# YIELD RESPONSE OF SOYBEAN AND COWPEA TO ROCK PHOSPHATE FERTILIZER BLEND AND RHIZOBIAL INOCULATION ON TWO BENCHMARK SOILS OF NORTHERN GHANA

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**DOCTOR OF PHILOSOPHY** 

IN

SOIL SCIENCE

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BY

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#### DECLARATION

I hereby declare that this submission is my own work toward the PhD and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.



## DEDICATION

This dissertation is dedicated to my dear wife Dr. Mrs Ruth Owusu Ofori and daughter Elizabeth Sarah Ofori.



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### ABSTRACT

Two field experiments were conducted on a Gleyic Lixisol and a Ferric Luvisol at Bontanga and Nyankpala, respectively during the 2012 and 2013 cropping seasons. The aim of the study was to assess the effects of Rock Phosphate Fertilizer Blend (RPFB) and rhizobial inoculation on yield of soybean and cowpea. The effect of combined application of low rates of nitrogen fertilizer and RPFB on N and P uptake in soybean and cowpea were also determined. The experiments were laid out in a randomized complete block design with split-plot arrangement of the treatments which were replicated three times. At each experimental site, inoculated (+Rh) and uninoculated (-Rh) treatments constituted the main plots. The following treatments were assigned as sub-plots on the Gleyic Lixisol at Bontanga: To = 0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>; T<sub>1</sub> = RPFB 34.35 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>; T<sub>2</sub> = RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>; T<sub>3</sub> = TSP 34.35 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and T<sub>4</sub> = TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> while To = 0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>; T<sub>1</sub> = RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> <sup>1</sup> and T<sub>4</sub> = RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> + 25 kg N ha<sup>-1</sup>; T<sub>3</sub> = TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and T<sub>4</sub> = RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> + 25 kg N ha<sup>-1</sup>; T<sub>3</sub> = TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>

Phosphorus fertilizer application and rhizobial inoculation increased soybean nodulation on the Gleyic Lixisol. Application of TSP 34.35, 68.70 and RPFB 68.70 kg  $P_2O_5$  significantly increased nodule dry weight of soybean while cowpea nodule dry weight increased with the application of TSP 68.70 and RPFB 34.35 kg  $P_2O_5$ . The application of TSP 68.70 kg  $P_2O_5$  fertilizer significantly increased soybean shoot dry matter yield by 84% while RPFB 68.70 kg  $P_2O_5$  gave an increase of 25% relative to the control. Similarly, cowpea shoot dry matter yield increased by 81 and 67% due to application of TSP and RPFB, respectively compared to the control. The TSP 68.70 kg  $P_2O_5 + 25$  kg N and RPFB 68.70 kg  $P_2O_5 + 25$  kg N treatments also increased soybean grain yield on the Ferric Luvisol by 74 and 9%, respectively over the control while that of cowpea was increased by 53 and 51%, respectively.

Soybean grain N and P uptake on the Ferric Luvisol were significantly increased by 78 and 120%, respectively over the control as a result of the application of TSP 68.70 kg  $P_2O_5 + 25$  kg N. Application of RPFB 68.70 kg  $P_2O_5 + 25$  kg N did not significantly increase soybean grain N and P uptake. Cowpea grain N and P uptake were also increased by 40 and 81%, respectively over the control due to application of TSP 68.70 kg  $P_2O_5 + 25$  kg N. The RPFB 68.70 kg  $P_2O_5 + 25$  kg N treatment gave an increase in grain N and P uptake of 44 and 74%, respectively over the control.

Value-cost ratio analysis indicated that TSP 68.70 kg  $P_2O_5 + 25$  kg N is a promising treatment combination for profitable soybean and cowpea production on the Ferric Luvisol than RPFB 68.70 kg  $P_2O_5 + 25$  kg N.

Results of this study have shown that, application of triple superphosphate and 25 kg N better enhances soybean N and P uptake than rock phosphate fertilzer blend. For cowpea, application of both triple superphosphate and rock phosphate fertilizer blend equally enhanced grain N and P uptake. The study further revealed that in the short term, application of TSP 68.70 kg  $P_2O_5 + 25$  kg N on the Ferric Luvisol is more profitable for soybean and cowpea production than RPFB 68.70 kg  $P_2O_5 + 25$  kg N.



#### **CHAPTER ONE**

#### **1.0 INTRODUCTION**

Biological nitrogen fixation (BNF) through legume-rhizobium symbiosis has received much attention by researchers in sub-Saharan Africa as it offers an environmentally friendly alternative or supplement to the use of chemical nitrogen fertilizers by resource-poor farmers (Ellafi *et al.*, 2011). Phosphorus (P) and nitrogen (N) are important nutrient elements for symbiotic N<sub>2</sub> fixation because of their effects on nodulation and the N<sub>2</sub> fixation process (O'Hara *et al.*, 2002). The functional role of phosphorus in legumes such as promoting root development, enhancement of root nodulation and increasing grain yield have been demonstrated in many reports (Tang *et al.*, 2001; Uchida, 2000). Symbiotic nitrogen fixation requires large amounts of phosphorus due to the energy-consuming nature of the process (Schulze *et al.*, 2006) and the energy generating metabolism which depends strongly on soil P availability (Plaxton, 2004). Large increases in legume yield due to addition of phosphorus fertilizer have been reported by many authors (Kamara *et al.*, 2007; Uzoma *et al.*, 2006; Yakubu *et al.*, 2010).

Soybean and cowpea, as leguminous crops, are able to meet a significant portion of their nitrogen requirement when they are cultivated in the presence of effective and compatible strains of bradyrhizobia. According to Bekere and Hailemariam (2012), the absence of these strains in soils makes inoculation very essential in ensuring the presence of an effective bradyrhizobia population in the rhizosphere. Soil nutrient composition is influenced in many ways by soil microorganisms such as *B. japonicum* found in inoculants and other rhizobacteria which promote plant growth by enhancing plant nutrient uptake (Saharan and Nehra, 2011).

Crop production in sub-Saharan Africa is mostly limited by nitrogen and phosphorus as these two elements are deficient in most soils (Mburu *et al.*, 2011). Grain yield of legumes as well as their contributions to the nitrogen economy of farming systems through BNF are commonly limited by nutrient deficiencies particularly phosphorus (Sinclair and Vadez, 2002). Soil nutrient deficiency has been a major constraint to efforts by both government and non-governmental organizations to improve food security and curb hunger in Africa (Mburu *et al.*, 2011).

In spite of the huge economic and nutritional benefits associated with the production of grain legumes in northern Ghana, crop yields are mostly limited by low soil fertility and deficiencies in organic matter and macronutrients such as phosphorus and nitrogen (CSIR-RELC, 2005). Leguminous crops such as soybean and cowpea have important nutrient requirements and other production factors which farmers need to meet for maximum yields. The problem of low crop yields as a result of low soil fertility can be partly corrected with the application of phosphorus and nitrogen fertilizers (Daria *et al.*, 2012). The problem of low soil fertility results in low crop yield which directly affects the livelihood of smallholder farmers. Moreover, increasing yield of oil seed crops such as soybean has the potential to save Ghana foreign exchange by way of import substitution of about 35,000 - 63,000 metric tons of soybean demanded by the local market annually as processed soy meal worth 37.1-66.7 million US dollars (Daria *et al.*, 2012).

In recent times, there has been much focus on direct application of rock phosphate to soils due to its potential as an alternative to water-soluble phosphate fertilizers such as single and triple superphosphate, which have become much more expensive (Akande *et al.*, 2010). "Yara legume" is a rock phosphate fertilizer blend with crop nutrition formula; 18% P<sub>2</sub>O<sub>5</sub>, 13% K<sub>2</sub>O, 31% CaO, 4% S, 2% MgO, 0.6% Zn manufactured by Yara Ghana Ltd for legume production in northern Ghana. However, scientific research regarding the short term effects of this fertilizer blend on growth and yield of soybean and cowpea in low N environment is lacking. Moreover, not much is known on the extent to which application of this fertilizer blend influences P uptake and use efficiency of soybean and cowpea and its cost effectiveness.

The main objective of this study, therefore, was to explore the possibility of increasing soybean and cowpea grain yields through the application of rhizobia inoculants and rock phosphate fertilizer blend.

Based on the hypothesis that application of rock phosphate fertilizer blend and rhizobia inoculants will stimulate plant N accumulation for enhanced grain yield of soybean and cowpea in low N environments, the specific objectives of this study were to:

i. evaluate the effect of rhizobial inoculation and rock phosphate fertilizer blend application on nodulation, biomass and grain yields of soybean and cowpea; ii. assess the effect of rock phosphate fertilizer blend and low rate of nitrogen fertilizer input on N and P uptake and P use efficiency of soybean and cowpea; iii. determine the short term cost effectiveness of combined application of rock phosphate fertilizer blend and low rate of N fertilizer for soybean and cowpea production in the study area.

#### **CHAPTER TWO**

#### 2.0 LITERATURE REVIEW

#### 2.1 Importance of soybean and cowpea

Grain legumes play important and significant roles in natural ecosystems, agriculture, agro-forestry and industries. In northern Ghana, several efforts have been made by both governmental and non-governmental organizations to promote grain legume production due to their potential to increase smallholder farmers' income levels and improve on their household's nutritional status (Mbanya, 2011). There have been numerous agricultural intervention programmes in northern Ghana including the Youth in Agriculture Programme, Alliance for a Green Revolution in Africa (AGRA), United States Agency for International Development (USAID) and several others that were focused primarily on promoting both the production and utilization of grain legumes by way of value chain enhancement (Etwire *et al.*, 2013a).

Soybean [*Glycine max* (L). Merril] and cowpea [*Vigna unguiculata* (L.) Walp] are important legumes cultivated in Ghana. Soybean is gradually becoming an economically important crop as it has become one of the most cultivated grain legumes in sub-Saharan Africa. According to Rao and Reddy (2010), among all the grain legumes, soybean has the greatest potential of producing the cheapest source of food protein as well as other essential nutrients for farm households. Apart from serving as a feed source for both fish and livestock, it is an equally good source of protein in human diet (Masuda and Goldsmith, 2009). Studies by El-Agroudy *et al.* (2011) indicate that soybean contains 30% oil with no cholesterol, 40% protein as well as important vitamins needed for healthy growth in humans. Reports by Ugwu and Ugwu (2010) also indicate that soybean has some benefits over other legumes such as groundnut and cowpea due to its low susceptibility to pests and diseases, better storage quality and larger leaf biomass which benefits subsequent crops by way of soil fertility improvement. Although soybean is a new crop in Ghana compared to other legumes, it has become much more popular and has been accepted by many farmers in Northern Ghana (Etwire *et al.*, 2013a). The crop plays a significant role in the rural economies of farm households in Ghana, most especially in the eastern part of northern Ghana (Akramov and Malek, 2012). According to SRID (2012), the Northern Region contributes about 70% of the nation's total area under soybean cultivation and 77% of national production.

