UNIVERSITY FOR DEVELOPMENT STUDIES

EVALUATION OF THE EFFECTS OF LIMING, RHIZOBIUM INOCULATION AND PHOSPHORUS FERTILIZER ON GRAIN YIELD AND YIELD COMPONENTS OF SOYBEAN (*Glycine max* (L.)) MERRILL) IN THE GUINEA SAVANNAH ZONE OF GHANA

BY

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(UDS/MCS/0023/14)

DISSERTATION SUBMITTED TO THE DEPARTMTNT OF AGRONOMY, FACULTY OF AGRICULTURE, UNIVERSITY FOR DEVELOPMENT STUDIES, IN PARTIAL FULFILMENT FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN CROP SCIENCE

MARCH, 2020

DECLARATION

Student

I hereby declare that this Thesis is the result of my own original work and that no part of it has been presented for another degree in this University or elsewhere. Works of others, which served as sources of information, have been duly acknowledged.

Signature.. Date..

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SUPERVISORS'

We hereby declare that the preparation and presentation of the Thesis were supervised in accordance with the guidelines on supervision of Thesis laid down by the University for Development Studies.

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ABSTRACT

An experiment was conducted to evaluate the combined effect of liming, inoculation and phosphorus fertilizer on growth, nodulation, nitrogen fixation, yield components and grain yield of soybean (*Glycine max* (L.) MERRILL) in the Guinea savannah of Ghana. The study was conducted between December 2015 and April 2016 in front of the greenhouse of the University for Development Studies at Nyankpala Campus. The experiment was laid out in a split-plot design with four replications. Liming (Organic, Inorganic and Control) was the main plot and soil amendment (Phosphorus, Inoculation, Phosphorus + Inoculation and Control), the subplots. The experimental soil had an initial pH of 4.5 and low cation exchange capacity (CEC) of 2.6 meq/100 g. $CaCO_3$ treatments increased soil pH to 8.5 and CEC to 4.1 meq/100 g and leaf ash increased soil pH to 9.4. The study showed that, liming with $CaCO_3$ at 18 g/10 kg of soil of pot increased plant height by about 58% over the other liming materials used. Inorganic lime ($CaCO_3$) at a rate of 18g per pot performed higher in all parameters than oil palm leaf ash and control. Phosphorus at 148 kg per hectare TSP also recorded better results among the soil amendments in all parameters except number of nodules per plant and grain yield of the soybean. There was interaction between liming and soil amendment effects on days to fifty percent flowering, leaf area index, fresh shoot weight and grain yield. Inorganic lime ($CaCO_3$) at 18 g / 10 kg pot of soil and phosphorus fertilizer at 148 kg/ha TSP gave soybean plants better chance to harness soil nutrients and had an influence on vegetative growth and eventually on grain yield by about 52% over the other combinations. It is therefore recommended that, liming and phosphorus fertilizer applications should be adopted by farmers growing soybean in northern Ghana.

ACKNOWLEDGEMENTS

I am first and foremost thankful to the most gracious and most dependable God for His divine inspiration and protection. My profound gratitude goes to my selfless supervisors, Prof. Israel K. Dzomeku and Dr. Ahmed Mahama Rufai, Associate Professor and Senior Lecturer, respectively in the Agronomy Department whose constructive criticisms, inspiration, dedication and efficient supervision have led to the success of this project work. I am also grateful to all lecturers of the Agronomy Department for their criticisms and suggestions during my seminar presentations. My warmest gratitude also goes to Professor Samuel Adjei Nsiah, Ghana Country Coordinator, International Institute of Tropical Agriculture (IITA) for his objective and insightful comments as well as sponsoring my Thesis work, Mr. Nuhu Inusah for the pieces of advice and material assistance during the collection of my data. Special thanks also go to my brother Mr Yussif Mashud for his encouragement and material assistance during the course of this study. I say big kudos for their encouragement and rich experience they shared with me during the study. Many thanks also go to Mohammed Adam, a final year student of the Faculty of Education for his encouragement and help in the typing of this thesis. I also express my sincere appreciation to Mr. Abu Amadu, and Sulemana Jamal who are teachers of Gushegu Senior High School for their suggestions and encouragements during the study. I am highly indebted to my friend and role model, Mr. Yahaya Damba, Assistant Lecturer in the Biotechnology Department of the University for Development Studies for his encouragement, rich experience, moral and spiritual support during the study. Finally, I wish to thank all my course mates, especially Mr. Wuni Mawiya for their constructive advice and encouragement.

DEDICATION

To my late father Alhaji Issifu Abiwoyi Olalere, lovely mother, Hajia Salimatu Salawudeen, my wife, Yussif Rahinatu and our beautiful daughters Taskin Dupe Ibrahim and Azeeza Ayo Ibrahim for their patience, support and prayers.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

%	Percentage
≤	Less than or equal to
≥	Greater than or equal to
ACT	Palm leave ash control
ADF	Air Defense Force
AI	Palm Leave ash + Inoculation
$Al(OH)_3$	Aluminium (III) hydroxide
Al^{3+}	Aluminium ion
ANOVA	Analysis of Variance
AP	Palm Leave ash + Phosphorus
HCO^{3-}	Bicarbonate
BNF	Biological nitrogen fixation
B	Boron
C	Carbon
C1	Chlorine C_3 plant
$Ca(OH)_2$	Calcium hydroxide
Ca^{2+}	Calcium ion
$CaCO_3$	Calcium Trioxocarbonate (IV)
CaO	Calcium Oxide
CO_3^{2-}	Carbonate ion
CEC	Cation exchange capacity
cm	Centimeter

CO	Carbon monoxide
CO_2	Carbon dioxide
Cu	Copper
CGR	Crop growth rate
CSIR	Council for scientific and industrial research
Cu^{2+}	Copper ion
DAP	Diammonium Phosphate
EA	Exchangeable Acidity
Fe	Iron
Fe^{2+}	Iron ion
FAO	Food and Agriculture Organization
FYM	Farm Yard Manure
H	Hydrogen
H^+	Hydrogen ion
H_2O	Water
H_2PO_4	Dihydrogen phosphate
ha	Hectare
HPO_4	Hydrogen phosphate
H	Hydrogen
IAEA	International Atomic Energy Agency
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IITA	International Institute for Tropical Agriculture
K	Potassium

kg	Kilogram
kg/ha	Kilogram per hectare
LAI	Leaf area index
LAD	Leaf area duration
LAR	Leaf area ratio
LRF	Lime Requirement Factor
LSD	Least significant differences
Mg	Magnesium
Mg^{2+}	Magnesium ion
Mn	Manganese
Mn^{4+}	Manganese ion
Mo	Molybdenum
MoFA	Ministry of Food and Agriculture
N	Nitrogen
N-P-K	Nitrogen, Phosphorus, Potassium
N_2	Nitrogen gas
Na	Sodium
NAR	Net assimilation rate (NAR)
Ni	Nickel
ns	Not significant
O	Oxygen
^{o}C	Degree Celsius
O.C	Organic Carbon

OM	Organic matter
P_2O_5	Phosphorus pentoxide
PAS	Permissible Acid Saturation,
pH	Hydrogen Concentration
P	Phosphorus
ppm	Parts per million
RCBD	Randomized complete block design
RCGR	Relative crop growth rate
S	Sulphur
SARI	Savannah Agriculture Research Institute
SMB	Soil Microbial Biomass
ton	Tonnes
TSP	Triple Supper Phosphate
UDS	University for Development Studies
US$	United State dollar
USA	United State of America
USDA	United State Department of Agriculture
WAP	Weeks after planting
Zn	Zinc

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Soybean (*Glycine max* (L.) Merrill) is the world's leading source of oil and protein. It has the highest protein content of all food crops and is second only to groundnut in terms of oil content among food legumes (Fekadu *et al*., 2009; Alghamdi, 2004). Economically, soybean is an important leguminous crop worldwide (Plahar, 2006). It plays a very important function in the natural ecosystem and agriculture where its ability to fix atmospheric N_2 in symbiosis with rhizobium makes it a very good colonizer of low-N environments (Graham and Vance, 2003). Soybean is a pea plant belonging to the botanical family leguminosae. Like all other peas, beans, lentils and peanuts, which include some 500 genera and more than 12,000 species, it belongs to the subfamily papilionideae (Shurleff and Aoyagi, 2007).

Soybean is an important global legume crop that grows in the tropical, sub-tropical and temperate climates like peas, beans, lentils and peanuts. It has been called "yellow jewel", "great treasure", "nature's miracle protein" and "meat of the field" (Noureldin *et al*., 1998). Soybean is a multipurpose crop which is drought-tolerant and is grown for oil production, human food, livestock feed, industrial purposes, and recently for bio-energy (Mathu *et al*., 2010). Soybean is well-known as an important source of protein in the human diet and animal ration; containing substantial amounts of all the essential amino acids, oil, minerals and vitamins (Tefera, 2010). It is an economically important leguminous crop in Ghana widely cultivated in different agro-ecologies, yet its production still lags behind annual consumption (Plahar, 2006).

Soybean was introduced in Ghana in 1910 (Plahar, 2006) and was used by local farmers in the northern sector which leads in soybean production in Ghana. Mean acreage under soybean cultivation per farmer in the northern part of Ghana was 3.4 acres in 2006 with individual farm size holdings ranging from 0.5 acres to 80 acres. In 2006, the southern sector production was still comparatively at the rudimentary stages except for Ejura Farms of about 300 acres and a few satellite farmers (Plahar, 2006). In northern Ghana, where the largest production of soybean occurs in the country, the average yield is about 2.5 tonnes/ha (Singleton *et al*., 1992) as compared to that of USA which is 4.6 tonnes/ha (Richard *et al*., 1984).

Ghana's current production is about 15,000 metric tonnes of soybean grain annually (MoFA and CSIR, 2005), but total domestic demand for cooking oil, seasoning and animal feed cake is estimated at nearly 30,000 metric tonnes per year (ADF, 2004). Promotion of the nutritional and economic values of the crop is being done in Ghana by the Ministry of Food and Agriculture, and this has resulted in rapid expansion in production during the past decade (Sarkodie-Addo *et al*., 2006).

Soybean, like any other legumes, also improves soil fertility by converting atmospheric nitrogen into the soil for its own use which also benefits subsequent crops in rotation. Fixation of atmospheric nitrogen in the root nodules contribute up to 70% of the total nitrogen uptake by the plant (Weber, 1966).

Biological nitrogen fixation (BNF) offers an economically attractive and ecologically sound means of improving crop yield, reducing external N inputs and enhancing the quality of soil resources which consequently reduce the dependence on mineral fertilizers that could be costly and unavailable to smallholder farmers. Leguminous crops such as soybean hold promise in this regard. Solomon *et al.* (2012) reported that legumes, including soybean, can obtain between 50% and 80% of their nitrogen requirements through BNF.

1.2 Problem statement

Despite the numerous benefits of the soybean, the grain yield per unit area is low in Ghana, being an average of 2.5 tonnes/ha (Singleton *et al*., 1992), the USA produces 4.6 tonnes/ha (Lawson *et al*., 2008), Argentina and Brazil produce 3.32, and 2.30 tonnes per hectare on the average respectively (Norman *et al.*, 1995). This low yield of soybean has been associated with poor soil fertility and inappropriate soil bacterium strains for roots nodulation (Singh and Rachie, 1987). Unavailability of *Bradyrhizobium* spp. and deficiency of phosphorous, potassium, molybdenum and sulphur in the soil are some of the contributing factors to the low yield of soybean (Singh and Rachie, 1987). Low fertility status of most of the cultivated tropical soils has been identified as a major factor causing low crop yield (Shiferaw *et a*l., 2004; Byerlee, 2007). Inherently poor or nutrient-depleted soils are characterized by low soil organic matter, plant unavailable phosphorus and total nitrogen especially in the Savannah and Transition zones of Ghana (FAO, 2005).

Inherently poor and declining soil fertility, soil acidity, poor management practices and low agricultural input use are the major causes of low soybean yields (Kanyanjua *et al*., 2002; Kimani *et al.*, 2004; Okalebo *et al.*, 2006; Njeru, 2009). Effects of soil acidity are many; the most important being the retardation of plant growth through toxicity of Aluminium (Al) and Hydrogen (H) ions, unavailability of other plant nutrients, mainly nitrogen and phosphorus, and reduction of microbial activity in the soil (Ano and Ubochi, 2007). Reduced availability of nitrogen (N) and phosphorus (P) in predominantly acidic soils is responsible for reduced soybean performance through reduced photosynthesis and early root development, low microbial activity and poor nitrogen fixation, leading to low yields (Amba *et al*., 2011; Kamara *et al*., 2007).

Soil acidity has long been known to induce N deficiency in legumes if the crop depended solely on symbiotic N_2 fixation. Aluminium and manganese toxicity, as well as calcium and phosphorus deficiency in acid soils, inhibit rhizobium growth and root infection resulting in symbiotic failure (Negi *et al*., 2006; Zahran, 1999; Keyser and Bambara and Ndakidemi, 2010).

Phosphorus is the most limiting nutrient for the growth of leguminous crops in the tropical and subtropical regions (Ae e*t al*., 1991). The low availability of phosphorus nutrition in soils has become the limiting factor for plant and root growth (Okada *et al*., 2004; Zafar *et al*., 2011 and Othman *et al*.,1991).

Phosphorus is one of the essential nutrients for legumes growth and BNF (Mhango *et al.,* 2008). Phosphorus deficiency can limit nodule number,leaf area, and biomass and grain development in legumes (Schulze *et al*., 2006). Phosphorus affects root development and hence uptake of nutrients and water. Phosphorus, apart from its effect on nodulation process and plant growth, has also been found to exert some direct effects on soil Rhizobia (Singleton *et al*., 1992). Phosphorus deficiency in soybean result in poor nodulation, reduced seed viability, and decreased percentage of fully developrd seeds (Bishnoi *et al*., 2007).

1.3 Justification of the study

Soil pH can significantly influence plant growth by affecting the composition of the soil solution and the availability of essential and non–essential elements (Dayton, 1991). The addition of lime to acidic solution produces Ca^{2+} which is essential to plant growth and when lime is applied to the soil, it can raise the pH of the soil at which bacteria species can act best (Guo *et al*., 2009, Negi, 2006). As such, economically feasible and sustainable agriculture production in poor soil as a result of soil pH, liming is required. Liming will increase the soil pH and provide Ca^{2+}, Mg^{2+} and decrease aluminium (Al^{3+}) and iron (Fe^{3+}) toxicity thereby stimulating microorganism activity and crop growth (Kanyanjua *et al*., 2002; Kisinyo *et al*., 2012).

Phosphorus fertilizer application to soybean is an important step in attaining high yield under low soil (Martin, 2005). P enhances both nodulation and N_2 fixation (Israel, 1987). Singh and Sale (2000) reported that, P fertilization stimulate root growth, photosynthesis and increase hydraulic conductibility of roots. Chiezey (2001) reported that, significant yield increase in soybean with P application on savannah soil and similar reports were made elsewhere by other workers on soybean (Alpha *et al* ., 2006). According to Fatima *et al*. 2007, the efficiency of *Rhizobium* is influenced by the availability of phosphorus in the soil as it is directly involved in growth. Phosphorus has a key role in the energy metabolism of all plant cells and particularly in nitrogen fixation as an energy requiring process (Dilworth, 1991). To obtain high seed yield of soybean, good nodulation and high and long lasting N_2 fixation activity are very important. Shahid *et al*. (2009) reported that, seed production in soybean can be increased by 70-75% when the proper bacterial strains were used to inoculate soybean seeds. Similarly, the higher nodulation due to inoculation resulted in higher N_2 fixation by Rhizobium and eventually the number of pods per plant which bring about higher grain yields as a whole.

To obtain high seed yield of soybean, good nodulation and high and long lasting N_2 fixation activity are very important. Shahid *et al*. (2009) reported that seed production in soybean can be increased by 70-75% when the proper bacterial strains were used to inoculate soybean seeds. Similarly, the higher nodulation due to inoculation resulted in higher N_2 fixation by Rhizobium and eventually the number of pods per plant which bring about higher grain yields as a whole. Moreover, Tahir *et al*. (2009) pointed out that Rhizobium inoculation significantly increased numbers of pods per plant, dry matter yield and seed yield by 85, 62 and 41%, respectively, over the control. Symbiosis between soybean and B. japonicum could be the possible explanation to the above findings.

Therefore, there is the need for the evaluation of liming, inoculation and phosphorus fertilizer on growth, nodulation, nitrogen fixation and grain yield of soybean.

1.4 Objectives of the study

1. To evaluate the effect of liming ($CaCO_3$, oil palm leaves ash and control), phosphorus fertilization and inoculation on growth, nodulation, nitrogen content and grain yield of soybean.
2. To assess the effect of soil amendments (phosphorus and rhizobium inoculation) on growth, nodulation, nitrogen content and grain yield of soybean.
3. To evaluate the combined effect of liming and soil amendment on growth, nodulation, nitrogen content and grain yield of soybean.

CHAPTER TWO

2.0 Literature review

2.1 Fertility of tropical soils

Soil is a natural medium for plant growth and the most valuable natural resource a nation possesses (Obeng, 2000). Tropical soils vary from young volcanic or alluvial soils to some of the oldest (Oxisols, Ultisols and less leached Alfisols), most highly weathered and leached soils in the world (Giller, 2001). The highly weathered and leached soils which are predominant in the tropics (covering half of the land area) resulted from various parent materials, high temperatures and rainfall. These soils are highly susceptible to degradation resulting in low fertility that poses serious constraints to poverty alleviation and sustainable food security in many parts of the tropics. In the face of increasing population growth and concomitant decline in the area of land available for expansion of agriculture, many developing countries are confronted with diverse challenges of increasing agricultural production (FAO, 2002).

The previous act of leaving a land to fallow for 10-12 years can no longer be accommodated as a result of this population pressure. Sadly, there is scarcely any productivity-enhancing investment accompanying increase in land use intensity. The extent and severity of land degradation in developing countries is not sufficiently known. Oldeman (1994) reports that, an assessment carried out by the Global Assessment of soil Degradation (GLASOD), indicates that in Africa about 65% of agricultural lands are exposed to some degree (slight to extreme) of degradation.

Soil erosion by water and wind, depletion of soil nutrients, salinity, waterlogging, acidification and deforestation are the major agents of land degradation. The impact of such soil degradation is difficult to reverse, depending on the severity of the effect on the soil (Salako, 2001). The high level of nutrient depletion and soil degradation in many smallholder systems, coupled with the high fertilizer prices that limit farmer's capacity to replenish soil fertility necessitate alternative nutrient management systems for the rehabilitation and reversal of soil degradation. In the case of Ghana, soils developed from highly weathered parent materials (FAO, 2005). Alluvial and eroded shallow soils are common to all agro-ecological zones, most of which are inherently infertile, or infertile as a result of human activities (MoFA, 1998).

2.2 Soils of northern Ghana

Soil is a natural medium for plant growth and the most valuable natural resource that a nation possesses (Obeng, 2000). There are, however, different types of soils with different suitability rating for the healthy growth of plants. Fertile soil is essential for producing healthy plants with high yields and nutritious products. The physical and chemical characteristics of the soil are significant indicators of soil quality that can directly or indirectly influence the healthy growth of plants and the quality of its product (Peters, 2002). The type of soil in any locality depends on several factors. These include, the parent rock, the climate, the relief, the drainage, the living organisms on the land and the time taken for a particular parent material to break down into soil (Obeng, 2000). Such is the importance of the climate among these factors that in Ghana, soil zoning is put into two based on the two major distinct vegetation zones, namely forest and savannah of which Northern Region of Ghana falls within the savannah zone (Obeng, 2000).

The soils of Ghana are developed from a highly weathered parent material (FAO, 2005). Alluvial and eroded shallow soils are common to all agro-ecological zones. Most soils are inherently infertile, or infertile as a result of human activities (Oppong-Anane, 2006). The Northern Region is covered by a tropical climate marked by the alternation of dry and rainy seasons. It experiences a mono-modal rainfall pattern, beginning in May and ending in October, with an average annual rainfall of 750 to 1050 mm. The dry season is between November and April. Temperatures are high almost throughout the year with the highest of 37°C in March and April. However, lower temperatures are experienced between November and February, during the period of the North-Easterly Trade Winds.

Geologically, the region is characterized with sedimentary rocks, predominantly the voltaian sandstones, shales and mudstones. The soils derived from the above parent materials range from groundwater laterite, savannah ochrosols, sandy soils, alluvial soils and clay. These types of soils vary in terms of physical and chemical composition and therefore influence the quality of plant product separately.

In the northern Ghana Guinea savanna, the soils have low accumulation of organic matter in the surface horizon owing to high temperatures, which results in a rapid rate of decomposition. Thus, the soils are notoriously low in nutrient status with phosphorus and nitrogen being particularly deficient in almost all soils (Jones and Wild, 1975). The soils in northern Ghana are described generally as savannah ochrosols and groundwater laterites. They are formed over granite and Voltaian shales (Abubakari *et al.*, 2012). Over the years, the physical, biological and chemical compositions of the soil within the three northern Regions have experienced drastic changes. This is due to diverse and changing land uses that characterized the savannah landscape (Abubakari *et al*., 2012).

The northern half of Ghana is dominated by Luvisols which are described as having a mixed mineralogy, high nutrient content and good drainage (Bridges, 1997). The percent organic matter and nitrogen are particularly low in the Savannah and transition zones (FAO, 2005). It is generally recognized that most of Ghana's soils have low fertility with the following ranges of nutrients organic matter (0.6 – 2.0%), total nitrogen (0.02 – 0.05%), available P (2.5 – 10.0 mg kg^{-1} soil), available Ca (45-90 mg per soil) and a pH range of 4.5 – 6.7 (AQUASTAT; FAO, 2005) which are responsible for low food production. In order to sustain soil and crop productivity, it is necessary to explore alternative soil fertility replenishing strategies that are different from what small-scale farmers are used to, which will be effective and affordable to support improved livelihoods. Table 1 provides information on soil characteristics by administrative regions.

Table 1: Average soil fertility status of seven administrative regions of Ghana

REGION	SOIL PROPERTY				
	pH	OM (%)	Total N (%)	Available P (mg/kg soil)	Avail Ca (mg/kg soil)
Greater Accra	5.4 – 8.2	0.1 –1.7	0.05 – 0.9	0.8 – 144.0	14 - 470
Western	3.8 – 7.1	1.0 –5.7	1.0 – 5.7	0.4 – 11.3	28 - 420
Ashanti	4.3 – 7.8	1.5 – 3.0	0.1 – 0.3	0.1 – 12.0	50 - 100
Brong Ahafo	3.5 – 6.7	0.3 – 1.7	No data	0.1 – 64.0	16 - 140
Northern	**4.5 – 6.7**	**0.6 – 2.0**	**0.02 – 0.05**	**2.5 – 10.0**	**45 - 90**
Upper West	6.0 – 6.8	0.5 – 1.3	0.01 – 0.07	2.0 – 7.4	52 - 152
Upper East	5.1 – 6.8	1.1 – 2.5	0.06 – 0.14	1.8 – 14.8	44 – 152

Source: Data from FAO, 2005

2.3 Origin and distribution

The first domestication of soybean was recorded in North China around the 11th century BC (Hymowitz and Newell, 1981). Soybean is native to Eastern Asia, mainly China, Korea and Japan, from where it spread to Europe and America and other parts of the world in the 18th century (Ngeze, 1993). Evidence in Chinese history indicates its existence more than 5,000 years ago, being used as food and a component of drugs (Norman *et al.*, 1995). Some researchers have suggested Australia and Eastern Africa as other possible centres of origin of the genus *Glycine* (Addo-Quaye *et al.*, 1993). It is widely grown on large scale in both the temperate and tropical regions such as China, Thailand, Indonesia, Brazil, the USA and Japan; where it has become a major agricultural crop and a significant export commodity (Evans, 1996).

Soybean was first introduced to Africa in the early 19th century, through Southern Africa (Ngeze, 1993) and is now widespread across the continent (Wikipedia, 2009). However, Shurtleff and Aoyagi (2007) have stated that, it might have been introduced at an earlier date in East Africa, since that region had long traded with the Chinese. The same report indicates that, soybean has been under cultivation in Tanzania in 1907 and Malawi in 1909.

In Ghana, the Portuguese missionaries were the first to introduce the soybean in 1909. This early introduction did not flourish because of the temperate origin of the crop (Mercer Quarshie and Nsowah, 1975). However, serious attempts to establish the production of the crop in Ghana started in the early 1970s. This was as a result of collaborative breeding efforts of Ghana's Ministry of Food and Agriculture (MoFA) and the International Institute of Tropical Agriculture (IITA) (Tweneboah, 2000). The crop is a drought tolerant leguminous grain that grows in areas where maize and common beans are grown. It grows to a height of 60 – 120 cm, maturing in 3 to 6 months depending on the variety, climate, and location (Mathu *et al.*, 2010). Depending on the variety, the crop can be grown from 0 – 2,200 m altitude and under rainfall ranging from 300 to 1,200 mm (Mathu *et al.*, 2010). Soybean grows well in both sandy and heavy textured soils over a wide range of soil pH 5.5-8.5 (Nieuwenhuis and Nieuwelink, 2002; Kamara *et al.*, 2007).

2.4 Importance of soybean

Soybean, a native crop of China, is much widely spread as it is found in nearly every country in sub-Saharan Africa where Nigeria is the largest producer. The crop is a drought-tolerant leguminous grain that grows in areas where maize and common beans are grown. It grows to a height of 60 – 120 cm, maturing in 3 to 6 months depending on the variety, climate, and location (Mathu *et al*., 2010). Depending on the variety, the crop can be grown at 0 – 2,200 m altitude and under rainfall ranging from 300 to 1,200 mm (Mathu *et al*., 2010). Soybean grows well in both sandy and heavy textured soils over a wide range of soil pH 5.5 – 8.5 (Nieuwenhuis & Nieuwelink, 2002; Kamara *et al*., 2007). Soybean has a high commercial value and high concentration of protein (about 40%), calcium, phosphorus, fiber, and in addition it is cholesterol free (Imas & Magen, 2007). Moreover, it provides food, cash and animal feed, Like other leguminous crops, soybean has impact on soil improvement as the canopies cover the soil and protect it from recurrent erosion, and add nitrogen from the atmosphere through biological N fixation (Nieuwenhuis & Nieuwelink, 2002; Imas & Magen, 2007). Soybean contributes to sustainable cropping systems by improving soil fertility through biological nitrogen fixation. It also provides useful crop residues for animal feed or when left in the field to decompose, it increases the organic matter content of the soil (Soko, 2000). Most African countries, including Ghana, can reduce expenditures on inorganic fertilizers through exploitation of atmospheric BNF (Giller, 2001). This is particularly important for resource poor farmers whose economics of inorganic fertilizer use is not attractive, a situation worsened by the escalating prices of fertilizers.

