KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CROP AND SOIL SCIENCES

EVALUATION OF NITROGEN FIXATION POTENTIAL OF COWPEA VARIETIES AND EFFECT OF RESIDUE NITROGEN FOR MAIZE PRODUCTION

BY

JULIANA FATAAH

SEPTEMBER , 2015

EVALUATION OF NITROGEN FIXATION POTENTIAL OF COWPEA

VARIETIES AND EFFECT OF RESIDUE NITROGEN FOR MAIZE PRODUCTION

KNUST

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 M.Phil. in Agronomy (Crop Physiology).

JULIANA FATAAH (B.ED AGRICULTURE)

SEPTEMBER, 2015

DECLARATION

I hereby certify that this thesis has not been submitted for a degree to any other university and it is entirely my own work and all help and references have been duly acknowledged.

Juliana Fataah	…………..………	……………………
Student	Signature	Date
Dr Joseph Sarkodie-Addo	………………………	……….……………
Supervisor	Signature	Date

Certified by:

Dr Enock A. Osekre	…..…………………	……………………
Head of Department	Signature	Date

KNUST
NYANSAPƆ WƆSANE NO BADWENMA

ABSTRACT

Field experiments were conducted in the major and minor seasons in 2014 to study the nitrogen fixing potentials of ten cowpea varieties and to determine the effect of residual fertility on maize growth and yield. The experimental design was Randomized Complete Block with four replications. Each replication had 10 cowpea plots and a reference plot of maize. Data collected were plant height, number of leaves, number of branches, stem girth, emergence, plant stand, days to 50% flowering, plants harvested, shoot dry weight, nodule number, nodule dry weight, percentage nodule effectiveness, number of pods per plant, number of seeds per pod, 100 seed weight, harvest index and grain yield per hectare. After the cowpea was harvested, the residues were incorporated back in to the soil and maize was sown on all plots. A control plot received the normal fertilizer recommended rates. Data collected on maize were plant height, number of leaves, stem girth, shoot dry weight, number of cob per plant, number of seeds per cob, 100 seed weight, harvest index and grain yield per hectare. The results showed that all the varieties nodulated freely with the native rhizobia in the soil. „Asetenapa‟ variety fixed the greatest amount of nitrogen and the maize grain yield was greater in the „Asetenapa‟ residue incorporated plots. The grain yield from the fertilizer applied treatments was not significantly higher than any of the residue incorporated treatments. The results indicated that if cowpeas are cultivated on plots and their residues are effectively recycled, the field would be fertile enough to support maize yields similar to the application of recommended rates of fertilizer for maize production.

DEDICATION

This thesis is dedicated to my lovely husband, Paul Diyoh, my daughters and all family members who in diverse ways helped me to go through this course successfully.

ACKNOWLEDGEMENT

I wish to express my profound gratitude to the Almighty God for his protection and guidance throughout the course. My heartfelt thanks go to my supervisor, Dr Joseph Sarkodie-Addo of the Department of Crop and Soil Sciences, Faculty of Agriculture, KNUST, for his kind advice, guidance, support and constructive suggestions during this study and also who helped in the statistical analysis of the experimental data. I am grateful to the Legumes Division of the Crops Research Institute, Fumesua for providing me with the planting materials.

I also thank the staff of the Crops Research Institute, Legumes Department for their support, especially Frank Bosompem and Paul Marno.

I extend my special thanks to the staff of the Soil Science laboratory of the Department of the Soil Science for the soil sampling analysis.

Finally I am indebted to Mr Eric Amankwaah and all course mates for their advice, love and support.

TABLE OF CONTENTS

KNUST
NYANSAPƆ WƆSANE NO BADWENMA

LIST OF TABLES

KNUST

CHAPTER ONE

INTRODUCTION

Cowpea (*Vignaunguiculata*(L).Walp) is an important crop in many countries of tropical Africa, Asia and South America (Singh *et al*., 1997).Both grains and leaves are edible products of cowpea which are rich and cheap source of high protein. They supplement the low quality cereals or root tubers consumed in tropical Africa (Kitch *et al*.; 1998, Karikari and Molatakgosi, 1999). On average cowpea contains 23-25% protein, 50-67% starch in dry weight basis (Quin, 1997). From a single planting, one may be able to have several products such as leaves, immature pods, mature seeds and immature seeds.

Cowpea is a leguminous crop and is able to fix nitrogen from the atmosphere. Approximately 80 percent of the atmosphere is nitrogen gas (N_2). Unfortunately N_2 is unusable by most living organisms. The terrestrial flux of N from biological N_2 fixation has been calculated to range from 139-170kg/ha/yr (Burns andHardy, 1995; Paul, 1988). Nitrogen depletion in maize-based system of West Africa savannah is estimated to be 3680kg/ha/yr (Sanginga *et al*., 2000) and it has been obvious since the mid -1990s that fertilizer use is necessary if sustainable agricultural production in smallholder farms is to be raised to levels that can sustain the growing population.

Successful maize production depends on the correct application of production inputs that will maintain the environment as well as agricultural production. One of such inputs which is very important to increase maize production is the use of chemical nitrogen fertilizers but adverse effects associated with the use of inorganic fertilizers on the environment has called for the need to look for other alternatives.

In contrast to expensive chemical N-fertilizers, the use of nodulated legumes is often a more attractive and practical alternative. According to Giller (2001) if only legumes grains are harvested and the residues are effectively recycled, net nitrogen accrual from the incorporation of legumes residue can be as much as 140kg/N/ha depending on the legume. There is, however, a dearth of reliable estimate of N_2 fixation by these legumes and hardly any quantitative information is available on their residual N benefits to subsequent crops.

Maize is the most important cereal crop produced in Ghana and it is also the most widely consumed staple food in Ghana with increasing production since 1965 (FAO 2008; Morris *et al.*,1999). Maize accounts for more than 50 percent of the total cereal production in the country. The bulk of maize goes into consumption and is the most important crop for food security. The crop has now risen to a commercial crop on which many agro- based industries depend for raw materials (Iken and Amusa, 2004). It is the most important cereal in the world after wheat and rice with regards to cultivated areas and production (Purseglove, 1992; Osagie and Eka, 1998). According to IITA (2001) report, maize contains 80% carbohydrate, 10 percent protein, 3.5 percent fiber and 2 percent mineral. It also contains vitamin B and iron. According to Khawar *et al*. (2007), maize has a variety of uses. The starch extracted from the grain is used in making confectionary and noodles. Maize can be used as forage, feed for livestock and for making silage after fermentation of corn stocks. The crop is a multipurpose crop because every part of it has economic values. The grain, leaves, stalk, tassel and cob can be used to produce a large variety of food and non- food products (IITA 2009). For instance, the oil present in corn (rich in embryo) is far and widely used for cooking and manufacturing of soaps. Sticky gums contains dextrin used for sealing envelops and labels. Corn starch is well recognized for its use in cosmetics and pharmaceutical industries as diluent. Corn seeds are functional in making alcohol, the stem fibers for manufacturing of papers. The silk improves blood pressure and support liver functioning as well as producing bile and is also a potent antioxidant that guards body from harming radicals responsible for cellular damage and/ or cancer (Dilip and Jhariya, 2013). For the varied importance of maize, production needs to be increased to satisfy its demand by the increasing population. Farmers are obliged to the use of artificial fertilizers to increase production which has its own implications such as high cost of production and environmental pollution. Leguminous crops can be grown as a substitute to fertilizer in the next farming season.

The residual effect on the subsequent crop production especially maize which is widely cultivated throughout the world in the tropical Africa (Myers, 1988) is very important, hence the need for this research.

The main objective of this study was to determine the N_2 fixation potential of some improved cowpea varieties and estimate the amount of N_2 in their residues for succeeding maize crop.

The specific objectives were:

i. To evaluate the nodulation and amount of nitrogen fix by selected cowpea varieties.

ii. To determine the amount of residue N that would be available to succeeding crop.

iii. To determine whether the residue N is capable of supporting maize growth and yield.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin, Production and Uses of Cowpea

Cowpea (*Vignaunguiculata*(L).Walp) is believed to have originated and domesticated in South Africa and later spread to East and West Africa and Asia. It reached south-west Asia in 2300 B.C (Purseglove, 1992) but was not cultivated intensively in India until the late 18th century (Perrino *et al.*, 1993). However, the earliest intensive cultivation may have been by the Greeks and the Romans in Southern Europe in the 8th century B.C (Tostiaa and Negri, 2002; Perrino *et al*., 1993).

Cowpea has so many names including crowder-pea, southern pea and most popularly known as black-eyed bean. It is a native of Africa, with West Africa particularly Nigeria being a major center of diversity (Ng and Padulosi, 1997). Four culti-groups of cowpea are recognized namely: biflora or *catjang*, which is characterized by small erect pods and found mostly in Asia, *unguiculata,* which is the commonest type, *sesquipedalis* or yardlong is mostly found in Asia and characterized by its very long pods which are consumed as green snap „bean" and lastly the *textilis* found in West Africa which was used for fibers due to its long peduncles. Cowpea is a herbaceous warm-season annual that is similar in appearance to common bean except that the leaves are generally darker green, more shiny and less pubescent. The plant growth habit can be erect, semi erect, prostrate (trailing) or climbing depending on the genotype.

Most cowpea is grown in Africa particularly in Nigeria and Niger which accounts for 72% of world cowpea production (FAOSTAT, 2012). The Sahel region also contains other major producers such as Burkina Faso, Ghana, Senegal and Mali.

More than 5.4 million tons of dried cowpea are produced worldwide, with Africa producing nearly 5.2 million. Nigeria, the largest producer and consumer accounts for 61% of production in Africa and 58% worldwide (IITA, 2009). Cowpea has considerable adaptation to high temperatures and drought compared to other crop species (Hall *et al*., 2002; Hall2004). As much as 1000kg ha^{-1} of dry grains has been produced in the sahelian environment with only 181mm of rainfall and high evaporation demand (Hall and Patel,1985).

Dry grain yields above 7000kg per ha have been achieved in large field plots with guard rows in the valley of California (Sanden 1993), where growers often obtained yields above 4000 kg per ha. Clearly cowpea is both responsive to favorable growing conditions and capable of growing under drought, heat and other abiotic stresses. The crop is mainly grown in warm climate since they require warm soil temperatures for good establishment. They are adapted to a wide variety of soils from heavy to light textured and from humid tropics to semi –arid tropics. The duration of cowpea growth varies widely in different genotype but environmental conditions also seem to affect it. According to Moody (1985), the duration from sowing to flowering may range from 38 to142 days. Most cowpeas are in generally quantitative short day plants with a tendency to flower as days become shorter.

According to the Food and Agriculture Organization of the United Nations (FAO), as of 2012, the average cowpea yield in West Africa was estimated 483kg/ha^{-1}(FAO, 2012), which is still below the estimated potential production yield (Kormawa *et al*., 2002).

The crop is one of the most widely cultivated legumes, mainly in the savannah and transition zones of Ghana (Asante et al., 2006). A long-term drought in the Sahelian zone of West

Africa has caused many farmers in this part of Africa to shift more of their production to cowpea because of its drought tolerance (Duivenbooden *et al*., 2002). Because of the shift in production and the adaptation of new varieties and improved production system, worldwide production has gone up from an annual average of about 1.2 million tonnes during the decade of 1970s to 3.6 million tonnes per annum (during the five-year period spanning 1998 to 2003, (FAO, 2008). The FAO estimates that nearly 4 million metric tons of dry grains is produced annually in about 10 million ha worldwide (FAO, 2008).

Singh *et al*. (1997) estimated slightly higher than FAO estimates, with worldwide production of 4.5 million on 12 to 14 million ha. With this figure, 70% production occurs in the drier Savanna and Sahelian zones of West and Central Africa where it is usually grown as an intercrop with millet, sorghum and less frequently as a sole crop or intercropped with maize, cassava or cotton (Langyintuo *et al*., 2003).

The nutritional composition of cowpea grain is important because it is eaten by millions of people who otherwise have diet lacking in protein, minerals and vitamins. In a study of 100 cowpea breeding lines in the IITA collection, seed protein content ranged from 23-32% of seed weight (Nielson *et al*., 1993).

Cowpea grain is also a rich source of minerals and vitamins (Hall *et al*., 2004) and it has one of the highest levels of folic acid, a crucial vitamin B that helps prevent spinal tube defects in unborn children.

The crop can be used at all stages of growth as a vegetable crop and the leaves contain significant nutritional value (Ahenkora*et al.*, 1998; Nielson *et al*.,1993). The tender green leaves are an important food source in Africa and are prepared as a pot herb like spinach.