Cowpea as a leguminous crop forms an integral part of many cropping systems in Africa as a whole and Ghana in particular (Fening and Danso, 2002). In sub-Saharan Africa, cowpea is the food legume commonly grown by smallholder farmers. This is mainly because it is relatively adapted to drought and soils with low nutrient status (Pule-Meulenberg *et al.*, 2010). Cowpea grain contains 23% protein and 57% carbohydrate, while the leaves contain 27 - 34% protein (Belane and Dakora, 2009). Interestingly, cowpea is freely nodulated by some members of the family *Rhizobiaceae* with *Rhizobium* and *Bradyrhizobium* as typical examples (Mpepereki *et al.*, 1996). Studies have shown that about 66% of the nitrogen requirement of cowpea is obtained from symbiotic nitrogen fixation in soils of Botswana (PuleMeulenberg and Dakora, 2009) and up to 99% in Ghanaian soils (Naab *et al.*, 2009). The basis of the importance of cowpea in cropping systems in sub-Saharan Africa lies in its observed N contribution to cropping systems through its symbiotic relationship with species of *Rhizobium* and *Bradyrhizobium*. Studies by Belane and

Dakora (2009) reported cowpea grain yield of 1.5 t ha<sup>-1</sup> in Ghana while over 2.6 t ha<sup>1</sup> have been obtained for the crop in South Africa (Makoi *et al.*, 2009).

#### 2.2 Factors limiting legume nitrogen fixation and yield

#### 2.2.1 Excessive soil moisture

The intensity of rainfall in the humid tropics can be so high that even upland sandy soils can become flooded for some time. The length of time that the soil remains flooded will depend on how easily the soil can be drained (Giller, 2001). Free oxygen can be readily used up in a waterlogged soil if temperatures are high with much organic substrate present, and this will create anaerobic conditions in the soil within hours (Giller, 2001). Survival of rhizobia during long periods of flooding is of great significance in rotational cropping systems involving legumes. The population size of rhizobia nodulating Siratro in sandy soils was found to be generally larger  $(10^2 - 10^4)$ cells g<sup>-1</sup> soil) when the soil was moist or fully waterlogged than when the soil was dry (< 10 -10<sup>3</sup> cells g<sup>-1</sup> soil) (Toomsan, 1990). However, in a heavy textured soil, numbers of soybean bradyrhizobia multiplied during flooded cultivation of a rice crop but this declined when the soil was drained (Simanungkalit et al., 1995).

The ability of soybean bradyrhizobia to survive for more than five years in soils that were periodically flooded for rice cultivation was confirmed by Roughley et al. (1995). On the contrary, large reductions in the numbers of rhizobia nodulating chickpea which are generally fast growing rhizobia have been found after paddy rice cultivation (Toomsan *et al.*, 1982). This clearly indicates a great variability in the tolerance of legume - nodulating rhizobia to waterlogging stress. NC

Waterlogging and flooding have a profound effect on soybean by suppression of plant growth and yield (Matsunami et al., 2007) and this has been largely attributed to the effect of waterlogging on the process of nitrogen fixation in soybean (Jung et al., 2008). Application of nitrogen fertilizer however, was able to restore growth and yield

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of soybean (Shimamura *et al.*, 2006), suggesting that yield reduction in soybean under waterlogging stress was mainly caused by depression of nodule nitrogen fixation. Moreover, the suppression of growth in soybean plants caused by water logging was found to be greater in plants dependent on  $N_2$  fixation than plants supplied with nitrate (Bacanamwo and Purcell, 1999), suggesting the sensitivity of  $N_2$  fixation to the effects of waterlogging than plant growth. However, the degree of tolerance to water logging may differ widely among different legume cultivars as demonstrated in pigeon pea (Chauhan *et al.*, 1997).

#### 2.2.2 Soil moisture stress (drought)

Desert soils harbour rhizobial populations and the presence of nodules on legumes growing in such environments is indicative of the ability of rhizobia to survive in soils with limiting moisture conditions (Waldon *et al.*, 1989). Rhizobia population density is, however, lowest under the most dried conditions and increases with a decrease in the moisture stress (Tate, 1995). According to Orchard and Cook (1983), rhizobia survival and activity in the soil is dependent on how they are distributed among microhabitats and soil moisture dynamics. There is pronounced variability in the ability of rhizobial strains to survive in dry soils (Issa and Wood, 1995). Strains that survive under greater water stress are those that are able to retain less water in their cells (Al-Rashidi *et al.*, 1982). Rhizobia undergo morphological changes as an immediate response to water stress as seen in *R. meliloti* which showed irregular morphology at low water potential (Busse and Bottomley, 1989).

The change in rhizobial cell morphology due to water stress leads to a reduction in infection and nodule formation in legumes. Low soil water content was identified as the cause of soybean's lack of response to rhizobial inoculation in soils where native population of R. *japonicum* was very high (Hunt *et al.*, 1981). A favourable

rhizosphere environment is necessary for a successful legume-rhizobium symbiosis; the rate at which the symbiosis is inhibited by stress and the magnitude of the stress effect are, however, dependent on the plant's phase of growth and development as well as the severity of the stress. Williams and de Mallorca (1984) found out that mild water stress conditions caused a reduction in only the number of nodules on soybean roots, while both nodule number and size were reduced by moderate and severe water stress.

Drought stress has a drastic effect on legume  $N_2$  fixation as rates of  $N_2$  fixation have been found to be more sensitive to reduced soil moisture content compared to other physiological processes such as photosynthesis and transpiration (Serraj *et al.*, 1999). A number of legumes from both temperate and tropical regions have been found to exhibit a reduction in  $N_2$  fixation when subjected to soil moisture stress. These include legumes such as *Arachis hypogaea* (Simpson and Daft, 1991), *Vicia faba* (Guerin *et al.*, 1990), *Glycine max* (Kirda *et al.*, 1989), *Vigna* sp. (Pararjasingham and Knievel, 1990) and the shrub legume *Adenocarpus decorticand* (Moro *et al.*, 1992). The pronounced effect of soil moisture stress on  $N_2$  fixation is due to the fact that processes such as nodule formation, growth and activity are more sensitive to moisture stress than general metabolism in root and shoot (Albrecht *et al.*, 1994).

Moreover, the plant's growth stage also determines its nodulation and  $N_2$  fixation response to water stress. Studies by Pena-Cabriales and Castellanos (1993) indicate that water stress occurring at vegetative growth stage had a detrimental effect on  $N_2$ fixation than when it occurred at the reproductive stages where the plant had very little chance of recovery. There is possibility of selection of rhizobial strains with varied sensitivity to moisture stress due to the existence of a wide range of moisture levels in ecosystems where legumes are capable of fixing nitrogen.

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The sensitivity of rhizobia strains to moisture stress varies among strains as seen in cowpea rhizobia (Osa-Afina and Alexander, 1982), *R. leguminosarium* (Fuhrmann *et al.*, 1986), *R. meliloti* (Busse and Bottomley, 1989) and *B. japonicum* (Mahler and Wollum, 1980). Optimization of soil moisture for growth of the host plant will result in maximum N inputs into the soil through N<sub>2</sub> fixation as the macro-symbiont (host plant) is more sensitive to moisture stress effects than the micro-symbiont (Tate, 1995).

#### 2.2.3 Soil phosphorus deficiency

Phosphorus (P) availability is a major limiting factor to crop production on 40% of arable land in the world, particularly in the tropics and subtropics where the soils are highly weathered with pronounced P deficiency (Vance, 2001). Phosphorus is one of the several elements whose deficiency has been found to affect the N<sub>2</sub> fixation process as a principal yield - limiting nutrient along with nitrogen (Pereira and Bliss, 1989). Soil P deficiency is common in most west African soils (Adetunji, 1995) and investigations by Weber *et al.* (1996) indicate that legumes growing in the moist Guinea savanna zones of West Africa require about 30 kg P ha<sup>-1</sup> for optimum growth and N<sub>2</sub> fixation.

The significant variation in the ability of rhizobia to grow under low soil P levels (Beck and Munns, 1984) is mainly as a result of variation in phosphorus uptake systems efficiency (Smart *et al.*, 1984). There is also variability in the ability of rhizobial strains to store phosphorus as polyphosphate (Smart *et al.*, 1984). Soil P deficiency can lead to rhizobial P deficiency particularly under acidic conditions when there is precipitation of dissolved P salts in the presence of aluminium (Graham, 1992). High concentrations of calcium are required for growth of rhizobia under low

P concentrations (Beck and Munns, 1984) but deficiency in calcium often comes with phosphorus deficiency in acid soils (Giller, 2001).

Phosphorus is an essential element for both nodulation and N<sub>2</sub> fixation (Ssali and Keya, 1983). Phosphorus content in nodules range between 0.72 and 1.2% (Hart, 1989) as nodules serve as strong sinks for P. Plants dependant on N<sub>2</sub> fixation will, therefore, have a higher requirement of P than those supplied with combined nitrogen (Robson, 1983). Phosphorus supply has a direct effect on processes such as nodulation, specific nodule activity and N<sub>2</sub> fixation (Nkaa et al., 2014). Nodulated legumes have higher P requirement due to higher ATP requirements for the function of nitrogenase (Ribet and Drevon, 1996). Phosphorus is also needed for signal transduction and nodule development (Al-Niemi et al., 1997). Acute P deficiency can prevent nodulation by legumes. Phosphorus and sulphur are required for nodule metabolism and these tend to be concentrated in the nodules during times when the plant is deficient in these nutrients (O'Hara et al., 1988 a). The ability of nodulated plants to capture nutrients particularly phosphorus is limited, since nodulated plants have less well-developed rooting system than non - nodulated plants (Cassman et al., 1980). Phosphorus concentrations in nodules of soybean and pea (*Pisum sativum*) can be very high (6 mg  $g^{-1}$  dry weight), with shoot P ranging between (2 to 3 mg g<sup>-1</sup> dry weight) (Israel, 1987). Under low soil P conditions, application of P fertilizer on species dependant on N<sub>2</sub> fixation caused an increase in nitrogenase activity, nodulation and total plant N accumulation (Robson et al., 1981). Stimulation of N<sub>2</sub> fixation in clover and pea following application of P is mainly via enhancement of shoot growth, which ultimately has an influence on nodule formation and development (Jakobsen, 1985; Robson et al., 1981). Phosphorus deficiency has a more direct impact on nodule functioning as seen in soybean and Stylosanthes (Israel, 1993). According to Al-Niemi

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*et al.* (1997), nodule bacteriods may suffer P limitation even under adequate P supply conditions.