Most grain legumes, including soybean are relatively high value crops compared to most cereals, such as maize and rice. Thus, households that incorporate soybean in their cropping systems can generate more cash income from sales of the crop (Chirwa, 2007). Furthermore, there are potential markets in the region and beyond for soybean. Therefore, the crop can greatly contribute to the economy's narrow foreign exchange earnings base if its production can be increased, especially under smallholder farm conditions (Chirwa, 2007).

2.5 Uses of soybean

Soybeans have many industrial uses and importance for human use. The bean has an oil content of about 20%, protein content of 40-45% and carbohydrate of 30% and total sugar of about 10%. The bean is also a good source of calcium, phosphorus, copper, potassium, magnesium and thiamine. According to Dugje *et al.* (2009) soybean is more protein-rich than any of the common vegetable or legume food sources in Africa. It has an average protein content of 40%. The seed of soybean also contain about 20% oil on a dry matter basis, and this is 85% unsaturated and cholesterol-free. Borget (1992) has stated that, soybean contributes to the feeding of both humans and domestic animals and that, it has various nutritional and medicinal properties as well as industrial and commercial uses. The crop can be used for soil conservation, green manuring, compost and nitrogen fixation. Soybean can be cooked and eaten as a vegetable as well as processed into soy oil, soy milk, soy yogurt, soy flour, tofu and tempeh (Rienke and Joke, 2005; MoFA and CSIR, 2005). In the US, the major biofuel for transportation is soybean biodiesel, which displaces petroleum and diesel. Soybean has a great potential for the alleviation of protein energy malnutrition (PEM) in Ghana because animal sources of proteins are scarce and expensive. In Ghana, soybean is used in the preparation of soy paste, soy milk, soy custard, soy kebab, soy cheese, soy palaver sauce and many others (MoFA, 2010).

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

Soybean oil is also rich and highly digestible, odourless and colourless which does not coalesce easily. It is one of the most common vegetable cooking oil used in food processing industries all over the world and it is also heavily used in industries, especially in the manufacture of paint, soap, typewriter ink, plastic products, glycerine and enamels (Rienke and Joke, 2005; Ngeze, 1993 and Wikipedia, 2009). Soybean is one of the most important feed stuffs for livestock either in the form of forage (as hay and silage) or as soybean meal (IITA, 1990). The cake obtained from soybean after oil extraction is also an important source of protein feed for livestock such as poultry, pig and fish. The expansion of soybean production has led to a significant growth of the poultry, pig and fish industries (Abbey *et al*., 2001; Ngeze, 1993; MoFA and CSIR, 2005). The haulms,the plant material left after the extraction of seed also provides good feed for sheep and goats (Dugje *et al*., 2009).

Soybean is said to contain some anti-nutritional substances that reduce the nutritional value of the beans and are dangerous to health and therefore need to be removed before they can be eaten. This is not a problem since these substances can be removed by simply soaking and or ‘wet’ heating the beans leaving a valuable product that is not harmful to humans (Rienke and Joke, 2005; Ngeze, 1993). Soybean is also reported to have many health benefits. It has been reported that regular intake of soy foods may help prevent hormone-related cancers such as breast cancer, prostate cancer and colon cancer (Wikipedia, 2009). It also relieves menopausal symptoms due to the oestrogen-like effect of soy flavones. Research also suggests that regular ingestion of soy products reduces the rate of cardiovascular diseases by reducing total cholesterol, low density lipoprotein cholesterol, and preventing plaque build-up in the arteries which could lead to stroke or heart attack (The Mirror, 2008). The high quality protein, low cholesterol oil and other nutritional values are beneficial in the treatment of nutritional diseases in children (MoFA and CSIR, 2005), diabetics and also very important protein source for vegans (Wikipedia, 2009).

2.6 Soybean as a protein supplement

Mahamood *et al.* (2009) reported that, soybean is a crop which has been proposed for the removal of the acute shortage of protein and oil worldwide. Uwaegbute (1992) reported that, soybean is one of the cheapest foods available to man when judged by the amount of protein, minerals, vitamins and energy obtainable per unit cost, and its high protein content makes it a very useful food for curing protein energy malnutrition. The grain legume proteins are usually the least expensive source for both rural and urban population, and nutritionally, the protein of soybean is similar to that of animal protein.

The amino acid analyses of soy bean protein and casein are remarkably similar (Masefield, l977). Norman (1978) reported that, the thought for utilization of soybean protein products in human foods has increased dramatically because of the population pressure on the food supply and the quest for alternative source of protein. This is more so in developing countries where there is great shortage of animal protein leading to a lot of nutritional hazards. A great effort has to be made to enrich some foods with soybean. The proteins of meat, poultry, fish, milk and eggs are very expensive compared with vegetable proteins, and soybean protein is superior to all other proposed protein supplement (Anazonwu, 1978). Norman (1978) also reported that, the protein content of soybean (40%) is considered higher than dairy products of 26.7%, as shown in table 2. The soybean by virtue of its high protein content and oil contents is valued as a high energy protein source.

Table 2: Nutrient content (%) of soybean compared to other food-stuffs

Food type	Water	Energy	Protein	Oil	Calcium	Iron
Common beans	10	334	25.0	1.7	110	8.0
Peas	10	337	25.0	1.0	70	5.0
Pigeon peas	10	328	26.0	2.0	100	5.0
Soybean	**8**	**382**	**40.0**	**20.0**	**200**	**7.0**
Meat	66	202	20.0	14.0	10	3.0
Milk	74	140	7.0	8.0	260	0.2
Egg	74	158	13.0	11.5	55	2.0
Ground Nuts	6	579	27.0	45.0	50	2.5
Wheat flour	13	346	11.0	1.6	20	2.5
Finger millet flour	12	332	5.5	0.8	350	5.0
Maize flour	12	362	9.5	4.0	12	2.5
Cassava flour	12	342	1.5	0.2	55	2.0
Plantain (banana)	67	128	1.5	0.0	7	0.5
Round potatoes	80	75	2.0	0.0	10	0.7
Sweet potatoes	70	114	1.5	0.0	25	1.0

Source: Malema, 2005 (Soybean Production and Utilization in Tanzania).

2.7 Botany and taxonomy of soybean

Soybean (*Glycine max* (L.) Merrill) is a pea plant belonging to the botanical family leguminosae. Like all other peas, beans, lentils and peanuts, which include some 500 genera and more than 12,000 species, it belongs to the subfamily papilionideae (Shurtleff and Aoyagi 2007). The genus *Glycine,* currently consists of two sub-genera, namely *Glycine* consists of seven perennial wild species confined to Southeastern Asia and *soja*,which comprises the domesticated and commercially important soybean, *Glycine max* and its wild ancestor, *Glycine soja.* Both are annuals and grow in the tropical, subtropical and temperate climates. They have 40 chromosomes (2n = 2x = 40) and are self-pollinating species with less than 1% out-crossing (Norman *et al.*, 1995; Shurtleff and Aoyagi 2007; IITA, 2009).

The genus name *Glycine* was originally proposed by Linnaeus in his first edition of Genera Plantarum; with the cultivated species first appearing in the edition, 'Species Plantarum', under the name *Phaseolus max* L. The combination, *Glycine max* (L.) Merr) was proposed by Merrill in 1917 and has since become the valid name for this useful plant (Wikipedia, 2009*)*.

According to recent taxonomical classification, soybean belongs to the genus *Glycine*, which has two subgenera: S*oja* and *Glycine*. Cultivated soybean (*G. max*) and its wild annual relative *G. Soja* belong to the subgenus *soja*. The subgenus *Glycine* contains 16 wild perennial species, mostly found in Australia. All of these species generally carry 2n = 40 chromosomes, except for *G. hirticaulis*, *G. tabacina* and *G. Tomentella*. Each subgenus has a different centre of diversity.

The subgenus *soja* is most diverse in the eastern half of north China, whereas maximum diversity for the subgenus *Glycine* occurs in Australia. Over the last two decades, a large germplasm of 16 perennial species of *Glycine* has been assembled by the US Department of Agriculture (USDA) (Wikipedia, 2009).

2.8 World production of soybean

Soybean production is increasing rapidly all over the world as a result of the numerous benefits derived from the crop. Current world production of soybean is 220 million metric tonnes of grain per annum, of which the seven leading producers are the USA 32%, Brazil 28%, Argentina 21%, China 7%, India 4%, Paraguay 3%, Canada 1% and others 4% (USDA, 2007). According to FAO data for 2005, the total land area under soybean cultivation in the world was 95.2 million hectares per annum and total production was 212.6 million tonnes annually. The three major producing countries were USA (29 million hectares), Brazil (23 million hectares), and Argentina (14 million hectares) (IITA, 2009).

Masuda and Goldsmith (2008), also gave the breakdown of world soybean production of 94 million hectares worldwide as follows: U.S.A. accounted for over 30 million, Brazil for almost 22 million, Argentina for 15 million, China for 9.2 million, India for 8.2 million, Paraguay for 2.2 million and Canada for 1 million hectares respectively. In relation to Sub-Saharan Africa, the same source showed that, soybean was grown on an average of 1.16 million hectares with an average production of 1.26 million tonnes of grain in 2005. African countries with the largest area of production were Nigeria (601 000 hectares), South Africa (150 000 hectares), Uganda (144 000 hectares), Malawi (68 000 hectares), and Zimbabwe (61 000 hactares).

Soybean was introduced in Ghana in 1910 (Plahar, 2006) and was used by local farmers in northern Ghana. The northern parts of the country lead in soybean production in Ghana. Mean acreage under soybean cultivation per farmer in the northern part of Ghana was 3.4 acres in 2006 with individual farm size holdings ranging from 0.5 acre to 80 acres. In 2006, production in southern Ghana was still comparatively at the rudimentary stages except for Ejura Farms of about 300 acres and a few satellite farmers (Plahar, 2006). The average soybean yields for northern Ghana (Northern, Upper West and Upper East Regions) was about 1.5 t / ha on the farmers' field compared to that of USA which was 4.6 t / ha (Lawson *et al*., 2008).

2.9 Morphological descriptions of soybean

Soybean is an annual, erect hairy herbaceous plant, ranging in height of between 30 and 183 cm depending on the genotype (Ngeze, 1993). Some genotypes have prostrate growth not higher than 20 cm or grow up to two meters high (Wikipedia, 2009). There are two types of growth habit of the soybean determinate and indeterminate types with six approved varieties grown in Ghana (Ngeze, 1993; CSIR and MoFA, 2005). The determinate genotypes grow shorter and produce fewer leaves, but produce comparatively more pods, while the indeterminate types grow taller, produce more leaves and more pods right from the stem to shoot. Also, the flowers are small, inconspicuous and self-pollinating and are borne in the axils of the leaves and are white, pink or purple (Ngeze, 1993). The stem, leaves and pods are covered with fine brown or gray hairs. The leaves are trifoliate, having three to four leaflets per leaf. The fruit is a hairy pod that grows in clusters of three to five each of which is five to eight centimetres long and usually contains two to four seeds (Rienke and Joke, 2005). Soybean seeds occur in various sizes, and in many, the seed coat colour ranges from cream, black, brown, yellow to mottle. The hull of the mature bean is hard, water resistant and protects the cotyledons and the hypocotyls from damage (Wikipedia, 2009; Borget, 1992).

Gary and Dale (1997) have described soybean growth and development in two main stages: the vegetative stage and the reproductive stage. The vegetative stage starts with the emergence of seedlings, unfolding of unifoliate leaves, through to fully developed trifoliate leaves, nodes formation on main stem, nodulation and the formation of branches. On the other hand, the reproductive stage begins with flower bud formation, through full bloom flowering, pod formation, pod filling to full maturity. The leaves fall before the seeds are mature (Infonet-biovision, 2012) and the grows to a height of 60-120 cm. It is well adapted to diverse environments and matures in 3-6 months depending on the variety, climate and location. Altitude influences temperature which in turn affects the initiation of flowering and maturity in soybean. Soybean improves soil fertility by fixing nitrogen from the atmosphere (Kasasa *et al*., 2000; Sanginga *et al*., 2003).

2.10 Soil and climatic requirements

2.10.1 Soil requirements

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

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

2.10.2 Climatic requirements

Soybean grows well in the tropical, sub-tropical and temperate climates (IITA, 2009). However, one of the important challenges facing crop physiologists and agronomists is to understand and overcome the major abiotic stresses in agriculture which reduces crop productivity and yield (Habibpor *et al*., 2011). Interest in crop response to environmental stresses has increased greatly in recent years because of severe losses that result from drought, heat and cold stresses (Diab *et al*., 2007).

Leguminous plants in association with *Rhizobium* species have the potential to fix large amounts of atmospheric N which contributes to the soil N pool provided that the N fixation is not restricted by other environmental or microbial factors (Achakzai *et al*., 2002). Rainfall, drought, salinity, acidity, low P and the presence of toxic ions hinder the establishment of symbiotic N fixation (Graham, 1992; Rajput *et al*., 2001).

The two important climatic determinants affecting BNF are temperature and light. Extreme temperatures affect N_2 fixation adversely because N_2 fixation is an enzymatic process. However, there are differences between symbiotic systems in their ability to tolerate high (> 35°C) and low (< 25°C) temperatures (Brockwell *et al*., 1991). The availability of light regulates photosynthesis, upon which BNF depends. This is demonstrated by diurnal variations in nitrogenase activity. Very few plants like cowpea can grow and fix N_2 under shade (Hungria and Vargas, 2000).

Yield of a soybean crop is a function of light interception, dry matter production, and partition of dry matter into the plant's seed. Optimal crop growth rate is achieved when leaf area index is large enough to intercept 95% of the sun light (Board, 2000). It was predicated that 19 to 25% yield loss was observed due to 44-56% shading of the crop by the weeds. Drought reduces the number of Rhizobia in soils, and inhibits nodulation and N_2 fixation (Napoles *et al*., 2009). Prolonged drought will promote nodule decay (Benjamin and Nielsen, 2006) and indicate that drought severely inhibits nitrogenase activity (Streeter, 2003), N_2 fixation and nodulation (Pimratch *et al*., 2008). As with other grain legumes, soybean is very sensitive to drought stress which leads to reduced yield and seed quality.

Sadeghipou and Abbasi (2012) reported that, water stress decreased number of pods per plant, number of seeds per pod, 100 seed weight and seed yield of soybean. Water stress increases the abortion of flowers and pods but also decreases fertilization values, photosynthates mobilization to seeds and seed filling period. The decrease in yield and yield components of soybean due to water stress has also been reported by other researchers (Mirakhori *et al*., 2009; Masoumi *et al*., 2011; Shafii e*t al*., 2011). In soybean, drought not only results in losses in CO_2 accumulation and leaf area development but also its symbiotic N_2 fixation is especially vulnerable to drought. With declining soil water content, soybean has decreased N_2 fixation rates in advance of declines of other physiological processes. This means a decrease in N availability to support cell and tissue development throughout the plant (Sinclair *et al*., 2007). Decrease in N_2 fixation with soil drying causes yield reductions due to inadequate N for protein production which is the critical seed product (Sinclair *et al*., 2007).

Rainfall, in terms of both quantity and distribution, affects the normal functioning of the crop as well as of the microbes. Heavy downpours resulting in waterlogging and long dry spells leading to moisture stress equally influence the efficiency of BNF activity and thus affect the amount of nitrogen fixed (Jung *et al*., 2008; Youn *et a*l., 2008). The detrimental effect of waterlogging is usually attributed to inadequate oxygen supply to sustain various root metabolisms for various crops including soybean. Decreased O_2 concentration in the rhizosphere during flooding affects nitrate assimilation. Firstly, nitrate could be used as an alternative to O_2 as an electron acceptor in hypoxic roots. Secondly, respiratory energy demands for N_2 fixation and assimilation is higher than those for nitrate uptake and assimilation (Bacanamwo and Purcell, 1999). Consequently, hypoxic roots of plants dependent upon N_2 fixation are strongly affected. Reyna et *al*. (2003) reported that, waterlogging reduced nitrogenase activity and irreversibly altered ultrastructures of cells in soybean root nodules. Normally, soybeans often do not fully recover from flooding injury and can reduce soybean yield by 17 to 43% at the vegetative growth stage and 50 to 56% at the reproductive stage (Oosterhuis *et a*l., 1990).

Yield losses are the result of reduced root growth, shoot growth, nodulation, nitrogen fixation, photosynthesis, biomass accumulation, stomatal conductance, and plant death due to diseases and physiological stress (vanToai *et al*., 2003).

2.10.3 Moisture requirement of soybean

Soybean requires optimum moisture for seeds to germinate and grow well. The optimum rainfall amount is between 350 and 750 mm, well distributed throughout the growth cycle

(Ngeze, 1993). Rienke and Joke (2005), and Addo-Quaye *et al.* (1993) have described two periods as being critical for soybean moisture requirement; from sowing to germination and flowering, and pod filling periods. During germination, the soil needs to be between 50% and 85% saturated with water, as the seed absorbs 50% of its weight in water before it can germinate. The amount of water needs increases, and peaks up at the vegetative stage, and then decreases to reproductive maturity. Large variation in the amount and distribution of soil water limits soybean yield. According to Bohnert *et al.* (1995) there are two major roles of water in plants, namely as a solvent and transport medium of plant nutrients, and as an electron donor in the photosynthetic reaction processes.

Troedson *et al.* (1985) reported that, soybean is quite susceptible to water stress, and usually respond to frequent watering by substantially increasing vegetative growth and yield. Jones and Jones (1989) defined water stress as the lack of the amount of soil water needed for plant growth and development and which, in certain cells of the plant may affect various metabolic processes. Direct impacts of drought stress on the physiological development of soybean depend on its water use efficiency (Earl, 2002).

In soybean management, water use efficiency is an important physiological characteristic related to the ability of the plant to cope with water stress. According to Passioura (1997) grain yield is a function of the amount of water transpired, water use efficiency and harvest index. Soybean, as a C_3 plant, is less efficient in water use due to high evapotranspiration and low photosynthetic rates. Pandy *et al.* (1984) found out that, increasing drought stress progressively reduced leaf area, leaf area duration, crop growth rate and shoot dry matter which lead to decreased hence soybean yield.

Drought stress, during flowering and early pod formation causes the greatest reduction in number of pods and seeds at harvest (Sionit and Kramer, 1977). Low soil moisture with high plant population may cause yield to decrease because of drought stress (Gary and Dale, 1997).

2.10.4 Temperature and photoperiod

Soybean is a legume species that grows well in the tropical, sub-tropical and temperate climates (IITA, 2007). Plant breeders have argued that within the soybean species, there are varieties which react differently to photoperiod and classified them as long day, short day and day neutral plants (Borget, 1992).

Rienke and Joke (2005) described soybean as being typically a short day plant, physiologically adapted to temperate climatic conditions. However, some have been adapted to the hot, humid, tropical climate. In the tropics, the growth duration of adapted genotypes is commonly 90-110 days and up to 140 days for the late maturing ones (Osafo, 1997). The relatively short growth duration is primarily due to sensitivity to day length. and this affects the extent of vegetative growth, flower induction, production of viable pollen, length of flowering period, pod filling and maturity characteristics (Norman *et al*., 1995). Most legumes require an optimum temperature of between 17.5°C and 27.5°C for development (Ngeze, 1993) but for soybean, the minimum temperature at which it develops is 10°C, the optimum being 22°C and the maximum about 40°C. The seeds germinate well at temperatures between 15°C and 40°C, but the optimum is about 30°C (Rienke and Joke, 2005). Addo-Quaye *et al.* (1993) have suggested the optimum temperature for soybean growth as between 23-25°C.

2.11 Agronomic practices in soybean cultivation

2.11.1 Sowing

The time for cultivation of soybean is dependent on the type of agro-ecological zone. Typically, in the Northern Region of Ghana, it has been suggested that the best time for soybean cultivation is mid - June to early July (SARI, 2005). Soybean yield depends on several factors e.g. seed germination and seedling vigour which are influenced by the conditions necessary for germination such as air, water and temperature. Crop establishment is another problem and is often cited as a production problem for soybean in both arid and semi-arid areas of Central and West Africa (ICRISAT, 1984).

2.11.2 Fertilizer application

Soybean plant has a nutrient dense, high protein seed and therefore requires high amount of nutrients for its growth (Lamond and Wesley, 2001). It is a legume that can meet its nitrogen needs by symbiotic relationship with nitrogen fixing bacteria of the species *Bradyrhizobia japonicum* from atmospheric nitrogen (Sarkodie-Addo *et al*., 2006). Generally, the plant will not benefit from supplemental nitrogen fertilizer applications, where there are indigenous populations of the appropriate *Bradyrhizobia* bacteria strains that cause effective nodulation of the roots and nitrogen fixation (Darryl *et al*., 2004). Gary and Dale (1997) have stated that, nitrogen fertilizer application circumvents the benefits of *Rhizobia* bacteria as the bacteria will not convert atmospheric nitrogen to ammonia for the when soil nitrogen is readily available to the plant. However, where soybean has not been grown recently, inoculation of the seed with specific *Bradyrhizobia* strains is essential for effective nitrogen fixation (Darryl *et al*., 2004).

The number of days to flowering glasshouse experiment was reported by Tairo and Ndakidemi (2013) reported that, flowering of soybeans starts at 41-44 days after planting, followed by pod formation at 46-49 days. According to them, field experiment gave 46-49 days and 51-54 days for flowering and pod formation respectively. Tairo and Ndakidemi (2013) indicated that, rhizobial inoculation increased plant height and the number of leaves per plant significantly in both glasshouse and field experiment. Dry shoot weight, number of pods per plant and grain yield of soybean increased when inoculation and phosphorus application were done (Tahir *et al*., 2009).

Matured plants beyond 45 days, the number of leaves declined significantly for all varieties and at all levels of P application during the major season and the number of leaves increased with age of the plant during the minor season (Karikari and Arkorful, 2015). Ayodele and Oso (2014) observed that, P application increased the number of leaves of cowpea. The availability of P can increase the intensity of nodulation and nitrogen fixation which could result in higher yield of the dry matter and seed yield of legume crops (Singh *et al*., 2011). Malik *et al.* (2006) reported that, soybean seed inoculation with *Rhizobium* in combination with phosphorus application at 90 kg per hectare performed better in grain yield under irrigated conditions.

Soybean can produce maximum seed yield with relatively low levels of available phosphorus in the soil. Phosphorus application is not likely to increase seed yield at soil phosphate concentrations above 12 ppm P (Bray-1 test). Also, most soils seldom need potassium fertilizer for soybean production, since K levels are generally high in both surface soil and subsoil. Potassium fertilizer is not required if soil test shows more than 124 ppm (Ferguson *et al.*, 2006).

According to Tahir *et al.* (2009) the number of nodules increased in soybean plants treated with inoculum, phosphorus and phosphorus-inoculum from 73 to 125, 93 and 140 respectively. Plant height increased from 64.69 to 88.16cm phosphorus+inoculated plants, 64.69 to 91.26cm in inoculated plants and 64.69 to 82.11cm in phosphorus treated plants (Tahir *et al*., 2009).

Linderman and Glover (2003) have stated that, of the basic nutrients N, P and K, N is supplied by the symbiotic bacteria in the nodules while the others come from the soil and will be taken into the plant in solution. The application of 40 mg P kg^{-1} soil significantly increased the shoot and root dry weight and the application of 0 and 20 mg P kg^{-1} soil, was significantly lower when compared to the application of 40 mg P kg^{-1} soil (Olaleye *et al*., 2012). The application of P at 40 mg P kg^{-1} soil significantly increased the number of nodules in all the cowpea genotypes studied, however the number of nodules was not significantly influenced by genotype. The increase in number of nodules per plant in cowpea was due to Phosphorus application (Agboola and Obigbesan, 1977).

2.11.3 Weeding

Weed management is essential for any field crop production system or agricultural production, especially for large monoculture areas which exert high pressure on the environment. Weeds are considered the number one problem in all major soybean producing countries and even with advanced technologies, producers note high losses due to interference by weeds. According to estimates, weeds alone, cause an average reduction of 37% in soybean yield (Oerke and Dehne, 2004). In the United States, weeds cause losses of several millions of US dollars annually and in Brazil, estimated expenses of US$ 1.2 billion on weed control represent between 3% and 5% of total production cost

(Vivian *et al*., 2013). Despite differences between soybean cultivars used worldwide and the main weed species which attack these cultivars, there are many resemblances in management practices and control.

Biotic stress of soybean shows a similar mechanism of yield loss. Among biotic stresses, weeds are most common biotic stress found in field crops and greater amount of money is used for weed control. Weeds reduce yield through competition with soybeans for water, light, and nutrients (Hoeft *et al*., 2000). Depending on weed species, weed population, and environmental conditions, a "critical period" exists in soybean development when weeds must be controlled to maintain yield (Hoeft *et al*., 2000). Failure to control weeds in the critical period results in reduced soybean vegetative dry matter and grain yield (Hagood *et al*., 1981). As with drought, reduced light interception and N deficiency, yield loss occurred as a result of reduced pod and seed numbers (Board and Kahlon, 2011).

2.11.4 Pests and diseases control in soybeans

Pests and disease affect soybean throughout the growing season. Seedling, leaf and stem diseases are generally the only problem in fields planted with soybean. Seed treatments with fungicides normally do a good job of controlling early season seedling diseases. Downy Mildew, *Septoria Blight* and Frogeye Leaf Spot, Pod and Stem Blight or Anthracnose are common diseases of the soybean (Mueller, 2012). Susceptibility of soybean varieties to diseases vary greatly and choosing a resistant variety is much more cost effective than fungicide applications. Plants deficient in K have weak stems, susceptible to some diseases and predisposed to aphid attack. The majority of soybean research has conclusively demonstrated that environmental stress affects yield through controlled sequential formation and growth of node, reproductive node, pod and seed (Board and Kahlon, 2011).

2.11.5 Harvesting and storage of soybeans

Lee *et al.* (2005) predicted four (4) different groups of soybean plant maturity with respect to days to first flowering. Generally, the soybean plants were grouped in maturity I, II, III and IV in which the first flower can occur in maturity group I few days before the actual predicted days and few days after the predicted days flowers can be observed in maturity group IV. According to them, maturity group I takes 28–33 days, maturity group II 34–38, maturity group III 37–47 and maturity group IV 43-55 days to observe the first flower. The predictions of first flowering dates are based on weather because flowering depends on both day length and temperature, so the predicted dates may occur slightly earlier in areas where temperatures are slightly warmer (Lee *et al*., 2005).

Harvesting of soybean is done at any time they are matured and the foliage of the plants is dried. The optimum harvest moisture range of soybean is 13% to 15% for maximum weight and to achieve minimum field losses before and during harvesting. Seeds of soybean are crushed and bruised when harvested at the moisture content more than 18% and many pods opened plants lodged when the harvesting moisture content is below 13%. Cleaning and handling seed of soybean at 10% moisture content can reduce germination when used as seeds for the next planting season. Proper drying and storage will maintain quality soybean and assure minimum losses.