The immature green pods are used in the same way as snap beans often mixed with cooked dry cowpeas or with other foods. Dry mature seeds are also suitable for canning and boiling. The foliage is an important source of high quality hay for livestock feed (Tarawali *et al*., 2002).

The fat content in cowpea ranges from1.4-2.7% (Nielson *et al*., 1993) while fiber content is 6% (Bressani, 1985). The protein in grain legumes like cowpea has been shown to reduce low-density lipoproteins that are implicated in heart diseases (Phillips *et al.*, 2003). Also the grain legume starch is digested more slowly than the starch from cereals and tubers; it produces fewer abrupt changes in blood glucose levels following consumption (Phillips *et al*., 2003). Protein isolates from cowpea grains have good functional properties, including solubility emulsifying and foaming activities (Rangel *et al.*, 2004) and could be a substitute for soy proteins isolates for persons (especially infants) with soy proteins allergies.

Careful positive attention to cowpea production would support 850 million people in the world with high incidence of under nourishment in sub-Saharan Africa as documented by FAO (2008). The haulm (dried stalks) of cowpea is a valuable by-product, used as animal feed (Singh *et al*., 1997). The crop also protects the soil against erosion due to its fast growing characteristic and as a broad leaf plant; it spreads to intercept the intensity of rain drops on the soil to reduce the effect of erosion.

Because of its superior nutritional attributes and versatility, adaptability and productivity, cowpea was chosen by the US National Aeronautical and Space Administration (NASA) as one of the few crops worthy of study for cultivation in space stations (Bubenheim *et al*.,1990; Ehlers and Hall, 1997).

2.2 Importance of nitrogen in crop production

Nitrogen is an essential plant nutrient. It is a key component in plant proteins and chlorophyll. It is the plant nutrient that is often most limiting to efficient and profitable crop production. Inadequate supply of available N frequently results in plants that have slow growth, depressed protein levels, poor yield and poor quality produce. Nitrogenstressed plants often have greater disease susceptibility compared to properly nourished plants. On the other hand, excessive N can be detrimental for growth and quality, inaddition to causing undesirable environmental impacts (Mikkelsen and Hartz, 2008). When N inputs to the soil system exceed crop needs, there is a possibility that excessive amounts of nitrate (NO_3^-) may enter either ground or surface water (O"Leary *et al.*, 2002). Nitrogen is the most important nutrient element required for crop production especially for cereals, which have been reported to be dominant in cultivated land in the world (Myer, 1988). For maximum grain yield to be realized in the Northern Guinea Savannah, addition of 120kgN per ha of inorganic fertilizer was required (Ogunlela and Ologunde, 1984). Grains legumes cause significant and positively yield effects on subsequent crops. For example, in sunflower, Steer and Seller (1990) found that the application of nitrogen fertilizer before floret initiation increased the concentration of palmitic and linoleic acids, but decreased those of stearic and oleic acids. Bauer and Carter (1986) and Kneip and Mason (1989) found that kernel breakage decreased and kernel density increased with nitrogen fertilizer. Juice purity in sugar beet is reduced by excessive nitrogen through increased in alpha-amino-nitrogen (Wiklicky, 1971).

Nitrogen is the most limiting factor for grassland productivity. It stimulates tiller development, increase leaf size and lengthens the period of green leaves (Rhykerd and

Noller, 1974). In warm-season grasses, many studies found that nitrogen fertilization caused higher beef gains in kg/ha (Perry and Baltens Perger, 1979).

Application to soils low in minerals nitrogen (N) will result in a loss of legume production and N-fertilizer of up to 160kg N/ha may be required to achieve seed yield similar to those of a well-nodulated crops (Gault *et al.*, 1984).

2.3 Challenges of nitrogen application

Although inorganic fertilizer is a convenient source of nitrogen for crop growth, its use is ultimately governed and regulated by economic and environmental considerations (Adeleke and Haruna, 2012). For instance, in Nigeria, government inconsistent policies on fertilizer subsidiary had led to the problem of high prices of fertilizers which was beyond what a peasant farmer could afford. It also led to adulteration of the material. Farmers were also faced with hoarding when subsidies are finally replaced (Haruna *et al.*, 2011). Recent studies have shown that the application of inorganic N fertilizer depletes soil organic carbon and N. Plants mostly depend on combined or fixed form of nitrogen, such as ammonia or nitrate. Much of this is provided to cropping system in the form of industrially produced nitrogen and the use of these fertilizers has led to worldwide ecological problems such as coastal dead zones.

3.4 Sources of Nitrogen to Crop Production

There are two main sources of nitrogen to crops; namely natural and artificial fertilizers.

Although the earths‟ atmosphere contain 78% N gas (N_2), most organisms cannot directly use this resource due to the stability of the compound. Plants instead depend upon combined or fixed form of nitrogen, such as ammonium or nitrate. Much of this is provided to cropping system in the form of industrially produced fertilizers and the use of these fertilizers has led to worldwide ecological problems such as coastal dead zone. Naturally plants get nitrogen through the decomposition of organic matter, the conversion of atmospheric nitrogen into compounds by natural processes such as precipitation, lightening and through biological nitrogen fixation (Vance, 2001). Plants also derived nitrogen from crop residues and animals manure.

3.5 Biological nitrogen fixation

Biological nitrogen fixation (BNF) is the process that changes inert N_2 to biological useful NH_3 through the action of micro-organisms. Biological Nitrogen Fixation is carried out by specialized group of prokaryotes. These organisms utilized the enzymes nitrogenase to catalyze the conversion of atmospheric nitrogen (N_2) to ammonia (NH_3). These prokaryotes include aquatic organisms such as *Cyanobacter,* free-living soil bacteria such as *Azotobacter*, bacteria that form associative relationship with plants such as *Azospirillum*, and most importantly bacteria such as *Rhizobium* and *Bradyrhizobium* that form symbiosis with legumes and other plants (Postgate,1982).

Micro-organisms that fix nitrogen require 16 moles of adenosine triphosphate (ATP), to reduce each mole of nitrogen (Hubbell and Kidder, 2009). These organisms obtain this energy by oxidizing organic molecules. Associative and symbiotic nitrogen fixing microorganisms obtain these compounds from their host plants‟ rhizospheres (National Research Council 1994; Hubbell and Kidder 2009). Some species of *Azospirillium* are able to form close association with several members of the *Poaceae* (grass), including cereals crops such as rice, wheat, corn, oats and barley. These bacteria fix appreciable amount of nitrogen within the rhizosphere of the host plants. Cowpea can fix about 40kgN/ha from nodules in the presence of the right rhizobia strain which can satisfy the crop nitrogen (N) requirements (Singh 1997).

In symbiotic nitrogen fixation these organisms fix nitrogen by partnering with a host plant. The plants provide sugars from photosynthesis that are utilized by nitrogen fixing bacteria for the energy it needs for nitrogen fixation. In exchange for these carbon sources, the microbes provide fixed nitrogen to the host plant for its growth and also beneficial for subsequent crop to be cultivated. One example of this type of nitrogen fixation is the water fern Azolla‟s symbiosis with a cyanobacteria *Anabaena azolla*. This symbiosis has been used for at least 1000 years as a bio- fertilizer in water paddies in South-east Asia. Rice paddies are typically covered with Azolla"bloom" whichfix up to 600kg N $ha^{-1}yr^{-1}$ during the growing season (Fattah, 2005). Another example is the symbiosis between A*ctinorhizal* trees and shrubs, such as Alder (Alnusspp) with the Actinomycete Frankia. These plants survive in nitrogen-poor environment. Actinorhizal plants are found in many ecosystems including alpine, xeric, chaparral, glacial till, riparian coastal dune and arctic tundra environment (Benson and Silvester, 1993).

The symbiotic partners described above play an important role in the worldwide ecology of nitrogen fixation, by far the most important nitrogen fixing symbiotic associations are the relationships between legumes and rhizobium and *Bradyrhizobium* bacteria. Important legumes used in agricultural systems include alfalfa, beans, clover, cowpea, lupines, peanut, soybean and vetches. Of the legumes in agricultural production, soybeans are grown on 50% of the global area devoted to legume production. Biological nitrogen fixation is an efficient source of nitrogen (Peoples *et al*., 1995). The total annual terrestrial inputs of N from BNF as given by Burns and Hardy (1975) range from 139 million to 175 million tons of N, with symbiotic associations growing in arable land accounting for 25 to 30% (35 to 44 million tons of N).

Cowpea like all other legumes, has ability to fix atmospheric nitrogen through its nodules, this makes it an important component of traditional intercropping systems of the dry savannas in Saharan Africa (Blade *et al*., 1997).

Legumes are very important both ecologically and agriculturally because they are responsible for a substantial part of the global flux of nitrogen from atmospheric N_2 to fixed forms such as ammonia, nitrate and organic nitrogen. Atmospheric N_2 fixed symbiotically by the association between rhizobium species and legumes represent a renewable source of N for agriculture (Peoples*et al*.,1995). The crop can fix about 240kg $ha^{-1}$of atmospheric nitrogen and make available about 60-70kg ha^{-1} nitrogen for succeeding crops grown in rotation with it (Akins and Afuakwa, 2008).Values estimated for various legumes crops and pasture species are often impressive commonly falling in the range of 200-300kg of N $ha^{-1}yr^{-1}$ (Peoples *et al*., 1995)

2.6 Factors affecting biological nitrogen fixation

Several environmental conditions limit factors to the growth and activity of N-fixing plants. The most problematic environments for rhizobia are marginal lands with low rainfall, extremes of temperature, acidic soils of low nutrients status and poor water holding capacity (Bottomley, 1991).

Salinity is a serious threat to agriculture (Cordivilla *et al.*, 1994). Increasing salt concentrations may have a detrimental effect on soil microbial populations as a result of direct toxicity as well as through osmotic stress. The depressive effect of salt stress on N_2 fixation by legumes is directly related to the salt – induced decline in dry weight and N content in the shoot (Cordivilla, 1995). Application of salt or drought decreases nodules permeability. This decrease is associated with a contraction of nodules inner-cortex cells and an increase in acid abscissic acid content of the nodules (Irekti and Drevon, 2003).

Additionally, it has been argued that the limitations of O_2 diffusion imposing structural modifications due to salinity are compensated for by the decrease of nodule growth and the formation of a large number of small nodules facilitating the O_2 entry in the nodules by increased contact area with external medium (L‟taief *et al*., 2007). It is well known that some free-living rhizobia are capable of survival under drought stress or low water potential (Fuhrmann *et al.*, 1986). Moderate moisture tension slowed the movement of *R. trifolii* (Handi, 1970).

The migration of bacteria ceases when water-filled pores in soil become discontinuous. Optimization of soil moisture for growth of the host plant, which is generally more sensitive to moisture stress than bacteria, results in maximal development of fixed- nitrogen inputs into the soil system by rhizobium-legume symbiosis (Tate, 1995). High temperature and heat are major problems for biological nitrogen fixation of leguminous crops (Michiels *et al.*, 1994). High root temperature strongly affects bacterial infection and N-fixation in several legumes including soybean, peanut, cowpea and beans.

High soil temperature will delay nodulation or restrict it to the subsurface (Graham, 1992). For most rhizobia, the optimum temperature range for growth in culture is 28 to 31^{0}C and many are unable to grow at 37^{o}C (Graham, 1992). However, 90% of cowpea rhizobium strains obtain from the hot, dry environment of the Sahel savannah grew well at 40^{o}C (Werner and Newton, 2005). It appears that every legume and rhizobium has optimum temperature relationship which is around 30^{o}C for clover and pea, between 35^{o}C to 40^{o}Cfor soybean, peanut and cowpea and between 25^{o}C to 30^{o}C for common bean (Long, 2001).

Soil acidity also affects N-fixation. Legumes and their rhizobia exhibit varied responses to acidity. Some species like *Lucerne* (*M. sativa)* are extremely sensitive to acidity while others such as *Lotus tenuis* tolerate relatively low pH (Correa and Barneix, 1997). The failure of legumes to nodulate under acid-soils conditions is common especially in soils of pH less than 5.0. The inability of some rhizobia to persist under such conditions is one cause of nodulation failure (Carter *et al*., 1994). Nutrient deficiency stress has great impact on N-fixation. The effect of salt stress or acidity on calcium availability and the initial stages of nodule formation will affect the net nodulating capacity of legumes.