It is very necessary for leguminous plants to develop mechanisms that will lead to improved P acquisition and utilization, due to the high P requirement for legume symbiosis. There are different mechanisms by which leguminous plants employ as adaptation to acquire P under low soil P conditions (Graham and Vance, 2000). These adaptive mechanisms include acidification of the rhizosphere by releasing H<sup>+</sup> ions, production and release of acid phosphatase, changes in root morphology and enhanced P acquisition via mycorrhizal symbiosis (Vance *et al.*, 2003). Although most of these mechanisms are not clearly understood (Graham and Vance, 2000), most legumes have been found to be heavily dependent on mycorhizas for efficient uptake of P (Hayman, 1986). Both nodulation and N<sub>2</sub> fixation have been reported to be stimulated by mycorrhizal symbiosis between legume plant roots and soil fungi, particularly in low P soils (Redecker *et al.*, 1997).

#### 2.2.4 Soil mineral nitrogen

The percentage nitrogen derived from atmosphere (%Ndfa) by a legume is determined by other factors apart from the symbiotic association between the legume cultivar and its micro-symbiont. It is also a function of the interaction between plantavailable soil N and legume growth (Unkovich and Pate, 2000). Soil nitrate and N<sub>2</sub> fixation are the two main sources from which legumes meet their N requirements for growth. Nodulation and the N<sub>2</sub> fixation processes have been found to be inhibited by high soil nitrate levels (Herridge *et al.*, 1984; Peoples *et al.*, 1995 b). High concentrations of soil nitrate, normally arising as a result of excessive tillage and application of N fertilizer have been shown through experiments to delay the nodule formation and onset of N<sub>2</sub> fixation, thereby causing a reduction in % Ndfa and the amount of N fixed (Salvagiotti *et al.*, 2008). Application of fertilizer N to groundnut reduced the crop's level of reliance on fixed N (Maskey *et al.*, 2001). On the contrary, strategies that are known to (i) reduce concentrations of soil nitrate such as rotation of legumes with N - depleting crops (Peoples and Baldock, 2001), or (ii) increase competition for soil mineral N such as legume-cereal intercrop (HauggaardNielsen *et al.*, 2003) will increase % Ndfa. Data reviewed by Salvagiotti *et al.* (2008) indicate that a relatively small amount of N was fixed by soybean after application of N fertilizer at rates of 200 - 300 kg N ha<sup>-1</sup>.

There have been attempts to overcome the inhibitory effects of nitrates on N<sub>2</sub> fixation through breeding for nitrate tolerant genotypes of soybean (Streeter and Wong, 1988). This strategy was, however, not successful due to the relatively low grain yields obtained from the selected lines (Herridge and Rose, 1994). Investigation by Streeter (1985) suggests that, nitrate inhibition of N<sub>2</sub> fixation appears to have a localised effect, and that the inhibition is likely to diminish with a reduction in the concentration of nitrate in the immediate surroundings of the nodules . The use of polymer coated urea which releases N slowly into the soil may increase the chances of N<sub>2</sub> fixation not being limited by N fertilizer application (Salvagiotti *et al.*, 2008).

#### 2.2.5 Soil temperature

High soil temperature is a major problem for legume growth through its effect on nitrogen fixation in both tropical and subtropical areas (Michiels *et al.*, 1994).

Rhizobial strains' ability to survive and persist in soils for long periods is influenced by soil temperature (Mohammadi *et al.*, 2012). For most rhizobia strains, growth in culture conditions requires an optimum temperature range of 28 to 31°C while many are not able to grow at 37 C (Graham, 1992). On the contrary, cowpea rhizobia isolated from the Sahel - Savanna of West Africa grow at 37 °C, with more than 90% of these strains being able to grow well at 40 °C (Werner and Newton, 2005). Rhizobia strain adaptation to high temperature has been reported by Hartel and Alexander (1984). Karanja and Wood (1988), found that a high proportion of the strains that survived and persisted at 45° C became ineffective in fixing nitrogen, and the loss in symbiotic effectiveness was attributed to plasmid curing. Bacterial infection and N<sub>2</sub> fixation in legumes such as soybean (Munevar and Wollum, 1982), cowpea (Rainbird *et al.*, 1983) and peanut (Kishinevsky *et al.*, 1992), are strongly affected by high temperatures within the rhizosphere.

Rhizobia nodulating different legumes have varied temperature ranges within which they can fix N effectively. For example, the critical temperature for  $N_2$  fixation is between 25 and 30 °C for common bean, while that of cowpea, soybean and peanut ranges between 35 and 40 °C (Long, 2001). Nodule formation and nitrogen fixation depend on the strain nodulating the legume as well as the plant genotype (Munevar and Wollum, 1982). Rhizobia survival is higher in well-aggregated soils exposed to high temperature than non - aggregated soils and is favoured by dry rather than moist conditions (Graham, 1992).

Cowpea rhizobia were also found to have a reduced infectivity following storage at  $35^{\circ}$ C. High soil temperatures have been linked to the frequency of non-infective isolates in soil and as noted by Martínez-Romero *et al.* (1991), such non-infective isolates actually out-numbered those that were able to cause infection in the rhizosphere of bean. The effect of temperature on rhizobia is dependent both on rhizobia strain and soil type. For example, the susceptibility of *R. leguminosarum bv*.

*trifolii* than *Bradyrhizobium* sp to high soil temperatures was remediated in sandy soil upon addition of montmorrilonite and illite (Mohammadi *et al.*, 2012). Competition for nodulation is also greatly influenced by soil temperature (Triplett and Sadowsky, 1992) and this to a lesser extent may be as a result of a delay in nodulation or restriction of nodules to sub - surface region which is temperature – induced.

#### 2.2.6 Population size and effectiveness of indigenous soil rhizobia

According to Peoples *et al.* (2009), a symbiotic relationship between legumes and soil bacteria is established when there is nodule formation as a result of soil rhizobia infecting the roots of legumes. In Africa, many soils with the potential to produce soybean do have low levels of nitrogen fixation activities, and are often not able to support high soybean grain yields unless inorganic N fertilizers or rhizobial inoculants are applied (Abaidoo *et al.*, 2007). Specific *Bradyrhizobium* species is required for nodulation of soybean. Bradyrhizobia populations that are compatible with soybean are often not present in soils with no soybean cropping history

(Abaidoo *et al.*, 2007). In their assessment of the population size of indigenous *Bradyrhizobium* sp. in Africa using the most probable number (MPN) technique, Abaidoo *et al.* (2007) found that between 43 and 79% of soils from countries in West and East Africa had less than 10 bradyrhizobia cells  $g^{-1}$  soil that nodulated TGx soybean. In the same study, the population size for cowpea *Bradyrhizobium* spp. in soils sampled from 19 sites in Ghana ranged from 2 - 792 cells  $g^{-1}$  soil.

Counts of indigenous cowpea bradyrhizobia population in Ghanaian soils by Fening (1999) indicate that soils in Ghana do have cowpea bradyrhizobia populations in the range of  $0.6 \times 10^1$  to  $31.0 \times 10^3$  cells g<sup>-1</sup> soil. The symbiotic effectiveness of these bradyrhizobia isolates were comparable to plants fertilized with inorganic nitrogen

fertilizer and others even showed superiority in effectiveness relative to the standard TAL 169 strain used, which suggests that these native isolates may be useful strains for the production of cowpea inoculants. Legume response to rhizobial inoculation as well as the capacity of the legume to derive N through nitrogen fixation is dependent on population size of effective indigenous rhizobia (Thies *et al.*, 1991). Indigenous soil rhizobial population is also influenced by biological, climatic, physical and chemical soil factors that affect survival and infectivity of bradyrhizobia as well as nitrogen fixation efficiency which ultimately have an effect on the growth and performance of the legume host (Woomer *et al.*, 1988)

Survival of rhizobia is also influenced by stressful edaphic factors including soil salinity, pH, acidity, temperature, low clay content, high concentration of heavy metals and fallowing (Howieson and Ballard, 2004). Singleton and Tavares (1986) asserted that in cases where the introduced rhizobia is able to overcome competition by the native strain, it was not always a sufficient condition to enhance  $N_2$  fixation when indigenous soil rhizobia population was above a certain threshold with strains that were effective.

#### 2.3 Approaches to enhancing legume growth and yield

#### 2.3.1 Rhizobial inoculation

As the principal source of nitrogen in the soil, legume nitrogen fixation plays an important role in sustainable land management since it serves as an efficient and effective means of nitrogen supply for plant growth under favourable environmental and atmospheric conditions (Hungria and Vargas, 2000). Host legume efficiency is influenced by several factors such as soil moisture, available soil N as well as effective

and competitive indigenous rhizobia population (Palmer and Young, 2000). Rhizobia could be manipulated for soil fertility and legume crop productivity improvement through rhizobial inoculation technology (Keyser and Li, 1992). Peoples *et al.* (1995) indicated that inoculation can result in large populations of rhizobia being established even under adverse soil conditions, thereby improving nodulation and nitrogen fixation. Research by the Food and Agricultural

Organization (FAO) of the United Nations and International Atomic Energy Agency (IAEA) during the period 1972-1998 showed that application of rhizobium inoculants with suitable strain of rhizobium at sowing is a good agronomic practice for maximum legume yield (Hardarson and Atkins, 2003). This is often

recommended in two different situations: (1) in soils with low indigenous rhizobial population (Catroux *et al.*, 2001) and (2) when the strains that are present are inefficient in fixing nitrogen (Brockwell *et al.*, 1995).