2.12 Factors affecting soybean production

2.12.1 Soil acidity

Soil acidity is determined by the amount of hydrogen ion (H^+) activity in soil solution and influenced by edaphic, climatic, and biological factors (Carver and Ownby, 1995). Acidity refers to concentration of hydrogen cations in a solution (FAO, 2006). The pH values range from 0 to 14, in which below 7 indicates an acid solution, above 7 alkaline and 7 neutral solutions (Foth & Turk, 1972; Crawford, Singh & Breman, 2008). The pH values range from 0 to 14, in which below 7 indicates an acid solution, above 7 alkaline and 7 neutral solutions (Foth and Turk, 1972; Singh and Breman, 2008).

Soils that are acid have pH values less than 7 on the pH scale (SSSA, 1997). Theoretically, soil acidity is largely associated with the presence of hydrogen and aluminium ions in exchangeable forms (Brady, 2001; Fageria and Baligar, 2003). Thus, the higher the concentrations of these ions in soil solution, the higher the acidity. Most acid soils have been found to be low in fertility, have poor physical, chemical, and biological properties. Crop production on such soils is seriously constrained, particularly in areas where proper management measures have not been put in place (He *et al*., 2003). The natural pH of a soil depends on the nature of the material from which it was developed (TSO, 2010). In most soils, pH ranges from 2.0 to 11 (Batjes, 1995) and is used for classifications of soils (Landon, 1991; Soil Survey Staff, 1993; Kanyanjua *et al*., 2002). Table 3 shows classification of soils according to the level of pH.

Table 3: Classification of soil acidity according to the level of pH

Soil acidity class	pH range
Extremely acidic	< 4.5
Strongly acidic	4.5 – 5.0
Moderately acidic	5.0 – 6.0
Slightly acidic	6.0 – 6.5
Near neutral	6.5 – 7.0

Source: Kanyanjua *et al*. (2000)

In soils of low pH, containing high amounts of Al and Fe oxides, P is deficient in the soil solution because it is precipitated or surface-adsorbed with Al and Fe as insoluble compounds (Kanyanjua *et al*., 2002). Several other essential plant nutrients, which are present in the soil solution as cations are deficient in soils with low pH.

Soil acidity is one of the most important soil factors which affect plant growth and ultimately limit crop production and profitability (Fageria, 2009). Soil acidity is one of the most prevalent problems in the production of food and fiber because about 40% of the world’s arable land is affected by this problem (Zdenko, 2003). These effects include injury on plant roots therefore reducing water and nutrient uptake, reduced availability of essential plant nutrients, toxicity of Al and manganese (Mn); and survival of microorganisms in the soil (Crawford *et al*., 2008; Onwonga *et al*., 2008).

In order to be able to produce crops in acid soils, several means to correct nutrient deficiency can be adopted. These include liming, addition of organic matter, and fertilization with mineral fertilizers (Onwonga *et al*., 2010; Masarirambi *et al*., 2012). Liming reduces Al^{3+} and H^+ ions as it reacts with water leading to the production of OH^- ions which react with Al^{3+} and H^+ in the acid soil to form $Al(OH)_3$ and H_2O. The precipitation of Al^{3+} and H^+ by lime causes the pH to increase, enhances microbial activity and nutrient availability (Onwonga *et al*., 2008). Hydrogen ions do not directly affect the growth of non - legume until the soil pH is below 3.4. Legumes are more sensitive to hydrogen ions, although it is actually the rhizobia that are affected rather than the plant. This affects the complex process of nodule formation which reduces the growth of legumes and the amount of nitrogen fixed – legumes may even show symptoms of nitrogen deficiency (Andréa *et al*., 2000). Hydrogen toxicity, decreases phosphorus availability and toxicities of some other trace elements and heavy metals (Opala, 2011).

Soybean as a leguminous crop relies on microbial nitrogen fixation as its source of N. However, under acid soils, the population of rhizobia bacteria is reduced and consequently nodulation and N fixation are impaired and this affects negatively on crop nutrition and yields. Therefore, liming acid soils for soybean production improves the soil's condition for microbial development. Mineral fertilizers increase nutrient availability in the soil solution since they are readily available and the addition of organic matter acts as supply of food for the microorganisms thereby enhancing their population and therefore mineralization (Crawford *et al*., 2008).

2.12.2 Aluminum and hydrogen toxicity

Aluminium toxicity is considered the most important growth-limiting factor for plants in acid soils (Foy *et al.*, 1978; Foy, 1984; Carver and Ownby, 1995; Jayasundara *et al.*, 1998). The primary response to aluminium stress occurs in the roots (Foy *et al.*, 1978; Foy, 1984, Taylor, 1988, Jayasundra *et al.*, 1998). Aluminium-injured roots are stubby and brittle. Root tips and lateral roots thicken and turn brown. The root system as a whole is affected, with many stubby lateral roots and no fine branching. Such roots are inefficient in absorbing nutrients and water (Foy *et al.*, 1978). The main symptom of Al toxicity is rapid inhibition of root growth. A number of mechanisms may cause this, including Al interactions within the cell wall, the plasma membrane, or the root cytoplasm (Taylor, 1988; Marschner, 1991; Horst, 1995; Kochian, 1995).

2.12.3 Nutrient solubility and availability

A high concentration of H^+ ions (low pH) in the soil can lead to nutrient deficiencies. Acidic conditions can lower the levels of phosphorus, calcium, magnesium and molybdenum, nutrients that are essential to plant growth (Forbes *et al.*, 1992).

2.12.4 Nitrogen

Nitrogen is a key element in plant growth, and plants need plenty of it in the growing season but too much nitrogen can actually slow plant growth because nitrogen not used by plants is washed (leached) out of the soil, which makes soil acid (Rebecca, 2004).The decomposition of plant residues and the return of larger amount of nitrogen are more rapid in the pH range of 6.0 to 7.2 rather than under acidic conditions which inhibits the growth and activity of symbiotic and other microbes. As a result, soils are often limed to pH of 6.0 or pH 6.5 to enhance nitrification. The process of atmospheric nitrogen fixation, both symbiotic and non-symbiotic, is also favoured by adequate liming.

2.12.5 Micronutrients

The special soil conditions that influence the availability of micronutrients is pH. It has been confirmed that with the exception of molybdenum whose deficiency decreases with increase in soil pH, the availability of the other micronutrients increases with decrease in soil pH or increase in soil acidity. The availability of the micronutrients; manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), and boron (B) therefore tends to decrease as soil pH increases. The exact mechanisms responsible for reducing their availabilities differ for each nutrient but can include the formation of low solubility compounds, greater retention by soil colloids (clays and organic matter) and conversion of soluble forms to ions that plants cannot absorb. Adverse effects of heavy metals on nodulation and N_2 fixation of legumes have been reported for clover and chickpea. Griller *et al.* (1989) suggested two possibilities to explain the mechanism by which the elevated metal concentrations eliminated N_2 fixation: (1) one or more of the metals present might have prevented the formation of N_2-fixing nodules by effective *Rhizobium* strains present in the soil or (2) the metal contamination might have resulted in elimination of the effective *Rhizobium* strains from the soil.

2.12.6 Microbial growth and activity

Low pH levels in soils can have a severe effect on the microorganisms that form symbiotic relationships with plants. A study examining the effects of soil pH on cowpea showed that poor growth of plants can sometimes be attributed to poor microbial activity

(Peet *et al*., 2003). Microorganisms in the soil aid plant growth by forming symbiotic relationships with roots thereby aiding in nutrient absorption. These relationships can be affected by low soil pH. In legumes, nodules are not produced at low pH levels lower than 5.0. Also, low soil pH can reduce the diversity of microorganisms and the numbers of rhizobia in the soil available for symbiotic relationships (Rengel, 2002).

2.12.7 Species diversity and richness of plants

The pH of soil can also affect plant species diversity and richness. A study done in the Blue Ridge region of the U.S. where highly acidic soils are prominent, concluded that sites with higher pH have more species richness. Also, the average density of species was twice as high in regions with high pH, as compared to regions with low pH. This condition was attributed to the more encouraging growing conditions associated with higher pH levels (Peet *et al*., 2003). The study also showed that species diversity was lower in regions with acidic soil. It was noted that this condition was probably due to the fact that plants must be highly specialized to survive in acidic conditions (Peet *et al*., 2003).

2.13 Ameliorating soil acidity

2.13.1 Organic amendment

During the decomposition of plant and animal debris, a whole range of organic compounds are released from the debris and/or synthesized by the decomposer microorganisms and aluminium can bind strongly with many of these compounds. Soil organic matter complexes with Al^{3+} and other polyvalent cations which can be grouped into two main categories (Stevenson and Vance, 1989); namely (i) well-defined biochemical compounds such as simple aliphatic organic acids, phenols, phenolic acids, hydroxamate siderophores, sugar acids and polymeric phenols and (ii) complex humic materials. While the decrease in exchangeable Al by liming is mainly a function of the rise of soil pH, the same is not always true for all OMs. There are other mechanisms involved in the reactions of Al with organic matter which are intricate and probably involve complex formation with low molecular weight organic acids, such as citric, oxalic and malic acids, and humic material produced during the decomposition of the organic matter and adsorption of Al onto the decomposing organic residues (Opala, 2011).

Ash is composed of many major and minor elements which trees need for growth. It contains 15% calcium, 2.6% potassium, 1.6% aluminium and 1.0% magnesium and iron, phosphorus, manganese, sodium and nitrogen are less than 1% in Ash (Muse and Mitchell, 1995). Most of these elements are extracted from the soil and atmosphere during the plant’s growth and they are essential in the production of crops and forages. The high content of calcium in the Ash gives ash properties similar to agricultural lime. Ash can also be a good source of potassium, phosphorus, and magnesium and it has about 0-1-3 (N-P-K) in commercial fertilizer (Risse and Gaskin, 2013).

In addition, wood ash is a good source of many micronutrients needed in trace amounts for adequate plant growth and contains few elements that pose environmental problems. Wood ash has a liming effect of between 8 and 90 percent of the total neutralizing power of lime, and can increase plant growth up to 45 percent over traditional limestone (Risse and Gaskin, 2013).

The major constraints to land application of wood ash are transportation costs, low fertilizer analysis, and handling constraints. Wood ash application is similar to lime application and both have benefit to crop productivity, but wood ash supplies additional nutrients. These materials are also alkaline and could cause crop damage if over applied or misused (Risse and Gaskin, 2013). Ash is an alkaline material with a pH ranging from 9 to 13 and contains 43% of $CaCO_3$ (Muse and Mitchell, 1995) whilst lime has a pH of 9.9, 31% Calcium, 5.1% magnesium and 100% $CaCO_3$ (Campbell, 1990).

2.13.2 Chemical amendment (liming)

Liming acid soils for the production of legumes may increase yield by improving the survival and growth of rhizobia in the rhizosphere, and by assisting in the formation of nodules by lowering the hydrogen ion activity and providing calcium. The extent to which these factors affect N_2 fixation is of considerable interest when assessing the lime requirements of legume crops. Hoyt and Nyborg (1972), showed that yield responses of rapeseed (*Brassica campestris* L.), barley (*Hordeum vulgare* L.) and alfalfa (M. *sativa* L.) to liming were correlated with the amounts of plant available Al and Mn in acid soil. However, they recognized that N fixation by alfalfa may be restricted by soil acidity even when toxic amounts of Al and Mn are not present and suggested that diagnosis of the need for liming should be based on pH and plant available Al and Mn.

The detrimental effect of low pH in the absence of toxic levels of Al and Mn on nodulation and N_2 fixation by alfalfa was demonstrated by Rice (1975) in greenhouse experiments. Amelioration of acid soils by surface application of lime and other materials is the main commercially available option. Lime application on surface soil generally does not have a rapid effect in reducing the subsoil acidity and mixing lime with the subsoil too is generally not economically feasible. Therefore, selecting and growing acid-tolerant cultivars may be a sustainable approach for the better growth and productivity of pastures and pulse crops on acid soils (Hynes and Mokolobate, 2001). The application of lime and fertilizer separately as well as in combination gave significantly higher number of pod bearing branches, shoot dry weight and taller soybeans than those crops grown without lime and fertilizer (Workneh *et al*., 2013).

Soils are limed to reduce the harmful effects of low pH (aluminium or manganese toxicity) and to add calcium and magnesium to the soil. The amount of lime needed to achieve a certain pH depends on (1) the pH of the soil and (2) the buffering capacity of the soil. The buffering capacity is related to the cation exchange capacity (CEC) and the higher the CEC, the more exchangeable acidity (hydrogen and aluminium) is held by the soil colloids. As with CEC, buffering capacity increases with the amounts of clay and organic matter in the soil. Soils with a high buffering capacity require larger amounts of lime to increase the pH than soils with a lower buffering capacity.

Lime reduces soil acidity (increases pH) by changing some of the hydrogen ions into water and carbon dioxide (CO_2). A Ca^{2+} ion from the lime replaces two H^+ ions on the cation exchange complex. The carbonate (CO_3^{2-}) reacts with water to form bicarbonate (HCO_3^-). These react with H^+ to form H_2O and CO_2. The pH increases because the H^+ concentration has been reduced.

2.13.3 Liming materials

Lime in general sense is any material that (1) contains calcium (Ca) or magnesium (Mg) and (2) will neutralize soil acidity. For example, calcium carbonate ($CaCO_3$) is a liming material because it contains Ca and the carbonate portion of the material (CO_3) will neutralize soil acidity (Mahler, 2000). Liming materials include limestone, burned lime, slaked lime, marl, oyster shells, slag, cement plant flue dust, mine tailings, sugar beet sludge, wood ashes, and paper mill lime sludge. Liming materials fall into the following four categories: carbonates, oxides, hydroxides, and by-product materials. In general, carbonate materials account for more than 90 percent of the lime used in the United States. Factors favouring carbonates over oxides and hydroxides include (1) ease of handling, (2) lower cost, and (3) the availability of many more sources. Although smaller amount of hydroxides and oxides are needed for raising soil pH, those two materials generally are used only when the grower requires a rapid pH change (Munroe and Murdock, 1993). Pulverized limestone is the most common material used to raise soil pH. Limestone consists either of calcium carbonate (calcitic limestone) or calcium/magnesium carbonate (dolomitic limestone).

2.13.4 Estimation of lime rate

Soil test helps determine the amount of lime required to raise soils to a desired pH. There is a need to perform a lime requirement test on all soils with a pH of 5.1 or lower. Soil pH is more critical for legumes such as alfalfa, lentils, soybean and peas than for cereals. Consequently, for soil testing less than pH 5.5 there is the need to perform a lime requirement test where legumes are grown. Numbers obtained from lime requirement soil tests are often meaningless when soil pH values exceed 5.6. Several different lime requirement tests have been developed to determine the amount of lime needed for improving crop yields. A lime requirement test is necessary for determining the correct amount of lime to apply because over-applications may decrease soil productivity. In addition to soil pH, soil texture, clay content, cation exchange capacity (CEC), base saturation, and other factors affect the amount of lime needed (Mahler, 2000). The level of soil acidity that is tolerable in any situation is determined by the permissible acid saturation (PAS) of the crop to be grown. If soil acid saturation exceeds the PAS, the excess acidity has to be neutralized by liming. If it is assumed that the neutralizing value of the lime available is 75% that of pure $CaCO_3$ (this will be dependent on purity and hardness, and in particular in fineness of the product) and that incorporation depth is 15cm, the lime needed per hectare to eliminate an exchangeable acidity of 1 meq/100g will be approximately 3000 kg If the neutralizing value of the lime is lower or higher than 75% the lime requirement factor will be adjusted accordingly (Taye *et al*., 2002).

Accordingly, lime requirement is calculated using acid saturation as follows;

LR = LRF (EA –PAS), where LRF = Lime Requirement Factor, PAS=Permissible Acid Saturation, EA= Exchangeable Acidity.

2.13.5 Liming time, placement and frequency of application

For crop rotations that include legumes like soybean, alfalfa or clovers, lime should be applied to allow enough time for reaction with the soil before the legumes are planted. Ideally, lime should be applied three to six months ahead of seeding the targeted crop. Applications as late as just before planting, with good soil incorporation, can still be beneficial on strongly acid soils. Some reduction in soil acidity will still occur although maximum pH increases are not normally reached until about one year after application of typical agricultural limestone. Placement is just as important as lime quality. Maximum contact with the soil is essential for neutralization of soil acidity. Most common liming materials are only sparingly soluble in water. For example, ammonium nitrate is about 84,000 times more soluble than pure calcium carbonate. Even if lime is properly mixed into the plough layer, it will have little reaction if the soil is dry. Moisture must be available for the lime-soil reaction to occur. Perhaps the best way to incorporate lime or any other material within the plough layer is to use two perpendicular passes of a combination disc followed by a chisel plough. Deep ploughing of lime does not achieve desirable mixing in the upper six to eight inches of soil.

However, because the plough or a heavy breaking disc inverts the lime, it can help to distribute the lime in the upper portion of the subsoil. Choice of tillage equipment will depend on the depth at which soil acidity neutralization is most needed. Good horizontal and vertical mixing of the lime provides the best results (Synder, 1987).

On soils low in magnesium, dolomitic limestone is the preferred form. Lime recommendations for raising soil pH are given in terms of pulverized limestone but other liming sources can be used. Lime is applied only if a need is indicated by the results of soil testing and the requirements of the plants being grown. Over liming can reduce nutrient availability, especially of micronutrients like iron, manganese, and zinc. Iron deficiency (chlorosis) of pin oak, for example, is common when soil pH is greater than 7.0 (McLean, 1971**).**

In soils of low pH, containing high amounts of Al and Fe oxides, P is deficient in the soil solution because it is precipitated or surface adsorbed with Al and Fe as insoluble compounds (Kanyanjua *et al*., 2002). Several other essential plant nutrients, which are present in the soil solution as cations become deficient.

2.13.6 Role of biological nitrogen fixation in cropping systems

Nitrogen compounds comprise 40 to 50 per cent of the dry matter of protoplasm, the living substance of plant cells (Dreyfus *et al*., 1987). For this reason, nitrogen is required in large quantities by growing plants and is indeed the key to soil fertility. The nutrient is needed by the plant as an integral part of all proteins, and is one of the main nutrients required for plant growth and photosynthesis which occur at high rates when there is sufficient nitrogen. A plant receiving sufficient nitrogen will typically exhibit vigorous plant growth and the leaves will also develop a dark green colour.

Nitrogen represents about 72% of atmospheric gases but it is required by the plants in form of ammonium (NH_4^+) and nitrate (NO_3^-). As microorganisms decompose organic matter, ammonium is released in a process called mineralization for plant uptake. In addition to organic N, plants are also supplied with inorganic N fertilizer for plant growth and development when soil N is deficient.

Legumes have the potential to contribute to the soil N budget through biological N_2 fixation (BNF), a process which is becoming more important for not only a as potential cheap alternative to mineral N fertilizers for providing N to crops but also in seeking more sustainable agricultural production (Boddey *et al*., 1997; Giller *et al*., 1997). Biological nitrogen fixation makes a significant contribution to N supply in cropping systems where legumes are grown in rotation or intercropped with cereals either as crops in their own right or as green manures. Evidence of N transfer from legume to cereal has been obtained in some intercropping and rotation studies through root excretion, N leached from leaves and leaf fall (Fujita *et al*., 1992; Stern, 1993; Ledgard and Giller, 1995; Yusuf *et al*., 2009). For example, Eaglesham *et al.* (1981) showed that, 24.9% of N fixed by cowpea was transferred to maize and also, up to 35% of N in maize grown after pigeon pea was shown by isotope dilution to be from nitrogen fixation and part of the fixed nitrogen was from below ground parts. Similarly, Mandimba (1995) revealed that, the nitrogen contribution of groundnut to the growth of maize in intercropping systems is equivalent to the application of 96 kg of N/ha at a ratio of plant population densities of one maize plant to four groundnut plants. Osunde *et al.* (2004) found that, without the addition of fertilizer, the proportion of N derived from N_2-fixation was about 40% in the intercropped soybean and 30% in the sole crop. For many farmers, BNF is therefore an essential cost-effective alternative or complementary solution to industrially manufactured N fertilizers for staple cereal crops (Carlson and Huss - Danell, 2003). Legumes such as soybean that have been subject to intense breeding efforts are very efficient at translocating their N into the grain ranging from 50-150 kg N/ha (Matusso *et al*., 2014) and even when the residues are returned to the soil there is generally a net removal of N from the field (Giller *et al*., 1994). Soybean residues at harvest are lignified (10% lignin) with C/N ratios of around 45:1 and these tend to immobilize N when they are added to the soil on short term and released for plant uptake in the long term (Toomsan *et al*., 1995). Specifically, soybean can fix 49-450 kg N/ha (Peoples and Crasswell, 1992) and net benefits ranging from 100 to 260 kg N/ha have been reported (Maphumo, 2011). Positive net N balances of up to 136 kg/ha for several legume crops such as cowpea, pigeon pea, green gram and groundnuts following seed harvest have been shown by Peoples and Craswell (1992). However, if crop residues are removed from the field, the net N balances for soybean ranges from 28 to 104 kg/ha. Some promiscuous soybean varieties that produce large quantities of leafy biomass have a greater potential to add N to the soil and are potentially more appropriate for cultivation by smallholder farmers than the recommended varieties grown on commercial farms in southern Africa requiring rhizobia inoculant (Mpepereki *et al*., 2000). While legumes can improve soil fertility through BNF, low soil fertility limits N fixation and the overall growth and yield of legumes grown on smallholder farms.

2.13.7 Nitrogen fixation in soybean

Soybean is a legume and normally provides itself with nitrogen through a symbiotic relationship with nitrogen fixing bacteria of the species, *Bradyrhizobium japonicum* (Sarkodie-Addo *et al*., 2006; Nastasija *et al*., 2008). Bacteria present in soybean root nodules will fix nitrogen from the atmosphere, normally supplying most or all the nitrogen needed by the plant. Soybean grown on soil where nodulated soybean has been grown in recent years will probably not require inoculation. However, if there is any question about the presence of rhizobium bacteria, inoculation is recommended (Darryl *et al*., 2004; Nastasija *et al*., 2008).

The amount of nitrogen that a plant can fix depends on the variety, the productivity of rhizobium bacteria, the soil and the prevailing climatic conditions. Soybean is capable of fixing between 60 kg and 168 kg of nitrogen per hectare per year under suitable conditions (Rienke and Joke, 2005). Soybean nitrogen requirements are met in a complex manner as it is capable of utilizing both soil nitrogen in the form of nitrate and atmospheric nitrogen, through symbiotic nitrogen fixation. In the symbiotic relationship, carbohydrates and minerals are supplied to the bacteria by the plant and the bacteria transform nitrogen gas from the atmosphere into ammonium and nitrate for use by the plant (Frazen, 1999).

Plant population is one factor that may influence how much residual nitrogen soybean is contributing to a cropping system. Estimated nitrogen fixation of determinate soybean was approximately increased from 200 to 280 kg ha^{-1} when plant population was increased from 48,500 to 194,000 plants ha^{-1} respectively (Ennin and Clegg, 2001). The process of nitrogen fixation requires the presence of the right species of the nitrogen fixing bacteria in the soil and they are often attracted to the roots by chemical signals from the soybean root (Rienke and Joke, 2005). Once in contact with the root hairs, a root compound binds the bacteria to the root hair cell wall. The bacteria release a chemical that causes curling and cracking of the root hair allowing the bacteria to invade the interior of the cells and begin to change the plant cell structure to form nodules. The bacteria live in compartments of up to 10,000 in a nodule called bacteroids.

The nitrogen fixation is aided by an enzyme called nitrogenase which takes place in an environment without oxygen, through a transfer compound called leghemoglobin. This results in a pink-red colour of nodule interiors, an indication of active fixation of nitrogen (Lindermann and Glover, 2003).

Ferguson *et al* (2006), reported that soybean plant will effectively utilize soil residual nitrate and nitrogen mineralized from soil organic matter, obtaining 25 to 75 percent of plant nitrogen with the balance supplied from symbiotic fixation. Legume nodules that are not fixing nitrogen usually turn white, grey or green and may actually be discarded by the plant. This may be as a result of inefficient rhizobium strain, poor plant nutrition, pod filling or other plant stresses. Nastasija *et al.* (2008) have outlined the following as limiting factors to N-fixation:

A temperature of 16^{o}C to 27^{o}C is ideal, while levels above or below this reduce bacterial activity and slow the establishment of the N-fixing relationship.

i. When soil N levels are too high, nodule number and activity decrease. Roots do not attract bacteria or allow infection; hence, nitrogen fixation is limited.

ii. Poor plant growth does not allow the plants to sustain nodules and plant growth, therefore sacrificing nodule activity.

iii. If soil pores are filled with water, and not air, there will be no nitrogen to be fixed.

Major amount of nitrogen is fixed by legume through the action of microorganism (Biological nitrogen fixation). Biological nitrogen fixation is a process used by microorganisms living in the soil to fix nitrogen in leguminous plants (Gregoire, 2003). It involves association of rhizobia and legumes. The rhizobium-legume symbiosis plays an important role in agriculture because it offers the ability to convert atmospheric molecular nitrogen into forms useable by the plant (Jensen and Nielsen, 2003). During nodulation, host plants excrete flavonoids and the bacteria nod-protein recognizes proper flavonoids and initiates synthesis of a nod-factor by a series of nod-genes products (Date and Halliday, 1987). The nod-factor in return initiates early processes of nodulation. The first nodules form within one week after seedling emergence and become visible as they increase in size. Ten to fourteen days later, the nodule bacteria are able to supply most of the plant’s nitrogen requirements. The nodules allow fixation of atmospheric nitrogen but are energetically expensive to develop and maintain (Shantharam and Mattoo, 1997). Hence the host suppresses the growth of most potential root nodules soon after the initial bacterial invasion of root hairs (Spaink, 1995). The host plant also further regulates nodule number in response to environmental factors such as the presence of nitrate or other sources of fixed nitrogen in the soil (Vandyk, 2003). The nodules which are bright in colour are effective while the nodules white in colour are ineffective, or have not yet developed to a stage at which they can fix nitrogen.

Soybean are nodulated by the slow growing *Bradyrhizobium japonicum* (Jordan, 1982), *Bradyrhizobium elkanii* (Kuykendall *et al*., 1992), *Bradyrhizobium liaoningese* (Xu *et al*., 1995) as well as the fast growing *Sinorhizobium fredii* (Scholla and Elkan, 1984). Promiscuous soybean varieties are known to nodulate with a wide range of rhizobium strains and therefore are likely to be widely adopted by farmers (Okereke *et al*., 2000; Fening and Danso, 2002; Okogun and Sanginga, 2003). The foregoing researchers have only dealt with the type of *Bradyrhizobium* that fix nitrogen with the soybean but they have not shown which one is more effective in fixing nitrogen under varying conditions of host and non-host factors. The effectiveness of BNF depends on the management of other inputs such as nutrient availability, population of rhizobia and soil pH (Jones and Giddens, 1985; Keyser and Li, 1992; Peoples *et al*., 1989; Ukovich *et al*., 2008).