Nitrogen fixation by Frankia actinorhizal symbiosis may be limited by low available P in the soil. Sanginga *et al*., (1989) observed increased N_2 fixation by *Casuarina equisetifolia*by adding phosphate to P-deficient soil. It has been established that soil NO_3 inhibit root infection (Abdel *et al*., 1996), nodule development and nitrogenase activity (Sayed *et al*., 1997). Danso *et al.* (1990) found that the inhibition of soybean N_2 fixation at higher N levels (83mg of N kg^{-1} of soil) was significantly reduced by a second inoculation. Application of soils low in minerals nitrogen (N) will result in a loss of legume production and N-fertilizer of up to 160kg N/ha may be required to achieve seed yield similar to those of a well-nodulated crops (Gault *et al.*, 1984).

Soil pH has great impact on nodulation. Worldwide, more than 1.5 g ha^{1} of acid soils limit agriculture production (Graham and Vance, 2000) and as much as 25% of the earth‟s croplands are affected by problems associated with soil acidity. Hungria and Vargas (2000) reported that there is a range of effects of soil pH on rhizobia but relatively few grow and survive well below pH values of 4.5 to 5.0. Acidity also influences both the growth of the legume and the infection process.

The use of herbicides, fungicides and other pesticides are potentially limiting factors to BNF. The herbicides sethoxydim, alachor, fluazifop butyl and metalachlor did not have detrimental effects on N_2 fixation or seed yields when added at the recommended rates for weed control in soybean plantation (Kucey *et al.,* 1988). However, paraquat significantly reduced the amount of N_2 fixed by soybean as measured by ^{15}N dilution method. Similarly, herbicides were reported to induce reduction in nodulation and fixation of soybean (Yoshida, 1990) and bean (Schnelle and Hensley, 1990).

Numerous (micro)-climatic variables, soil physical properties and agronomic management factors also play a part in controlling N_2 fixation; however, none of those factors should be considered in isolation as all are interconnected in the control of N_2 fixation. Virtually any environmental factor that negatively influences either the growth of rhizobia or the host plant itself has a dramatic impact on symbiotic N_2 fixation (Mohammadi *et al*., 2012).

2.7 Measurement of biological nitrogen fixation

There is no single correct method of measuring N_2 fixation. Each method has its own merits and demerits. Some of these methods are acetylene reduction assay, ^{15}N-isotopic technique, xylem-solute technique, total plant and soil N and Nitrogen-difference. In this study, the nitrogen difference technique was used. This method is based on the assumption that both nitrogen fixing plant and a non-nitrogen fixing plant (Giller and Wilson, 1991) take the same amount of nitrogen from the soil. So the difference in them will be the amount of N fixed by rhizobia through the air. This is the most simplest and inexpensive method.

The acetylene reduction assay (ARA) can be carried out on detached nodules, de-topped roots or whole plants in a closed vessel containing 10% acetylene. In this method, samples are taken by syringe and the ethylene produced by the reduction of acetylene is measured by injecting the sample in a gas chromotograph (Dixon and Wheeler, 1986). This method provides an instant measure of nitrogenase activity (but not necessarily of N_2 fixed) under the experimental condition. A problem that is inherent in ARA is the need to calibrate the rates of ethylene production with the actual rates of N_2 fixed. Also, nitrogen activity of some legumes decline considerably once nodules or roots are detached from the rest of the plant and it is also difficult to collect all nodules once roots are detached from the rest of the plants. It is also difficult to collect all nodules on plants with long roots. To minimize this limitation, the plants are confined to open ended chambers and ARA is done *in situ* (Barroquio*et al.*, 1986).

The N- solute analysis of xylem exudate is another method of estimating N_2 fixation. This is based on the assumption that nitrogen from BNF can be transported to the leaves in the form of ureide, allantoin and allantoin acid or in the form as asparagine and glutamine. In agricultural soils, where nitrate is the readily available form of N for plant growth, the solute derived from the soil mineral N will contain principally free nitrate organic products of nitrate reduction in the roots. This method is simple and virtually none-destructive. It is also relatively in expensive. It disadvantage is that it requires repeated measurements over a long period of time.

The ^{15}N isotope dilution method is another attractive method because one sampling can provide an estimate of BNF. It is used in plants but not in soil. The assumption in this method is that $^{15}N/^{14}N$ ratio of N absorbed from the soil or water is the same for the Nfixing plant and the non-fixing control. It is satisfied in soil when ^{15}N enrichment of soil N available to the N_2-fixing system is constant during the experiment. Either a non-fixing plant or available to theN_2fixing-plant or available soil N can be used as control but the validity of the control depends upon the percentage of N derived from the air (Ndfa). Fried and Broeshart (1975) reported that this method is based on the assumption that the reference plant absorbs N from the soil at the same enrichment as that absorbed by the legume.

Natural ^{15}N abundance method is based on the fact that soil has a higher ^{15}N than air. It is advantageous because of the stable isotopic composition of N sources. But the ^{15}N gradient observed with soil depth is a serious source of error but growing plants in pots avoids this problem.

^{15}N isotopic dilution technique is considered to be one of the most reliable methods for estimation of nitrogen fixation by nodulated legumes in the field (Danso, 1995; Mcneill *et* *al*., 1996).This method depends upon differences in the sources of N available to the plant. These sources are soil N, fertilizer N and atmospheric N (Fried *et al*., 1983). An advantage of this technique is that it assesses the integrated amount or proportion of nitrogen derived from the atmosphere through N_2 fixation in the field grown legumes crops (Reichard *et al*., 1987). The major limitation of this method in the developing countries is the high cost of instruments to measure and the use of expensive ^{15}N-labelled fertilizer (Peoples *et al.* 1989; Danso, 1995).

2.8 Future of biological nitrogen fixation

Biologically-fixed nitrogen could be directly "absorbed" by plants and keep the environment almost "untouched" (Cheng, 2008). Currently, approximately 2 tons of industrially-fixed nitrogen is needed as fertilizer for crop production to equal the effects of 1 ton of nitrogen biologically-fixed by legume crops. Therefore, biologically-fixed nitrogen influences the global nitrogen cycle substantially less, than industrially-fixed nitrogen (Cheng *et al*., 2005).

On the other hand, world population is now been increasingly relying on nitrogen fertilizers in order to keep up with the demands of food and economic growth rates.

However, less than 30% of synthetic fertilizers would actually be utilized; the unused chemicals sprayed on crops would be lost in the field and could subsequently cause serious environmental problems, let alone industrial pollution. Biological nitrogen fixation has the advantage of being environmental friendly and therefore would be ideal for sustainable agriculture.

Enormous progress in almost all aspects of biological nitrogen fixation has been made in the past century, especially in the recent two decades, in genetics and biochemistry, culminating in the determination of the crystallographic structures of both nitrogenase components. More studies are needed to be carried out in order to completely understand the nature of the process and make it more possible use of it. Biological nitrogen fixation is an important aspect of sustainable and environmentally friendly food production and longterm crop productivity. However, if BNF is to be utilized, it must be optimized. For efficient and effective BNF in agriculture, the host plant should be well managed through legumes for enhanced nitrogen fixation, effective strains should be selected to fix nitrogen and also good inoculation methods should be adopted for production and long-term crop productivity.

CHAPTER THREE

MATERIAL AND METHODS

3.1 Experimental Site

The research work was carried out at the Crops Research Institute (CRI) at FumesuaKumasi from June 2014 to December 2014. Fumesua is located within latitude 6$_o$ 41' N and longitude 1$_o$ 28' W. The area has bimodal rainfall pattern with the major season rains around April to June and minor season rains from August to November with annual rainfall of 1,345mm per annum. The temperature is usually high throughout the year with annual mean temperature between 22℃ to 31℃. The vegetation is that of humid forest type. The soil type is Ferric Acrisol Asuansi Series (Adu and Asiamah, 1992).

3.2 SOIL CHARACTERISTICS

The soil at the experimental site is well drained, sandy loam overlying reddish-brown and gravelly light clay. It belongs to the Kumasi series, Ferric Acrisol Asuansi. Composite soil samples were taken from the experimental site to a depth of 30cm.These samples were taken to the laboratory to determine the following properties (N,K, P,pH and Organic carbon). The samples were dried and sieved using a 2mm mesh sieve. The following properties were determined.

3.2.1 Organic Carbon

The Walkley-Black wet combustion procedure (Nelson and Sommers, 1982) was used to determine organic carbon.

3.2.2 Organic Matter

Percent organic carbon was multiplied by 1.724 (The Van Bemmelen factor) to get percent organic matter.

3.2.3 Soil pH

This was measured in 1:2.5 soils to water suspension by the use of a glass Electro calomel electrode pH meter (Mclean, 1962).

3.2.4 Total Nitrogen

The Macro Kjeldahl method described by Bremner and Mulvaney (1982) was used. A 10g soil sample (< 2mm in size) was digested with a mixture of 100g potassium sulphate, 10g copper sulphate and 1g selenium with 30mls of concentrated sulphuric acid. This was followed by distillation with 10ml boric acid (4%) and 4 drops of indicator and 15mls of 40% NaOH. It was then titrated with Ammonium sulphate solution. Based on the relation that 14g of nitrogen is contained in one equivalent weight of NH3, the percentage of nitrogen in the soil was calculated.

3.2.5 Potassium

The flame photometer method was used to determine the amount of potassium with ammonium acetate as the extractant.

3.2.6 Available phosphorous

The Bray-1 test method was used for the determination of phosphorus with dilute acid fluoride as the extractant (Jackson, 1958).

3.3 LAND PREPARATION

The land was previously cropped to soybean. The site for the experiment was mechanically cleared by slashing the vegetation and was ploughed and harrowed. The plots were laid out using tape measure, garden lines and pegs.

EXPERIMENT ONE: TO DETERMINE THE NITROGEN FIXATION POTENTIALS AND THE RESIDUE N CONTENT OF TEN IMPROVED COWPEA

VARIETIES

3.4 VARIETIES USED

Seeds of ten cowpea varieties used were obtained from the Crops Research Institute (CRI) of the Council for Scientific and Industrial Research (CSIR) at Fumesua, Kumasi. These varieties were „Bengpla‟, „Tona‟, „Asetenapa‟, „Nhyira‟, „Asomtem‟, „Soronko‟, „Hewale‟, „Adom‟, „Asomdwee‟ and „Videza‟.All the varieties are early maturing which is about 60 days. „Adom‟ and „Asomtem‟ are trailing and narrowed leaves, whereas the rest are erect with broad leaves. The seed of Bengpla is whitish in colour, Tono, Asetenapa, Asomdwe, Nhyira are red in colour while the rest are creamy in colour.

3.5 DESIGN AND EXPERIMENTAL LAY OUT

The experimental design was a Randomized Complete Block, with four replications (blocks). Each block consisted of 11 plots, each measuring 4m x 1.2m, giving a total of 44 plots. There was an alley of 2m between blocks and 1.5m between plots.

The experiment was carried out during the major season, April to July 2014. Three to four seeds per hole were sown on the 18^{th} June 2014 at a planting distance of 60cm x 20cm. Emergence of seedlings took place six days after sowing. Each replication had a plot of maize (4rows) of the variety Abotem planted as a non-fixing reference crop.

3.6. CULTURAL PRACTICES

3.6.1 Thinning

Thinning out was done to 2 stands per hill, 14 days after sowing, when the soil was moist and seedlings well established.

3.6.2 Weeding

Weeding was done manually using a hoe, in the 3rd and 6th week after sowing to control weeds. Each weeding operation was completed on the same day for all the blocks on the day of weeding.

3.6.3 Pest Management

Aphids were controlled on the 14th, 29th July and on the 12th August with Karate (25g lambda cyhalothrin EC) at 50ml per knapsack at the recommended 14 days interval to control the insects, till when pods were completely filled. In all, there were three times of spraying. Sunpyrifos (480g chlorpyrifos-ethyl) which is a post flowering chemical was also applied at the rate of 100ml per knapsack.

3.7 DATA COLLECTION

3.7.0 Vegetative growth

Sampling for growth (vegetative) analysis was done on 20, 35 and 50 days after sowing (DAS). At each sampling period, five plants on each plot were taken at random for the various parameters. These samples were taken from the border rows.

3.7.1 Plant stand

Plants in the two central rows were counted and recorded.

3.7.2 Plant height

Plant height was measured from the ground level to the highest tip of the stem for the five sampled plants. This was done using a metre rule at the various sampling periods. The average plant height was calculated for each treatment.