According to Herridge (2008), rhizobial inoculation is a form of insurance where the farmer by paying a small premium (cost of inoculation) is protected against the possibility of an N-deficient crop that could lead to a reduction in grain yield and income. Inoculation is a common agronomic practice for most soybean varieties cultivated in Brazil and USA (Hungria *et al.*, 2006 a). Studies by Ferreira and Hungria (2002), indicates that over 90% of the soils used for soybean cultivation contain relatively high populations of commercial rhizobia inoculants strains due to application of inoculants in these soils at some point in time. According to Alves *et al.* (2003), the economic benefit of rhizobia inoculation by way of N-fertilizer saving can be as much as \$ 2.5 billion per year.

Grain yields above 2 tons ha<sup>-1</sup> were obtained with soybean inoculation in Zimbabwe
(Mpepereki *et al.*, 2000). A similar success story cannot be said for soybean in subSaharan Africa, in spite of the fact that soybean is an important grain legume grown by smallholder farmers. In Ghana, the use of rhizobial inoculants as a means of enhancing nitrogen fixation in legumes especially soybean is fairly new and has not been well-exploited in the context of Ghana's agriculture.

#### 2.3.1.1 Response of legumes to rhizobial inoculation

A five-year comprehensive project by the Nitrogen Fixation in Tropical Agricultural Legumes (NifTAL) project which was aimed at determining the benefits of rhizobia inoculants application for legumes shows clear benefits associated with inoculation (Singleton *et al.*, 1992). Majority of the 228 standardized field trials conducted in more than 20 countries with 19 legume species gave significant response to inoculation when the trials were conducted in farmers' field and under intensive management with higher inputs (Singleton *et al.*, 1992).

Inoculation of legumes is done with the expectation that it will result in an increase in nitrogen fixation and grain yield. Although there may be dramatic increases in yield as a result of inoculation, legume response to inoculation may be limited by many factors such as: (1) excessive competition from native strains which result in failure of the inoculum to establish at normal inoculation rates or a reduction in the viability of the inoculum due to stresses such as temperature and dessication, (2) there may also be a limitation in plant yield potential by the environment such that N demand is met by available soil N or through nitrogen fixation by soil rhizobia which is less effective, (3) the presence of sufficient soil rhizobia population to meet the host demand for nitrogen when growth conditions are not limiting (Singleton *et al.*, 1997; Singleton and Tavares, 1986)

According to Theis et al. (1991), the response of legumes to rhizobial inoculation depends largely on the population of indigenous soil rhizobia, available soil N, and the crop's N demand. Inoculation of leguminous crops grown in soils with indigenous rhizobia population of 10 to 100 cells g<sup>-1</sup> soil was found to have increased the number of nodules per plant (Theis et al., 1991). There are varied opinions about the threshold population size of indigenous rhizobia below which inoculation is likely to benefit the farmer by way of increased nodulation and nitrogen fixation. According to Singleton and Tavares (1986), legumes responded positively to rhizobial inoculation by way of increased nodule number when the indigenous rhizobia population was less than 100 cells g<sup>-1</sup> of soil, but no corresponding increase in nodule dry weight when native rhizobia populations were effective. Howieson and Bullard (2004) after a comprehensive survey of literature came to the conclusion that a legume will respond positively to inoculation in soils with rhizobial population of less than 100 cells g<sup>-1</sup> soil. A slightly higher value of 300 rhizobia cells g<sup>-1</sup> soil was, however, proposed by Herridge (2008), as the threshold rhizobia population below which inoculation is likely to give beneficial results to the farmer. Studies by Hungria et al. (2006) also indicate that symbiotic performance and grain yield of soybean could be enhanced through successive reinoculation, even in soils with adequate numbers ( > 1000 rhizobia cells g<sup>-1</sup> soil) of effective rhizobia.

# 2.3.1.2. Response of soybean to rhizobia inoculation

Effective nodulation of soybean requires the presence of specific species of bradyrhizobium in the soil. Population of such compatible bradyrhizobia species are most of the time not available in soils, especially those with no soybean cropping history (Abaidoo *et al.*, 2007). Soybean introduced in many tropical soils for the first

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time required inoculation with *Bradyrhizobium japonicum* strains to ensure adequate and effective nodulation in these soils that contained high cowpea bradyrhizobia populations. Inoculation is a major constraint in the production of soybean genotypes which require specific bradyrhizobium species. It was to overcome this problem of specificity in soybean that the soybean breeding programme at the International Institute for Tropical Agriculture (IITA) in Nigeria developed promiscuous soybean varieties which were designated TGx (Tropical *Glycine* cross). These varieties had reduced nodulation specificity and therefore could nodulate effectively with native bradyrhizobium strain populations since the non promiscuous soybean did not do well under tropical climate (Abaidoo *et al.*, 2007).

Several experiments have been conducted using promiscuous soybean genotypes in many countries in West (Sanginga *et al.*, 1996), East and South Africa (Mpepereki *et al.*, 2000) without N fertilizer and inoculation and the results have indicated that the indigenous strains did not always meet the N requirements of the plants; in spite of the fact that these soybean genotypes were nodulated by indigenous rhizobia. Musiyima *et al.* (2005) obtained similar results using different promiscuous soybean varieties in Zimbabwe. The presence of nodules is not indicative of an effective nitrogen fixation that can enhance growth of the soybean plant significantly, since there is the possibility of ineffectiveness of indigenous bradyrhizobia strains in root nodules of promiscuous soybean (Zengeni and Giller, 2007). These results have generated a huge discussion on whether it is necessary to inoculate promiscuous soybean genotypes. There is variability in the effectiveness and population of indigenous bradyrhizobia in a given location (Fening and Danso, 2002). This has made it necessary for promiscuous soybean to be inoculated with foreign bradyrhizobia strains depending on the

indigenous bradyrhizobia population and their effectiveness in the locality (Okereke *et al.*, 2000) as well as the variety's degree of promiscuity (Sanginga *et al.*, 1999).

The significant role of bradyrhizobium inoculants application in enhancing nitrogen fixation in soybean on smallholder farms in Ghana was demonstrated by the N2Africa project which was aimed at putting nitrogen fixation to work for smallholder farmers in Africa. An assessment of the project's impact on biological nitrogen fixation from both agronomy and delivery and dissemination (D&D) trials conducted in northern Ghana between 2008 and 2012 revealed that, nitrogen fixation in soybean increased from 89 kg N ha<sup>-1</sup> in control treatments to 112 kg N ha<sup>-1</sup> where inoculants were applied at a rate of 5 g of inoculants kg<sup>-1</sup> of soybean seed (Ronner and Franke, 2012).

There have been varied responses of soybean to rhizobial inoculation in sub - Saharan Africa. In assessing the response of soybean to bradyrhizobia and phosphorus application in Ghana, Kumaga and Ofori (2004) observed significant increases in nodulation after inoculation of both promiscuous and non promiscuous soybean varieties and the increase in nodulation was attributed to high competitive ability of the bradyrhizobia inoculants used. Similar findings were also reported by

Okereke et al. (2000) for promiscuous soybean in the moist Savanna of West Africa.

Kumaga and Etu-Bonde (2004) through pot studies showed that nodulation and  $N_2$  fixation of promiscuous soybean may be increased when inoculated with effective bradyrhizobia. In a similar study, Thuita *et al.* (2012) found out that application of commercial rhizobia inoculants resulted in significant increase in growth and nitrogen fixation of promiscuous soybean in Kenyan soils.

In assessing the potential of commercial inoculants to improve cowpea yields in Kenya, Mathu *et al.* (2012) found out that rhizobial inoculation did not have a significant effect on nodulation, biomass yield and shoot N content in cowpea compared with controls. Cowpea, like many other promiscuous grain legumes, is not likely to respond positively to rhizobial inoculation unless inoculated with selected strains applied in high concentrations.

A study by Fening and Danso (2002), on the symbiotic effectiveness of 100 cowpea bradyrhizobia isolates in soils sampled from different ecological zones of Ghana indicates that, 26% of the isolates were effective in fixing nitrogen with cowpea, while 68% were moderately effective and 6% being ineffective. In Ghana, incorporation of cowpea residues into soils was estimated to supply 60 kg N ha<sup>-1</sup> soil nitrogen which was beneficial to subsequent crops (Dakora *et al.*, 1987).

# 2.3.1.3 Constraints to rhizobium inoculant technology use in Africa

Although numerous trials have indicated enhanced growth and grain yield response of many legume crops to rhizobium inoculation, there is variation in the adoption of the technology by farmers worldwide (Peoples *et al.*, 2009). While the technology has proved successful in the Americas, Europe and Australia where high quality inoculants are produced, the same cannot be said for Africa and Asia where inoculants production is less sophisticated and more fragmented with variable inoculant quality (Herridge, 2008). The major constraints in Africa and Asia as far as the use of inoculants is concerned have to do with small scale local production and distribution of inoculants (Sattar *et al.*, 1997) and poor quality of inoculants (Singleton *et al.*, 1997). Hoa *et al.* (2002) also suggested that farmers and agricultural extension staff may lack the requisite knowledge about inoculants coupled with the fact that researchers are not

able to convince farmers about the benefits of inoculation as a result of inconsistencies in crop response to rhizobial inoculation or researchers inability to show visible differences between uninoculated and inoculated treatments (Joshi, 1994).

According to Herridge (2008), addressing these limitations would require private sector investment in training and education especially in improving inoculant quality with special emphasis on research and development in rhizobial strain selection, inoculant production and application methods.

#### 2.3.2 Mineral nitrogen and phosphorus fertilization

Grain legumes are expected to meet their nitrogen requirements through nitrogen fixation and soil or mineral fertilizer N application. The antagonism between soil nitrate concentration and the  $N_2$  fixation process is a major constraint to N uptake in legumes (Streeter *et al.*, 1998). This is the case when nitrogen fixation is not impaired by any abiotic stress in the soil such as moisture, pH and soil temperature

(Purcell et al., 2004).

Nitrogen fertilizer application has been suggested as a means of increasing soil available N for legumes with high N demand such as soybean (Salvagiotti *et al.*, 2008). Soybean has the highest N requirement among grain legumes with each ton of soybean seed requiring the crop to assimilate approximately 100 kg N (Keyser and

Li, 1992). In most cases, the amount of N fixed by soybean is not enough to replace N export from the field through harvesting of grain, or which at best is close to neutral when N from below ground parts is included in estimating plant N balance (Salvagiotti *et al.*, 2008). In soybean, maximum nitrogen fixation occurs between the onset of podding (R3) and the beginning of grain filling (R5) stages in crop development and any deficit between the crop's N demand and N supply through nitrogen fixation must be met by uptake of N from other sources (Salvagiotti *et al.*, 2008).