The process of biological nitrogen fixation by legume nodules requires large amounts of P and its availability is a primary constraint to N_2 fixation (Danso, 1992; Better Crops, 1999; Sanginga, 2003; Kamara *et al*., 2007). Deficiencies of soil nutrients, especially P may restrict the development of a population of free-living rhizobia in the rhizosphere, limit the growth of the host plant, restrict nodulation itself, and cause an impaired nodule function (Better Crops, 1999; Danso, 1992). Moreover, limitation of mineral N in the soil tends to enhance fixation by legumes including soybean (Ukovich *et al*., 2008). The population and activity of rhizobia are highly influenced in acid soils, affecting directly N fixation (Jones and Giddens, 1985).

2.13.8 Factors influencing biological nitrogen fixation (rhizobia inoculation) in legumes

The introduction of superior strains of rhizobia into the soil does not guarantee a higher BNF and hence a higher yield (Lupwayi *et al*., 2000). However, in the absence of all other factors that affect nitrogen fixation, an introduced strain should be able to compete with the native rhizobia for nodulation. The efficiency and effectiveness of the introduced strain is limited by a number of factors and these factors have the tendency to influence the symbiotic relationship between the legume and the rhizobia. The success of inoculation, therefore, depends on a number of factors which are not excluded to indigenous rhizobia and N availability (Keyser and Li, 1992).

The presence and growth of rhizobia in the soil are affected by many factors. The growth and healthy activities of rhizobia depend on the initial population of the bacteria and the soil conditions that favor or hinder their development (Crop Focus, 2011). When the soil is limited with oxygen supply, the activity of rhizobia is reduced and ample oxygen availability in the soil will activate their activity.

The acidity of the soil and amount of nitrogen present in the soil affect the health of the bacteria, as it does to the soybean plants. Soil pH <5.6 or >8.0 creates a difficult environment for the bacteria to function efficiently and as well affect soybean productivity (Crop Focus, 2011). Nitrogen availability in the soil will also reduce the soybean-to-bacteria relationship. The plant may not initially need the bacteria due to excess residual nitrogen in the soil and in such cases, the soybean plant will not recognize the bacterial chemical reaction and thus will not initiate nodular tissue formation (Crop Focus, 2011).

2.13.8.1 Indigenous / native rhizobia

The amount of nitrogen fixed is usually high in soils with low mineral N but with sufficient water and enough of other nutrients capable of supporting plant growth (Unkovich *et al.*, 2008). Nodule formation and functioning is suppressed as the level of soil mineral N in the rhizosphere increases (Keyser and Li, 1992). Ideally, higher nodulation should increase the amount of nitrogen fixed but this could be limited by several environmental factors. For example, the legume – rhizobium symbiosis may not produce enough nitrogen during the early stages of growth to meet the N demand of the legume. Hence small application of chemical N is necessary to promote early growth (Keyser and Li, 1992). Nitrogen application at either vegetative or flowering stage can potentially increase pod and crop biomass by 44 % and 16 % respectively (Katulanda, 2011). There are several contradictory reports on the response of legumes to nitrogen application. There is a higher probability of obtaining positive response to inoculation when soil nitrate is low and legume has a high potential for growth and in the same way high soil nitrate can potentially hinder N_2 fixation (Peoples *et al.*, 1995). Response of legumes to nitrogen application depends on the time of application and the rates of application (Yinbo *et al.*, 1997).

2.13.8.2 Nutrient management systems and their deficiencies in legume - rhizobia symbiosis

In Rhizobium-legume symbiosis, the essential mineral nutrients are those required for the normal establishment and functioning of the symbiosis. Based on this definition adapted from Arnon *et al* (1939), the following chemical elements C, H, O, N, P, S, K, Ca, Mg, Fe, Mn, Cu, Zn, Mo, B, C1, Ni and Co are known to be essential for the legume -

rhizobium symbiosis. Each essential nutrient has specific physiological and biochemical role with minimal nutrient concentrations required within both legumes and rhizobia to sustain metabolic function at rates which do not limit growth (Graham *et al*., 1988).

Mineral nutrients influencing nitrogen fixation in leguminous plants can result in both positive and negative effects. For example, the presence of mineral nitrogen in the soil inhibits both nodule formation and nitrogenase activity (Sprent *et al*., 1988) though there are contradicting reports to this. Other researchers have reported the need for mineral nutrient to establish the plant before nodulation commences (Becker *et al*., 1991; Keyser *et al*., 1992; Hardarson, 1993; Carsky *et al*., 2001). The enhancing effect of low levels of combined nitrogen on N_2 fixation in legumes is related to the lag phase between root infection and the onset of N_2 fixation. Phosphorus (P) is second only to nitrogen as an essential mineral fertilizer for crop production. At any given time, a substantial component of soil P is in the form of poorly soluble mineral phosphates. A high phosphorus supply is needed for nodulation. When legumes dependent on symbiotic nitrogen receive an inadequate supply of phosphorus, they may suffer from nitrogen deficiency. Weisaney *et al.* (2013) reported that, the deficiency of phosphorous supply and availability remains a severe limitation to nitrogen fixation and symbiotic interactions.

Potassium and sulphur are not usually liming nutrients for nodulated legumes, although a K^+ supplement for osmo-adaptation has to be considered for growth in saline soils. Among mineral nutrients, boron (B) and calcium (Ca) are undoubtedly the nutrients with a major effect on legume symbiosis. Both nodulation and nitrogen fixation depend on B and Ca^{2+}, with calcium being more necessary for early symbiotic events and B for nodule maturation (Delgado, 1998). Copper (Cu) plays a role in proteins that are required for N_2 fixation in rhizobia. Copper deficiency decreased nitrogen fixation in subterranean clover. Iron is required for several key enzymes of the nitrogenase complex as well as for the electron carrier ferredoxin and for some hydrogenase. A particular high iron requirement exists in legumes for the heme component of haemoglobin (Tang *et al*., 1992).

Molybdenum is a metal component of nitrogenase; all N_2-fixing systems have a specific high molybdenum requirement. As reported by Brodrick *et al.* (1991) molybdenum deficiency induced nitrogen deficiency in legumes. Relying on N_2 fixation is widespread, particularly in acid mineral soils of the humid and sub humid tropics. A specific role for nickel in nitrogen fixing bacteria according to Buerkert (1990), is now well established with the determination that a nickel - dependent hydrogenase is active in many rhizobial bacteria. Ahmed *et al.* (1960) also reported that, cobalt is required for the synthesis of leghemoglobin and for the growth of legumes relying on symbiotically fixed nitrogen. It has been established that rhizobium and other N_2-fixing microorganisms have an absolute cobalt requirement whether or not they are growing within nodules and regardless of whether they are dependent on a nitrogen supply from N_2 fixation or from mineral nitrogen.

Therefore, in sustainable BNF in agriculture systems, the use of mineral fertilizers is one of the most important principles though it has to be done minimally and specifically according to the requirement of each farm location.

2.13.8.3 Optimization of N fixation

Biological N fixation presents economic, environmental, and agronomic benefits and could be used to a larger degree as an alternative to synthetic fertilizers (Silva and Uchida, 2000). However, nitrogen fixation in legumes requires the symbiotic interaction of plants with rhizobia bacterial. Increasing the quantity and efficiency of the N_2 fixation process could increase crop productivity and reduce fertilizer costs. Optimizing this symbiosis may require improving the selection of the host and rhizobia participating in this interaction. Breeding for improved cultivars of legumes may enhance the genetic potential of the plants in fixing nitrogen which can result in 10% increase in N_2- fixed relative to existing cultivars according to Giller and Cadish (1995).

Biological nitrogen fixation may be increased by repeated rhizobial inoculation (Vessey, 2004; Athar, 1998), use of more effective strains (Hynes *et al*., 1995), or co-inoculation with “helper organisms” such as mychorrhizae (Dileep-Kumar *et al*., 2001). The efficiency of N_2 fixation is not only dependent on the selection of the most robust strains of rhizobia but is also related to crop varieties and the interactions of specific strains with specific varieties. Good growth of the legume is also of importance (Keyser and Li, 1992). The environment also plays an important role because it is the soil and climatic condition that will determine the plant growth and indirectly nodulation and root development. (Giller and Cadish 1995). People *et al.* (1995) suggest that, conditions that will render the soil non-productive should be guarded against. The legume and the inoculum strain should be able to survive and function optimally in the environment in question.

2.13.8.4 Nitrogen availability

The amount of nitrogen fixed is usually high in soils with low mineral N_2 but with sufficient water and enough of other nutrients capable of supporting plant growth (Unkovich *et al*., 2008). Nodule formation and functioning are suppressed as the level of soil mineral N in the rhizosphere increases (Keyser and Li, 1992). Ideally, a higher nodulation should increase the amount of nitrogen fixed but this could be limited by several environmental factors. For example, the legume–rhizobium symbiosis may not produce enough nitrogen during the early stages of growth to meet the N demand of the legume. Hence a small application of chemical N is necessary to promote early growth (Keyser and Li, 1992). Nitrogen application at either vegetative or flowering stage can potentially increase pod and crop biomass by 44% and 16% respectively (Katulanda, 2011). There are several contradictory reports on the response of legumes to nitrogen application. There is a higher probability of obtaining positive response to inoculation when soil nitrate is low and the legume has a high potential for growth and in the same way high soil nitrate can potentially hinder N_2 fixation (Peoples *et al*., 1995). Response of legumes to nitrogen application depends on the time of application and the rates of application (Yinbo *et al*., 1997). Application of N fertilizer at the pod filling stage increases the proportion of plant N derived from the N_2 fixation (Yinbo *et al*., 1997).

2.13.8.5 Legume contribution in biological nitrogen fixation

Symbiotic nitrogen fixation by legumes plays an important role in sustaining crop productivity and maintaining fertility of marginal lands in smallholder farming systems. The most important nitrogen-fixing symbiotic associations are the relationships between legumes and rhizobium bacteria. Leguminous plants provide the major N input into the biosphere as a result of their ability to convert atmospheric N_2 to a form that can be assimilated by plants (Hardarson *et al*., 2003). By providing N through fixation, legumes reduce mineral N inputs and the cost of production. Nitrogen fixation is variable in different grain legumes. Some legumes such as Faba bean (*Vicia faba*) and Lupin (*Lupinus spp*) are known for their effectiveness (i.e. up to 200 kg N ha^{-1} of their N in one season) under suitable field conditions while soybean (*Glycine max*) can only fix on the average approximately about 100 kg N_2 ha^{-1} (Hardarson *et al*., 2003).

Sanginga (2003) reported the use of promiscuous soybeans for the development of sustainable cropping systems in the moist Savannahs of West Africa to alleviate the serious food production threat in N-depleted soils. The actual amounts of N_2 fixed by soybean and their residual N benefits to subsequent cereal crops varied between 38 and 126 kg N ha^{-1} when only seeds of soybean were removed from the plots while the net N accrual of soil nitrogen ranged between -8 and +47 kg ha^{-1} depending on soybean cultivar (Sanginga, 2003).

2.14 Need for nitrogen in soybean

A lot of contrasting reports have been published with regards to the response of legumes to nitrogen. Keyser *et al.* (1992) reported that, as the level of mineral N in the rhizosphere increases, nodule formation and functioning is suppressed, apparently resulting in low amount of nitrogen fixed. With all things being equal, higher nodulation should increase the amount of nitrogen fixed. However, this is dependent on several environmental factors. Panchali (2011) reported that, per adventure the legume–rhizobium symbioses due to such factors is not able to produce sufficient nitrogen during the early stages of growth to meet the plant N demand, then small application of mineral N becomes necessary.

Sosulski *et al.* (1989) suggested that, the high demand of N by annual legumes may require a high level of soil N to achieve maximum yield. Katulanda (2011) also confirmed a potential increase of pod and crop biomass by 44% and 16% respectively in response to nitrogen application at either vegetative or flowering stage. Kucey *et al.* (1989), Gan *et al.* (2003), and Osborne *et al.* (2006) were all in support of the use of nitrogen fertilizer to soybean at one stage of its growth or the other to boost its production. On the other hand, other researchers have not expressed support of N_2 use for soybean production. For example, Peoples *et al.* (1995) reported that, high response to inoculation in a low nitrate soil by a legume with high potential for growth cannot be underestimated, which implies that high soil nitrate can hinder N_2 fixation.

Schmitt *et al.* (2001) have also reported that, soybean fertilized with mineral N did not result in high grain yield and oil content. Barker and Sawyer (2005), Panchali (2011) and Gan *et al.* (2003) also reported that, the use of N for soybean at certain growth stages might not be advisable. The use of N_2 in soybean cannot be ruled out completely. Many factors (time of application, fertilizer type, rate of application and environment etc.) therefore, have to be put into consideration before conclusions can be drawn on these controversies.

2.15 Role of phosphorus in biological nitrogen fixation

Phosphorus is one of the essential nutrients for legume growth and BNF (Giller and Cadisch, 1995; Whitbread *et al.*, 2004; Mhango *et al.*, 2008). Phosphorus deficiency can limit nodule number, leaf area, and biomass and grain development in legumes.

Symbiotic nitrogen fixation has a high P demand because the process consumes large amounts of energy (Schulze *et al*., 2006) and energy generating metabolism strongly depends upon the availability of P (Plaxton, 2004). Several reports have documented that nodules are a strong P sink and nodule P concentration normally exceeds that of roots and shoots (Sa and Israel, 1991; Drevon and Hartwig, 1997).

Phosphorous affects root development and hence uptake of nutrients and water. Apart from its effect on the nodulation process and plant growth, phosphorus also has been found to exert some direct effects on soil rhizobia (Singleton *et al*., 1992). Singh and Sale (2000) reported that, P fertilization stimulates root growth, photosynthesis and increases hydraulic conductibility of roots. Phosphorus fertilizer application to soybean is an important step in attaining high yield under low soil P (< 10 mg kg-; Bray-1) (Aune and Lal, 1997; Martin, 2005). Soybean plant requires an application of 20-30 kg P_2O_5/ha during the growing season to sustain a high crop yield in low P soils. Soil phosphorus availability during plant seedling development is an important determinant of plant growth, N_2 fixation and grain formation of soybean (Vance, 2001). Low P availability in soils results in a decrease in shoot growth, affects the photosynthetic activity and limits the transport of photosynthates to nodules (Jakobsen, 1985) with significant decline in N_2 fixation by the plant (Israel, 1987).

There are inconsistent reports on the response of soybean to P application on highly weathered soils. Chiezey *et al.* (1991, 1992), and Chiezey (2001) reported significant yield increase in soybean with P application on savanna soils. Similar reports were made elsewhere by other workers on soybean (Anzaku and Azanaku, 2002; Alpha *et al*., 2006). However, Chiezey (1999), Erhabor *et al.* (1999), and Slaton *et al.* (1999) reported that, grain yield in soybean was not significantly influenced by P application. Kumaga and Ofori (2004) reported that, under on-farm conditions, increased application of phosphorus had quite prominent effects on nodulation and other growth and yield parameters of promiscuous soybean variety (naturally-nodulating) but it was almost the reverse in the case of non-promiscuous soybean variety (requires specific bacteria to nodulate), where only seed yield was increased. P application at 30 kg P/ha coupled with inoculation with Bradyrhizobia significantly favoured all the parameters studied in the two varieties.

The application of P in higher quantities under inoculated conditions proved beneficial only to non-promiscuous soybean variety and not the promiscuous soybean variety. A study conducted by Kamanga *et al.* (2010) in Dowa district, Central Region of Malawi reported that P fertilizer increased the yield of soybean but no reasons to this positive response were revealed. The report indicates that grain yields of P fertilized legumes were higher than yields of unfertilized treatments for soybean, pigeon pea, cowpea and groundnuts. Soybean showed response to P (20 kg/ha) with 0.5 t/ha extra grain yield than unfertilized plots. Fertilizer application increased biomass of these legumes. Soybean fertilized with P had 1.5 t/ha of biomass on top of the unfertilized treatment.

Similar studies by Khonje (1994) reported that, the population of *Bradyrhizobium* species and *Rhizobium* species in soils of Malawi are not uniform such that nodulation of promiscuous soybean will also depend on initial levels of indigenous populations of these nodule-forming bacteria. However, the study failed to ascertain why soybean variety surprisingly reduced nodulation after application of phosphate fertilizer at one of the sites in Malawi as compared to other sites used in the study which showed positive response to phosphate fertilizer application. In spite of these inconsistencies, the importance of P in soybean cultivation has been determined by many scientists (Vance, 2001; Mahamood *et al*., 2009; Shahid *et al*., 2009; Sharma *et al*., 2011).

Studies in Nigeria savanna showed that, uninoculated soybean required 24-39 kg P/ha at low soil P levels below critical limits to produce higher yields (Pal *et al*., 1989) and rhizobium inoculation increased the yield of promiscuous soybeans, particularly in soils having a low population of indigenous Bradyrhizobia (Olufajo,1990).

Soybean breeding program develops new varieties of soybeans that contribute to sustainable and profitable agriculture. High yields and valuable traits contribute to agricultural productivity. However, it is also pertinent to evaluate the newly developed varieties for their productivity and adaptability to the different agro-ecological zones characterized by different weather patterns, soil types and their responses to P fertilizer application (Chiezey *et al*., 2001). Soybean yields are limited by acidic and highly weathered soils low in available phosphorus. The mobility of phosphorus in soil is very limited, so soil exploration by roots is important in accessing soil P (Lynch and Brown, 2001). Olivera *et al.* (2004) reported that, phosphorus application to soybean increase plant biomass including nodule biomass and shoot P content due to the increased rate of nitrogen fixation. The biological system needs energy which provides hydrogen reductant and also the energy for ATP system in nitrogenase reactions.

In Malawi, low yield of legumes grown by smallholder farmers may be strongly linked to minimal use of P fertilizer (Mwalwanda *et al*., 2003) among other factors, and this was also identified in studies on the response of maize to legumes and N fertilizer in central Malawi (Robertson *et al*., 2005). Studies have shown that there are benefits of P fertilization in legume cropping systems.

Giller (2001) reported that, the application of P fertilizer can overcome the deficiency in soils that do not strongly adsorb P. Given the high variability of soil fertility in smallholder farming systems, soil testing remains the most precise available tool to (1) determine whether P deficiencies are the cause of low soybean yields, and (2) prescribe adequate P fertilization rates (Melgar *et al*., 1995).

Arable land tends to vary in soil fertility and this gives rise to varying responses to nutrient fertility management. The different crop species have varied responses to the different nutrient management interventions (Nyirenda, 1998) and thus it is worthwhile to test the response of different soybean varieties to nutrient management under on farm conditions. Root hairs, root tips and the outermost layers of root cells are the most pathways of P entering the plants (Rotaru, 2010). Once P is inside the plant roots, phosphorus may be stored in the root or transported to the upper part of the plants (Singh and Sale, 2000). During various chemical reactions, P is integrated into organic compounds, including nucleic acids (DNA and RNA), phospho-proteins, phospho-lipids; sugar phosphate compounds like adenosine triphosphate (ATP) (Bashir *et al*., 2011).

Nitrogen is reduced to NH_3 under consumption of ATP and redox equivalents, and is associated with the formation of H_2 as a by-product. Thus, adding P fertilizer may reduce stress in the symbiotic relation between root bacteria and legume plant by providing this energy. The enzyme that catalyses the reaction is called nitrogenase and consists of the dinitrogenase reductase protein (Fe protein) and the dinitrogenase (MoFe protein) which actually catalyses the reduction of N_2.

2.16 Effect of phosphorus on nodulation and nitrogen fixation of soybean

Plants absorb P as either the primary H_2PO_4 ion or smaller amounts of the secondary HPO_4 and since the former is more abundant over the range of soils prevailing for most crops, it is usually the principal form absorbed (Russel, 1988; Tisdale and Nelson, 1975).The phosphorus (P) content of soils is low compared to nitrogen and potassium (Tisdale and Nelson, 1975; Brady, 1990). The total phosphorus content of a soil does not indicate its fertility, what is important is the amount of available phosphorus (Yayock *et al.,* 1989). When soluble sources of phosphorus in fertilizers and manures are added to soil, they are fixed or are changed to unavailable forms and react to become highly insoluble forms (Brady, 1990).

For legumes, P enhances both nodulation and N_2 fixation (Israel, 1987). Phosphorus deficiency in soybean (*Glycine max)* can result in poor nodulation, reduced seed viability, and decreased percentage of fully developed seeds (Bishnoi *et al*., 2007). Borges and Mallarino (2000) speculated that, abundant rainfall during the growing season could have increased the soybean root mass at the surface, explaining a lack of response to phosphorus fertilization on low soil-test phosphorus soils for some environments. Plant growth was increased in acid soil with applied phosphorus with and without lime. This positive growth response of haricot bean for application of P in acidic soil may be related with better availability of P as the rates of P application increased (Mesfin *et al*., 2014).

Phosphorus (P) is the most limiting nutrient for the growth of leguminous crops in the tropical and subtropical regions (Ae *et al.,* 1991). Low phosphorus content of between the ranges 2-6 ppm have been reported for most savannah soils in Ghana (Nye, 1952). The effect of P on legume growth and development is a function of nutritional effects on nodulation (Gates, 1974). Nodules are strong sink for phosphorus and can increase in phosphorus concentration up to 50 % (Graham and Rosas, 1979) and dry weight up to 32.8 fold (Israel, 1987).

Cassman *et al.* (1980) found nodule dry weight of nitrogen fixing soybean to comprise 9% of the total plant dry weight and 61% of root dry weight at the highest rate of phosphorus application. Dadson and Acquaah (1984) and Assuah (1990) reported that, application of phosphorus up to 60 kg P/ha increased nodulation in soybean. It was observed that addition of mineral nitrogen increased the uptake of phosphorus from soil by plants and that the relative effect was greater when the level of phosphorus was low (Grunes, 1959). The effect of mineral nitrogen on phosphorus uptake has been attributed to various factors including increased root absorption capacity through increased root growth, increased cation exchange capacity of the roots, and salt effects (Grunes, 1959). White (1973) concluded that, at low levels of available phosphorus, the demand created by the plant's growth rate had an overriding influence on the rate of absorption of phosphorus by the roots whereas at high concentrations the rate of phosphorus uptake was dependent on concentration gradient.

Nitrogen supply accelerated the turnover rate between inorganic and organic pools of phosphorus in the root due to increased rate of plant growth resulting in increased rate of transport from root to shoot.

2.17 The need for inoculation in soybean cultivation

The presence of compatible rhizobia in the soil and their effectiveness are the determining factors for the need for inoculation. Poor nodulation of soybean by indigenous *bradyrhizobia* is one of the major constraints to the successful production of soybean in Africa (Singh and Rachie, 1987). Where no soybean crop has been grown before, it is usually necessary to inoculate with an efficient *Bradyrhizobium* strain to maximize yield when fertilizer nitrogen is not applied (Dadson and Acquaah, 1984). Significant responses to *bradyrhizobial* inoculation are observed when the crop is grown in areas where it has not been previously cultivated (Abel and Erdman, 1964; Kang, 1975) and yield increases as high as six-fold have been obtained (Bromfield and Ayanaba, 1980).

In some soils where soybean has been grown previously, continued inoculation may still be necessary (Rao *et al.*, 1985) apparently because of poor survival of introduced rhizobia. When the soil contains effective soybean *bradyrhizobia* or has produced adequately nodulated soybeans, inoculation may not produce significant increase in yield (Johnson *et al.*, 1965; Caldwell and Vest, 1970; Ham *et al.*, 1971). This lack of response occurred when the soil contained more than 103 *bradyrhizobia* per gram of soil (Weaver and Frederick, 1974; Singleton *et al.*, 1992). However, when the soil contained ineffective *bradyrhizobia*, application of effective *B. japonicum* in the inoculant produced much greater proportion of nodules even if the population of the effective strains was comparatively low (Robinson, 1969). Introduced inoculant strains must exhibit both saprophytic and symbiotic superiority, relative to indigenous strains, if they are to maintain yields in the absence of continued inoculation (Fuhrman and Wollum, 1989).

The Joint FAO/IAEA Programme of coordinated research showed that inoculation with a suitable strain of *rhizobium* at sowing was the single most useful agronomic practice in ensuring maximum legume yield (Gudni *et al.*, 2003). Since the desired type of N_2-fixing micro-symbiotic may not exist in the required amounts in a given soil, inoculation with an appropriate strain suited for a specific crop and soil conditions is often required (Gudni *et al*., 2003). The use of inoculation is therefore necessary when legumes are introduced into new regions.

However, Giller (2001) reported that, if the introduced legume crop can nodulate effectively with rhizobia that are present in the soil in sufficient numbers, then inoculation may not be necessary. The inoculation technology comes in different forms; powder or granular forms are common. The powder form is applied directly to the seeds before planting. The common problems with inoculations are their poor competitiveness with local strains; sensitivity to climatic and other stresses limiting their viability and number; and problems of packaging, transport and storage until end-use on the farm (Smith 1987; Bantilan and Johansen, 1995). Without refrigeration, the live microbial culture loses its potency fast making the use of the inoculants a difficult option under smallholder farming conditions in the tropics, especially in the rural communities (Smith 1987; Bantilan and Johansen 1995; Singleton *et al*., 1997; Montanez 2000).

This notwithstanding, inoculums have often been used to increase the number of desirable strains of rhizobia in the rhizosphere (Lupwayi *et al*., 2000). Fening *et al.* . (2002) confirmed that, only 6% of the indigenous rhizobia across Ghanaian soils are highly effective with 68% and 26% being moderate and ineffective, which therefore necessitate the need for the use of inoculation in Ghanaian soils. Therefore, inoculation at times can be used as a form of insurance against crop failures. Deaker *et al.* (2004) and Herridge *et al.* (2002) reported that, less problem is associated with over inoculation rather than not inoculating at all.

2.18 Effect of interaction of phosphorus and inoculation on nodulation, growth and seed yield of soybean.

The P content of the soil affects the efficiency of seed inoculation. Both P and inoculation work in the same direction as far as nitrogen fixation is concerned (Dhingrah *et al.*, 1988). In savannah soils where lack of phosphorus fertilization had a limiting effect on nodulation and yield, maximum benefit was derived from biological nitrogen fixation and maximum productivity when phosphorus fertilizer was applied (Olufajo and Adu, 1992). Olufajo and Adu (1992) and Raychaudhuri *et al.* (1997) found significant interaction between inoculation and phosphorus on nodule dry weight of soybean at the flowering stage. Similar interaction between inoculation and phosphorus on seed protein of soybean has been reported by Dadson and Acquaah (1984).