3.7.3 Number of branches

This was taken at the sampling periods. Branches of five sampled plants from each plot were counted and the average computed.

3.7.4 Total dry matter

The five sampled plants from each plot were put in labeled envelopes and oven dried to a constant weight at 60^{O}C for 72 hours, and then weighed, and the average weight calculated.

3.7.5Stem girth

The girths of the five samples were measured using a caliper just above the soil level and their average computed.

3.7.6 Nodule count and effectiveness

The five sampled plants were carefully dug out, retrieving detached nodules at each sampling period. The nodules were kept in labeled envelops and sent to the laboratory, washed and counted. Twenty nodules were sampled from the lot from each envelop to determine nodules effectiveness. Nodules were cut opened to determine apparent effectiveness, using a knife and hand lens. Nodules with pink or reddish colour were considered effective and fixing nitrogen, while those with green or colourless were considered ineffective. After this, the percentage (%) effective nodules were calculated.

3.7.7 Nodule dry weight

After the nodules were assessed for effectiveness, they were oven dried to constant weight at $60^{O}C$ for 72 hours. These were weighed and the average weight calculated.

3.7.8 HARVEST DATA

At harvest maturity, when about 85% of pods had turned brown, plants from the central rows on each plot were harvested for the yield analysis. Five plants from the border of each row were sampled, for pod number, number of seeds per pod, 100 seed weight, harvest index and total plant weight. These plants were carefully uprooted and put in labeled envelops. They were then oven dried for three days at $80^{O}C$ before weights taken.

3.7.9 Number of plants at harvest

Number of plants were counted from the two central rows of each plot and recorded.

3.7.10 Number of pods per plant

For pod number, five random plants were taken from each plot and all the pods plucked. These were then counted and the average pod number was calculated for each plot.

3.7.11 Number of seeds per pod

The number of seeds per pod was also determined by threshing the pod of the five plants from each plot, seeds were counted and the average calculated.

3.7.12 Hundred Seed weight

The 100 seed weight was determined by counting 100 seeds from the threshed seeds from each plot. These were weighed to represent the mean seed weight.

3.7.13 Harvest index

Seed weights of the five plants were divided by total plant weight of the five sampled plants of each plot to estimate the harvest index of each treatment.

3.7.14 Grain yield

Grain yield per hectare was determined by threshing the harvested plants from the 2 central rows. These were dried to about 10% moisture content and weighed, and the resulting weights in grams per square metre were converted to kilograms per hectare.

3.7.15 Nitrogen content of seeds and residues

Both seeds and residues of the five plants that were uprooted from the border rows of each plot were taken to the laboratory to determine their N content separately by the Kjedahl method described earlier. The same was done for the maize plants.

3.7.16 Nitrogen fixed

This was determined by subtracting the total N of the maize plots from those of the cowpea plots. This is the N difference method.

3.8 DATA ANALYSIS

All data was analyzed using the Analysis of Variance (ANOVA) and the treatment differences were compared using the Least Significant Difference (LSD) procedure at 5% level of probability.

EXPERIMENT TWO: TO DETERMINE THE CAPABILITY OF COWPEA RESIDUE NITROGEN FOR PROFITABLE MAIZE PRODUCTION

All haulms were carefully deposited back on their respective plots. To avoid contamination, weeds were killed after sowing by spraying with glyphosate on the 12th September as seeds of the maize variety Abotem were sown on 10th September (2014). Spacing was 75cm x40cm. Abotem is an extra early maize (80-85 days) recently released by the CSIR-CRI at Fumesua.

3.9 CULTURAL PRACTICES

3.9.1 Refilling

Seeds that did not germinate were refilled a week after planting on affected plots.

3.9.2 Thinning

Thinning was carried out to two stands per hill on all the plots a week after planting.

3.9.3 Weed control

Round up (360g SL glyphosate) was applied at the rate of (300ml) per knapsack two days after sowing. In the 3th and 4th weeks after planting, Atrazine 50%SC per knapsack was used to control weeds.

3.9.4 Fertilizer application

NPK(15:15:15) and Sulphate of ammonium were applied on the plots which were cultivated with maize in the major season at the rate of 5g per plant during the 2^{nd} and 4^{th} weeks after planting respectively.

3.9.5 Pest management

The insecticide called Power (25g lambda cyhalothrin E.C) at the rate of 50ml per knapsack was used to control insects just after tasseling.

3.10 DATA COLLECTION

3.10.1 Growth data

Five plants were sampled on the 20th, 40th and 60th day after sowing and the following parameters were taken.

3.10.2 Plant height

The plant height was measured from the ground level to the top of each of the five plants. This was done with the use of a metre rule at the various sampling periods. The average plant height was calculated for each treatment.

3.10.3 Number of leaves

Leaves of five sampled plants from each plot were counted and the average computed.

3.10.4 Stem Girth

The girths of the five samples were measured using a caliper at just above the soil level and their average computed.

3.10.5 Total Dry Matter

The total dry matter was taken at the three sampling periods. Five sampled plants from each plot were harvested, sun dried, weighed and the average weight calculated.

3.10.6 Harvest Data

At maturity, plants stand at harvest were counted and recorded. Five plants from the border rows of each plot were harvested separately to determine the number of cobs per plant, number of seeds per cob and mean seed weight.

3.10.7 Number of cobs per plant

Five plants were taken from each plot and all the cobs plucked. These were then counted and the mean recorded for each plot.

3.10.8 Number of seeds per cob

The number of seeds per cob was also determined by threshing the cobs of the five plants, seeds were counted and the average calculated.

3.10.9 Hundred Seed weight

The 100 seed weight was determined by counting 100 seeds from the threshed seeds from each plot. These were weighed to represent the mean seed weight.

3.10.10 Harvest Index

This was computed by dividing the seed weight of the five plants by the total dried weight of the plant (cobs and the trash) on each plot.

3.10.11 Grain yield

Grain yield per hectare was determined by threshing the harvested plants from the two central rows. These were dried and weighed. The resulting weights, in grams (g) per metre square were then converted to tons per hectare to obtain the average grain yield per hectare.

3.10.12 DATA ANALYSIS

All data was analyzed using the Analysis of Variance (ANOVA) and treatment differences were compared using the Least Significant Difference (LSD) procedure at 5% level of probability.

KNUST

CHAPTER FOUR

RESULTS

4.1 Soil chemical analysis

Table 4.1 Soil chemical analysis of the experimental site

Property	Value
□ organic carbon	1.87
□organic matter	3.22

nitrogen	0.11
potassium	0.24
Available p(mg/kg)	13.83
pH	5.93

The results of the soil chemical analysis is shown in Table 4.1. The soil was acidic, had adequate amount of organic matter and nitrogen. Potassium and available P were within the recommended standard.

Results of experiment 1

4.2 Days to emergence, flowering and maturity.

Table 4.2 Days to emergence, flowering and maturity of the cowpea varieties.

	Number of days to		
Variety	**Emergence**	**50%flowering**	**50% maturity**
Bengpla	6.00	65.75	42.00
Tona	6.00	67.50	42.50
Asetenapa	6.00	68.25	43.50

Adom	6.00	66.50	41.25
Nhyira	6.00	67.75	46.00
Asomdwee	6.00	68.00	40.75
Soronko	6.00	67.75	47.25
Videza	6.00	66.00	47.75
Hewale	6.00	67.50	42.50
Asomtem	6.00	67.50	42.00
LSD (5%)	NS	1.63	4.20
CV (%)	0.0	0.8	2

The results of days to 50% to emergence, days to 50% maturity are shown in Table 4.2. All treatments effect were statistically similar regarding days to emergence. Daysto 50% flowering was latest in the Asetenapa variety and this was significantly higher ($P < 0.05$) than the treatment effects of Bengpla, Adom and Videza only. Treatment effect of Asetenapa variety was also significantly higher than those of Bengpla and Videza only. All other treatment effects were similar.Days to 50% maturity was latestin Videza which was significantly higher ($P < 0.05$) than the effects all other varieties, except those ofSoronko and Nhyira varieties. Treatment effect of the Soronko variety was also significantly greater than the other varietal effects except that of Nhyira variety. All other varietal effects were similar.

4.3 Plant height

Plant height results are presented in Table 4.3. At the first sampling (20day), the height of Tona plant was the tallest, but this was significantly higher ($P<0.05$) than those of Bengpla, Videza, Asetenapa and Hewale varieties only. Plant height of Bengpla was the shortest, but were similar to those of Asetenapa, Videza and Hewale varieties. Results of the second sampling showed Adom variety producing the tallest plants and this was significantly higher (P<0.05) than all other treatment effects, except Tona. Videza plant produced the shortest plants though its effect was not different from those of Bengpla, Soronko, Hewale, Asommdwee and Nhyira varieties.

In the third sampling, Asentenapa variety produced the tallest plant and it was similar to all other varieties except Videza variety which was significant produced the shortest plants.

Table 4.3 Effect of cowpea variety on plant height at the 3 sampling periods

	Plant height (cm) at		
Variety	**20DAP**	**35 DAP**	**50 DAP**
Bengpla	9.81	21.55	31.15
Tona	12.46	26.75	32.80
Asetenapa	10.86	24.90	38.25
Adom	12.24	31.05	37.45
Nhyira	12.00	21.85	30.65
Asomdwee	11.97	20.70	32.75
Soronko	11.95	20.60	32.65
Videza	10.50	17.75	27.25
Hewale	10.40	19.93	29.85

Asomtem	12.14	25.75	34.58
LSD (5%)	1.51	4.72	8.83
CV (%)	3.0	6.6	7.7

KNUST

4.4 Number of leaves

Table 4.4 Effect of cowpea variety on the number of leaves at 3 sampling periods

	Number of leaves at		
Variety	**20DAP**	**35 DAP**	**50 DAP**
Bengpla	6.75	13.70	17.51
Tona	7.20	13.80	20.00
Asetenapa	6.55	11.70	19.72
Adom	7.85	13.95	27.63
Nhyira	6.45	11.60	20.81
Asomdwee	6.65	8.50	15.00
Soronko	6.25	11.10	22.64
Videza	8.25	9.80	19.42
Hewale	7.10	12.35	18.53

Asomtem	8.05	16.25	23.00
LSD (5%)	1.61	4.78	11.80
CV (%)	20.1	7.1	53.9

Results of leaf production by the varieties are presented in Table 4.4. During the first sampling, the Videza variety produced the greatest number of leaves but this was significantly higher than that of the Soronko variety only. All other treatment effects were similar. At the second sampling, the Adom variety produced the highest number which was significantly higher ($P < 0.05$) than that of Asomdwee variety only. All other varietal differences were not significant. In the third sampling, the Adom variety produced the greatest number of leaves which was significantly higher ($P < 0.05$) than that of Asomdwee variety only. All other treatment effects were similar.

4.5 Number of branches

Table 4.5 Effect of variety on the number of branches at the 3 sampling periods.

	Number of branches at		
Variety	**20 DAP**	**35 DAP**	**50 DAP**
Bengpla	0.00	4.60	5.50
Tona	0.00	4.15	6.75
Asetenapa	0.00	3.45	6.45
Adom	0.00	5.60	6.75
Nhyira	0.00	3.35	6.85

Asomdwee	0.00	2.25	5.90
Soronko	0.00	3.20	5.85
Videza	0.00	3.75	7.35
Hewale	0.00	3.80	6.85
Asomtem	0.00	4.82	5.80
LSD (5%)	0.00	1.98	NS
CV (%)	0.00	7.2	9.3

Varietal results for number of branches are presented in Table 4.5. In the first and third sampling occasions, varietal differences were not significant. During the second sampling, the Asomdwee variety produced the least number of branches, but this was significantly lower than those of Bengpla, Adom and Asomtem varieties only. All other varietal differences were not significant ($P > 0.05$).

4.6 Stem girth

Table 4.6 Effect of cowpea Variety on stem girth at 3 the sampling periods

	Stem girth (cm) at		
Variety	**20 DAP**	**35 DAP**	**50 DAP**
Bengpla	0.39	0.53	0.61
Tona	0.41	0.58	0.60
Asetenapa	0.36	0.51	0.63
Adom	0.36	0.60	0.67
Nhyira	0.35	0.48	0.55
Asomdwee	0.41	0.41	0.48

Soronko	0.42	0.52	0.60
Videza	0.33	0.43	0.58
Hewale	0.33	0.48	0.56
Asomtem	0.39	0.53	0.56
LSD (5%)	0.07	0.14	0.13
CV (%)	6.7	6.1	1.4

Stem girth results are shown in Table 4.6. At 20DAP, stem girth of Tona, Asomdwee and Soronko, were similar but each effect was significantly higher than all other varietal effects. All other treatment means were similar. At 35DAP, stem girth for Asomdwee was the least, and this was significantly lower ($P<0.05$) than those of Adom and Tona only. All other treatment effects were similar.