Although there are reports of observed significant yield responses of soybean to frequent N additions in cases where the N<sub>2</sub> fixation process could not meet crop N demand (Thies *et al.*, 1995), studies by Barker and Sawyer (2005) indicate inconsistent yield response of soybean to N fertilizer application at economically acceptable levels. As to whether N fertilizer application to legumes like soybean can prevent N limitations without having an effect on the crop's capacity to fix N<sub>2</sub> and the cost effectiveness of this option is a question that need to be answered through further research (Salvagiotti *et al.*, 2008). Although the general belief is that application of N fertilizers during late reproductive stages of crop growth (i.e. R3 to R5) should theoretically result in yield increases, measured responses in grain yield to fertilizer N applied at these stages have, however, been found not to be universal

#### (Gutiérrez-Boem et al., 2004).

Published data reviewed by Salvagiotti *et al.* (2008) indicate that soybean yield response to N fertilizer application depends on factors such as the yield potential of the environment and any constraints (biotic or abiotic) that negatively impacts on crop growth and associated N demand. According to Alves *et al.* (2003), the most feasible means of securing the required N supplies under such constraints is through the development of rhizobia strains with the capacity to fix nitrogen under such stressful conditions. Although N fertilizer application negatively impacts on N<sub>2</sub> fixation in legumes, this does not happen in all cases as low doses of 5 - 10 kg N ha<sup>-1</sup> as starter N have been found to enhance both N<sub>2</sub> fixation and grain yield of legumes grown in soils with low initial N (Hardarson and Atkins, 2003). Studies by Chen *et al.* (1992) and Starling *et al.* (1998) showed the effect of starter N on seed yield of soybean grown in soils with low N levels of 51 kg N ha<sup>-1</sup>(0 – 40 cm) and 20 kg N ha<sup>-1</sup>(0 – 15 cm) respectively. Several field studies have generally indicated negative or inconclusive results with starter - N application (Hardarson *et al.*, 1984; Herridge and Brockwell, 1988; Ying *et al.*, 1992). Singleton *et al.* (1999) after comparing grain yield of starter - fertilized and non - fertilized legumes, concluded that yields are enhanced by starter - N fertilization only on rare occasions.

There is variation in the response of grain legumes to starter - N application. Rennie and Kemp (1983 b) observed that application of 40 kg N ha<sup>-1</sup> resulted in a significant decrease in the amount of  $N_2$  fixed in some varieties but not others. Field grown common bean was twice as efficient in accumulating mineral N as soybean (George and Singleton, 1992).

The time of N fertilizer application can significantly influence legume yield as Yinbo *et al.* (1997) reported of an increase in pod yield of soybean from 13 to 44% following a split application of 25 kg starter N fertilizer with additional 50 kg N ha<sup>-1</sup> applied at flowering. Application of a second 25 kg N ha<sup>-1</sup> as top - dressing before flowering had no effect on yield compared to a 20% increase in yield when applied at flowering or during late pod formation. The rate of fertilizer N did not have an influence on the %Ndfa at harvest (84%) and total N fixation even increased under high N fertilizer rates.

Phosphorus (P) is an important nutrient for plant production due to its role in the plant's metabolic processes of growth and reproduction. According to Vance *et al.* (2000), phosphorus is the second most-limiting element for plant growth after nitrogen. Crop production on many soils across the world is commonly constrained by low P availability that limits plant growth. This is particularly the case in developing countries where farmers have restricted access to P fertilizers (Lynch, 2007).

Phosphorus functions as a key element with significant roles in many physiological and biochemical processes in plants (Nkaa *et al.*, 2014). It is an essential component of sugar phosphates; it is also involved in respiration and energy transfer via Adenosine Triphosphate (ATP); and it is also a major constituent of Ribonucleic acid (RNA), Deoxyribonucleic acid (DNA) as well as membrane phospholipids (Nyoki and Ndakidemi, 2014). According to Theodorou and Plaxton (1993), P is also involved in controlling key enzymatic reactions and in the regulation of metabolic pathways. Legumes tend to have a stronger requirement for P than cereals due to their less branched and less fibrous root systems. The nitrogen fixation process in legumes requires P for adequate growth and nodulation (Tang *et al.*, 2001). Though there are arguments on the direct roles of P in nodulation and nitrogen fixation (Miao *et al.*, 2007) the supply of P has been found to directly and positively stimulate nodulation in some legumes such as red clover (Hellsten and Huss-Danell, 2000).

At the physiological level, P deficiency has been found to reduce leaf expansion and shoot development and thereby reducing photosynthetic area and carbohydrate utilization (Ahloowalia *et al.*, 1995). Studies by Yakubu *et al.* (2010) in the SudanoSahelian zone of Nigeria indicate that application of P at a rate of 40 kg ha<sup>-1</sup> significantly increased N<sub>2</sub> fixation in cowpea, groundnut and bambara groundnut by 378, 169, and 138% respectively over the control. In earlier studies, application of P at rate of 20 - 40 kg ha<sup>-1</sup> caused a significant improvement in the performance of soybean (Kamara *et al.*, 2007) and cowpea (Uzoma *et al.*, 2006).

Although P in its bound form is quite abundant in many soils, it is, however, largely unavailable for plant uptake (Schachtman *et al.*, 1998). Crop yield on 40% of the world's arable land is limited by P availability (Vance, 2001). Phosphorus tends to be

in short supply particularly in coarse - textured sandy soils and in acid, heavy clay soils (Oxisols). Phosphorus is unavailable due to its ability to rapidly form insoluble complexes with Al, Fe, Ca and Mg cations and it is also incorporated into organic matter by microorganisms.

According to Vance (2001), to obtain sustainable management of P in agriculture requires that plant biologists discover mechanisms in plants that enhance P acquisition and making use of these adaptations to make plants acquire P with greater efficiency. There is also the need to develop P - efficient germplasm as well as advancement of crop management schemes that increase soil P availability.

According to Buresh *et al.* (1997), addition of P fertilizer could be best managed through addition of small rates (typically  $15 - 30 \text{ kg P ha}^{-1}$ ) in order to ensure most efficient recovery of P by the crop. Phosphorus recovery efficiencies in the first cropping season are between 20-25% and this is further reduced to 2-3% in the subsequent cropping season (Janssen *et al.*, 1987), with a buildup of residual P upon regular addition of P fertilizers. Addition of P with organic manures can enhance P availability by reducing the fixation of phosphate ions onto clay surfaces. In sandy soils however, oversupply of P can lead to deficiencies in Zn, but the use of animal manure can prevent these problems (Zingore *et al.*, 2008).

The response of legume species to P supply does not follow a general pattern. The P requirements of legumes for optimal growth and the magnitude of the effect that are exhibited by either low or high P at the level of the plant are determined by a number of factors including the legumes mode of acquiring N (Sanginga *et al.*, 1995), the genetic background of the plants (Sanginga *et al.*, 2000), the growing conditions of the

plant (Passarinho *et al.*, 2000) and the physiological stage at which plants are analysed (Pongsakul and Jensen, 1991).

### 2.4 Rock phosphate as P fertilizer source

Rock phosphate (RP) deposits in Africa are about 4.5 billion tons which represent about 75% of the world reserves (Sanginga and Woomer, 2009). Sedimentary sources of phosphorus abound in West Africa with about 16 major deposits in the

West African dry lands (van Kauwenbergh, 2006). According to Sanchez *et al.* (1997), this huge deposit of rock phosphate is a potential source of phosphorus and could be used as an alternate source of phosphorus to address the problem of phosphorus limitation in crop production. Rock phosphate has been tested on acidic soils in the tropics as a potential alternative to P - fertilizers such as single and triple super phosphate which are water soluble (Fardeau and Zapata, 2002). One major advantage in the use of rock phosphate by resource - poor farmers in Africa lies in its low price relative to the imported fertilizers. For instance, the price of finely ground Minjingu rock phosphate containing 67% of phosphorus in TSP ranges between \$200 - \$400 ton<sup>-1</sup> while that of TSP is \$ 1140 ton<sup>-1</sup> (Sanginga and Woomer, 2009).

Although farmers have been discouraged from using this valuable resource as a result of the limited success in the use of RP for direct application coupled with the fact that some of them are less reactive, there are some rock phosphates which are more reactive like the Tilempsi, Matam and Minjingu RPs in Mali, Senegal and Tanzania respectively which have a greater potential for direct application (Sanginga and Woomer, 2009). Moreover, Woomer *et al.* (1997) gave convincing evidence for the use of RP in East Africa. In comparing Tanzanian Minjingu RP (MRP) and imported TSP, it was realized that MRP was sold for \$115 ton<sup>-1</sup> whereas TSP was \$ 480 ton<sup>-1</sup> in Western Kenya. Moreover, MRP was 65% as effective as TSP and contained 69% much P on a unit basis thereby making MRP 45% effective but only at 24% of the cost.

#### 2.4.1 Factors affecting availability of P from rock phosphate

Direct application of rock phosphate to soils has received much attention in recent times due to its possible use as an alternative to soluble phosphate fertilizers which are more expensive (Akande *et al.*, 2010). According to Zapata *et al.* (2003), direct application of RP to soils is largely informed by the open and loosely consolidated nature of the microcrystal aggregates which have large surface area.

Most RP consists of Apatite as the main mineral with wide variation in its physicochemical and crystallographic properties (Chien *et al.*, 2010).

The solubility of RP is determined by the chemical and mineralogical nature of the P minerals in them and the ease with which the RP is able to release P for uptake by plants. Studies by Ghosal and Chakraborty (2012), indicate that the effectiveness of RP differs from one source to the other based on the mineral composition and the chemistry of the rock. Rock phosphate effectiveness is also affected by other factors such as environmental management, soil and the type of crop that is cultivated.

The reactivity of RP, according to Rajan *et al.* (1996), is a combination of the RP's properties upon which the rate at which the RP dissolves in a particular soil under a specified field condition. As the main constituent of RP is mineral apatite  $(Ca_5(PO4)_3X)$  where X is most of the time fluorine, its reactivity is dependent on the rate of its dissolution in acid as well the amount of P that is recovered (Ghosal and Chakraborty, 2012). The rate of reaction of the acid attack is also dependent on factors such as the concentration of the acid, reaction time, size and temperature of the particles (Hamadi *et al.*, 2012).