Apart from soybean, interaction between inoculation and phosphorus has been reported for other legumes. For instance, Giller *et al.* (1989) found that, inoculation together with phosphorus gave an increase in nodulation and nitrogen uptake of beans. In poor soil, Cobbinah *et al.* (1992) reported that, inoculation of *Leucaena leucocephala* with rhizobia and fertilization with P increased shoot N_2 content and a combined application of rhizobia and P fertilizer was as effective as fertilization with both P and N_2. Similar findings have been reported by Luyindula and Haque (1992) on *Sesbania* and *Leucaena*. Dhingrah *et al.* (1988) also found that, interaction between phosphorus and inoculation on lentil was significant and a combination of *Rhizobium* and 20 kg P_2O_5/ha gave yields equivalent to 40 kg P_2O_5/ha without *Rhizobium* inoculation.

2.19 Effect of nitrogen and phosphorous on nodulation, growth and yield of soybean

Phosphorous is an essential mineral nutrients required in relatively large amounts to maintain plant growth. It plays a major role in improving crop yield and quality (Raghotham, 1999; Abel *et al*., 2002). Plant height, grain yield, biomass yield and P uptake efficiency of soybean increases at high levels of P application (Sahoo and Panda, 2001; Manje *et al*., 2011). Phosphorous and potassium deficient plants often have slow growth, poor drought resistance, weak stems and are more susceptible to lodging and plant diseases (Jack and Sarah, 2001).

The application of P on soybean increases the amount of N_2 derived from the atmosphere by the soybean-*Bradyrhizobium* symbiotic system (Chien *et al*., 1993; Sanginga *et al*., 1996). Nitrogen nutrition in soybean is ensured by denitrogen fixation and mineral nitrogen assimilation, which is important for high vegetative growth, high productivity and high seed protein content of soybean (Ronis *et al*., 1985). Only 25 to 65 % of N_2 in soybean dry matter originates from symbiotic nitrogen fixation, the remainder comes from soil N_2 (Harper, 1974). Varvel and Peterson (1992) noted that, soybean plants act as sinks for soil N_2 and effectively use N regardless of source.

Therefore, N_2 fertilization could benefit soybean. Helms and Watt (1991), also found out that N_2 fertilization of soybean increases seed protein or oil concentration. Starter N_2 application is aimed at providing soybean with readily available soil N_2 during seedling development, and has been shown to increase soybean grain yield (Touchstone and Rickerl, 1986).

2.20 Lime

Lime are materials containing carbonates, oxides or hydroxides required to apply in acid soils to raise soil pH and in addition neutralize toxic elements in the soil. Soil pH is used to determine whether or not to lime a soil (TSO, 2010). Liming materials include $CaCO_3$, Ca, Mg $(CaCO_3)_2$, $Ca(OH)_2$, CaO and others, which vary according to their neutralizing value and degree of fineness (TSO, 2010). When lime is applied to the soil, Ca^{2+} and Mg^{2+} ions displaces H^+, Fe^{2+}, Al^{3+}, Mn^{4+} and Cu^{2+} ions from soil adsorption site resulting in increase in soil pH. Other than increasing soil pH, lime also supplies significant amounts of Ca and Mg, depending on the type. Indirect effects of lime include increased availability of P, Mo and B, and more favourable conditions for microbially mediated reactions such as nitrogen fixation and nitrification, and in some cases improved soil structure (Nekesa *et al*., 2005). For instance, application of lime significantly increased root and shoot yields in Nigeria (Anetor and Akinrinde, 2006), grain yields of soybean in Brazil (Kassel *et al*., 2000; Caires *et al*., 2006). Similarly, in Croatia Andric *et al.* (2012) reported increased soybean yield by 44% as a result of lime application. Moreover, Nekesa *et al.* (2011) in Western Kenya also found positive response of soybean grain yield to lime application either alone or combined with P fertilizer.

2.21 Combined effects of organic and inorganic fertilizers on soybean production

The importance of applying fertilizers in organic or inorganic form has been proven in various researches. However, use of manures alone has a slow but positive effect in releasing nutrients since they require microbial activity to decompose it. On the other hand, mineral fertilizers are of rapid nutrient availability but expensive and are easily leached from the soil. However, application of combined organic and inorganic fertilizers is a viable solution to restore, maintain soil fertility and increase crop yields (Danga *et al*., 2010; Sharief *et al*., 2010). Maheshbabu *et al.* (2008) in India found out that, combination of FYM and mineral fertilizer had a significant effect not only on soybean grain yield but also on its growth parameters. Also, Anetor and Akinrinde (2006) in Nigeria found out that, combined lime and organic fertilizer had a significant effect on the number of pods, pod weight and seed number of soybean. Similarly, in western Kenya, Nekesa *et al.* (2011) found out that, combined Diamonium Phosphate (DAP) or TSP and lime significantly increased soybean grain yields. Combined organic and inorganic fertilizers have also been reported to increase soybean yield by 12.9% in India (Maheshbabu *et al*., 2008), 19% in Indonesia relative to sole application of inorganic fertilizer (Yamika and Ikawati, 2012), and 50% against the sole application of organic fertilizer (manure) (Zerihun *et al*., 2013).

2.22 Effects of lime and phosphorus fertilizers on soil chemical properties

Soil chemical properties include pH, exchangeable acidity (H^+, Al^{3+}) and exchangeable bases (Ca^{2+}, Mg^{2+}, K^+ and Na^+). These properties influence availability of nutrients to crop, and therefore have the potential to reduce or increase crop yields. Application of soil amendments leads to improvement in soil chemical properties creating favourable conditions for crop nutrition, development and yield. In a comparative study of organic manures and NPK fertilizer in acids oil, Adeniyan *et al.* (2011), observed that, 5 tonnes/ha^{-1} of cattle manure significantly increased soil available P, pH, organic C and cation exchange capacity. Kheyrodin and Antoun (2012) obtained a significant increase in soil P, Ca and Mg contents in the 15–30 cm depth when manure was applied.

Application of 2 tonnes/ha of lime decreased exchangeable Al and increased pH, available Ca and Mg in Cameroon (The *et al*., 2001). Lime and P fertilizers significantly improved soil pH and available P as reported by Anetor and Akinrinde (2006) who also attributed the increased soil pH to lime which in turn reduced P fixation. Repsiene and Skuodiene (2010) found that, lime and manure, when applied sole or combined, had a significant effect in reducing Al, increasing Ca, pH, and Mg. Ademba *et al.* (2010), reported significant increase in soil total P, K, Ca, Mg with sole application of 10 tonnes per hectare of manure, 60 kg P_2O_5 ha^{-1} and 250 kg ha^{-1} of lime. In addition, the same study revealed that, lime and manure combined with DAP increased available P. In Nigeria, Ewulo (2005) reported that, application of 6 tonnes per hectare of cattle manure increased total soil P, K, Ca, Mg and cations exchange capacity (CEC), and decreased exchangeable acidity. Improved physicochemical properties of acid soils have been reported through combination of manure with N, P fertilizers and lime (Onwonga *et al.*, 2010). The improvement was attributed to the integrated effect of the amendments by improving soil pH, microbial activity, nutrient release from organic matter decomposition and improved soil structure as well. In addition, Kisinyo *et al.* (2012), reported significant positive effects on soil pH and available P in acid soils of Western Kenya with the application of lime and P fertilizer in sole or in combination.

2.23 Effect of lime and P fertilizer on N, P uptake and N_2 fixation of soybean

2.23.1 Nitrogen and phosphorus uptake by soybean plants

Nitrogen is a macronutrient also known as vegetative nutrient and mostly used by the plants and therefore, an important nutrient for soybean grain yield (Kamara *et al*., 2011). However, availability of N is highly affected by soil acidity and leaching. Acidity tend to reduce microbial-mediated processes and this results in poor organic matter decomposition, poor mineralization of nitrogen and consequently low N availability. Application of soil acidity amendments may improve soil conditions for mineralization to take place and increase N availability in the soil, its uptake and finally a positive influence on crop yield.

In Bangladesh, Jahangir *et al.* (2009) reported increased N uptake by soybean under P fertilizer application. Similarly, in India, Sharma *et al.* (2011) observed a significant increase in uptake of N in soybean under P fertilizer application. Additionally, Schmitt *et al.* (2001) found out that, application of manure increased significantly N uptake by soybean. Son *et al.* (2001) in a farmer's field experiment under moderate acidic soil, also reported that, application of organic resources alone and also when combined with inorganic resources recorded 5.81% and 5.83% N content, respectively, in the soybean grain. In addition, Tagoe *et al.* (2008) obtained increases of 10.1% and 40.6% in seed and plant total N content respectively with the application of manure respectively. Application of lime increased soil pH and favoured nitrogen fixation resulting in a significant 3.1% increase in plant N concentration where N_2 (Caires *et al.*, 2006).

Phosphorus is an important plant macronutrient that makes up to about 0.2% of a plant's dry weight (Schachtman *et al.*, 1998). Phosphorus is present in seed and fruit in large quantities and is essential for seed formation. Phosphorus has also been reported to be a root growth stimulant and it is associated with early crop maturity (Abbas *et al.*, 2011). In acidic soils, most plant nutrients tend to be unavailable but lack of P is said to be the one that largely affects crop growth, absorption of water and other nutrients, hence low crop yields (Crawford *et al.*, 2008). Application of manure, lime and P fertilizers improve soil chemical, physical and biologic properties. They reduce P fixation by Al and iron (Fe) oxides in the soil, and increase availability of P which increases its uptake by crop (Crawford *et al*., 2008; Kisinyo *et al.*, 2012).

Anetor and Akinrinde (2006), reported 65.6% increase in P uptake by early growing soybean variety with the application of 2 tonnes/ha^{-1} lime. This was attributed to increased availability of P in the soil, enlarged proliferation of roots and to reduction of Fe and Al activity in the soil.

2.23.2 Effects of lime and p fertilizer on soil microbial biomass

The Soil Microbial Biomass (SMB) is the active component of the soil organic pool, playing an important role in nutrient cycling, plant nutrition and functioning of different ecosystems. It is responsible for organic matter decomposition thus affecting soil nutrient content (Onwonga *et al*., 2010). As such, the biomass is both a source and sink of the nutrients C, N, P and S contained in the organic matter (Lin *et al*., 2010; Basu *et al*., 2011).

Soil microorganisms are significant determinants of organic matter decomposition, soil nutrient status, crop health, and overall crop productivity (Basu *et al*., 2011). Soil MB is undoubtedly a valuable tool for understanding and predicting changes in soil fertility management and associated soil conditions such as nutrient dynamics and soil reactions (Sharma *et al*., 2004). However, changes in soil conditions (plant or animal residues) will determine how fast the microbial biomass responds (Onwonga *et al*., 2010).

Therefore, understanding soil microbial biomass dynamics is particularly critical in the management of acid soils, to reverse declining soil organic matter content and to restore soil fertility. Soil amendments have been used and reported as improving SMB.

2.24 Growth analysis

Plant growth analysis is an explanatory, holistic and integrative approach to interpreting plant form and function. It uses simple primary data in the form of weights, areas, volumes and contents of plant components to investigate processes within and involving the whole plant (Evan, 1996; Hunt, 1978). The most common growth functions are crop growth rate (CGR), leaf area index (LAI), leaf area duration (LAD), net assimilation rate (NAR), leaf area ratio (LAR) and relative crop growth rate (RCGR). These are normally calculated from total shoot dry weights and leaf area indexes recorded over a given period (Clawson *et al.*, 1986).

Crop growth rate is a dynamic character that determines the final yield in cereal and legume crops. Ball *et al.* (2000) have reported that, high population of soybean ensures early canopy closure, maximizes light interception, crop growth rate and crop biomass, resulting in increased yield potential. Crop growth rate depends on LAI and NAR, the latter depending on light-intercepting efficiency and photosynthetic efficiency of the leaf (Kokubun, 1988). Increasing plant population reduces the amount of time that it takes to reach 95% light interception levels that correspond to LAI levels of 3.2 to 3.5 (Higley, 1992).

Pod and seed number are the most important yield components of soybean. However, leaf area index, leaf area duration and dry matter accumulation during the reproductive period strongly influence the yield components (Liu *et al.*, 2004). Malone *et al.* (2002) have reported that, leaf area index values of at least 3.5-4.0 in the reproductive stages are required for maximum potential yield of soybean.

Stern and Donald (1961) stated that, leaf area index influences crop growth rate and that dry matter production by a crop also increase as the leaf area index increases until a maximum value is attained; thereafter as the leaf area index increases further, the rate of dry matter production will decline. This is because the lowermost leaves become so heavily shaded that, photosynthetic contribution becomes less than respiration.

2.25 Agricultural importance of leguminous crops

The term "grain legumes" or "pulses" refer to leguminous plants producing dry edible seeds (Howieson *et al*., 2000). Major grain legume species traditionally grown in the tropics include cowpea (*Vigna unguiculata* (L.)Walp.), black gram (V. *mungo* (L) Hepper), green gram (V. *radiata* (L.) Wilczek) and common bean (*Phaseolus vulgaris* (L.)).Others are lima beans (P. *lunatus*), pigeon pea (*Cajanus cajan* (L.) Mill sp.), groundnut (*Arachis hypogaea* L.), bambara nut (*Vorandzeia subterranean* L.), chick pea (*Cicer arientum* L.) and soybean (*Glycine max (*L.) Merr.) (Raemaekers, 2001).

Grain legumes are well known to contribute significantly towards reducing poverty, improving food security, improving nutrition and health, and sustaining the natural resource base (Rusike *et al*., 2013). Biological Nitrogen fixation (BNF) abilities of legumes is an important method for sustainable crop-land management and is a very good source of providing N to plants under favourable atmospheric and environmental conditions (Hungria and Vargas, 2000; Chen *et al*., 2002). Mahamood *et al.* (2009) reported that, soybean is a crop which has been proposed for the removal of the acute shortage of protein and oil worldwide.

The ability of legumes to fix atmospheric N_2 in symbiosis with rhizobia strains makes them excellent colonizers of low-N environments (Graham and Vance, 2003). However, rhizobia strains differ in their N_2 fixation efficiency and effectiveness. Likewise, grain legumes vary in their N_2 contributions to cropping systems depending on the proportion of plant N removed in harvested seed and that from fixation (Salvagiotti *et al*., 2008). The efficiency of the legumes to fix N biologically is affected by various factors such as soil moisture, temperature, available soil nutrients, biotic and abiotic stresses and the presence of efficient, competitive rhizobia strains, cropping systems and field management practices (Thies *et al.*, 1995; Palmar and Young, 2000; Kiers *et al.*, 2003). Ojiem *et al.* (2007), Nyemba and Dakora (2010) and Mhango (2011) reported, 22 to 124 kg/ha of total N fixed by groundnut under different cropping systems while Adu-Gyamfi *et al.* (2007) reported that, biological N_2 fixation of 20 to 118 kg/ha by pigeon pea. Common bean ranks low compared to most other legumes with reported N_2 fixation of less than 31 kg/ha/year (Hardarson *et al*., 1993; Ojiem *et al*., 2007). Efforts to optimize nodulation and BNF in grain legumes are critical challenges because of widespread increase in soil degradation in Africa.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The experiment was conducted between October 2015 and April 2016 in-front of the greenhouse of the University for Development Studies at Nyankpala in the Tolon District of the Northern Region of Ghana. Nyankpala is located at an altitude of 183 m above sea-level, and latitude 09 25’ and 00 58’ longitude of the equator. It has a monomodal rainfall pattern with annual mean rainfall of 1000 – 1200 mm which is fairly distributed from April-November (SARI, 2004). Temperature distribution is uniform with mean monthly minimum of 21.9^{o}C and maximum of 34. ^{o}C. It has a minimum relative humidity of 53% and a maximum of 80% (SARI, 2004).

3.2 Experimental design and lay-out

The experiment was laid in a split-plot design, with four replications. The main plot factor was lime (Calcium Carbonate, Oil palm leaf ash and Control) and the sub-plot factor was phosphorus and inoculant amendments (Phosphorus at 148 kg P_2O_5 /ha TSP, Inoculant at 5 g/1000/seed, Phosphorus at 148 kg P_2O_5/ha TSP-Inoculant at 5 g/1000/seed and control). Each replication consisted of twelve pots and a total of forty eight pots were used for the experiment.

3.3 Soil sampling

Soil samples were taken from three sites namely farms behind the fence of SARI main office, farming for the future Garden of UDS and farms in-front of the UDS library. Purposive sampling was used to identify the farms that have records of soil acidity and simple random sampling was used to pick soil samples from the three selected sites. At each site, twenty core soil samples were taken with a soil auger from 0–15cm horizon into black polythene bags and packed in a box and transported to SARI's soil laboratory for analysis.

3.4 Filling of pots

Soil taken from the farm behind the fence of SARI main office were dried under room temperature and sieved using a 2-mm mesh sieve. The sieved soils were then filled into the pots at 10 kg per pot. Four (4) pots each were filled with soil incorporated with calcium carbonate ($CaCO_3$) as inorganic liming material, oil palm leaf ash as organic liming material and Control. $CaCO_3$ was applied at the rate of 2 tonnes/ha and the same amount was applied for the oil palm leaf ash. At this liming rate, the amount of lime that was applied was 18g per pot at a crop spacing of 60 cm x 10 cm with two plants per stand.

3.5 Inoculation of seeds

The inoculant of the *Bradyrhizobium japonicum* stain USDA110 was obtained from IITA in Tamale-Ghana and applied at the rate of 10 g inoculant/1000 seed. Water was used to moisten the seeds to ensure that all the applied inoculum stick to the seed and the required quantity of inoculant was suspended in 200 ml of water. The moist seeds were gently mixed with inoculant so that all the seeds received a thin coating of the inoculant. Seeds were allowed to air-dry for a few minutes and were then sown at the required rate and spacing. Pots with un-inoculated seeds were planted first to avoid contamination. Seeds were immediately covered with soil after sowing to avoid death of bacterial cells.

3.6 Agronomic practices

Sowing was done in the first week of December, 2015 with three seeds per hole and the holes were fairly covered with soil to ensure a good contact between the seed and the soil. The seeds were later thinned to two plants per hole at a crop spacing of 60 cm x 10 cm. Manual weeding was done at any time weeds were seen in the pots to avoid competition with the soybean plants. Weeds were removed by hand and regular watering was carried out to prevent wilting of plants and keeping the required moisture content for proper growth of the soybean plants. Liming and inoculation was done before planting was carried out. Phosphorus applied at the rate of 148 Kg per hectare of Triple Supper Phosphate (TSP) and 1.33 g of TSP fertilizer was applied per pot at three weeks after planting. Harvesting and threshing of the soybean were done in April, 2016.

3.7 Soil analysis

3.7.1 Soil pH

A 20 g soil sample was weighed into a 100 ml plastic beaker before planting and after harvesting, and 50 ml of distilled water was added to the soil sample. The solution was stirred thoroughly and allowed to stand for 30 minutes. The pH meter was calibrated with buffer solutions at pH 4.0 and 7.0 and the pH of the soil was read by immersing the electrode of the meter into the upper part of the suspension. The pH of the soil sample which was 3.5 was recorded.

3.7.2 Extractable soil minerals

Nitrogen level in soil was analyzed by taking a small amount of the soil sample mixed with universal extracting solution. The extracting solution removes minerals from the soil and soil was filtered from the suspension. The soil suspension was tested with nitrate test reagents. A colour change in the solution occurred which was used to compare to standards printed colour chart. The same procedures were used for phosphorus and potassium levels in the soil but phosphorus and potassium test reagents were used respectively. Colour changes were used to compare to a standard printed on a colour chart for determination of the various level of the phosphorus and potassium in the soil. Ammonium acetate extractable levels of these elements as described by Walworth (2011), was used to determine the major exchangeable cations (K, Ca, Mg, and Na) using units of cmol$^+$/kg.

3.7.3 Available phosphorus

The readily acid-soluble forms of phosphorus were extracted with Bray No.1 solution. (HCl: NH_4 F mixture) (Bray and Kurtz, 1945; Olsen and Sommer, 1982). Phosphorus in the sample was determined with a spectrophotometer by the blue ammonia molybdate with ascorbic acid as a reducing agent. A 5 g soil was weighed into a 100 ml extraction bottle and 35 ml of Bray's no.1 solution (0.03M NH_4F and 0.025M HCl) was added. The bottle was placed in a reciprocal shaker and shaken for about 10 minutes and filtered through Whatman No.42 filter paper. An aliquot of 5ml of the filtrate was pipetted into a 25 ml flask and 10 ml of colouring reagent (ammonia paramolybdate) was added followed by a pinch of ascorbic acid.

After mixing well, the mixture was allowed to stand for 15 minutes to develop a blue colour. The colour was measured using a 21D spectrophotometer at 660 nm wavelength. The available phosphorus was extrapolated from a standard curve.

3.8 DATA COLLECTED

3.8.1 Plant height

To evaluate the effect of the treatments on soybean growth and development, three plants per pot were randomly selected and tagged before harvest and their heights measured using a measuring tape at 5, 8 and 11 weeks after planting (WAP) . Plants were measured between the highest photosynthetic tissue and ground level (Cornelissen *et al*., 2003).

3.8.2 Number of leaves per plant

At 5, 8, and 11 WAS, the number of leaves per plant per pot were counted and recorded.

3.8.3 Leaf area index

Leaf area index (LAI) was determined at 5, 8 and 11WAS. This was done by detaching all opened leaves from six sampled plants from each pot. Ten (10) leaves were picked from each pot and weighed and their fresh weight recorded. A cork borer of 0.01 m diameter was used to punch through ten leaves after sticking them together and the circular disc of leaves were recorded. The leaf area index was then calculated using the relation: Leaf Area Index = $\frac{\text{Leaf area}}{\text{Ground cover}}$

3.8.4 Days to 50% flowering

Plants were monitored closely to count the number of days taken for half of the plants in a pot to flower. The date was recorded as days to 50% flowering.

3.8.5 Number of nodules per plant

Six soybean plants at eight weeks after sowing were carefully uprooted from each experimental pot by digging around the plant using a spade. The roots were washed under tap water to remove all attached soil particles and other debris from the roots and the nodules. The nodules were then detached from the roots and counted.

3.8.6 N umber of effective nodules

Six soybean plants at eight weeks after sowing were carefully uprooted from each experimental pot by digging around the plant using a spade and then washing the roots and the nodules with tap water to remove all attached soil from them. The nodules were then detached from the roots and a blade was used to cut the nodules to observe the nodule colour to assess the effectiveness of the nodules.

3.8.7 Shoot fresh and dry weight

Six plants from each pot that were used for nodule count were used for the fresh weight of the soybean plant. The root system of the plant was cut off and the shoot weighed to determine the fresh weight of the plant. These plants were put into envelopes and dried in an oven at 70 °C for 72 hours. The dry weights of the shoots were determined and recorded.

3.8.8 Days to podding and number of pods per plant

The plants were monitored daily after the first flowers appeared to observe the first plant on which pods were formed. The date on which a first pod was found on a plant was recorded and used to estimate the number of days after sowing the pods were formed. At one week to harvesting, the number of pods on each plant was counted.

3.8.9 Grain yield

At maturity, all pods were harvested and threshed and the seeds obtained per pot were weighed. This was converted into kilograms per hectare and recorded for analysis.

3.10 Statistical analysis

A split-plot analysis of variance (ANOVA) was used to analyze data collected. The analysis was done using statistical software program GENSTAT version 10.3DE. Means were separated using the least significant difference (LSD) at $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Soil chemical analysis

The pH, Ca^{2+}, K^+, Na^+, total extractable bases, exchangeable acidity, cation exchange capacity and % base saturation differed significantly ($p < 0.05$) with soil treated with liming materials except for N, organic carbon, organic matter and Mg^{2+} which were not significant ($p > 0.05$). The Ash control consistently produced the highest soil properties while main control and $CaCO_3$ control had similarly low amount of soil properties in the soil analysis except Ca^{2+} and pH (Table 4).

The results of the analysis showed that the soil was acidic with a pH of 3.5 and sandy. The nitrogen, phosphorus and potassium levels were low but potassium level and pH values rose after the application of the liming materials to the soil (Table 4). Soils with poor fertility status and sandy loam soils need soil amendments such as $CaCO_3$ and ash to improve the release of nutrients for the proper growth and development of crops.

Table: 4 Some soil properties influenced by liming

	Soil Physical and Chemical Properties												
	pH	Ca^{2+}	K^+	Na^+	Mg^{2+}	T.E.B	Ex.A	CEC	%B.S	%N	% O.C	% O.M	Av P
l	7.5	2.8	18	1.30	1.70	24.0	0.05	24.0	99.8	0.05	0.56	0.96	9.8
trol	8.5	8.2	0.7	0.42	1 .00	10.0	0.04	10.3	99.7	0.042	0.48	0.83	12.3
ol	6.3	2.2	0.6	0.40	0.80	4.0	0.13	4.1	97.3	0.05	0.60	1.04	16.2
	0.001	0.001	0.013	0.11	0.12	0.03	0.001	0.03	0.001	0.25	0.25	0.25	0.72
	0.64	1.82	11.0	0.94	1.23	13.6	0.023	14.0	0.45	0.014	0.16	0.27	18.8
	5	24.0	99.2	81.0	63.0	62.4	21.0	62.1	0.3	16.6	16.6	16.6	85.3

Values (mean ± se) were separated at $p < 0.05$.

4.2 Plant height

Plant height was recorded at three different stages of growth (5, 8 and 11 WAS). There was a significant effect ($p < 0.001$) of liming (Fig 1) and soil amendments (Fig 2) on the plant height of the soybean plants. Liming with $CaCO_3$ increased plant height by about 80% over the ash and control treatments. Plant height remained greater throughout the experiment with the $CaCO_3$-treated pots over ash and control treatment, but ash treated plants were significantly higher than the control plants.