During the third sampling, stem girth for Adom was the greatest, but this was significantly higher than that of the Asomdwee variety only. Treatment effects of the Asomdwee variety was significantly lower than that of the Asetenapa variety only. All other treatment effects were similar.

4.7 Shoot dry weight

Table 4.7 Effect of cowpea variety on shoot dry weight at the 3 sampling periods

	Shoot dry weight(g) at		
Variety	**20 DAP**	**35 DAP**	**50 DAP**
Bengpla	2.11	3.87	9.40
Tona	1.90	6.30	16.63
Asetenapa	1.59	4.55	12.80
Adom	2.00	7.17	20.00
Nhyira	1.88	3.53	18.20
Asomdwee	1.53	2.57	5.60
Soronko	1.78	2.91	18.2
Videza	1.84	2.44	7.74
Hewale	1.81	3.36	11.10
Asomtem	1.71	4.88	12.41
LSD (5%)	NS	1.13	3.42
CV (%)	25	14.4	31.30

Results of plant dry weight are shown in Table 4.7. At 20DAP, varietal differences were not significant (P>0.05). At 35DAP, Adom produced the greatest dry matter of 7.17g, which was significantly higher than all other varietal effects, except Tona. The Videza variety produced the least dry matter weight which was significantly lower than all other varietal effects, except those of Hewale, Soronko, Asomdwee and Nhyira varieties.

At 50DAP, Adom produced the greatest dry matter which was significantly higher than all other varietal effects except Nhyira and Soronko.

4.8 Nodule number

Table 4.8 shows the number of nodules produced by the cowpea varieties. At 20DAP, the Videza variety produced the greatest number of nodules (18.75) and this was significantly higher ($P < 0.05$) than those of Bengpla, Nhyira and Soronko. All other varieties differences were not significant. At 35DAP, Asetenapa produced the greatest number of nodules per plant, but this was greater than Bengpla variety only. All other varietal differences were not significant.

Nodule number at 50DAP was not significantly affected by cowpea varieties.

Table 4.8.Effect of cowpea variety on nodules number at the 3 sampling periods

	Number of nodules per plant at		
Variety	**20 DAP**	**35 DAP**	**50 DAP**
Bengpla	8.75	8.05	10.31
Tona	16.80	12.40	15.30
Asetenapa	16.70	16.85	17.22
Adom	14.80	12.05	13.90
Nhyira	8.50	9.60	10.71
Asomdwee	17.15	15.40	14.60
Soronko	8.50	10.70	18.61
Videza	18.75	15.40	18.42
Hewale	10.50	9.00	9.80
Asomtem	10.1	15.35	16.10
LSD (5%)	9.00	8.31	NS
CV (%)	45	38	17

4.9 Nodule dry weight

The results of nodule dry weight are presented in Table 4.9. „Hewale‟ variety produced the greatest nodule dry variety weight at 20DAP, which effect was significantly higher than that of Adom, Soronko and Videza varieties only. All other varietal differences were not significant. At 35 and 50DAP, varietal differences for nodule dry weight were not significant.

Table 4.9.Effect of variety on nodule dry weight at 3 the sampling periods

	Nodules dry weight (g) at		
Variety	**20 DAP**	**35 DAP**	**50 DAP**
Bengpla	0.02	0.05	0.03
Tona	0.03	0.11	0.10
Asetenapa	0.02	0.07	0.10
Adom	0.01	0.05	0.04
Nhyira	0.06	0.04	0.10
Asomdwee	0.60	0.50	0.11
Soronko	0.01	0.05	0.06
Videza	0.01	0.07	0.05
Hewale	0.12	0.04	0.07
Asomtem	0.10	0.08	0.10
LSD (5%)	0.10	NS	NS
CV (%)	23	28	31

4.10 Nodule effectiveness, plants stand and plants harvested.

Table 4.10. Effect of variety on nodule effectiveness, plant stand and number of plant

Variety	**% nodule**	**Plants stand**	**Number of plants**

	Effectiveness at 35 DAP		**at harvest**
Bengpla	100.00	66.2	51.2
Tona	97.50	71.2	56.2
Asetenapa	96.50	60.8	45.8
Adom	97.50	69.0	54.0
Nhyira	98.75	61.0	46.0
Asomdwee	98.75	58.5	41.0
Soronko	95.00	70.5	55.8
Videza	97.50	60.2	45.5
Hewale	98.75	62.2	47.2
Asomtem	96.25	70.0	55.0
LSD (5%)	NS	NS	NS
CV (%)	1.1	5.3	6.6

Table 4.10 shows the results of percent nodule effectiveness, plant stand and number of plants at harvest. At 35DAP, percent nodule effectiveness was not significant. Plant stand and number of plants at harvest were also not significantly different among the various varieties.

4.11 Number of pods, number of seeds and trash weight

Table 4.11.Effect of cowpea variety on the number of pods, number of seeds per pod and trash weight

Variety	Number of pods per plant	Number of seeds per pod	Trash weight (g)
Bengpla	7.58	18.52	84.90

Tona	7.87	14.52	88.11
Asetenapa	6.90	12.72	81.52
Adom	8.10	11.02	119.60
Nhyira	10.95	12.40	115.2
Asomdwee	6.30	16.52	82.81
Soronko	7.38	8.77	112.20
Videza	7.95	12.75	77.42
Hewale	13.65	11.82	169.11
Asomtem	6.65	15.55	91.50
LSD (5%)	4.85	6.17	NS
CV (%)	16.8	5.5	20.9

The results of number of pods, number of seeds per pod and trash weight are presented in Table 4.11. There were no significant differences among the varieties in the trash weight. Hewale produced the greatest number of pods, which was significantly higher than all other treatment effects, except that of the Nhyira variety. All other treatment differences were not significant ($P > 0.05$). With the number of seeds per pod, the Bengpla variety produced the greatest effect of 18.52 seeds and this was significantly higher than those of Adom, Hewale and Soronko varieties. Treatment effect of Soronko variety which was the lowest was significantly lower than those of Asomtem and Asomdwee varieties only. All other treatment differences were not significant ($P > 0.05$).

4.12 One hundred seed weight, harvest index and grain yield

Table 4.12 Effect of cowpea variety on 100 seed weight, harvest index and yield.

Variety	100 seed weight (g)	Harvest index	Yield (kg/ha)
Bengpla	12.1	0.18	949
Tona	12.0	0.28	1327
Asetenapa	12.7	0.20	437
Adom	12.1	0.16	1067
Nhyira	10.7	0.28	1016
Asomdwee	12.4	0.19	685
Soronko	13.4	0.17	758
Videza	13.0	0.17	1131
Hewale	12.2	0.21	702
Asomtem	12.4	0.39	874
LSD (5%)	2.1	0.17	6.22
CV (%)	20.5	13.1	27.2

Table 4.12 shows the results of 100 seed weight, harvest index and yield. The Soronko variety produced the greatest 100 seed weight and this was significantly higher than that of Nhyira variety. All other treatment effects were similar. Harvest index was greatest in the Asomtem variety and this effect was significantly higher than all other varietal effects except those of Tona, Nhyira varieties. Other varieties recorded similar harvest indices.

Grain yield results was greatest in the Tona variety, but this was significantly higher than those of Asetenapa, Asomdwee and Hewale varieties only. Grain yields of Adom and Videza varieties were also significantly higher than that of Asetenapa. All other treatment differences were not significant

4.13 Seed N, residue N and N fixed

Table 4.13 Effect of cowpea on nitrogen content of seed, residue and fixed N

Variety	**Seed N (%)**	**Residue N (%)**	**Fixed N (%)**
Bengpla	4.14	0.84	2.39
Tona	3.56	1.90	2.86
Asetenapa	4.33	2.23	3.96
Adom	3.63	1.79	2.83
Nhyira	3.85	1.94	3.20
Asomdwee	3.70	2.49	3.60
Soronko	3.56	1.68	2.64
Videza	4.37	2.20	3.97
Hewale	3.63	1.83	2.86
Asomtem	3.85	1.39	2.65
LSD (5%)	0.86	1.09	1.63
CV (%)	1.8	4.0	8.1

The results of seed N, residue N and fixed N are presented in Table 4.13. Videza produced the greatest seed N which was significantly higher ($P < 0.05$) than all other treatment except Bengpla and Asetenapa only. All other varietal differences were not significant.

The Asomdwee variety left the greatest amount of N in the residue and this was similar to Asetenapa and Videza varieties only. All other treatment effects were similar. Fixed N was greatest in Videza variety, but this effect was significantly higher than all other varietal effects except Nhyira, Asomdwee and Asetenapa varieties. All other treatment differences were not significant.

RESULTS OF EXPERIMENT 2

4.14 Plant stand, emergence and tasseling.

Table 4.14. Effect of cowpea residue on plant stand, emergence and days to 50% tasseling

Residue	Emergence	Plant stand	50% tasseling
Bengpla	6.00	46.8	41.25
Tona	6.00	39.8	41.00
Asetenapa	6.00	37.0	40.50
Adom	6.00	38.0	41.25
Nhyira	6.00	38.5	40.50
Asomdwee	6.00	38.5	41.25
Soronko	6.00	41.2	41.00
Videza	6.00	40.0	41.25
Hewale	6.00	39.5	41.00
Asomtem	6.00	40.0	41.00
fertilizer	6.00	28.2	41.00
LSD (5%)	NS	10.6	NS
CV (%)	0	4.6	0.5

Results of maize plant stand, days to emergence and tasseling are shown in Table 4.14. Residue incorporation and fertilizer application did not significantly affect days to emergence and tasseling. Plant stand of maize did not differ in all residue incorporated plots (Table 4.14). Plant stands in fertilizer applied plots was the lowest. This effect was significantly lower than the Asetenapa, Adom, Nhyira and Asomdwee residue plots.

4.15 Plant height

Table 4.15.Effect of cowpea residue on maize plant height at the three sampling periods

	Plant height (cm) at		
Residue	**20DAP**	**40DAP**	**60 DAP**

Bengpla	29.8	107.1	198.7
Tona	27.4	115.8	196.9
Asetenapa	30.6	117.6	198.0
Adom	32.7	110.6	193.0
Nhyira	27.5	104.5	196.1
Asomdwee	30.1	94.9	190.8
Soronko	27.4	101.9	215.4
Videza	27.5	105.6	189.8
Hewale	27.1	94.9	179.4
Asomtem	25.4	90.8	184.2
fertilizer	24.5	111.8	198.5
LSD (5%)	5.6	NS	23.8
CV (%)	4.8	5.5	5.4

Maize plant height results are shown in Table 4.15. At 20DAP, Adom residue plots produced the tallest plants, which effect was significantly higher than all other treatment means except Asetenapa residue plots. Treatment differences were not significant at 40DAP sampling. At 60DAP, Soronko residue plots produced the greatest plant height, but this was significantly higher than only the Hewale residue plots. All other effects were similar.

4.16 Number of leaves

Results of maize number of the leaves are presented in Table 4.16. Treatment differences were not significant (P>0.05) on all sampling occasions.

Table 4.16. Effect of cowpea residue on the number of leaves at the three sampling periods.

	Number of leaves at		

Residue	20DAP	40DAP	60DAP
Bengpla	7.50	11.00	10.20
Tona	7.25	11.20	9.97
Asetenapa	7.50	11.30	10.30
Adom	7.25	11.10	10.62
Nhyira	7.25	11.45	10.30
Asomdwee	6.75	10.90	10.65
Soronko	7.50	11.20	11.00
Videza	7.00	10.90	10.40
Hewale	7.00	10.75	10.35
Asomtem	7.25	10.85	10.00
fertilizer	6.50	11.30	11.05
LSD (5%)	NS	NS	NS
CV (%)	43.8	8.5	3.0

4.17 Stem Girth

Table 4.17.Effect of cowpea residue on stem girth at the three sampling periods

	Stem girth(cm) at		
Residue	**20DAP**	**40DAP**	**60DAP**
Bengpla	6.20	15.17	17.12
Tona	6.45	15.39	17.92
Asetenapa	7.30	16.22	18.39
Adom	6.85	18.02	17.09
Nhyira	6.22	17.02	17.02
Asomdwee	6.00	16.95	10.32
Soronko	5.52	15.60	18.19

Videza	5.82	15.72	17.28
Hewale	5.40	15.12	18.86
Asomtem	5.17	12.60	16.03
fertilizer	5.17	17.40	21.45
LSD (5%)	1.54	3.96	2.98
CV (%)	9.00	2.2	3.1

Maize stem girth results are shown in Table 4.17. At 20DAP, Asetenapa residue plots produced the greatest stem girth, and this was significantly higher than all other treatment effects, except the Adom residue treated plots.

