to genotype variations as they were grown under similar conditions. Varietal differences in nodule production has been reported in other legumes including cowpea (Tour, 2003; Addu, 2003), soybean (Sarkodie-Addo *et al.*, 2006; Konlan, 2003), groundnuts (Martinson, 2006; Addo, 2014, unpublished data). Additional observations from the nodulation data showed that the varieties produced more nodules than the lines. It has been generally said that varieties that are highly nodulating are generally more adapted to the environmental conditions than those that support production of lesser number of nodules. Indeed, Werner and Newton (2005) stated that fluctuations in soil pH, nutrient availability,

temperature and water status affect metabolic activities of rhizobia, and their ability to cause nodulation. Thus, in the present studies, the varieties that are more adapted than the soybean lines were highly nodulating.

Soybean varieties that produced highest nodule numbers had lower nodule dry weights as compared to those that produced fewer nodules (Tales 4.5, 4.7). Salentuya 1 variety produced the greatest number of nodules, but line TGX 1990-8F which produced one of the lowest numbers of nodules recorded the greatest total nodule dry weight of 0.364g. Other workers have reported such negative correlations in their studies (Sarkodie-Addo, 1991; Addu, 2003; Hume and Shelp, 1988). The probable reason is that varieties that produced greater number of nodules bear small sized nodules, and hence low dry weight, whilst those that produce fewer nodules produce larger nodules, hence greater nodule dry weight.

Nodule effectiveness ranged between 31 to 59%. Results showed that despite the Salentuya 1 being the largest nolulating variety, only 40% of the nodules were effective. Jengumah variety recorded the greatest nodule effectiveness of 59%. Giller (2001) had stated that the fact that more nodules have been produced does not mean efficient nitrogen fixation.

5.2. NITROGEN FIXATION AND GROWTH AND GRAIN YIELD OF SOYBEAN

The amount of N fixed was greatest in the Anidaso variety and least in line TGX 1990-5F. The results showed that the varieties fixed different amount of N. The differences in N fixation among the varieties studied was probably due to genotypic variations as they were grown under similar conditions. Varietal differences in N fixation and nodule

production have been reported in other legumes including cowpea (Tour 2003; Addu, 2003), soybean (Sarkodie-Addo *et al.,* 2006), groundnuts (Konlan 2011; Konlan *et al.,* 2013) and Bambara groundnuts (Berchie, 2010). Additional observations showed that all the varieties fixed greater N than the lines, probably because they are better adapted than the lines (Werner and Newton, 2005). The levels of N fixed in the present studies were lower than had been reported for soybean in other studies (Giller 2001; Sarkodie-Addo, 1991).

Nitrogen fixation and grain yield results showed that although the Anidaso variety fixed the largest amount of N, its grain yield was the second lowest (Tables 4.12, 4.13). Conversely, line TGX 1990-8F fixed N that was lower than among the varieties, but its grain yield was the greatest among all the lines and varieties studied. These results indicate that the 'Anidaso' variety could not translate the large amount of N fixed into grain yield production. Tour (2003) observed from his studies that the cowpea varieties that fixed largest amount of N produced the lowest grain yield. Fataah (unpublished data, 2015) also reported that the cowpea varieties that fixed most N did not produce the greatest grain yield. Notwithstanding several reports showed N fixation to be positively correlated with grain yield in several legumes (Keyser and Li, 1992; Sarkodie-Addo, 1991; Hume and Shelp, 1988; Sarkodie-Addo *et al.,* 2006).

5.3. SOYBEAN RESIDUE N AND MAIZE PRODUCTION

Results of N left in soybean residue (Table 4.13) showed that Anidaso that fixed the largest amount of N left also the greatest amount of N in the residue. This amount was significantly greater than the N content in the residue of all the soybean lines. Growth in

terms of dry matter production (Table 4.18) was greatest in the fertilizer-applied treatment. Indeed, total dry matter from this treatment was about 150% greater than the Anidaso variety which left the greatest amount of N in their residue. This was probably due to the readily available nutrients from the fertilizer (Abbas *et al*., 2003). The plants from the residue incorporated plots had to wait for decomposition and mineralization of the residues before they could use the nutrients to grow. Hence any factor that will affect the above processes for example; drought, high C: N ratio, temperature as well as the amount of N used by the microbes themselves will affect the availability of N to the maize plants (Donnelly *et al*., 1990; Handayanto *et al*., 1997)

Maize grain yield results indicated no significant difference among all treatments. This means grain yield from the residue incorporated plots was similar to that of the fertilizer-applied treatment. Additionally, yields from the residue-incorporated treatments are similar to yields obtained by most farmers who apply both NPK and ammonia as top dressing in maize production. The present results indicate that if farmers will incorporate trash of legumes unto their fields, there would not be any further need to apply fertilizer, and this would not lead to any significant reduction in grain yield. Apart from this addition of organic residues would improve soil structure, drainage, lower bulk density and improve microbial activity. Such system would be more sustainable and environmentally friendly than the present application of chemical fertilizers. According to Giller (2001), if after harvesting grains, and legume residue are effectively recycled, N acquired from such a practice can be as much as 140 kg/ha depending on the legume.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

Results indicated that all soybean varieties and lines nodulated with the soil existent cowpea rhizobia. Differences in nodulation and N fixation could be attributed to genotypic differences among the varieties. Nitrogen fixation improved soybean growth and grain yield although varieties that fixed the most N did not produce the greatest grain yield. The Anidaso variety left the greatest amount of 14.3 kg/ha of N in the residue for succeeding crop. Soybean residue incorporated treatments produced similar grain yields as that which received the normal recommended fertilizer rates for maize.

The results indicate that if farmers would recycle the soybean residue unto their fields, application of chemical fertilizers may not be necessary. It is recommended that studies be repeated in all maize growing regions to verify results before recommending the technology to farmers.

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APPENDIX

Initial soil properties at the experimental site

Sample identification	0-30cm depth
% total nitrogen	0.07
Available phosphorus(mg/kg)	7.26
Exchangeable potassium(cmol/kg)	0.20
% organic carbon	1.12
% organic matter	1.93
pH	5.96