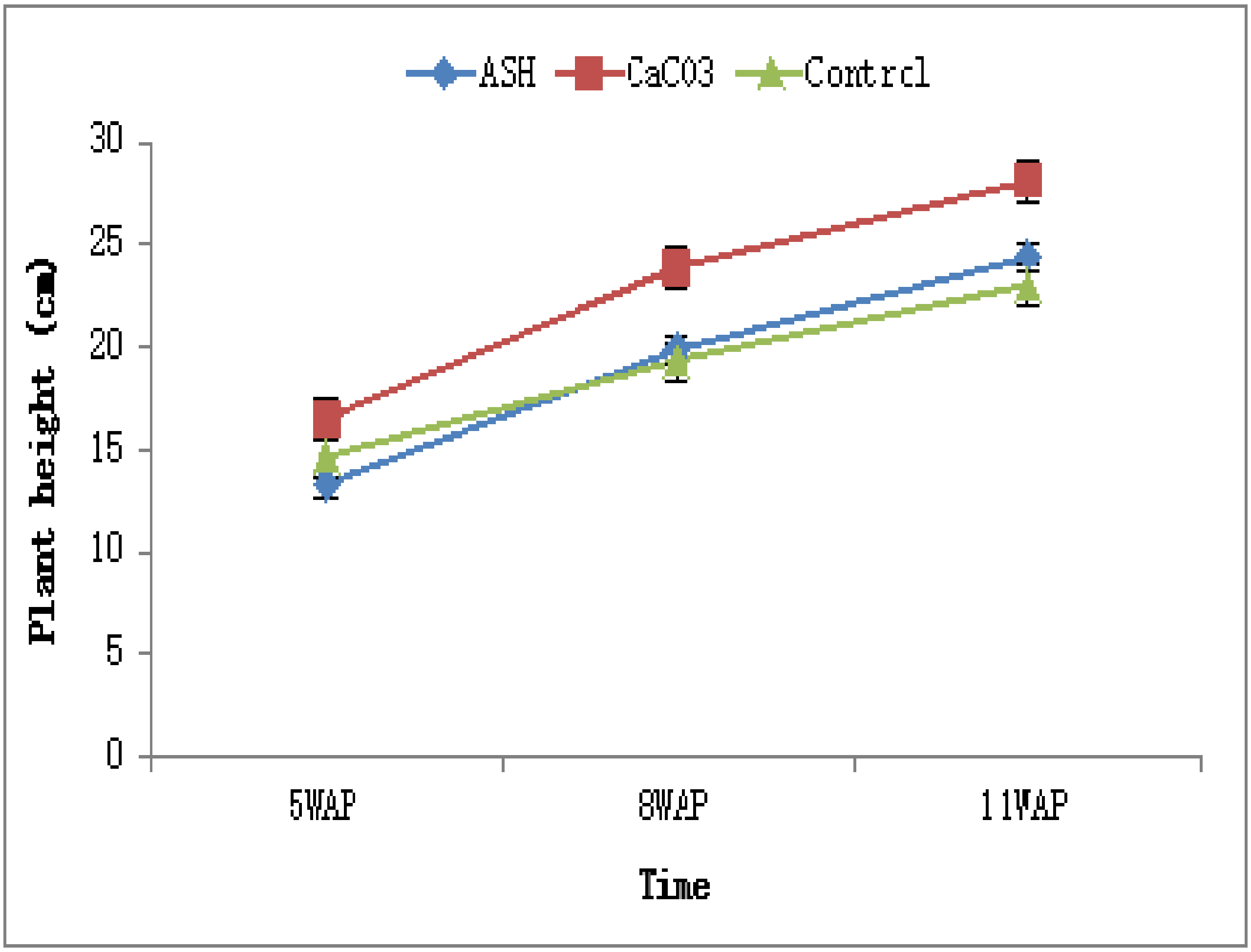


Figure 1: Effect of liming on plant height at 5, 8, and 11 WAS. Bars represent standard error of differences (SED).

Plant height was significantly ($p < 0.001$) influenced by soil amendment. At all the three stages of measurement of plant height, phosphorus consistently did better than the rest of the amendments used (Fig 2). The use of phosphorus fertilizer resulted in about 25% increase in plant height over the use of a combination of phosphorus and inoculation, inoculation only and the control treatment at the three stages of measurement (5, 8 and 11 WAS). At 11WAP, Inoculated plants, inoculated-phosphorus and Control gave almost the same plant height (Fig 2).

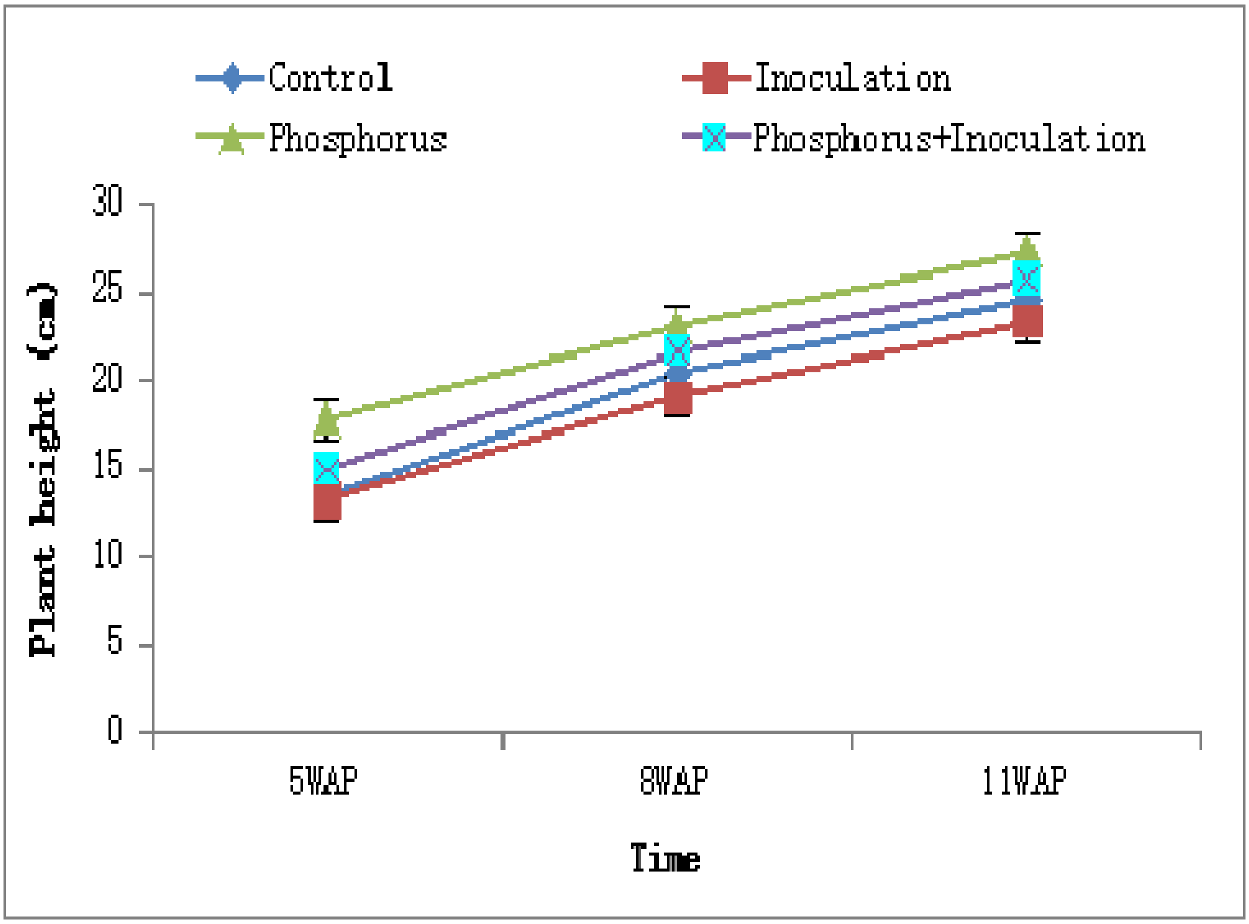


Figure 2: Effect of soil amendment on plant height at 5, 8 and 11 WAS. Bars represent standard error of differences (SED).

4.3 Number of leaves per plant

There was significant difference (p = 0.002) in the number of leaves per plant at the three timing of counting of the number of leaves when different liming materials were applied to the soybean plants (Fig 3). CaCO3 treatment recorded the highest number of leaves throughout the experiment. CaCO3 treated pots resulted in about 15% in increase in the number of leaves than control and it also did better than the ash treatment by about 41%. Control pots also did better than the ash treated pots.

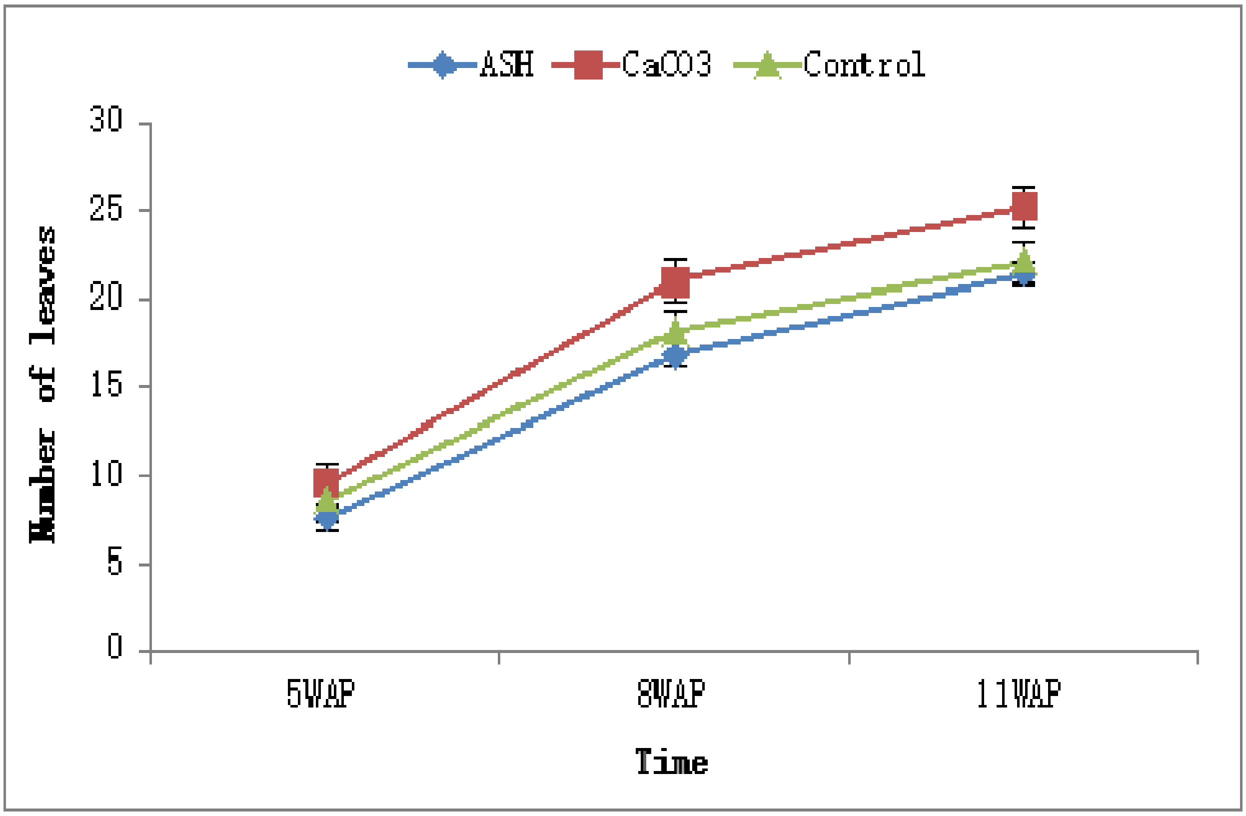


Figure 3: Effect of liming on the number of leaves per plant at 5, 8 and 11 WAP. Bars represent standard error of differences (SED).

Soil amendment affected the number of leaves per plant significantly (p = 0.008) at 5 WAP and 8 WAP but no significant differences was recorded at 11 WAP. Phosphorus treated plants consistently recorded the highest number of leaves per plant by about 35% while Control, Inoculation, and Phosphorus-Inoculation recorded similar but low number of leaves as compared to Phosphorus application throughout the experimental period (Fig 4).

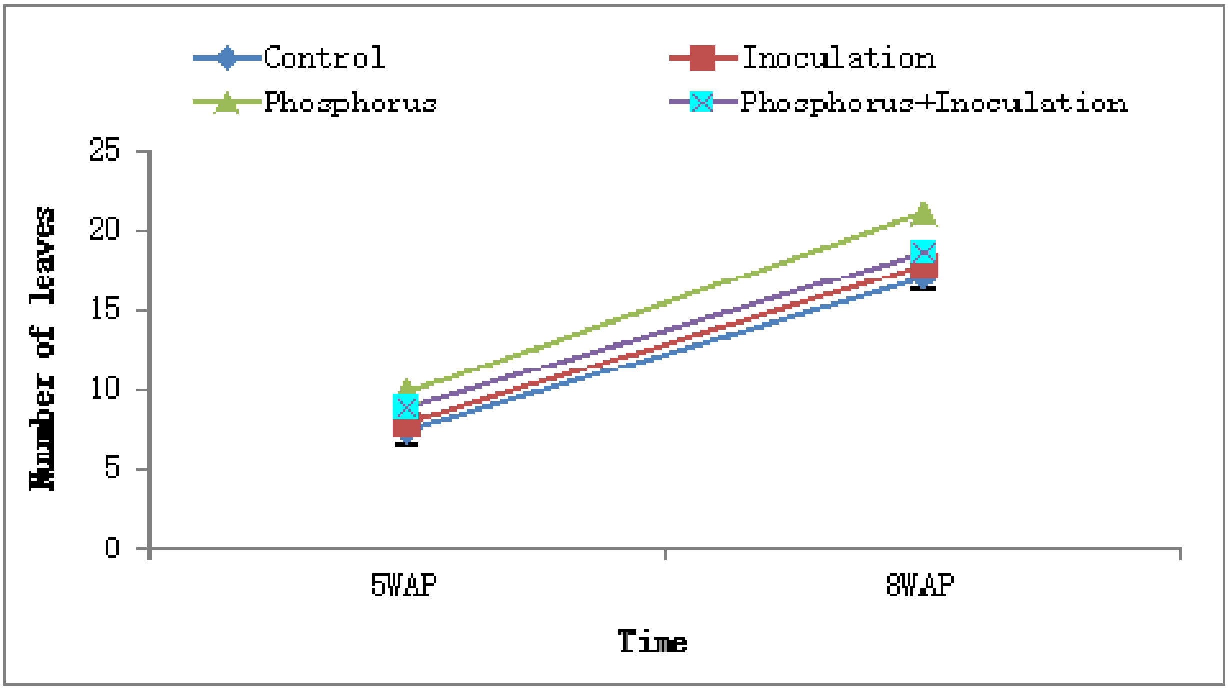


Figure 4: Effect of soil amendment on the number of leaves per plant at 5, 8 and 11 WAP. Bars represent standard error of difference (SED).

4.4 Leaf area index

At 5 WAP, there was no interaction effect between liming and soil amendment. However, liming ($p < 0.001$) and soil amendment ($p < 0.002$) significantly affected leaf area index (Fig 5). There was significant ($p < 0.001$) effect of liming and soil amendments as well as the interaction effect of liming and soil amendment on the leaf area index of the soybean plants at 8 and 11 WAP. Plants grown with $CaCO_3$ treated plants recorded higher leaf area index over Ash and Control.

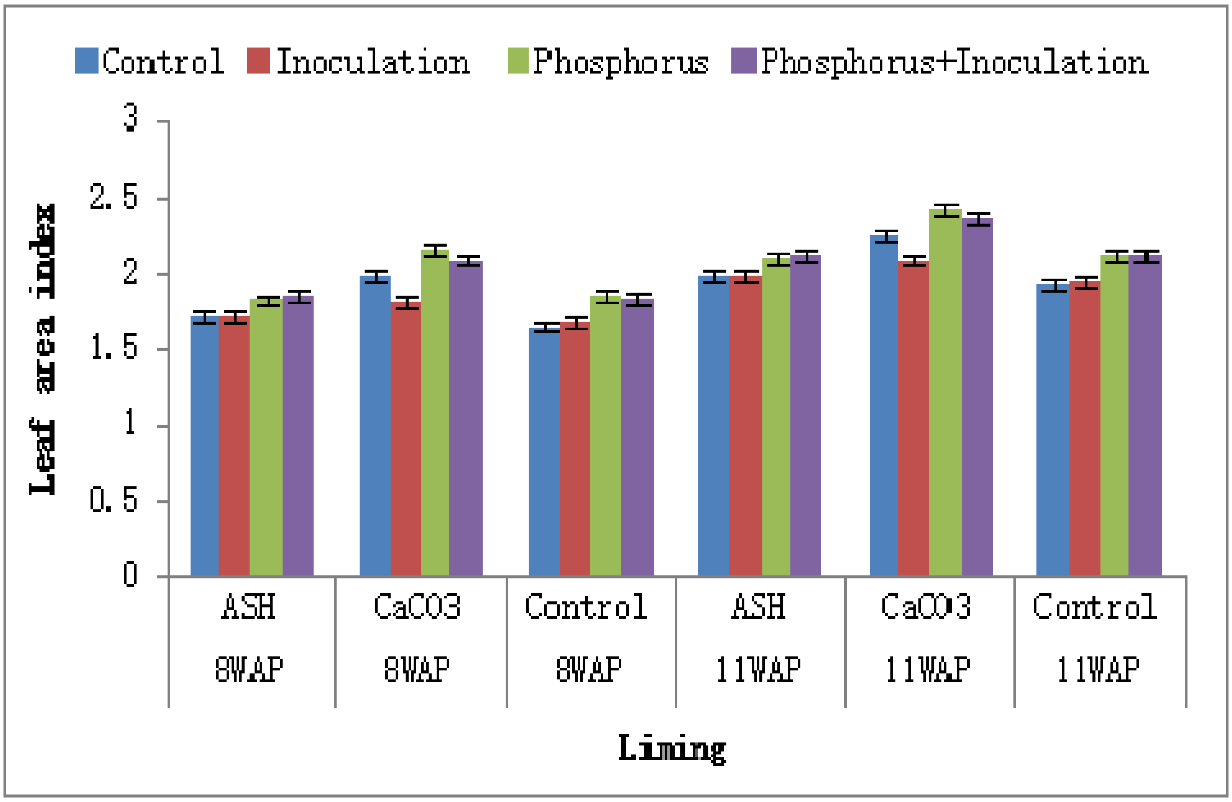


Figure 5: Effect of liming on leaf area index at 8 and 11 WAP. Bars represent standard error of difference (SED).

Leaf area index was significantly ($p < 0.002$) influenced by soil amendment. In all the three timing of measurement of leaf area index, phosphorus fertilizer treatment did better than the other soil amendments used throughout the experiment (Fig 6). As stated above, phosphorus did well throughout the experiment followed by Phosphorus-Inoculation, Inoculation then Control but at 8 and 11 WAP, Control recorded a better leaf area index than Inoculation.

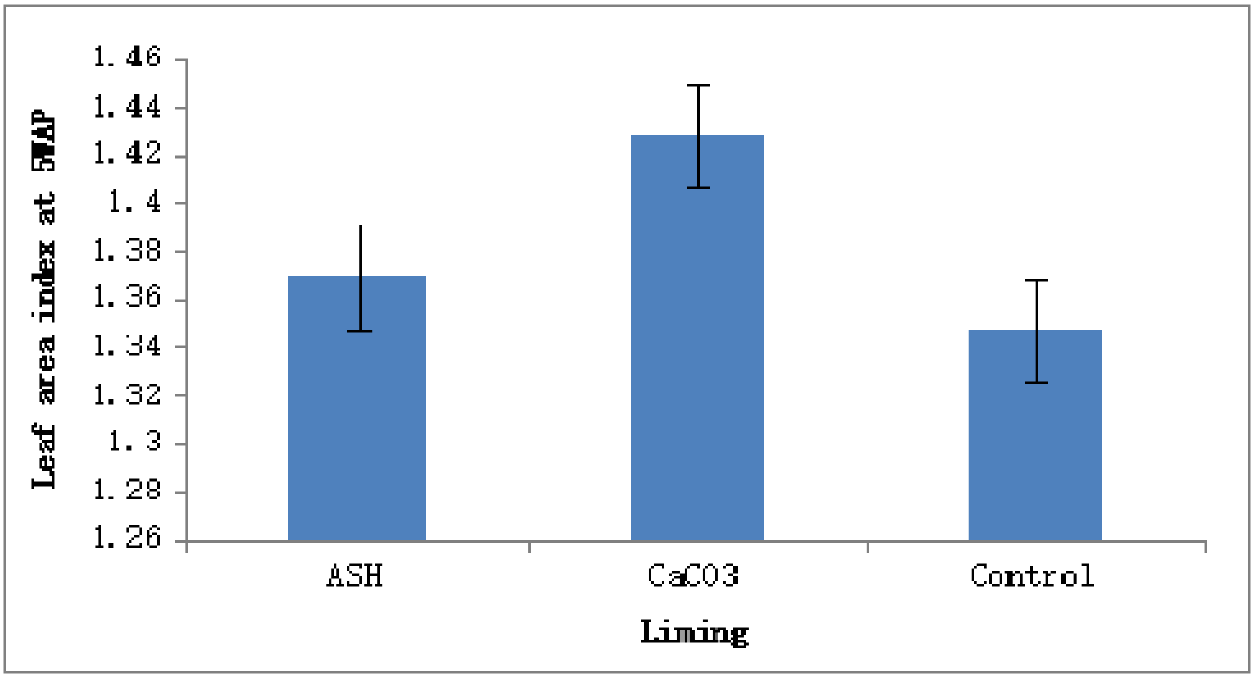


Figure 6: Effect of soil amendment on leaf area index at 5 WAP. Bars represent standard error of difference (SED).

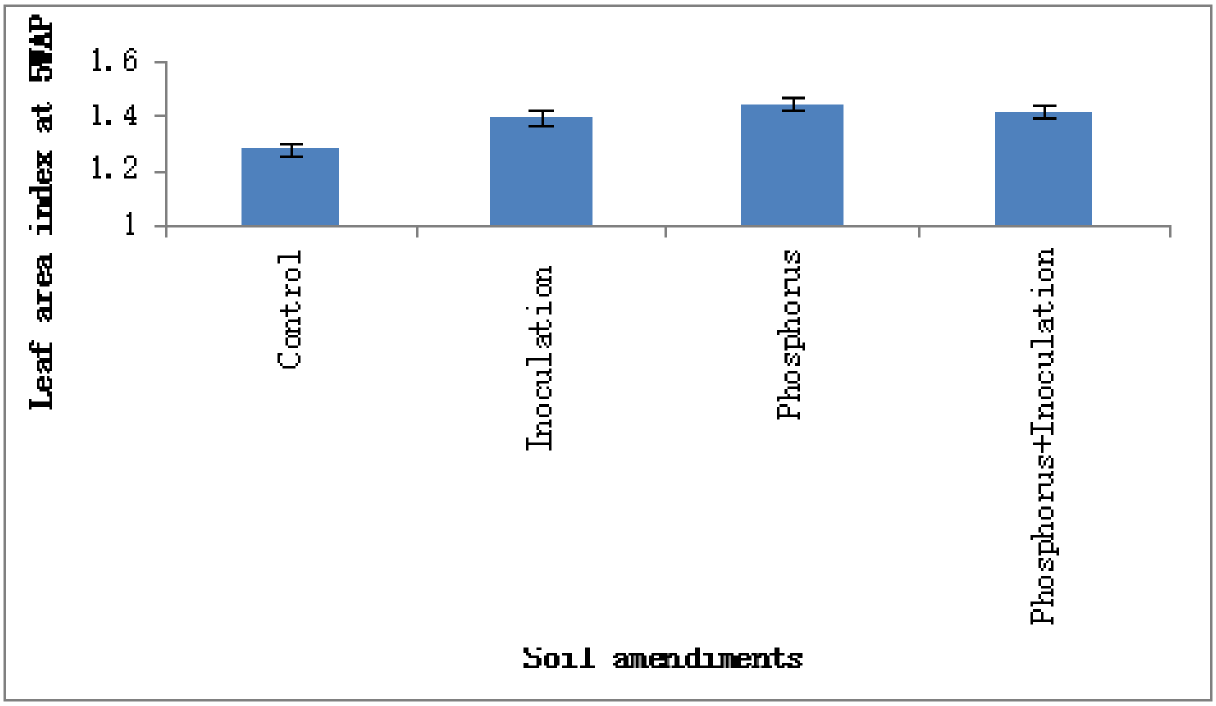


Figure 7: Effect of soil amendment on leaf area index at 5 WAP. Bars represent standard error of difference (SED).

4.5 Fresh straw weight

There was significant ($p < 0.001$) effect of liming and soil amendment used as well as the interactive effect of liming and soil amendment on the fresh shoot weight of soybean plants. Plants grown with $CaCO_3$ incorporated with Phosphorus recorded about 80% fresh shoot weight over Ash and Control in different liming treatments. However, Control treated soybean plants recorded the highest fresh shoot weight when plants were treated with different soil amendments (Fig 8).

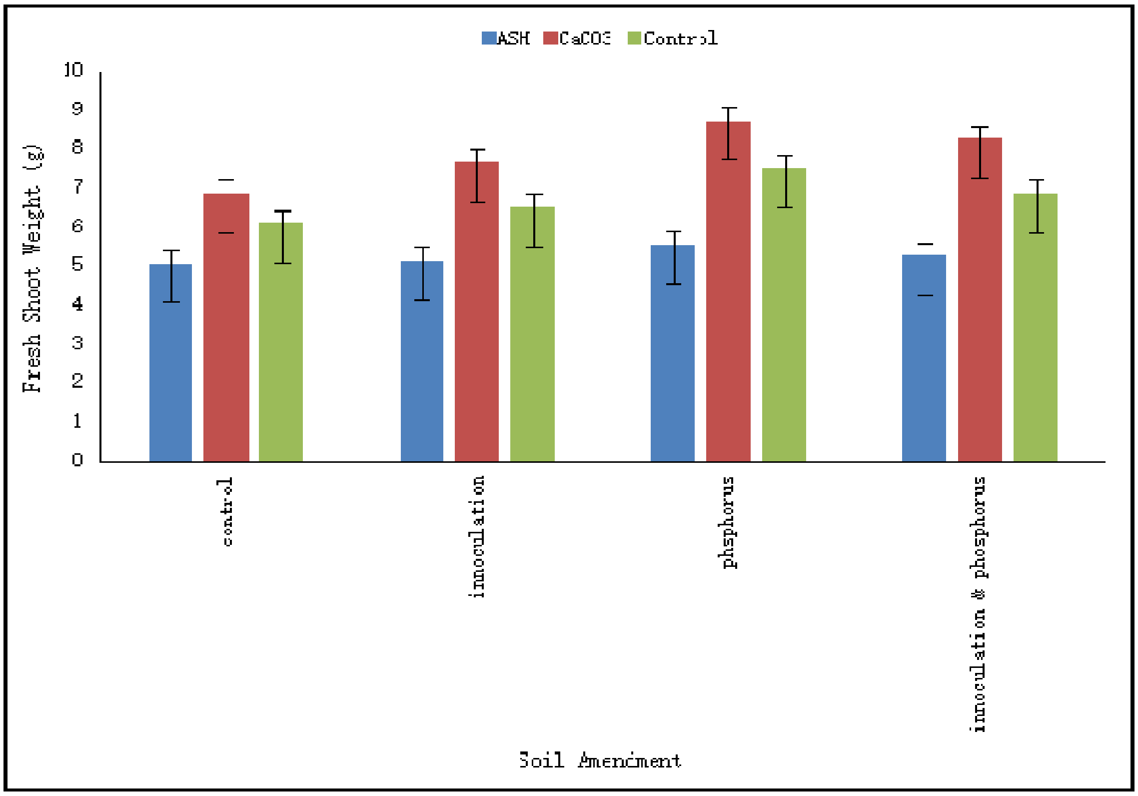


Figure 8: Effect of liming on fresh shoot weight of plants. Bars represent standard error of difference (SED).