At 40DAP, Adom, Asomdwee, Nhyira and fertilizer applied plots produced greater stem girth than the Asontem residue plots only. Other treatment effects were similar.

At 60DAP, the fertilizer applied plots produced the greatest stem girth and this was significantly higher than all other treatment effects, except Hewale residue applied plots.

4.18 Plant dry matter

Table 4.18.Effect of cowpea residue on the dry matter of maize at the three sampling periods

	Maize plant dry matter(g) at		
Residue	**20DAP**	**40DAP**	**60DAP**
Bengpla	1.65	34.8	58.27
Tona	1.68	50.6	62.15
Asetenapa	1.38	38.6	79.12

Adom	1.47	43.1	72.72
Nhyira	1.60	46.3	55.85
Asomdwee	1.73	46.9	64.87
Soronko	1.08	33.5	76.01
Videza	1.40	39.5	59.42
Hewale	1.52	31.5	54.95
Asomtem	1.15	24.6	31.92
fertilizer	4.03	52.0	93.67
LSD (5%)	2.79	22.1	40. 91
CV (%)	24.9	31.5	43.38

Maize dry matter results following incorporation of cowpea residues are presented in Table 4.18. All residue incorporated plots did not differ significantly in maize dry matter production. Maize dry matter from fertilizer applied plots was significantly higher than that which received Soronko and Asontem residue only. At 40DAP, the fertilizer treatment supported the greatest maize dry matter yield, and this was greater than all other treatment residue effects, except Tona and Asomdwee residue plots. Among the residue applied treatments, Asomtem plots supported the least maize dry matter, and this was lower than those of Tona and Asomdwee plots only. At 40 and 60DAP, the fertilizer-applied treatments plots supported the greatest maize dry matter and this was higher than that of Asontem residue plots only. All other treatment differences were not significant.

4.19 Plants harvested, number of cobs and number of seeds per cob

Number of plants harvested from all residues incorporated plots were similar (Table 4.19). Plants harvested from the fertilizer applied treatment were the lowest and this was significantly lower than all other treatment effects except the Asetenapa, Adom and Nhyira plots.

Number of cobs produced did not differ significantly among all treatments (Table 4.19). Number of seeds per cob was lowest in the Asomdwee, which was significantly lower than all other treatments effect except Asomtem plots. The fertilizer applied treatment effect was greater than those of Asomtem and Asomdwee residue incorporated plots only.

Table 4.19. Effect of cowpea residue on maize plants at harvest, number of cobs per plant and number of seeds per cob.

Residue	No of plants harvested	No of cobs per plant	No of seeds per cob
Bengpla	39.0	1.0	390
Tona	34.8	1.0	404
Asetenapa	32.0	1.0	478
Adom	32.5	1.0	384
Nhyira	33.5	1.0	409
Asomdwee	33.8	1.0	298
Soronko	36..2	1.0	404
Videza	34.0	1.0	434

Hewale	34.5	1.0	379
Asomtem	35.5	1.0	345
fertilizer	23.3	1.0	408
LSD (5%)	10.3	NS	53
CV (%)	4.9	0.0	7.2

KNUST

4.20. One hundred seed weight, harvest index and grain yield

Table 4.20 Effect of cowpea residue on 100 seed weight, harvest index and seed yield.

Residue	100 seed weight (g)	Harvest index	Yield (t / ha)
Bengpla	24.38	0.48	1.66
Tona	24.00	0.55	1.93
Asetenapa	25.00	0.42	2.60
Adom	24.12	0.58	2.21
Nhyira	25.88	0.55	2.00
Asomdwee	23.62	0.43	1.82
Soronko	28.00	0.59	1.65
Videza	24.38	0.56	2.43
Hewale	23.38	0.56	1.87

Asomtem	25.50	0.46	1.23
fertilizer	24.62	0.43	2.54
LSD (5%)	NS	NS	1.36
CV (%)	4.4	13.2	33.9

The results of 100 seed weight, harvest index and seed yield are presented in Table 4.20. Treatment difference for 100-seed weight and harvest index were not significantly different. Asetenapa residue plot produced the greatest yield which was significantly higher than the Asomtem residue plot only. All other treatment effects were similar.

CHAPTER FIVE

DISCUSSION

5.1. DIFFERENTIAL GROWTH AMONG COWPEA VARIETIE

There was general increase of the height among all the cowpea varieties at the three sampling periods and this could be attributed t the fact that all the varieties were determinate and the height increases until the onset of reproductive growth where the plants growth remain constant (Singh and Rachie, 1985). Taller plants can compete well with weeds for solar radiation than shorter ones.

The number of leaves did not reduce as it is assumed that senescence and abscission normally set in with age, rather the number of leaves increased. This could be due to the amount of moisture in the soil at that time and the fertility status of the soil since nitrogen influences vegetative growth. Furthermore, if there are more leaves, it means more growth and yield would be enhanced. This is because photosynthesis in such plants would be greater (Gardner, *et al.*, 1985). The varieties Tona, Adom, Nhyira and Videza which recorded the greatest number of leaves also recorded the greatest grain yields.

Those varieties with greater number of branches produced higher yields. This shows the importance of branches which normally correlate with yields. The more the branches the more will be the number of leaves and hence interception of greater solar radiation which means greater rates of photosynthesis and greater reproductive growth. The varieties Tona, Asetenapa, Adom andVideza which produced the greatest number of branches produced the greatest grain yields.

5.2. EFFECT OF COWPEA VARIETIES ON NODULES NUMBENODULE DRY WEIGHT, PERCENTAGE EFFECTIVENESS AND N- FIXATION

FAO (1989) reported that the number of nodules formed on the root system of a leguminous plant depends firstly on the genetic condition of the host plant, secondly on the Rhizobium strain used and thirdly on the environmental conditions of growth and that with exceptions, it is assumed that nodules are capable of fixing nitrThe present results showed that the Asetenapa variety which produced the greatest number at 25 and 35DAP, and the second greatest at 50DAP did not record the greatest nodule weight. This indicates that in this study, nodule number did not correlate with their dry weight. Furthermore, Soronko produced the greatest nodules number at 50DAP, but its nodule dry weight was among the lowest. This might be due to the fact that nodules of Soronko were smaller in sizes. Addu (2003) and Sarkodie-Addo (1991) have reported similar observations, where nodule number correlated negatively with nodule dry weight. Effectiveness of nodules can be detected by the degree of pink red coloration of N-fixing bacteriods tissue inside each nodule. White or green nodules are inactive. When more nodules are formed and are effective, it is assumed that the amount of fixed N would be high and would reflect the output of present and successive crops, when especially if it is a cereal. However, the present results showed that though the Bengpla variety recorded 100% nodule effectiveness, it was the poorest in N fixation (Tables 4.10 and 4.13). Also the Asetenapa variety fixed the second greatest amount of N, although it recorded the lowest nodule effectiveness. These results indicate more to nodule effectiveness in N fixation. Nitrogen fixation has been known to depend on a host of factors including extremes of soil temperature (Bottomly, 1991), salinity (Cordivalla *et al*., 1994), nature of rhizobium- legume symbiosis (Tate, 1997), soil acidity (Corea andBarneix, 1997) and soil nitrate (Abdel *et al.*, 1996).

5.3 EFFECT OF COWPEA VARIETY ON N- FIXATION AND GRAIN YIELD

Grain yield results (Table 4.12) shows varietal differences. Tona produced the greatest grain yield of 1327 kg/ha, whilst Asontem produced the lowest yield of 437 kg/ha. Since they were all growing under the same conditions, the differences can be ascribed to genotypic variations.

N-fixed data showed that the Asetenapa and Videza varieties fixed the largest amount of N (Table 4.13). Comparing with grain yield results (Table 4.12), Videza yielded 1,131 kg of grain per hectare, which was not the greatest, whilst the Asetenapa variety produced the lowest grain yield of 437 kg/ha. However Tour (2003), observed from his studies that cowpea lines which fixed the largest amount of N produced the lowest grain yields. This means that in this present study as well as Tour observation, the varieties involved could not translate the greater amount of N into grain yield. It must be noted, however, that several studies have reported positive correlation between nitrogen fixation and grain yield (Caldwell and Vest, 1990; Sarkodie-Addo, 1991; Hume and Shelp, 1990; Sarkodie-Addo *et al*., 2006; Giller, 1991).

5.4 Effect of residue N of cowpea on growth and yield of maize

When the growth parameters of maize planted on the plots that were previously cultivated with cowpea were compared to that of the fertilized plot, there was no consistent pattern between the fertilized plot and the residue incorporated plots. Whilst in some of them, the residue incorporated treatment effects were greater than the fertilized treatment, in others, the opposite was the case. However, in maize dry matter, the fertilized treatment effect was consistently greater than the residue incorporated plots (Table 4.18). This observation shows that the cowpea residue decomposed early and released their N to the growing maize plants. Several works have reported that decomposition and mineralization of organic matter are dependent on several factors including C:N ratio, temperature, lignin content and soil moisture. However, cowpea residue, as all legumes residue, has low C:N content and very little lignin. This made decomposition to be faster and release of N for maize growth. This is probably the reason why the residue incorporated treatments produced similar effects as the fertilizer applied treatments.

Maize yield data (Table 4.20) showed that the greatest yield was obtained from Asetenapa residue incorporated plot. Indeed, the fertilizer applied plot supported grain yield which was not significantly different from any of the residue incorporated plots. Asetenapa residue plot produced the greatest yield which was significantly higher than the Asomtem residue plot only. Additionally, the least yield 1.23 t/ha obtained from the Asomtem residue plot is similar to yield from most maize farmers in the country. The present results suggest that incorporation of cowpea residue to soil will not only reduce cost of production, as fertilizers would not be used, but also maize yield would not be sacrificed. This would be a more sustainable farming practice. According to Giller (2001), if after harvesting grains and legumes residue are effectively recycled, net nitrogen accrual from such practice can be as much as 140 kg N/ha depending on the legume.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

The results indicated that all the varieties nodulated freely with the naturalized rhizobia in the soil. Variation in nodulation was probably due to genotypic differences. Videza and Asetenapa were the top nodulating varieties. The varieties differed in the amount of N fixation; Asetenapa, Videza and Asomdwee varieties supported greater N fixation than other varieties.

Additionally, residue N differed among varieties; Asetenapa, Videza and Asomdwee varieties left the greatest amount of N in their residues.

The greatest maize grain was recorded in the Asetenapa residue incorporated plots. Grain yield, from the fertilizer applied treatment was not different from any of the cowpea residue incorporated treatments.

The results suggest that if farmers would plant cowpea and incorporate all the residue into the soil, there would be no need to apply fertilizer. This obviously would reduce cost of production without sacrificing grain yield.

It is recommended that the studies be repeated in other maize growing regions in Ghana for verification of results before recommending the technology to farmers.

KNUST

REFERENCES

Abdel-Wahab H. H., Zahran H.H., and Abd- Alla M. H. (1996). Root hair infection and nodulation of four legumes as affected by the form and application of nitrogen fertilizer. Folia Microbiol. 41:303-308.

Addu, G. (2003). Evaluation of promiscuous nitrogen fixation in some 13 soybean experimental lines.BSc. Thesis.Nkrumah University of Science and Technology, 40 pages.

Adu, S.V. and Asiamah R.D. (1992). Soils of the Ayensu-Densu Basin. Central, Eastern Region and Greater Accra Regions. S.R.I. C.S.I.R., Memoir # 9, Kwadaso-Kumasi, Ghana.

Adeleke M. A. and Haruna I.M. (2012). Residual Nitrogen Contributions from Grains Legumes to the Growth and Development of Succeeding Maize Crop. ISRN Agronomy, volume 2012 (2012), 5 pages.http://dx.doi.org./10.5402/2012/213729.

Agyei-Wiredu, P. (1996). Effect of Leaf development on growth and yield of cowpeavarieties. BSc thesis, Kwame Nkrumah University of Science and Technology,Kumasi.