Other factors that enhance the reactivity of RP are the increasing rate at which carbonate replaces phosphate as well as the replacement of Ca by Mg and Na within the apatite's structure and a decrease in the particle size (Ghosal and Chakraborty, 2012). A decrease in soil pH has also been found to increase the effectiveness of RP(Chien *et al.*, 2010; Prochnow *et al.*, 2006).

Studies by Asomaning *et al.* (2006) indicates that P availability from RP is mainly influenced by (a) the inherent differences among the RP sources and (b) properties of the soil. Soils with a higher P fixing capacity also enhance the release of P from

RP as reported by Chien et al. (2010).

Although application of phosphate rock directly to the soil may be an economically attractive alternative to the use of imported soluble fertilizers, direct application requires that it is applied at higher rates as a result of its lower solubility and reduced P content, which consequently increases the transportation cost (Omamo, 1998).

The effectiveness of rock phosphate as a direct amendment to soils have been found to be controlled by factors such as the chemical composition of the rock deposit, its particle size, reactive properties of the soil and most importantly by the rate at which isomorphic substitution of carbonate for phosphate occurs within the crystalline structure of the apatite (Mokwunye, 1995). Dissolution of applied phosphate rock depends on specific soil and moisture conditions with site - specific crop responses (Vanlauwe *et al.*, 2000 b).

#### 2.4.2 Approaches to enhancing solubility of rock phosphate

The application of RP directly to soils has several advantages such as the low input cost and the fact that it releases P to the soil at a much slower rate (Sale and Mokwunye, 1993). Rock phosphate as P fertilizer also has higher content of Ca which ranges

between 24 - 33% as stated by Zin *et al.* (2005). This makes its application advantageous in liming soils in the long term by way of increasing soil pH and cation exchange capacity. The soil's physical and chemical characteristics are also improved which ultimately leads to improved plant nutrition (Chien and Menon, 1995 b).

In spite of these advantages associated with PR, its low solubility has been a major disincentive for its recommendation as an alternate P source for crops. This is mainly due to the fact that a larger portion of P dissolved is immediately adsorbed and immobilized, with only a fraction (30-50%) of the dissolved P being available for uptake by plants (Bolan *et al.*, 1990).

According to Bado (1998), rock phosphate sometimes may fail to dissolve for the release of phosphorus during its first time of application, while in others its phosphorus use efficiency can exceed that of triple super phosphate. The release of P by rocks of poorer quality can be readily improved by partial acidulation thereby increasing P availability by about 10% compared to mineral fertilizers, although this benefit does not greatly inhibit the release of P over several years (Sanginga and Woomer, 2009). Other approaches to increase the solubility of RP involve the application of P - solubilizing microorganisms (Gupta *et al.*, 2007). The capacity of P - solubilizing microorganisms to solubilize P is determined by their ability to reduce pH of the soil through the release of organic acids such as citrate, lactase and succinates. These acids can either dissolve mineral phosphates directly through anion exchange or chelate the cations mainly calcium as well as Fe and Al ions associated with phosphate. By this process, insoluble phosphorus is converted into soluble monobasic (H<sub>2</sub>PO<sub>4</sub><sup>-</sup>) or dibasic (HPO<sub>4</sub><sup>2-</sup>) forms leading to increased P availability (Gyaneshwar *et al.*, 2002).

Chien *et al.* (1996) also suggested the compaction of RP with water-soluble P fertilizers as another means of enhancing the effectiveness of RP but this method as well as acidulation and compaction with water-soluble P are all expensive processes and would result in an increase in the farmer's fertilizer input cost.

One cost effective method of improving the solubility of RP is co-application with onfarm organic fertilizers such as farmyard manure. In assessing the agronomic effectiveness of natural rock phosphate with poultry manure, Agyin-Birikorang *et al.* (2007) indicated that application of poultry manure with RP at a ratio of 1:1, rendered the RP equally effective as TSP in agronomical terms.

Studies by Mokwunye (1995) and Buresh *et al.* (1997) revealed that large application of rock phosphate at one time had a positive residual effect on crop yields for several continuous cropping seasons and this have been used as a justification for the use of rock phosphate to improve soil P status. From the view point of Sanginga and Woomer (2009), RP can become widely accepted and used by farmers in Africa if the fertilizer industry is able to improve on the solubility of non reactive RPs by way of co granulation or partial acidulation as the decision by farmers to apply rock phosphate is determined by its cost - effectiveness (Buerkert *et al.*, 2001).

#### 2.5 Phosphorus fertilizer nutrient use efficiency

Legume cultivation in the tropics and sub-tropics mostly occur on soils with low P content and this is mainly due to processes such as weathering, erosion and P fixation (Wang *et al.*, 2010). This therefore makes low P availability one of the major limiting factors for legume production in these areas. According to Fageria *et al.* (2008), a plant is said to be nutrient efficient when it is able to give a relatively higher yield for a unit

of nutrient supplied or taken up compared to other plants growing in a similar agroecological environment.

Phosphorus use efficiency (PUE), Phosphorus utilization efficiency (PUtE) and Phosphorus uptake efficiency (PUpE) are among the major agronomic indices that have been used to describe phosphorus fertilizer nutrient use efficiency. According to Mosier *et al.* (2004), PUE or agronomic efficiency of phosphorus (AEP) is the crop yield (kg) per kg of P nutrient applied. This efficiency index gives an indication of the increase in productivity that is gained by using a particular nutrient. Phosphorus uptake efficiency or P recovery efficiency (PRE) is the amount of nutrient (kg) in harvested crop per kg nutrient applied. This efficiency index determines how much nutrient is taken out of the system compared to its nutrient input. Phosphorus utilization efficiency or physiological efficiency of phosphorus (PEP) is the yield (kg) per kg nutrient taken up by the plant. This gives an indication of the efficiency at which P taken up by the plant is utilized for producing economic yield.

Studies by Mosier et al. (2004) indicate that in the short term, all these fertilizer use efficiency indices increase with a reduction in the amount of fertilizer applied even to levels well below optimum economic levels. One might be led to falsely conclude that application of the lowest fertilizer rates results in the most efficient cropping system while this is not the case.

Monitoring P fertilizer use efficiency in legumes is necessary for optimization of soil available P for plant growth as well as minimize the effect of P fertilizer application on the environment. This is especially necessary for P fertilizers that are water soluble as they represent a majority of P fertilizers used for crop cultivation worldwide (Chien *et al.*, 2012).

Phosphorus recovery efficiency from soils varies depending on the method used. According to Syers *et al.* (2008), the recovery of applied P fertilizer and residual soil P normally ranges between 50 to 90% when estimated by the balance method. Chien *et al.* (2011), however argued that recovery of P fertilizer using the difference method ranges from 10 to 25%. The recovery of P fertilizer according to this method can however be lower or higher depending on factors such as P fertilizer rate applied, soil available P level, and P sorption capacity of the soil. Soils with relatively high P sorption capacity will have almost 0% P fertilizer recovery for low rates of P applied due to sorption of P on soil surfaces (Stevenson, 1986), while the recovery could be much higher than 25% in soils with low P sorption capacity.

There is genetic variability in plant P efficiency and P efficient plants have several adaptive mechanisms by which they are able to grow well in soils with low P (Wang *et al.*, 2010). Among these mechanisms are modifications in root morphology and architecture, secretion of phosphatases and organic acids into the rhizosphere and root symbiosis (Vance *et al.*, 2003; Gahoonia and Nielsen, 2004 a). For soybean, root architecture and morphology, root exudates and root symbiosis have been found to be the most important traits for efficient uptake of P fertilizer (Wang *et al.*, 2004; Cheng *et al.*, 2008).

In assessing P use efficiency of soybean as affected by phosphorus application and inoculation, Shah *et al.* (2001) reported that inoculation increased PUE and PUpE of soybean while P fertilizer application decreased PUE. They also established that P application and inoculation effect on yield of soybean was mainly through their effects on PUE. Ogoke *et al.* (2006) corroborated this finding by reporting that P application decreased both PUE and PUtE in soybean in the moist savanna zone of West Africa.

Sanginga *et al.* (2000) also reported that P use efficiency in cowpea decreased with increasing P application. In contrast to PUE, PUpE however increased with the amount of P applied. Other yield variables such as dry matter production, shoot to root ratio, total shoot N and N fixed were strongly related to P uptake efficiency.

According to Bar-Josef (1991), there are three ways by which plants can improve on their access to both native and applied soil P. These include i. increasing their absorptive areas, ii. favorable modification of absorption to increase uptake from low nutrient concentrations and iii. by modification of the rhizosphere to increase availability of nutrients (Bar-Josef, 1991). Plant genotypes that are P efficient are able to adjust their shoot to root ratio under different P levels. These plants when under P stress also invest more energy in proliferation of their root biomass to increase the area in contact with soil root.

Fertilizer efficiency in tropical farming systems is different from that in the temperate regions due climatic factors such as high temperature and intensive rainfall coupled with highly weathered and highly permeable soils with low capacity for retention of ions (Baligar and Bennett, 1986 a)

According to Baligar and Bennett (1986 a), fertilizer use efficiency in tropical farming systems is influenced by (a) soil factors (b) efficiency of crops, (c) climatic factors, (d) nature of fertilizer materials, (e) fertilizer application practices and (f) fertilizer efficiency modifiers. The soil factors do have significant influence on transformation, fixation (adsorption) and losses of N, P and K through leaching. The major soil factors include texture, proportion and amounts of clay, organic matter content, the cation exchange capacity, and concentration of ions on the exchange complex, the capacity of the soil to release ions, soil pH, soil moisture, soil temperature, soil aeration and soil

compaction. Use efficiency of applied fertilizer varies depending on the type fertilizer nutrient. Nitrogen efficiency is about 50% or lower with that of phosphorus varying between 10-30 % while that of potassium is between 20-40% (Baligar and Bennett, 1986 b).

#### 2.6 Value - cost ratio analysis

Legume production in northern Ghana is limited by low soil fertility and phosphorus deficiency has been identified as a major constraint to sustainable crop production in this region (CSIR-RELC, 2005). Most small scale farmers cannot afford the high cost of using water-soluble P fertilizers. This therefore calls for the need to identify other cheaper sources of P fertilizers to increase crop production. Rock phosphate- based fertilizers offer such cheaper means of increasing crop production on P deficient soils. Yara legume, a rock phosphate fertilizer blend is one of such fertilizers manufactured by Yara Ghana Ltd. for legume production in northern Ghana.