4.6 Dry straw weight

Dry shoot weight (biomass) varied significantly ($p < 0.001$) among liming materials treated to soybean plants on the field (Fig 9). The application of $CaCO_3$ to soil significantly increased the shoot dry weight by about 86%. Control and Ash treated soybean plants shoot dry weight was significantly lower when compared to the application of $CaCO_3$ to the soil but Ash treated soybean plants dry weight were more than Control treated soybean plants.

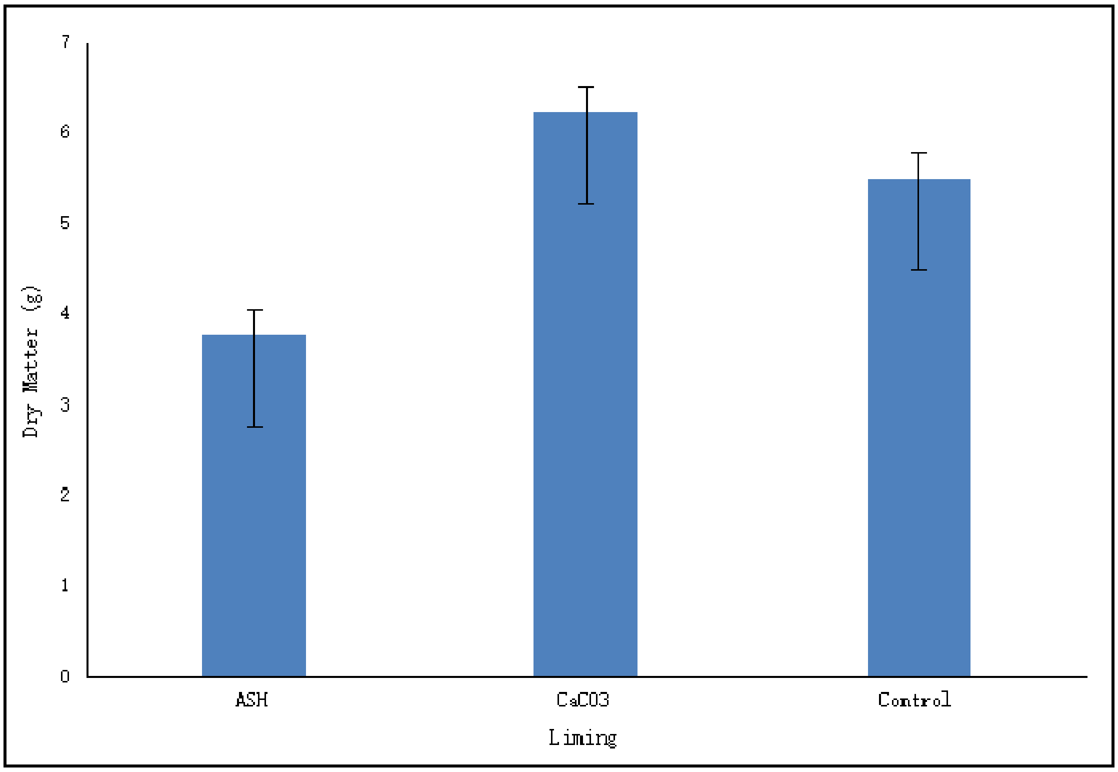


Figure 9: Effect of different soil amendment on dry shoot weight of soybean plants. Bars represent standard error of difference (SED).

Dry shoot weight was significantly ($p < 0.001$) influenced by soybean plants treated with different soil amendment. The use of Phosphorus fertilizer resulted in increased dry shoot weight by about 13% over the use of the other soil amendments. Inoculated plants produced similar dry shoot weight to Inoculation-Phosphorus when soybean plants were treated with different soil amendments. Control recorded the least weight of dry shoot weight among the different soil amendment treatments used (Fig 10).

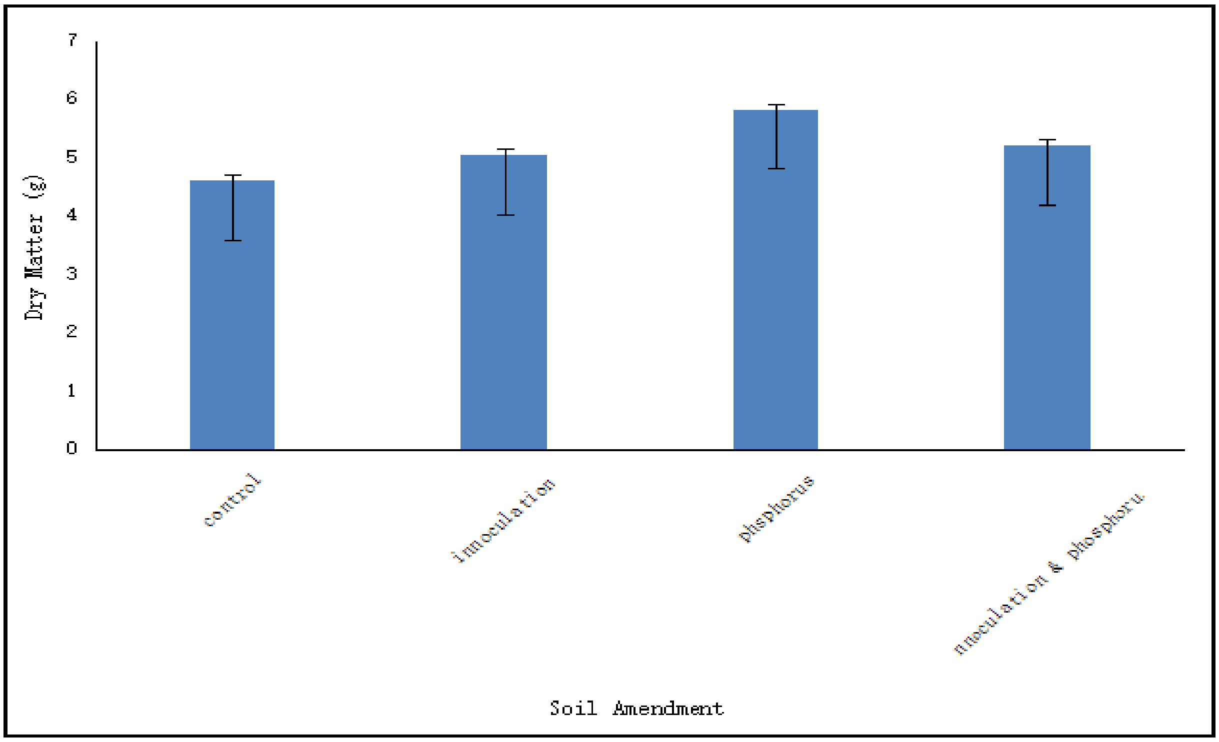


Figure 10: Effect of soil amendment on dry straw weight of plants. Bars represent standard error of difference (SED).

4.7 Days to 50 % flowering

There was significant (p = 0.029) interaction among treatment in different liming materials and soil amendment. $CaCO_3$ treated plants recorded shorter days to flowering and Control registered the longest number of days to 33.3% flowering within the liming materials. Inoculation-Phosphorus and Phosphorous treated plants recorded few days to flowering in the soil amendment treatment whilst Control and Inoculation recorded the longer days to 50% flowering (Fig 11).

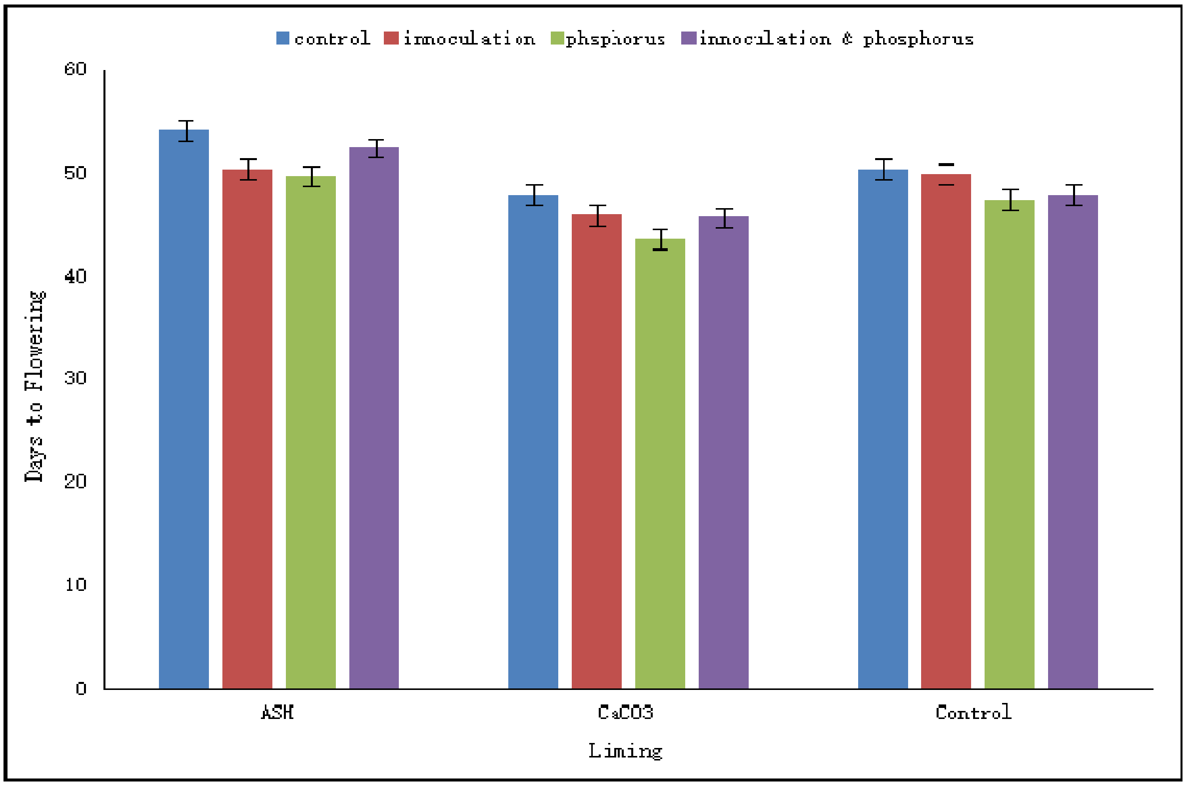


Figure 11: Effect of liming and soil amendment on days to 50% flowering. Bars represent standard error of difference (SED).

4.8 Days to podding

The number of days to podding among treatments varied significantly ($p < 0.001$) among the liming materials and soil amendment in the experiment (Table 5). Shorter days to podding were observed in $CaCO_3$ treated plants and Ash recording the highest days to podding with the different liming materials. Phosphorous treated plants within the soil amendment treatments recorded shorter days to podding, and Control and Inoculation recorded the higher number of days to podding which was same.

4.9 Number of nodules

There were significant ($p \leq 0.05$) effects of liming and soil amendment on the number of nodules formed by the soybean plants. However, the interactive effect of liming and soil amendment was not significant ($p \geq 0.05$) for the number of nodules. When soybean plants were treated with different liming materials, higher number of nodules was recorded with soils incorporated with $CaCO_3$ over Ash and Controls (Table 5).

Number of nodules per plants produced similar results with Inoculation and Phosphorus +Inoculation among soil amendment treatment. Soybean plants treated with either Inoculation or Phosphorus-Inoculation produced more nodules compared to Control and Phosphorus (Table 5).

4.10 Effective nodulation

Number of effective nodulation was significantly different ($p < 0.001$) among liming materials and soil amendment treated plants. Soybean plants treated with different liming materials produced higher number of effective nodulations in soils incorporated with $CaCO_3$ over Ash and Control. Plant treated with inoculant performed significantly higher than Control and Phosphorus among soil amendment treatments. Inoculation application gave the highest effective nodulations similar to Inoculation-Phosphorous treatment and lowest numbers of nodules were recorded by Control and Phosphorous treatments (Table 5).

Table 5: Effects of liming and soil amendments on days to Podding, number of nodules and number of effective Nodules.

Treatment	Parameters		
	Days to podding	Number of nodules/plant	Effective nodules
Liming material			
Ash	77.31	14.81	10.81
$CaCO_3$	66.6	26.3	18.9
Control	70.6	18.3	14.6
Soil amendment			
Control	71.8	15.4	11.1
Inoculation	73.6	22.4	17.8
Phosphorus	69.3	19.2	12.6
Phosphorus-Inoculation	71.5	22.3	1.5
Liming	460.2***	7.2**	9.5**
Soil amendment	72.3***	14.8***	19.1***
Liming *Soil amendment	2.4 ns	1.7 ns	1.4 ns

*** indicate significant difference at $p \leq 0.05$ and ns indicates non significance at $p \geq 0.05$

4.11 Grain yield

There was a significant ($p < 0.001$) effect of liming and soil amendments as well as the interaction effect of liming and soil amendment on the grain yield of soybean plants. Plants grown in $CaCO_3$ treated with phosphorus soil amendment increase grain yield by about 52% over the treatment with Ash and the Control. Phosphorus-treated plants produced significantly higher yields in Ash and $CaCO_3$ treatments but similar results were observed between Inoculation and Inoculation-Phosphorus treatment under Control under liming. In the main Control pots however, soybean plants recorded higher grain yield when plants were treated with Phosphorus and Inoculation-Phosphorus (Fig 12).

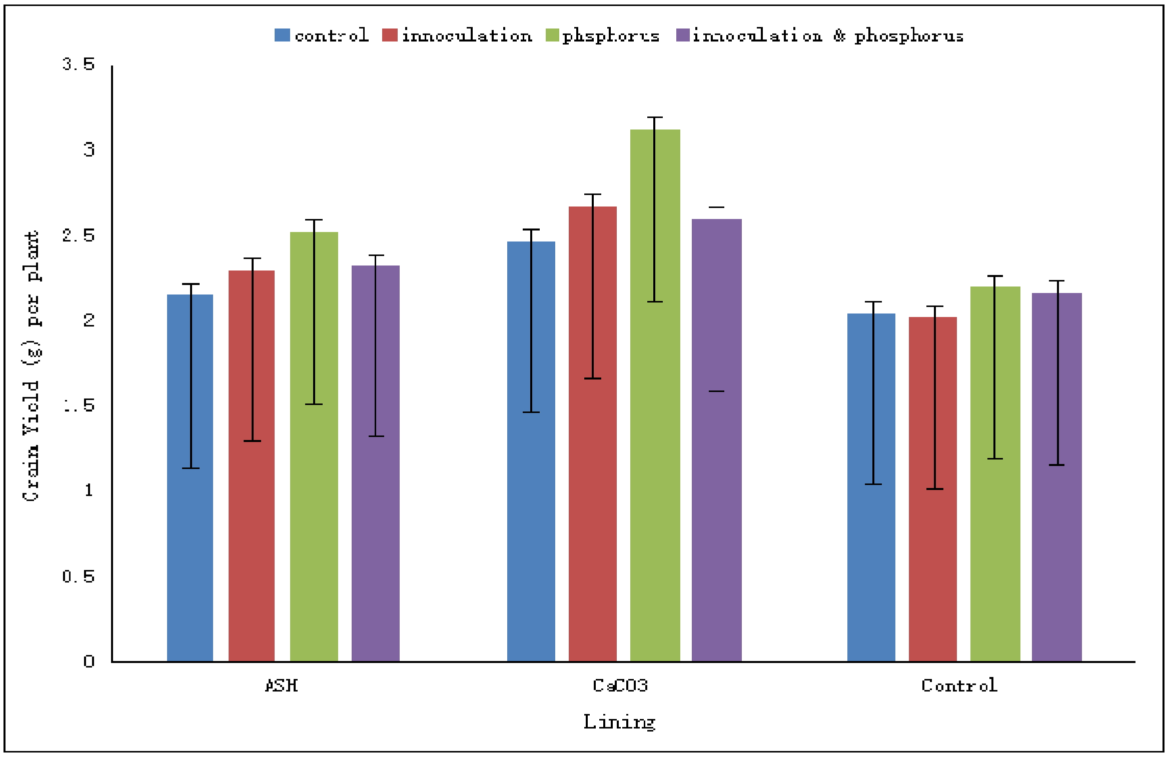


Figure 12: The interaction effect of liming and soil amendment on grain yield at harvest. Bars represent standard error of difference (SED).

4.12 Correlation between days to 50% flowering and days to podding

There was strong positive correlation between days to podding and days to 50%flowering (Table 6). The higher the days to 50% flowering, the higher the days to podding and the vice versa.

TABLE 6: Correlation between grain yield, number of pods per plant, days to podding, days to 50% flowering, effective nodulation and nodule count.

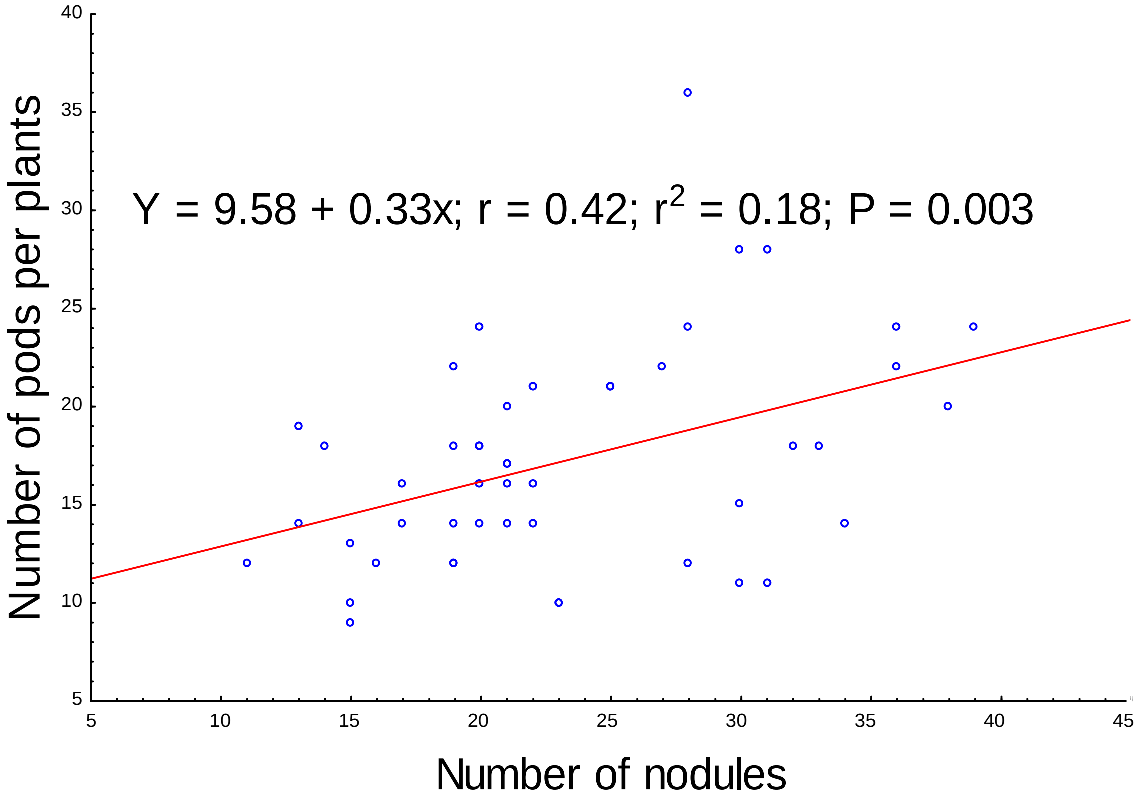
	Grain yield	No of pods/plt	Days to podding	Days to 50% flowering	Effective nodulation	Nodule count
Grain yield	1.000					
No of pods/plt	0.472	1.000				
Days to podding	-0.507	-0.805	1.000			
Days 50% flowering	-0.392	-0.708	0.747	1.000		
Effective nodulation	0.238	0.492	-0.477	-0.516	1.000	
Nodule count	0.320	0.598	-0.566	-0.629	0.892	1.000

4.13 Correlation between number of nodules and number of effective nodules

Number of nodules correlated positively with number of effective nodules and this correlation was significant (Table 6). Effective nodulation correlated positively and strongly with nodule count implying, the more the nodule count, the more the effective nodulation and the vice versa.

4.14 Correlation between number of nodules and number of pods per plants

Number of nodules correlated positively with number of pods per plant (Fig 13). The positive correlation was moderate between number of nodules and number of pods per plant with a correlation coefficient of 0.42.



$Y = 9.58 + 0.33x;\ r = 0.42;\ r^2 = 0.18;\ P = 0.003$

Figure 13: Correlation between number of nodules and number of pods per plant

4.15 Correlation between number of pods per plant and grain yield

Grain yield correlated positively with number of pods per plant (Table 6).This means that,the higher the number of pods per plant, the higher the grain yield, and the lower the number of pods per plant, then, the lower the grain yield.

4.16 Correlation between dry shoot weight and grain yield

The increase in dry shoot weight influenced grain yield marginally between these two parameters of soybean tested. Correlation was positive and significant (P = 0.001) (Fig 14). The correlation coefficient was 0.46 (46%) when dry shoot weight and grain yield were correlated.

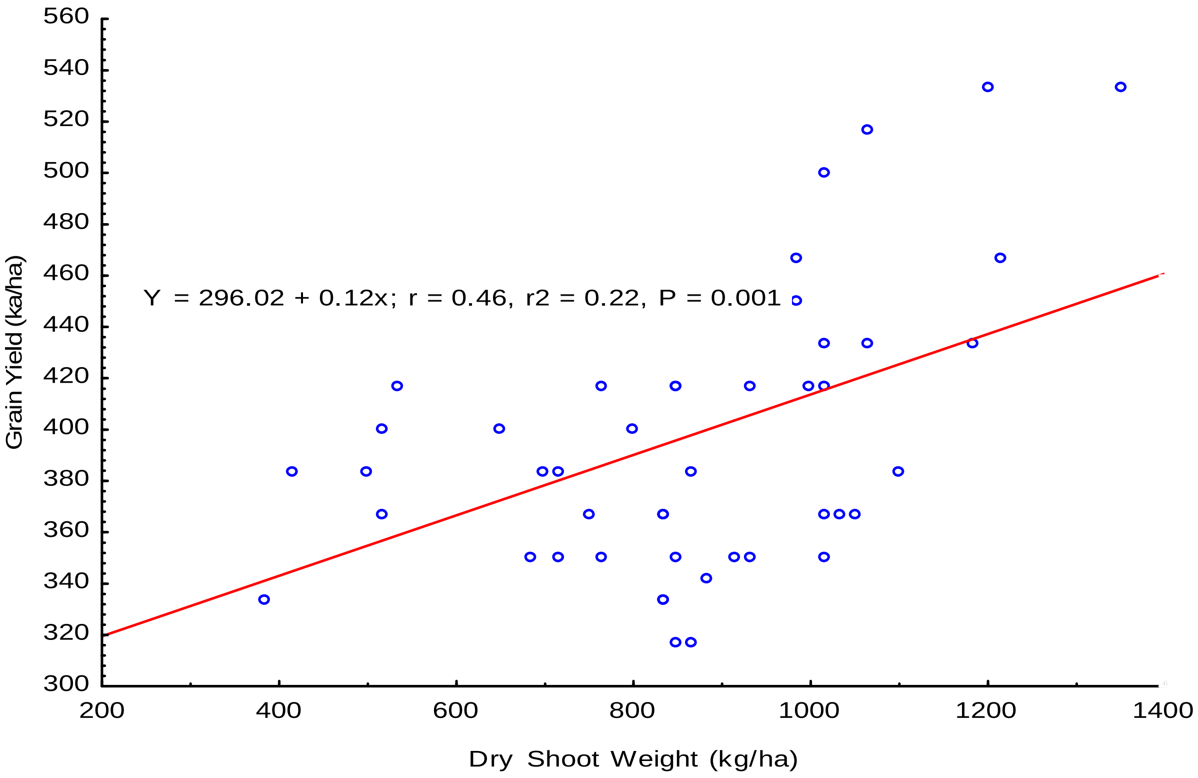


Figure 14: Correlation effect of dry shoot weight per hectare to grain yield per hectare. Positive r value showed positive correlation

4.17 Plant chemical analysis

Nitrogen concentration of soybean plants was not significantly ($p < 0.05$) with Control recording low levels of N among liming treatments. No statistical difference was observed with both liming and soil amendment treatments (Fig 15). Liming and soil amendment application consistently produced the highest level of Nitrogen than the Control (reference plant) but showed similar results to each other.

Liming with Phosphorus ($CaCO_3$+P) recorded the highest Nitrogen level with 2.25% and limning and Phosphorus-Inoculation ($CaCO_3$+P+I) was the least in Nitrogen content with 1.84%.

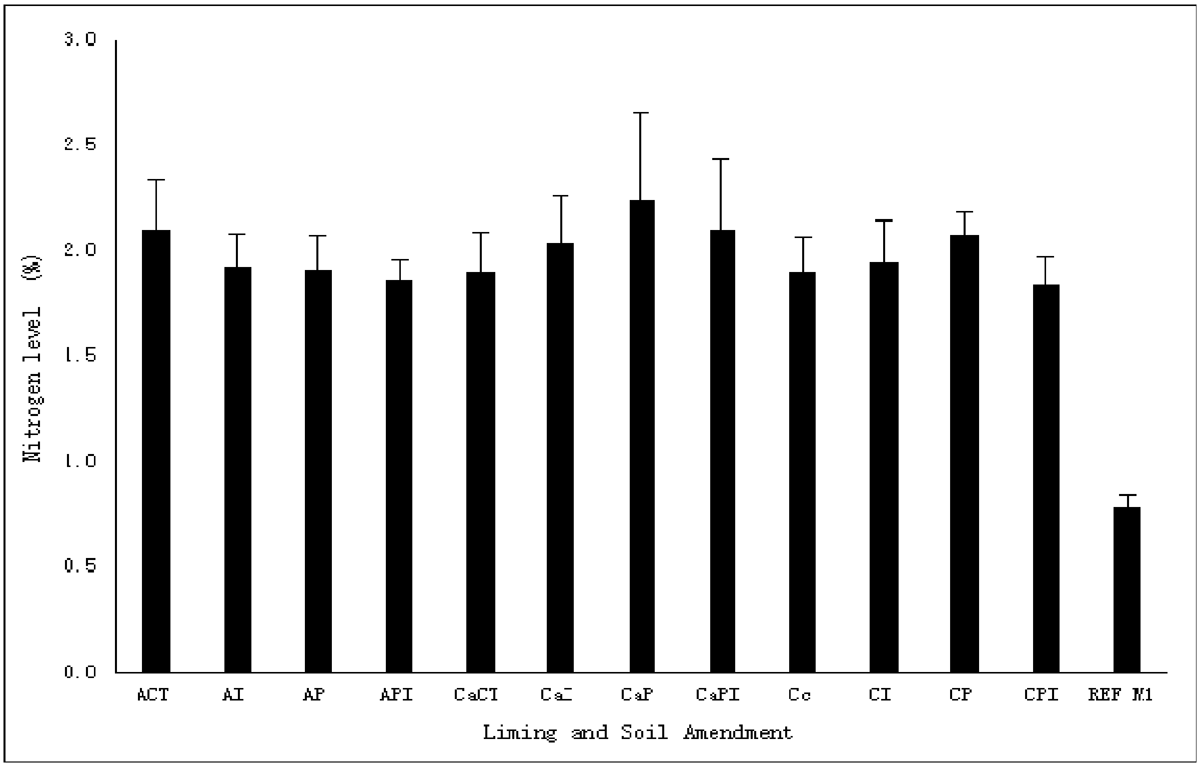


Figure 15: Effect of liming and soil amendment on nitrogen level of plants. Bars represent standard error of difference (SED).