Agyeman K.., Berchie, J. N. Osei Bonsu I., Tetteh Nartey E. and Fordjour; J. K. (2014). Growth and yield performance of Improved Cowpea (Vigna unguiculata L.) Varieties in Ghana.Volume 2.Published by Science and Education Centre of North America.

Ahenkora K, Adu-Dapaah H. K. and Agyemang A (1998) Selected Nutritional components and sensory attributes of cowpea (Vigna unguiculata(L.) leaves.Plant Food HumNutr 52:221-229.

Akins, C.A. and Afuakwah J. (2008). Nitrogen fixation by lupins in Western Australia. @ 2008‐ 2015 researchgate net.

Akowuah P., Sarkodie-Addo J. and K. Boa (2012). Effect of some Agronomics practicesto increase maize yield in Ghana.Journal of Science and Technology. Vol. 32,No.2. Kwame Nkrumah University of Science and Technology.KNUST.

Asante, I. K., Adu-Dapaah, H. and Addison P. (2006). Variation in crude protein and mineral elements in 32 cowpea (Vigna unguiculata (L). accessions in Ghana Jnl. Agric Sci.39:83-86.

Asiamah, R. D. (1998). Soils and Soil Suitability of Ashanti Region.UNESCO/FAO. S.R.I. Technical Report No. 193. Kwadaso-Kumasi.

Bauer, P. J. and Carter P. R. (1986). Effect of seedling date, planting density, moisture and availability, and soil nitrogen fertility on maize kernel breakage susceptibility. Crop Sci. 17: 362-366.

Barorquio W., Daroy M., Tirol A., Ladha J and Watanabe I. (1986). Laboratory acetelene reduction assay for relative measurement of N2-fixing activities associated with fieldgrown wetland plants. Plant Soil 90: 358-372.

Benson D.R. and Silvester, W.B. (1993). Biology of Frankia strains, actinomycetesymbionts of actnorhizal plants. Microbiological Reviews 57, 293-319.

Blade, S.F, Shetty, S.V. R, Terao, T. and Singh, B.B. (1997). Recent developments incowpea cropping systems research. In: Singh, B.B. Mohan RAJ. D.R., Dashiell, K.E. and Jackai, L.E.N. (eds) Adavnces in Cowpea Research. International Institute ofTropical Agriculture and Japan International Research Center for AgriculturalSciences.

Boa- Amponsem, K. (2000). No- till Annual Report Mimeo CSIR-CRI, Kumasi; Ghana.

Bottomley P. (1991). Ecology of Rhizobium andBradyrhizobium in Biological nitrogen fixation. eds Stacey G., Burris R. h., Evans H. J. (Chapman & Hall, New York, N.Y.), PP.292-347.

Bressan(1985). Nutritive value of cowpea. In: Singh SR, Rachie KO (eds) CowpeaResearch, Production and Utilization, Wiley, New York, pp 353-359.

Bubenheim, D. L., Mitchell C. A. and Nielsen S. S. (1990). Unity of cowpea foliage in crops production for spaces, in: Janick J., Simon J.E. (eds), Advances in new crops Timber press, pp.535-538.

Burns, R. C. and Hardy R.C. (1975). Nitrogen fixation in bacteria and higher plants (Springer Verlag, New York N.Y). Tarawali S. A., Singh B.B., Kormawu P. M. Tamo M (eds) Challenges and Opportunities for Enhancing Sustainable Cowpea Production.

Caldwell, B.E. and Vest G. (1970). Effect of Rhizobium japonicum strains on soybean yields.Crop Science. 1,19-21.

Carsky, R.J., Vanlauwe B. and Lyasse O. (2002).Cowpea rotation as a resource management technology for cereal-based terms in the Savannas of West Africa. In: Fatokun CA., Tarawali S.A, Singh B.B., Kormawa P. M., Tamo M. (eds) Challenges and Opportunities for Enhancing Sustainable Cowpea Production. International

Institute of Tropical Agriculture, Ibadan, Nigeria, pp.252-266.

Carter, J. M., Gardner W. K. and Gibson A. H. (1994). Improved growth and yield of faba bean (vicia faba cv. Fiord) by inoculation with strains of Rhizobium leguminosarum biovar viciae in acids soils in south-west Victoria. Aust. J. Agric. Res.45:613-623.

Cheng, Q., Yang J., Day A., Dowson-Day M. Dixon R. (2005). Evolutionary implication of nitrogenase-like genes in plants kingdom and prospects for nif gene transfer in model eukaryotes. In: Wang YP et al., eds.Biological Nitrogen Fixation, Sustainable Agriculture and the Environment. Pp. 387-389.

Cheng, Q. (2008). Perspectives in Biological Nitrogen Fixation Research.. Department ofBiochemistry, Redox Biology Center, University of Nebraska-Lincoln, Nebraksa 68588 (U.S.A.).

Cordivilla, M. P. (1995). Growth stage response to salinity in symbiosis Vicia faba-Rhizobium leguminosarum bv. Viciae.Plant physiol. 14:105-111.

Cordovills, M. P., Ligero F. and Lluch C. (1994). The effect of salinityon N fixation and assimilation in Vicia faba. J. Exp. Bot.45:1484-1488.

Correa, O.S. and Barneix A. J. (1997). Cellularmechanism of pH tolerance in Rhizobium loti.

World J. Microbiol.Biotechnol. 13:153-157.

Danso, S. K. A. (1995). Assessment of biological nitrogen fixation. Fert. Res. 42:33-41

Dixon, R. O. D. and Wheeler C.T. (1986). Nitrogen Fixation in plants. New York: Chapman and Hall.

Dilip, K. and Aditya N.J. (2013). Nutritional, Medicinal and Economical importance of corn: A mini Review. Department of Natural Products, NIPPER, Sector- 67, SASNagar (Mohali)- 160-602, Punjab, INDIA

Dilworth, M. J. (1996). Acetylene reduction by nitrogen fixing preparations from Clostridium pasteurianum. Biochem. Biophys, Acta 127:285

Duivenbooden van H., Abdoussalam S. and Mohamed A.B. (2002).Impact of climate change on agricultural production in the Sahel.Part 2.Case study for groundnut and cowpea in Niger. Climate Change 54:349-368.

Egbe, O. M, Alibo and Nwueze I. (2010). Evaluation of some extra early and earlymaturing cowpea varieties for intercropping with maize in Southern Guinea

Egypt. J. Microbiol.

Ehlers J.D., Fery R.L., and Hall A.E. (2000).Cowpea breeding in the U.S new varieties andimproved germplasm. In:Fatokun CA, Tarawali SA,Singh BB, Koramawa PM, Tamo M(eds) challenges and opportunities for Enhancing Sustainable cowpea production.International Institute of Tropical Agriculture. Ibadan, Nigeria pp.62-77

Ehlers J.D. and Hall A.E. (1996). Genotypic classification of cowpea based on responses toheat and photoperiod. Crop Sci 36:673-679.

Ehlers J.D., Hall A.E. (1997).Cowpea (Vigna unguiculata (L) Walp). Field crops Res53:187-204.

FAO (2006). Food and Agriculture Organization.Production Year Book.

FOA (1989) Technical Paper 2: Biological Nitrogen Fixation FAO Corporate Document Repository. K . Mulongy (1989).

FAO Statistical Databases (2008). FAOSTAT: Agriculture Data. Available online: http://faostat.fao.org.

Fattah,Q. A. (2005).Plant Resources for Human Development" Third InternationalBotanical Conference 2005.Bangladesh Botanical Society. Dhaka, Bangladesh Fried, M., Danso, S. K. A. andZapataF. (1883) . The methodology of measurement ofN2-fixation by non- legumes as inferred from field experiments with legumes, Can. J. Microbiol. 29:1053-1062.

Fried,M. and BroeshartH. (1975).An independent measurement of the amount ofnitrogen fixed by legumes crops.Crop and Soil. 43:707-711.

Fried, M., Danso, S. K. A. and Zapata, F. (1983). The methods of measurement of Nitrogen Fixation by non-legumes as inferred from field experiments with legumes. Can. J. Microbial. 29:1053-1062.

Fuhrmann, J. Davey C. B. and Wollum A. G. (1986). Desiccation tolerance in clover rhizobia in sterile Soils. Soil Sci. Soc. Am. J. 50:639-644.

Gardner, F., Pearce R. B. andMitchell, R. L. (1995).Physiology of Crop Plant. IowaState Univ. Press,Ames, IA,.

Gault, R.R., Chase, D.L., Banks, L.W. and Brockwell J. (1984). Remedial measures to salvage unnodulated soybean crops. Journal of the Australia Institute of Agricultural Science, 50, 244-246.

Giller, E.K. and Wilson, J.K. (1991).Nitrogen fixation in tropical cropping systems. C.A.B. International. Willingford..Oxon,UK.

Giller, K. E. (2001). Nitrogen fixation in tropical cropping systems.Second edition.CABI Wallingford, UK.405 pages.

Graham P.H. and Vance C.P. (2000). Nitrogen fixation in perspective, an overview of research and extension needs. Field Crops Res. 65: 93-102.

Graham, P. H. (1995). Stress tolerance in Rhizobium and Bradyrhizobium and nodulation under adverse soil conditions. Can. J. Microbiol. 38:475-484.

Hall, A. E. (2004). Breeding for adaptation to drought and heat in cowpea. Eur J. Agron 21:447-454.

Hall, A. E., Ismal A. M., Ehler J. D., Marfo K. O., Cisse N, Thiaw S. and Close T.J. (2002). Breeding cowpea for tolerance to temperature extremes and adaptation to drought. In:

Fatokun C. A., Tarawali S. A., Singh B. B., Kormawa P. M., Tamo M. (eds). Challenges and Opportunities for Enhancing Sustainable Cowpea Production International Institute of Tropical Agriculture, Ibadan, Nigeria pp. 14-21.

Hall, A. E. and Patel P . N. (1985). Breeding for resistance to drought and heat. In: Singh SR, Rachie K.O. (eds) Cowpea Research, Production and Utilization. Wiley, New York, pp. 137-151.

Handi,Y. (1970). Soil water tension and the movement of Rhizobia. Soil Biol. Biochem. 3: 121-126.

Haruna, I.M., Maunde, S. M., and Yahuza, S. (2011). Growth and calyx yield of roselle (*Hibiscus sabadariffa* L) as affected by poultry manure and nitrogen fertilizer rates in the southern guinea savanna of Nigeria. Canadian journal of Pure and Applied Sciences 5 (1):1345-1348.

Hubbell, D.H. and Kidder, G. (2009).Biological Nitrogen Fixation.University of Florida IFAS Extension Publication SL 16. 1-4

Hume, D. J. and Shelph B.J. (1990). Superior Performance of the Hup-Bradyrhyzobium janonicum strain 532C in Ontario Soybean field trials can. J. Plant Sci 70:661-666.

Hungria M, and Vargas M.A.T. (2000). Environmental factors affecting N_2 fixation in grain legumes in the tropics with emphasis on Brazil. Field Crops Res. 65: 93-102.

IITA (2001). International Institute of Tropical Agriculture, Ibadan, Oyo State. Annual Report on Maize Production.

IITA (2009). International Institute of Tropical Agriculture, Ibadan, Oyo State. Annual Report on maize Production.

Iken, J. E., and Amusa, N. A. (2004). Maize Research and Production in Nigeria. Afr. J. Biotechnol., 3(6):302-307 key, N.E.

Irekti, H., and Drevon J. J.(2003). Acide abcissque et conduntance a" la diffusion de l"oxygen" ne dans les nodosite"s de haricot soumises a"un, choc salin. In:J.J. Drevon, B. Siffi, editor fixation symbiotique de l"Azote et De"veloppment Durable dans les Bassin Mediterrane"en, INRA Editions les colloques, vol.100.

Jackson, M. L. (1958). Soilchemical analysis. Prentice-Hall, Inc. Edgewood cliffs, New Jersey pp.151-154.

Karikari, S.K. and Molalakgosi G. (1999). Response of cowpea (Vigna Unguiculata (L) . Walp.Varieties to leaf harvesting in Bostwana. UNISAWA JOURNAL of Agriculture 8: 5-11.

Khawar, Jabran, Zahid Ata and Muhammad Farooq (2007). Maize: Cereal with a variety of Uses. DAWN-Business.http://wwwdawn. Com./2007/03/12/ebr 5.htm.

Kitch, W., Boukes C, Endondo and Murdock (1998).Farmer‟s acceptability criteria in breeding Cowpea. Experimental Agriculture 34: 475-488.