Farmers will be willing to use most recommended fertilizer intervention packages if it is proven to increase their crop yield at a relatively cheaper cost than other fertilizers. The value - cost ratio (VCR) analysis has been used by many researchers as a means of determining the economic profitability of the use of a particular intervention in a farming enterprise. In agricultural terms, VCR refers to the ratio of the farm output relative to the input costs expressed in monetary terms. Morris (2007) defines economic viability as when the value cost ratio is greater than or equal to 2. A value - cost ratio of 1 is considered as break-even point, where the monetary value of the farm output is equal to the cost of input. The enterprise is said to run at a loss when the VCR is less than 1, in which case the value of the output does not meet the input cost.

#### 2.7 Summary of literature review

Soybean and cowpea are major source of income to many smallholder farmers in northern Ghana. In spite of the huge economic and nutritional benefits associated with the cultivation of these legumes, their production is mostly limited by several factors including soil mineral nitrogen, soil P deficiency, high soil temperature, population size and effectiveness of indigenous soil rhizobia. Soil P deficiency is a major limiting factor to increased grain legume production due to the important role that phosphorus plays in the nitrogen fixation process and plant growth. Although P fertilizer application has been advocated as a means through which farmers can improve on their farm yields and income, most farmers are not able to apply the right quantities of these fertilizers or do not apply them at all. This is due to the high cost of these fertilizers which are mostly imported and this has necessitated the need to obtain other P fertilizer sources that is affordable to the smallholder farmer.

Rock phosphate is one of such P fertilizer sources. "Yara legume" is a rock phosphate fertilizer formulation for legume production in northern Ghana. Application of this fertilizer blend is expected to increase legume grain yield through alleviation of phosphorus and other nutrient deficiencies. The short term effect of this fertilizer on yield of soybean and cowpea has, however, not been thoroughly investigated against the background that P release from rock phosphate is much slower than water soluble fertilizers such as triple superphosphate. Moreover, the effect of rock phosphate fertilizer application on P uptake and use efficiency, stimulation of plant N uptake in low N environments and its economic implications for farmers in soybean and cowpea production need to be well-documented.

Application of lower rates of nitrogen fertilizer is another means of increasing soil available N for legumes with high N demand such as soybean in low soil N environments. Although there have been significant yield responses to N fertilizer application in cases where plant N demand could not be solely met by the N<sub>2</sub> fixation process, some studies indicate that yield response of soybean to N fertilizer is not consistent. Much information is, therefore, needed on whether N fertilizer application to legumes such as soybean and cowpea can alleviate plant N limitations in a costeffective manner without necessarily compromising on the crop's capacity to fix nitrogen.



#### 3.1 Description of the experimental sites

In this study, field experiments were carried out at two locations (Figure 3.1) in northern Ghana to assess soybean and cowpea productivity as influenced by rock

phosphate fertilizer blend and rhizobia inoculants application. Laboratory studies were conducted at the soil microbiology laboratory of Soil Research Institute,

Kwadaso, Kumasi and the Chemistry laboratory at the Faculty of Agriculture, Kwame Nkrumah University of Science and Technology, Kumasi.

# 3.1.1 Bontanga experimental site

The first field experiment was conducted from February to May 2012 at the

Bontanga Irrigation Project site near Nyankpala in the Kumbungu district of the Nothern Region. This area was selected because it is a farming hub for the local community with farmers engaged in soybean and cowpea cultivation among other crops such as rice and vegetables. The area lies within the interior Guinea Savanna zone of Ghana (Latitude 09° 36' 12.0" N, Longitude 001° 01' 54.2" W) at an altitude of 108 m above sea level. The climatic condition of the area is warm semi-arid with annual mono-modal rainfall between 800 and 1200 mm which occurs between May and September (SARI, 2002). This is followed by 7 months of dry season characterized by the North - East Trade Winds (Harmattan) which are dry and dusty with associated low relative humidity during this period. The area has mean monthly temperature range of 26 °C to 32 °C with March and April as the warmest months. The soil is classified as a Gleyic Lixisol (FAO, 1998). The site had previously been cultivated to upland rice.

# 3.1.2 Nyankpala experimental site

The second experiment was conducted from July - November, 2013 at the Savanna Agricultural Research Institute (SARI) experimental field at Nyankpala in the Tolon district of the Northern Region. The area lies within the interior Guinea Savanna zone of Ghana (Latitude 09°23' 35.9" N, Longitude 001° 00' 22" W) at an altitude of 185

m above sea level. The soil is sandy loam and is derived from Voltaian sandstone and classified as a Ferric Luvisol (FAO, 1998). The site had been cultivated to maize during the previous planting season.

#### 3.2 Soil sampling and sample preparation

Prior to establishment of the experimental fields, an auger was used to collect 15 core soil samples from a depth of 0 - 20 cm at 5 m interval along a Z - plane on each of the experimental fields. Samples from each field were bulked and thoroughly mixed in a bucket after which sub-samples were put in plastic bags and sent to the laboratory. At the laboratory, the soils were air dried, crushed and passed through a 2 mm sieve mesh. The sieved soil was mixed thoroughly and stored in polyethylene bags for laboratory analysis. Samples of fresh moist soil were kept frozen in a refrigerator and later used for the determination of available soil nitrate (NO<sup>-</sup><sub>3</sub>-N) content. Those to be used for estimation of the indigenous soil rhizobia population were kept at 4 °C.





Fig. 3.1 Regional map of northern Ghana showing locations of the study sites

# **3.3 Determination of soil chemical properties**

# 3.3.1 Soil pH

Soil pH was determined using a glass electrode pH meter (WAG-WE 30200, United Kingdom) in a 1: 2.5 (soil: distilled water ratio). Twenty grams of the soil sample was weighed into a 100 ml plastic beaker. Using a measuring cylinder, 50 ml of distilled water was added to the soil, stirred thoroughly and left to stand for 30 minutes. The pH meter was first calibrated with buffer solutions at pH 4.0 and 7.0, after which the soil pH was determined by immersing the electrode into the upper portion of the suspension and the reading displayed was recorded.

# 3.3.2 Total nitrogen

Determination of soil total nitrogen was done by using the macro Kjeldahl method involving digestion and distillation as described by Soils Laboratory Staff (1984). A 0.5 g soil sample was weighed into a Kjeldahl digestion flask and 5 ml distilled water was added. Five millilitres of concentrated sulphuric acid and selenium mixture were added after 30 minutes, mixed carefully and digested for 3 hours. The digest was diluted with 50 ml distilled water and left to cool. The digest was made to 100 ml with distilled water and thoroughly mixed. A 25 ml portion of the digest was transferred to the reaction chamber of the Kjeldahl apparatus and 10 ml of 40% NaOH solution was added. The mixture was then distilled and the distillate was collected in 2 % boric acid. Using Bromocresol green as an indicator, the distillate was titrated against 0.1 *M* HCl solution. A blank distillation and titration were also done to account for traces of nitrogen in the reagents and the water used.

Calculation:

%

Ν

M□□a □ b□□1.4□mcf □ v s□ t

ADH

where:

 $M = \text{concentration of HCl used in titration} \quad a = ml$ HCl used in sample titration b = ml HCl used in blank titration s = weight of air-dried sample in grams mcf =moisture correction factor (100 + % moisture) / 100 1.4 = 14 x 0.001 x 100% (14 = atomic weight of nitrogen) v = total volume of digest, t = volume of aliquot taken

for distillation

#### 3.3.3 Soil organic carbon

The modified Walkley and Black procedure by Nelson and Sommers (1982) was used to determine soil organic carbon. The procedure involved wet combustion of the organic matter with a mixture of sulphuric acid and potassium dichromate. This was followed by titration of the excess dichromate against ferrous sulphate solution. One gram soil was weighed into a conical flask. A reference sample and a blank were also included. Ten millilitres of 0.166 M potassium dichromate solution was added to the soil and the blank flasks. To this, 20 ml of concentrated sulphuric acid was added from a measuring cylinder, swirled and left to stand for 30 minutes on an asbestos sheet. Distilled water (250 ml) and 10 ml concentrated orthophosphoric acid were added, swirled and allowed to cool. The solution was then titrated against 1.0 M ferrous sulphate solution using 1ml of diphenylamine as indicator.

Calculation:

% Organic C = M

 $\Box 0.39 \Box \Box V_1 \Box$ 

V<sub>2</sub>□□mcf s

where:

M = molarity of ferrous sulphate solution

 $V_1$  = volume (ml) of ferrous sulphate solution required for blank titration

V = volume (ml) of ferrous sulphate solution required for sample titration  $^{2}$ 

 $0.39 = 3 \ge 0.001 \ge 100 \ \% \ge 1.3$  (3 = equivalent weight of C) mcf

- = moisture correction factor (100 + % moisture) / 100
- 1.3 = a compensation factor for incomplete combustion of the organic matter.

s = weight of air - dry sample in grams

#### 3.3.4 Available phosphorus

All forms of available phosphorus in the soil that were readily soluble under acidic conditions were extracted with Bray 1 solution (HCl: NH F mixture) as described by

4

Bray and Kurtz (1945). Phosphorus in the sample was determined using a Spectrum lab 23A (Lemfield Medical, England) spectrophotometer by measuring the colour intensity developed by the blue ammonium molybdate with ascorbic acid as a reducing agent. A 5 g portion of the soil sample was weighed into 100 ml extraction bottle with 35 ml of Bray 1 solution (0.03*M* NH F and 0.025*M* HCl) was added. The

bottle was placed in a Stuart (United Kingdom) reciprocal shaker and shaken for 10 minutes after which it was filtered through a Whatman No. 42 filter paper. A 5 ml aliquot of the filtrate was pipetted into 25 ml flask and 10 ml colouring reagent (ammonium paramolybdate) was added followed by a pinch of ascorbic acid. The contents were well-mixed and allowed to stand for 15 minutes to develop a blue colour. The colour intensity was measured using a Spectrum lab 23A (Lemfield Medical, England) spectrophotometer at wavelength of 660 nm. The available phosphorus was extrapolated from a standard curve.

A standard series of 0, 1.2, 2.4, 3.6, 4.8 and 6.0 mg P  $L^{-1}$  was prepared by pipetting 0, 10, 20, 30, 40 and 50 ml of 12.0 mg P  $L^{-1}$  in 100 ml volumetric flask respectively and made to volume with distilled water.