Phosphorus level varied significantly ($p < 0.01$) among liming and soil amendment treatments applied to plants (Figure 16). Ash (Inoculation-Phosphorus) treated plants significantly increased the phosphorus content in the plants than the other treatments and, Ash (Inoculation) significantly reduced phosphorus content in the plants. The phosphorus content in plants treated with ACT, CPI, CaCT, CI, CaP, CaPI, CP and Cc showed similar results.

Where: ACT= Oil palm leaf ash control, CPI= Control-phosphorus-Inoculation, CaCT = $CaCO_3$, CI = Control-Inoculation, CaP = $CaCO_3$, CaPI = $CaCO_3$-Phosphorus-Inoculation, CP = Control-Phosphorus and Cc= Control-control.

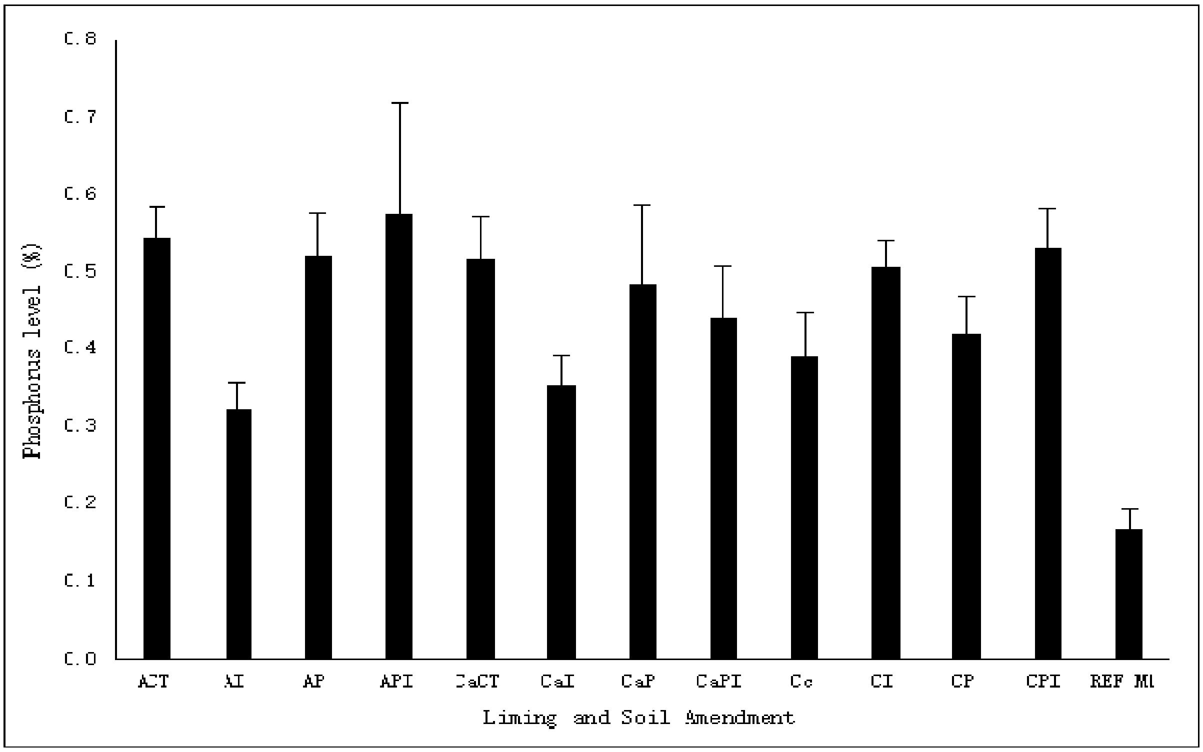


Figure 16: Effect of liming and soil amendment on phosphorus level of plants. Bars represent standard error of difference (SED).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Plant height

Plant height was significantly affected by liming materials, and the use of $CaCO_3$ influenced plant height significantly in soybean plants as compared to oil palm leaf ash and control as shown in figure 1. This could be due to good response of chemical or inorganic liming material to neutralize acidic soils and that could lead to the release of available nutrients for growth of the plants. This confirmed the work of Workneh *et al* (2013) who reported that, application of lime separately as well as in combination with nitrogen fertilizer gave significantly taller soybean plants than those crops grown without lime.

Application of soil amendment to soybean plant gave significant variations in plant height. Plants that received phosphorus as well as the combination of phosphorus and inoculation gave taller plants than those plants grown without phosphorus (Fig 2). The results indicate that, plants that received inoculation (*Bradyrhizobium inoculum)* had no effect on plant height (Fig 2). This result agreed with the findings of Mesfin *et al.* (2014) who reported that, plant growth was increased in acid soil treated with Phosphorus fertilizer. This growth response of soybean to the application of P in acidic soil may be related with better availability of P in P fertilizers which increased P content in the soil.

5.2 Number of leaves per plant

The results indicate that, adding different liming treatments to soybean plants positively affected the number of leaves per plant and significant variation existed at 5 WAP and 8

WAP, but did not show any significant differences at 11 WAP as shown in figure 3. The $CaCO_3$ consistently produced the highest number of leaves per plant and control gave similar results as Ash treatment. This finding supported the work of Mesfin *et a*l. (2014) who reported that, plant growth was increased in acid soil treated with and without lime. The increased number of leaves per plant could also be attributed to the neutralization of acidic soil by liming material. This confirmed the findings of Crop Focus (2011), who reported that, soil pH < 5.6 creates a difficult environment for the bacteria to function efficiently as well affect soybean productivity.

Soil amendment affected the number of leaves per plant significantly but no significant differences were recorded at later stages of the plants growth (Fig 4). Phosphorus treated plants consistently recorded significantly highest number of leaves per plant while low numbers of leaves were recorded for the control, there was no difference among inoculation and phosphorus-inoculation.

5.3 Leaf area index

The results show that liming treatments affected leaf area index of soybean plants positively and significant variation existed at 5 WAP, 8 WAP and 11 WAP (Fig 5). This confirmed the findings of Crop Focus (2011), who reported that soil pH < 5.6 creates a difficult environment for the bacteria to function efficiently as well affect soybean productivity.

Soil amendment affected leaf area index per plant significantly but no significant differences were recorded at later stages of the plants growth (Fig 6). Phosphorus treated plants consistently recorded significantly highest leaf area index per plant while low numbers of leaf area index were recorded for the control, inoculation and phosphorus-inoculation with no difference among them.

5.4 Fresh straw weight

Fresh straw weight produced per plant was significantly ($p < 0.05$) affected by liming and soil amendment treatments (Fig 7). $CaCO_3$ in combination with different soil amendment techniques produced significantly higher fresh shoot weight than their counter parts control and Ash treatments. The results, therefore, show that the liming have unequal or irregular growth pattern in soybeans plants treated with lime as well as fresh shoot production potential. This could be due to good soil growth conditions because liming release nutrients to the soil component in neutralizing the acidic soil and making growth nutrients available at different rate for soybean plants. This confirmed the findings of Campbell (1990) and Muse and Mitchell (1995), who reported that, $CaCO_3$ varied in percentage concentrations in Ash and lime.

Fresh straw weight of soybean significantly ($p < 0.05$) varied with plants treated with different soil amendment. Phosphorus and phosphorus-inoculation treated plants in different liming techniques gave significantly higher fresh shoot weight than control and inoculation which gave similar results (Fig 7). The results showed that fresh biomass increased with P application rate, yielding the highest fresh biomass . This result agreed favorably with the observations reported by Singh *et al* (2011)., and Ayodele and Oso (2014) that, legume biomass increased when phosphorus fertilizer was applied to the plants. There was fair correlation between dry shoot weight and grain yield with a correlation coefficient of 46% (Fig 14).

5.5 Dry straw weight

Dry shoot weight was significantly ($p < 0.001$) influenced by different liming materials treated to soybean plants on the field (Fig 8). The treatment of $CaCO_3$ to soil significantly increased the shoot dry weight than control and Ash treated soybean plants. Shoot dry weight was significantly lower when compared to the application of $CaCO_3$ to the soil but Ash treated soybean plants dry weight were more than control treated soybean plants.

Dry shoot weight was significantly ($p < 0.001$) influenced by soybean plants treated with different soil amendment used in this study. The use of phosphorus fertilizer resulted in increased dry shoot weight over the use of phosphorus- inoculation but inoculation produced similar dry shoot weight to inoculation and phosphorus treated soybean plants. Control recorded the least weight of dry shoot weight among the different soil amendment methods used in this study (Fig9). The significant increase in dry straw weight due to the application of P in the study agrees with Asia *et al.* (2005) who reported of a 20.7% increase in biomass due to phosphorus application.

5.6 Days to 50% flowering

Flowering of soybean plants was significantly affected by liming and soil amendment. Inorganic lime ($CaCO_3$) in combination with different soil amendments reduced the number of days to 50% flowering and the organic lime (Ash) in combination with the different soil amendment increased the days to 50% flowering as compared to the control (Fig 10). This confirmed the finding of Lee *et al.* (2005) that, days to flowering in soybean plants are based on the environment and that the first flower can occur few days before or after the actual predicted days to flowers There was a strong correlation between days to flowering and days to podding (table 6).

The reduction of days to flowering by inorganic lime ($CaCO_3$) treatment was contrary to the results of Tairo and Ndakidemi (2013) who observed that, flowering of soybeans starts at 41-44 days after planting.

There were fewer days to first flowering recorded by inorganic lime treated soybean plants and highest days recorded for organic lime treatments. This could be due to the plant growth nutrients differences in these two materials and the availability of such nutrients to the soybean plants. This assertion is supported by Risse and Gaskin (2013) who reported that, wood ash and lime treatments to plants gave similar results and both had benefits to crop productivity but wood ash supplied additional nutrients. These additional nutrients from the ash may have caused a delay in the onset of flowering in the ash-treated plants compared with those treated with $CaCO_3$ since with the relatively better nutrition,the ash-treated plants would not be forced to shorten their phenology as a way of circumventing a nutrient deficiency stress.

Phosphorus-treated plants with different liming techniques generally gave reduced number of days to flowering than inoculation and phosphorus and inoculation treated plants. Days to flowering in soil amendment control was significantly higher than in inoculation, phosphorus-inoculation and phosphorus treatments. This indicates that phosphorus and inoculation application to soybean plants can reduce the number of days to flowering even though phosphorus application reduced the number of days to flowering more than inoculation.

5.7 Days to podding

Liming affected the number of days to podding of the soybean plants significantly (Table 5). Inorganic lime reduced the podding days by four (4) and organic lime increased the podding days by seven (7). This could be due to the additional nutrients liming supplied to the soil which make the plants to exhibit the longer number of days to podding. The days to podding recorded by $CaCO_3$ (inorganic lime), control (zero lime) and organic lime (Ash) were 67, 71 and 77 days respectively. This result contradict the findings of Tairo and Ndakidemi (2013) who reported that, the number of days to podding were 46-49 days for glasshouse and 51-54 days for field experiment. There was strong positive correlation between days to flowering and days to podding (table 6).

The days to podding was significantly affected by application of soil amendment (Table 5). Control and phosphorus-inoculation gave similar number of days to podding which was significantly different from inoculation and phosphorus treated plants. Phosphorus treated plants recorded 3 days fewer to the control treated plants and inoculation recorded 2 days more to the days recorded by the control. The results indicated that, phosphorus reduced days to podding and inoculation increased days to podding. However, plants treated with phosphorus and inoculation together did not indicate any effect on the number of days to podding. This could be attributed to the neutralization effect of inoculation and phosphorus which balance the separate effect of the two treatments on soybean plants.

5.8 Number of nodules per plant

The results indicate that the number of nodules produced by inorganic lime ($CaCO_3$) treatment, control and organic lime (Ash) were 26, 18 and 15 respectively (Table 5).

Inorganic lime ($CaCO_3$) gave a significant higher number of nodules than control and organic lime (Ash) and more nodules were obtained in the control treatment than in the treatment with Ash. There was very strong correlation between number of nodules and effective nodulation with a correlation coefficient of 92% (Fig 13).

Inorganic lime ($CaCO_3$) increased number of nodules by 44% and organic lime (Ash) reduced number of nodules by 26%. This could be attributed to the amount of liming substances inorganic or organic lime can supply the soybean plants. This confirmed the report of Muse and Mitchell (1995) and Crop Focus (2011) that ash has pH ranging from 9 to 13 and soil with pH greater than 8.0 creates an unfavorable environment for the bacteria to function efficiently and also affect soybean productivity respectively.

Number of nodules recorded by control, phosphorus, inoculation and phosphorus-inoculation were 15, 19, 22 and 22 respectively (Table 5). The number of nodules produced under these treatments were far fewer than the 125, 93 and 140 nodules obtained by Tahir *et al.* (2009) in soybean plants treated with inoculant, phosphorus and phosphorus-inoculant respectively. The inoculation of soybean plants with bacteria has increased the number of nodules by 45.5% and plants that received phosphorus fertilizer only increased effective nodulation by 133.5%. However, plants treated with inoculation and phosphorus fertilizer gave similar numbers of nodules which are higher than that of the control. This could be attributed to the presence of bacteria and availability of phosphorus. This confirmed the finding of Singh *et al.* (2011) who reported that, availability of P can increase the intensity of nodulation. The positive effect of *Rhizobium* inoculation on nodulation has been confirmed by Elkoca *et al.* (2005) who reported a significant increase in nodulation by native soil *Rhizobium* population in single inoculation conducted on a legume. Phosphorus is also known to initiate nodule formation, increase the number of nodule primordial and is essentia for the development and functioning of formed nodules (Waluyo *et al*., 2004 and Tagoe *et al*.,2008).Several researches have reported that the supply of phosphorus plays important role in the establishment, growth and function of nodules (Abbasi *et al*., 2008; Tagoe *et al*., 2008 and Shu-jie *et al*., 2007).

5.9 Effective nodulation

The results indicated liming affected effective nodulation significantly. There was very strong positive correlation between number of nodules and effective nodulation with a correlation coefficient of 92% (Fig 13). Inorganic lime ($CaCO_3$) increased effective nodulation by 29% and organic lime (Ash) reduced effective nodulation by 26%. This could be attributed to the amount of liming substances that inorganic or organic lime can supply the soybean plants. This confirmed the report of Muse and Mitchell (1995), and Crop Focus (2011), that Ash has pH ranging from 9 to 13 and soil with pH greater than 8.0 creates a unfavorable environment for the bacteria to function efficiently as well affect soybean productivity respectively.

The inoculation of soybean plants with rhizobium has increased effective nodulation by 60.4% and plants that received phosphorus fertilizer only increased effective nodulation by 13.5%. However, plants treated with inoculation and phosphorus fertilizer gave similar results as plants treated with inoculants. This could be attributed to the presence of bacteria and availability of phosphorus. This confirmed the finding of Singh *et al.* (2011) which revealed that, availability of P can increase the intensity of nodulation.

5.10 Grain yield

Grain yield was influenced by liming material used and inoculation and phosphorus fertilizer applied. Inorganic lime ($CaCO_3$) and phosphorus treated plants recorded the highest yield of grain compare to the other combinations of liming and soil amendment treatments. This might be due to increased pH by inorganic lime ($CaCO_3$) and P availability to the soil created better environmental conditions for plant growth. This confirms an earlier report that environmental stress affects yield through controlled sequential formation and growth of node, reproductive node, pod and seed (Board and Kahlon, 2011).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The study showed that liming and soil amendment promoted soybean plants growth and development in the pot experiment. Soybean plants treated with inorganic lime (CaCO3) and phosphorus fertilizer gave the highest plant height.

It also established that, liming and soil amendment enhanced number of leaves, days to 50% flowering, days to podding, dry shoot weight, hence gave good field practices. This gave plants a better chance to harness soil nutrients, and liming and phosphorus fertilizer had an influence on vigorous vegetative growth and eventually into yield.

6.2 Recommendations

The study recommended that acidic soils should be limed to improve the availability of plant growth nutrients before soybeans can be planted to achieve maximum yield. This may be achieved by adding 2 tonnes per hectare of wood Ash or 18 grams per plant of $CaCO_3$ to increase the pH of the soil. The ash will add micro nutrients to the soil in addition to the macronutrients it adds to the soil. These will improve the overall growing conditions of the soil for better soybeans production.

It is recommended that the used of inoculants in the production of soybean should be encouraged because the incorporation of the inoculants into the soil promoted plant vegetative growth of soybean. The inoculants promoted nodules in the soybean plants which promoted the use of atmospheric nitrogen of the environment within the experimental set up.

Application of phosphorus fertilizer promoted vegetative growth and yield of the plants. It is therefore recommended that farmers should use $CaCO_3$ and phosphorus fertilizer for soybean production under acidic condition to obtain higher grain yield. The study recommended that in future field trials should be done on acidic soils for better recommendation to farmers.

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APPENDICES

Appendix 1: ANOVA for plant height at 5 WAP

	DF	SS	MS	V.r	P
Replication	3	5.23	1.74	0.46	
Liming	2	82.13	41.1	10.88	<0.001
Residual	6	30.71	5.12	1.47	< 0.001
Soil Amendment	3	159.23	53.08	14.07	<0.001
Liming * Soil Amendment	6	46.21	7.70	2.04	0.088
Error	27	93.81	3.48		
Total	47	417.31			

Degree of freedom (DF), Sum of squares (SS), Mean sum of square (MS), V ratio (V) and P-Value (P).

Appendix 2: ANOVA for plant height at 8 WAP

	DF	SS	MS	V.r	P
Replication	3	10.73	3.60	0.46	
Liming	2	198.04	99.02	12.76	<0.001
Residual	6	119.46	719.91	3.94	
Soil Amendment	3	108.23	36.08	4.65	<0.001
Liming * Soil Amendment	6	25.95	4.33	0.56	0.761
Error	27	136.56	5.06		
Total	47	598.98			

Degree of freedom (DF), Sum of squares (SS), Mean sum of square (MS), V ratio (V) and P-Value (P).

Appendix 3: ANOVA for plant height at 11 WAP

	DF	SS	MS	V.r	P
Replication	3	30.083	10.02	1.38	
Liming	2	219.19	109.65	15.11	<0.001
Residual	6	119.04	19.84	4.45	
Soil Amendment	3	99.42	33.14	4.57	0.009
Liming *Soil Amendment	6	15.71	2.62	0.36	0.898
Error	27	120.38	4.46		
Total	47	603.92			

Degree of freedom (DF), Sum of squares (SS), Mean sum of square (MS), V ratio (V) and P-Value (P).

Appendix 4: ANOVA for Leaf area index at 5 WAP

	DF	SS	MS	V.r	P
Replication	3	0.0167	0.0056	1.50	
Liming	2	0.056	0.028	7.50	< 0.001
Residual	6	0.028	0.005	2.03	
Soil Amendment	3	0.197	0.066	17.66	< 0.001
Liming *Soil Amendment	6	0.033	0.0054	1.46	0.222
Error	27	0.061	0.002		
Total	47	0.42387			

Degree of freedom (DF), Sum of squares (SS), Mean sum of square (MS), V ratio (V) and P-Value (P).

Appendix 5: ANOVA for Leaf area index at 8 WAP

	DF	SS	MS	V.r	P
Replication	3	0.019	0.0062	2.32	
Liming	2	0.649	0.324	121.91	< 0.001
Residual	6	0.028	0.005	2.10	
Soil Amendment	3	0.383	0.128	47.98	< 0.001
Liming * Soil Amendment	6	0.070	0.011	4.27	0.003
Error	27	0.060	0.002		
Total	47	1.21			

Degree of freedom (DF), Sum of squares (SS), Mean sum of square (MS), V ratio (V) and P-Value (P).

Appendix 6: ANOVA for Leaf area index at 11 WAP

	DF	SS	MS	V.r	P
Replication	3	0.0122	0.0041	1.0	
Liming	2	0.645	0.3223	119.23	< 0.001
Residual	6	0.028	0.0046	2.03	
Soil Amendment	3	0.391	0.1308	48.21	< 0.001
Liming * Soil Amendment	6	0.0667	0.0111	4.11	0.002
Error	27	0.0614	0.0022		
Total	47	1.2033			

Degree of freedom (DF), Sum of squares (SS), Mean sum of square (MS), V ratio (V) and P-Value (P).

Appendix 7: ANOVA for Number of leaves at 5 WAP

	DF	SS	MS	V.r	P
Replication	3	30.32	10.076	2.71	
Liming	2	32.00	16.00	4.31	0.022
Residual	6	45.33	7.56	2.64	
Soil Amendment	3	42.06	14.02	3.78	0.02
Liming * Soil Amendment	6	25.00	4.17	1.12	0.371
Error	27	77.19	2.86		
Total	47	251.812			

Degree of freedom (DF), Sum of squares (SS), Mean sum of square (MS), V ratio (V) and P-Value (P).

Appendix 8: ANOVA for number of leaves at 8 WAP

	DF	SS	MS	V.r	P
Replication	3	40.50	16.50	1.52	
Liming	2	145.79	72.90	6.70	0.004
Residual	6	82.38	13.73	1.34	
Soil Amendment	3	108.50	36.17	3.32	0.031
Liming * Soil Amendment	6	73.88	12.31	1.13	0.336
Error	27	276.62	10.25		
Total	47	736.67			

Degree of freedom (DF), Sum of squares (SS), Mean sum of square (MS), V ratio (V) and P-Value (P).

Appendix 9: ANOVA for Number of leaves at 11 WAP

	DF	SS	MS	V.r	P
Replication	3	30.75	10.25	0.95	
Liming	2	136.79	68.40	6.32	0.005
Residual	6	5623.2	937.2	1.13	
Soil Amendment	3	61.42	20.47	1.89	0.150
Liming * Soil Amendment	6	59.71	9.95	0.92	0.494
Error	27	272473.2	832.3		
Total	47	40607.3			

Degree of freedom (DF), Sum of squares (SS), Mean sum of square (MS), V ratio (V) and P-Value (P).

Appendix 10: ANOVA for Days to 50 % flowering

	DF	SS	MS	V.r	P
Replication	3	180.75	60.250	38.05	
Liming	2	276.5	138.25	87.32	< 0.001
Residual	6	21.5	3.583	3.15	
Soil Amendment	3	92.42	30.81	19.46	< 0.001
Liming * Soil Amendment	6	19.33	3.2	2.04	0.089
Error	27	30.75	1.41		
Total	47	621.25			

Degree of freedom (DF), Sum of squares (SS), Mean sum of square (MS), V ratio (V) and P-Value (P).

Appendix 11: ANOVA for Days to podding

	DF	SS	MS	V.r	P
Replication	3	20.250	6.750		
Liming	2	939.54	469.77	784.93	< 0.001
Residual	6	6.13	1.02	2.02	
Soil Amendment	3	109.08	36.36	60.76	< 0.001
Liming * Soil Amendment	6	7.29	1.22	2.41	0.089
Error	27	13.63	0.50		
Total	47	1095.92			

Degree of freedom (DF), Sum of squares (SS), Mean sum of square (MS), V ratio (V) and P-Value (P).

Appendix 12: ANOVA for number of nodules per plant

	DF	SS	MS	V.r	P
Replication	3	130.90	43.63	2.06	
Liming	2	1122.04	561.02	26.53	<0.001
Residual	6	461.13	76.85	8.98	
Soil Amendment	3	382.73	127.58	6.03	0.002
Liming * Soil Amendment	6	85.96	14.33	0.66	0.669
Error	27	231.06	8.56		
Total	47	2419.48			

Degree of freedom (DF), Sum of squares (SS), Mean sum of square (MS), V ratio (V) and P-Value (P).

Appendix 13: ANOVA for number of effective nodules

	DF	SS	MS	V.r	P
Replication	3	5.50	1.80	0.17	
Liming	2	497.17	248.58	23.17	<0.001
Residual	6	164.13	27.35	3.85	
Soil Amendment	3	426.50	142.17	13.25	< 0.001
Liming * Soil Amendment	6	57.50	9.58	0.89	0.511
Error	27	192.06	7.11		
Total	47	1340.67			

Degree of freedom (DF), Sum of squares (SS), Mean sum of square (MS), V ratio (V) and P-Value (P).

Appendix 14: ANOVA for number of pods per plant

	DF	SS	MS	V.r	P
Replication	3	119.23	39.743	3.19	
Liming	2	663.54	331.74	26.67	<0.001
Residual	6	330.96	55.16	18.72	< 0.001
Soil Amendment	3	224.40	74.80	6.01	0.002
Liming * Soil Amendment	6	23.79	3.97	0.32	0.923
Error	27	79.56	2.95		
Total	47	1441.48			

Degree of freedom (DF), Sum of squares (SS), Mean sum of square (MS), V ratio (V) and P-Value (P).

Appendix 15: ANOVA for fresh straw weight

	DF	SS	MS	V.r	P
Replication	3	10.40	3.46	17.81	
Liming	2	56.03	28.01	18.02	< 0.001
Residual	6	4.72	0.79	11.87	
Soil Amendment	3	10.53	3.51	18.02	< 0.001
Liming * Soil Amendment	6	2.22	0.37	1.90	< 0.001
Error	27	1.79	0.07		
Total	47	84.50			

Degree of freedom (DF), Sum of squares (SS), Mean sum of square (MS), V ratio (V) and P-Value (P).

Appendix 16: ANOVA for dry straw weight

	DF	SS	MS	V.r	P
Replication	3	291169	97056	20.29	
Liming	2	1412082	7060411	147.58	< 0.001
Residual	6	3.74	0.62	8.62	
Soil Amendment	3	25.71	85698	17.91	< 0.001
Liming *Soil Amendment	6	27605	4601	0.96	0.466
Error	27	1.95	0.072		
Total	47	77.30			

Degree of freedom (DF), Sum of squares (SS), Mean sum of square (MS), V ratio (V) and P-Value (P).

Appendix 17: ANOVA for grain yield

	DF	SS	MS	V.r	P
Replication	3	0.238	0.079	7.64	
Liming	2	3.06	1.53	146.81	< 0.001
Residual	6	0.09	0.01	1.54	
Soil Amendment	3	0.99	0.33	31.69	< 0.001
Liming * Soil Amendment	6	0.34	0.057	5.51	< 0.001
Error	27	0.26	0.01		
Total	47	4.97			

Degree of freedom (DF), Sum of squares (SS), Mean sum of square (MS), V ratio (V) and P-Value (P).