Kneip, K.R. and Mason S.C. (1989). Kernel breakage of density of normal and opaque-2 maize grain as influenced by irrigation and nitrogen. Crop Sci. 29: 158-163.

Kormawa,P.M., ChinJ.N. and Manyong V. M. (2002). Cowpea demand and Supply patterns in West Africa: the case study of Nigeria. In: Fatokun, C.A. Tarawali, S.A., Singh B. B., Kormawa, P. M. and Tamo, M. (eds). Challenges and Opportunities for Enhancing Sustainable cowpea production.International Institute of Tropical Agriculture.

Kucey, R. M. N., Chaiwanaupt, P. Snitwongse, P.and Bookerd, N. (1988). Nitrogen fixation (^{15}N dilution) with soybean under Thai field conditions.III Effect of Bradyrhizobium japonicum strain and herbicides in north-east Thailand. J. Gen. Appl. Microbiol. 34:243-253.

L‟taief, B. Sifi B., Zaman-Allah M., Drevon J, and Lachaa M. (2007). Effect of salinity on root-nodule conductance to oxygen diffusion in the Cicer arietinum-Mesorhizobium cicer symbiosis. J. Plant phhysiol., 164:1028-1036.

Langyintuo, A..S., Lowenberg-Deboer, J, Faye M., Lambert, D. Ibro, G., Moussa, B.,Kergna, A. Kushwaha, S., Musa, S. and Ntoukam, G. (2003).Cowpea supply and demand in West and Central Africa. Field crops Res. pp.82, 215-231

Long, S. R. (2001). Genes and signals in the Rhizobium- legume symbiosis. Plant physiol., 125:69-72.

Martenensson, A.M. and Ljungren, H.D. (1984). A comparison between

KNUST

acetylenereduction methods, the isotope dilution method and total nitrogen difference method for measuring nitrogen fixation in lucerne (*Medicago sativa* L.).

Mclean, M. L. (1962). Soil pH and lime requirement in methods of soil analysis.Part 2.Chemical and microbial properties. Agronomy and monograph No 9, 199-244.

Mcneill, A. M., C .J. Pillbean, H. C. Harris and Swift R. S. (1994). Seasonal variation in the suitability of different methods for estimating biological nitrogen fixation by grain legumes under rain fed conditions. Aust. J. Agric. Res., 47: 1061-1073

Michiels, J., Verrth C., and Vanderlyden J. (1994). Effects of temperature stress on bean nodulating Rhizobium strains. Appl. Environ. Microbiol. 60:1206-1212.

Mikkelsen, R. and Hartz T.K. (2008).Nitrogen Sources of Organic Crop Production. Better crops/vol. 92 (2008, No. 4).

Minchin, F.R., Witty J.F. andMytton. L. R. (1994). Measurement of nitrogenase activityin legume root nodules: In defense of acetylene reduction assay. Reply. Plant Soil,158:163-167

Mohammadi, K., Sohrabi Y., Heidari G., Khalesro S. and Mahammad M. (2012). Effective factors on Biological Nitrogen Fixation.African Journal of Agricultural Research Vol.7 (12). Pp. 1782-1788. Available online at http://www.academicjournals.org/AJ

Moody, K. (1985). Evaluation of various weed control in cowpea. Plant and Soil. Volume 85. Issue, 2 PP. 267-277.

Morris, M. L., Tripps R and Dankyi A.A. (1999). Adaptation and impact of improved Maize Production Technology. A case study of the Ghana Grains Development Project, Economics Program Paper.99-01.Mexico, D.F., CIMMYT.Available online http://www. Cimmyt.org/Research/economics/map/research results/program.

Papers/pdf/Epp/2099.01 pdf.

Myers, A. (1988). " Cereal cropping" Plant and soil.Volume 1. 174:,30-33.

Nelson, D. W. and Sommers, L.E. (1982). Total carbon, organic carbon and organic matter. Part 2, chemical and microbial properties. Agronomy monograph No 9.

Nielson, S.S. Brandt W.E., and Singh B.B. (1993).Genetic variability for nutritional composition and cooking time for improved cowpea lines. Crop Science 33:469-472

Ng, N. Q. and S. Padulosi (1997). Origin, taxonomy, and morphologyof *Vigna unguiculata* (L.) Walp. In: Singh B.B., Mohan Raj D.R, Dashiell K.E, Jackai LEN (eds).

Advances in cowpea Research co-publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences

(JJIR-CAS) .Sayce, Devon. U, pp.1-12.

Ogunlela, V.B. and Ologunde, O. (1984). Response to rate timing and method offertilization by maize in the Southern Guinea Savannah of Nigeria, Nigeria Agricultural Journal, vol.19-20, pp. 64-72.

Osagie, A.U. and Eka O.U.(1998). Nutritional Quality of plant Foods.Post- Harvest Research Unit, University of Benin, Benin pp. 34-41.

O‟ Leary, M. Rehm G. and Schmitt M. (2002). Understanding nitrogen in soils. University of

Minnesota. Extension edu. Contact Nutrient Management: nutmgmt @umn.

Paul, E. A. (1988). Towards the year 2000: directions for future nitrogen research in Advances in nitrogen cycling in agricultural ecosystems. Ed. Wilson J. R. (CAB International, Wallingford, United Kingdom), pp. 417-425.

Peoples, M.B., Herridge, D.E., and Ladha, J.K. (1995). Biological nitrogen fixation: An efficient source of nitrogen for sustainable agriculture production. Plant Soil, 174: 3-28.

Peoples, M.B., Faizah A.W., Rerkasem B. and Herridge D.F. (1989).Methodsof evaluating Nitrogen Fixation by Nodulated Legumes in the Field. ACIAR Monograph No. 11, 76pp. , Canberra.

Peoples, P. B. and Gibson,A.H.(1989).Nitrogen assimilation, metabolism and utilizationin soybean. Pp.196-211. In A. J. Pascale (ed). World Soybean Research conf IV Proc.Buenos Aires, Argentina.

Perrino, P., Laghetti G., Spagnoletti zeuli P.L. and Monti, L.C. (1993).Diversification of cowpea in the Mediterranean and other centers of cultivation.Genetic resourcesand crop evolution, 40, 121-132.

Perry Jr., L.J. and Balten Sperger (1979). Leaf and stem yields and forage quality of three N- fertilized warm-season grasses. Agron. J. 71:355-358.

Peyraud, J. C. and Astigarra L. (1990). Review of the effect of nitrogen fertilization on the chemical composition, intake, digestion andnutritive value of fresh herbage consequences on anjimal nutrition and N balance. Animal Feed Science and Technology, volume 72, ISSUES 3-4 pages 235-259.

Phillips,R.D., Mcwatters K..H., Chinna, M.S., Hung Y.C., Beuchat, L.R., SefaDadeh, S., Sakyi-Dawson, E., Ngoddy, P., Nnanyelugo, D. and Enwere, J. (2003). Utilization of Cowpea for human food. Field crops Res.82, 193-213.

Postgate, J. R. (1982).The fundamentals of Nitrogen Fixation. New York, NY:Cambridge University.

Purseglove, J.W. (1992). Tropical Crops: Monocotyledons. Longman Scientific and Technical, New York, pp. 300-305.

Quin, F. M. (1997). Advances in cowpea research. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Resarec Centre for Agricultural Sciences (JIRCAS). Ibadan, Nigeria.

Rangel, A. Saraiva K., Schwengber P. Narciso M. S. Domont G.B., Ferreira S.Tand Pedrosa C. (2004). Biological evaluation off a protein isolate from cowpea (*Vigna unguiculata*) seedsFood chem. 87:491-499.

Reichardt, K.G. Hardsarson, ZapataF., KirdaC. and S.K.A. Danso S. K. A. (1987).Sitevariability effect on field measurement of symbiotic nitrogen using 15N isotope dilutionmethod.Soil Biol. Biochem., 19: 405-409.

Rhykerd, C.L. and Noller S.C.(1974). The role of nitrogen in forage production, pp.416424. In D. A. Mays (ed). Forage fertilization, American Society of Agronomy, Madison, WI.

Salvagiotti , F, Cassman K.G., Specht J.E., Walters D.T., Weiss A. and Dobermann A. (2008). Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. Field Crops Res. 108:1-13.

Sanden, B. (1993). Blackeye varietal and irrigation cut off trial. In: University of California Dry Bean Research. Progr Rep California Dry Bean Advisory Board, Dinuba, CA, pp.120-121.

Sanginga, N. Danso S. K.A., Bower and G. D. (1989). Nodulation and growth response of Allocasuarina and Casuarina species to phosphorus fertilization. Plant Soil 111:125-132

Sanginga, N., Lyasse O. and Singh B. B. (2000). Phosphorus use efficiency and nitrogen balance of cowpea breeding lines in low P of the derived savanna zones in West Africa. Plant and Soil 220:119-128.

Sarkodie-Addo, J., Adu-Dapaah H.K. Ewusi-Mensah N., and Asare E. (2006). Evaluation of medium- maturing soybean (Glycine max (L) Merill) lines for theirnitrogen fixation potentials. Department of Crop Science, Kwame NkrumahUniversity of Science and Technology, Kumasi.KNUST.

Sarkodie-Addo, J. (1991). Evaluation of Bradyrhizobium japonicum isolates from Ontario soybean fields. MSc. Thesis, University of Guelp. 130 pages

Sayed , W. F., Wheeler C. T., Zahran H. H..and Shoreit A. A. M. (1997). Optimizing the conditions for the isolation of Frankia from nodules of casuarina. Egypt. J. Microbiol.

Schewenke, G.D., Peoples, M. B., Turner, G.L. and Herridge, D.F. (1998). Does nitrogen fixation of commercial dryland and chickpea and soybean in North-Weat New South Wales maintain or enchance soil nitrogen. Aust. J. Expt. Agric. 38-61-70.

Schnella, H. A. and Hensley D. A. (1990). Effects of pesticides upon nitrogen fixation and nodulation by dury bean. Pestic. Sci. 28:38-88.

Singh, B.B. (1997). Performance of promising cowpea varieties at Minjibir pp.14-15 IITA, Annual report 1997. Project II Cowpea cereals systems improvement in the savanna.

Singh, S. R. and Rachie, K. O. (1985). Cowpea Research , Production and Utilization: Wily Interscience Publications, John Wily and Sons Ltd. Chichester, 460 pages.

Singh,B.B. MohanD.R. Dashiell, K.E and Jackai, L.E.N. (1997).Advances incowpea Research, IITA, Ibadan Nigeria, International Institute of Tropical Agriculture

Steer. B.T. and Seiler G.(1990) Charges in fatty acid composition of sunflower (Helianthous annus L). Seeds in response to time of nitrogen application, supply ratesand defoliation, J. Sci. Food Agric SI: 11-26

Tarawali,S.A., Singh B.B., and Gupta (2002).” Cowpea as a key factor for a new approach to integrate crop- livestock systems in dry savannas of West Africa,” in Challenges and Opportunities for Enhancing Sustainable Cowpea Production, Proceedings of the world cowpea conference III held at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Tate, R.L. (1995). Soil microbiology (symbiotic nitrogen fixation) (John Wiley & Sons, Inc. New York, N.Y.). pp. 307-333

Tostiaa, N. andNegri V. (2002). Efficiency of three PCR based markers in assessing genetic variation among cowpea *(Vigna unguiculata spp. unguiculata*) landraces. Genome. 45: 656-660.

Tour, S. (2003). Response of 10 early maturing cowpea lines to promiscuous nodulation and nitrogen fixation.Bsc.Thesis, Kwame Nkrumah University of Science and Technology.46 pages.

Vance,C. (2001). Symbiotic nitrogen fixation and phosphorus acquisition.Nutrition in a world of declining renewable resources.Plant physiology 127:391-397.

Weaver,R.W. and S.K.A. Danso S.K. A.(1994).Dinitrogen fixation. Pp 1019-1994. In Weaver R.W., J.S. Angle and P.S. Bottomley (eds). Methods of soil analysis.Part 2.American Society of Agronomy, Madison, W 1 USA.

Werner, D.,and Newton W. E. (2005). Nitrogen fixation in agriculture, forestry, ecology and the environment. Springer Publication.

Wicklicky, L. (1971). The Processing Quality of Sugar Beet. Zucker 21: pp.667-672.

Yoshida, S. (1990). Growth nodulation and nitrogen fixation of soybean plants with seeds treated with kasugamycin. Soil Sci. Plant Nutr 36: 283-288.

KNUST

NYANSAPɔ WɔSANE NO BADWENMA