35□15□□a

 $\Box b \Box \Box mcf s$ 

Calculation:  $P(mg kg_{-1}) = .$ 

where:

 $a = mg P L^{-1}$  in the sample extract

 $b = mg P L^{-1}$  in the blank mcf =

moisture correction factor

35 = volume of extracting solution

15 =final volume of sample solution

s = sample weight in grams

# 3.3.5 Available nitrogen

# **3.3.5.1 Preparation of extract**

Twenty millilitres of  $0.5 M \text{ K}_2\text{SO}_4$  was added to 5 g of wet soil sample and shaken for one hour and filtered. One millilitre aliquot of this was taken for the determination of  $NO_3^-$  - N. Soil moisture was determined to allow for the expression of  $NO_3^-$ -N on dry weight basis.

# 3.3.5.2 NO<sub>3</sub><sup>-</sup> N- salicylic acid method

One millilitre each of the extract solution (sample) and a standard were transferred into marked test tubes using a pipette. One ml of 5% salicylic acid solution was then added to each of the test tubes, after which the contents was mixed on a vortex mixer and left to stand for 30 minutes. Ten millilitres of 4 *M* sodium hydroxide solution was added, mixed and was left for an hour for full colour development. The absorbance of the coloured solution and the standard were determined on a

Spectrophotometer at a wavelength of 410 nm against a reagent blank solution using 1 ml of concentrated sulphuric acid. Nitrate - N concentration in the sample was calculated as follows:

NO<sup>-</sup><sub>3</sub>- N (
$$\mu$$
g g<sup>-1</sup>) =  $\underline{\qquad}^{C\Box V}_{W}$ 

where:

 $C = corrected concentration (\mu g ml^{-1})$ 

V = volume of extract (ml)

W = weight of sample (g)

# 3.3.6 Extraction of exchangeable cations

Calcium, magnesium and potassium in the soil were determined in 1.0 *M* ammonium acetate (NH OAc) extract. Ten grams of soil sample was transferred into a leaching

tube and leached with 250 ml of buffered 1.0 *M* ammonium acetate (NH<sub>4</sub>OAc) solution at pH 7.

# 3.3.6.1 Determination of exchangeable calcium and magnesium

A 25 ml portion of the 1.0 *M* ammonium acetate extract was transferred into a conical flask and the volume made to 50 ml with distilled water. A 1 ml portion of potassium

ferrocyanide (2%), 1 ml of hydroxylamine hydrochloride, 1 ml of potassium cyanide (2%), 10 ml of ethanolamine buffer and 0.2 ml Eriochrome Black T solutions were added. The mixture was titrated against 0.01 M ethylene diamine tetraacetic acid (EDTA) to a pure turquoise blue colour. A 20 ml 0.01 M EDTA and 25 ml of 1.0 M ammonium acetate solutions were added to provide a standard blue colour for titration. The titre value was again recorded. The titre value of calcium was subtracted from this value to get the titre value for magnesium.

#### **3.3.6.2 Determination of calcium**

A 25 ml portion of 1.0 M ammonium acetate extract was transferred into a 250 ml conical flask and the volume made to 50 ml with distilled water. One millilitre portion each of hydroxylamine hydrochloride, potassium cyanide (2%) and potassium ferro cyanide (2%) were added. This was followed by the addition of 4 ml of 8 M potassium hydroxide and a spatula of muroxide indicator. The solution obtained was then titrated against 0.01 M EDTA solution to a pure blue colour.

Twenty mililitres of 0.01 M calcium chloride solution was titrated with 0.01 M EDTA in the presence of 25 ml 1.0 M ammonium acetate solution to provide a standard pure blue colour and the titre value of calcium was recorded.

Calculation:

Mg = (Ca + Mg) - Ca

Concentration of Mg or Ca (cmol c kg-1) = \_\_\_\_\_0.01  $\Box V_a$  $\Box V_b \Box 1000$ 

0.10 W

where:

W = weight (g) of air - dry soil

V = volume (ml) of 0.01 *M* EDTA used in the sample titration a

V = volume (ml) of 0.01 *M* EDTA used in the blank titration b

0.01 =concentration of EDTA used

# 3.3.7 Determination of exchangeable potassium

Potassium in the percolate was determined by flame photometry (Cottenie, 1980). A standard series of potassium concentrations were prepared by diluting 1000 mg L<sup>-1</sup> potassium solution to 100 mg L<sup>-1</sup>. This was done by taking a 25 mg portion into a 250 ml volumetric flask and made to volume with water. Portions of 0, 5, 10, 15 and 20 ml of the 100 mg L<sup>-1</sup> standard solution were put into separate 200 ml volumetric flasks. To each of the flasks, 100 mls of 1.0 *M* NH OAc solution was added and

made to volume (200 ml) with distilled water. The standard series obtained was 0,

2.5, 5.0, 7.5, 10.0 mg L<sup>-1</sup> for potassium. Potassium was measured directly in the percolate using a Jenway PFP 7 (United Kingdom) flame photometer at wavelengths of 766.5 nm.

Calculation:

Exchangeable K (cmol c kg-1 soil) =  $250\square \square a \square$ 

b mcf 10 39.1 s

4

where:

 $a = mg K L^{-1}$  in the diluted sample b =

mg K  $L^{-1}$  in the diluted blank sample s =

SAN

air - dried soil sample weight in grams

mcf = moisture correcting factor

#### 3.4 Determination of soil physical and biological properties

# **3.4.1 Particle size distribution**

This was determined by the Bouyoucos hydrometer method (Bouyoucos, 1936). A 40 g soil was weighed into 250 ml beaker and oven dried at 105 °C over night. The sample was placed in a desiccator to cool, after which the oven dry weight was taken. A 100 ml of dispersing agent sodium hexa - metaphosphate was added to the soil. It was then placed on a hot plate and heated until the first sign of boiling was observed. The content of the beaker was weighed into a shaking cap and fitted to a shaker and shaken for 5 minutes. The sample was sieved through a 50 µm mesh sieve into a 1.0 L cylinder. The sand portion was dried and further separated using graded sieves of varying sizes into coarse, medium, and fine sand. These were weighed and their weights recorded. The 1.0 L cylinder containing the dispersed sample was placed on a vibration-less bench and then filled to the mark. It was covered with a watch glass and allowed to stand overnight. The hydrometer method was used to determine the silt and the clay contents. The cylinder with its content was agitated to allow the particles to be in suspension. It was then placed on the bench and hydrometer readings taken at 40 seconds and 6 hours interval. At each hydrometer reading, the temperature was also taken.

The percent sand, silt and clay were calculated as follows:

% Clay = corrected hydrometer reading at 6 hours x 100/weight of sample

% Silt = corrected hydrometer reading at 40 seconds x 100/weight of sample - % clay.

The various portions were expressed in percentage and the textural triangle was used to determine the soil textural class.

# 3.4.2 Determination of indigenous soil rhizobia population and number of viable bradyrhizobia in the commercial inoculant

The Most Probable Number (MPN) enumeration procedure by Woomer et al. (1990) was used to estimate the indigenous soil rhizobia populations and the number of viable bradyrhizobia cells  $g^{-1}$  of the commercial inoculant.

# 3.4.2.1 Planting of seeds and inoculation in plastic growth pouches

Soybean and cowpea seeds of high viability (95 – 100%) were surface sterilized in 95% ethanol for one minute followed by three minutes wash in 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The seeds were washed in five changes of distilled water and soaked in water for 3 hours for the seeds to imbibe water. The seeds were then transferred into Petri dishes lined with moist filter paper after which it was covered with aluminium foil and kept in an incubator at a temperature of 26 °C to pregerminate. Using sterilized forceps, seeds of similar radicle length were transferred aseptically onto the trough of the paper wick in the growth pouches filled with 30 ml of Broughton and Dilworth N – free plant nutrient solution (Appendix 1). To prevent the growing radical from pushing the seed out of the pouch, a hole was made in the trough of the wick using a fine- tipped sterile forceps while the wick was wet and the radicle was inserted into the hole during planting. For the estimation of indigenous soil rhizobia population, 30 healthy plants

were selected for inoculation with dilutions from the soil sample when the plants were 7 days old.

For the estimation of viable bradyrhizobia cells in the commercial inoculant, fifty plants were selected for inoculation with serial dilutions made from the inoculant.

A five - fold serial dilution of the soils to be tested was made by weighing 100 g of soil into 400 ml of sterilized distilled water and shaken vigorously by hand for five minutes to obtain a homogeneous mixture. For the commercial inoculant, a ten-fold serial dilution of the inoculant was made by weighing 10 g of the inoculant into a 1 L flask containing 90 ml of sterile distilled water. Using a micropipette, 1 ml each of the six dilution series ( $5^{-1}$  to  $5^{-6}$ ) from the soil sample was inoculated at the base of the four replicates of plants in each set of dilution, starting from the highest dilution down to the lowest in the series using the same pipette. Similarly, 1 ml each of the

dilution series  $(10^{-1} \text{ to } 10^{-10})$  from the commercial inoculant was inoculated at the base of each one of the four replicates in each set. One pouch from each set was left uninoculated to serve as the control. The plants were observed periodically while nutrient solution was replenished when necessary. The final observation was made after 3 weeks for the presence (+) or absence (-) of nodules (Appendix 2 - 6). The most probable number of bradyrhizobia cells g<sup>-1</sup> of soil and the commercial inoculant were estimated using the MPNES software developed by Woomer et al. (1990).

#### 3.5 Plant tissue analysis

# 3.5.1 Total phosphorus

A 0.5 g portion of plant sample was weighed into a crucible and placed in a muffle furnace at a temperature of 500  $^{\circ}$ C for three hours. The ash sample was used to prepare

ash solution by adding 10 ml of 5.3*M* HNO<sub>3</sub> to the sample. The ash solution was placed on a hot plate till first sign of boiling was observed. The solution was then filtered into a 100 ml receiving flask. The filter paper was washed twice with 10 ml of distilled water and made up to volume (100 ml). Fifty millilitres portion of the filtrate was measured into a 100 ml flask and 10 ml each of ammonium molybdate and ammonium vanadate solution were added and was made to volume with distilled water. The solution was shaken thoroughly and allowed to stand for 30 minutes for colour development. The absorbance of the yellow colour developed was read on a spectrophotometer at a wavelength of 470 nm. The P content of the observed absorbance was determined from the standard curve. A standard series of 4, 8, 12, 16, and 20  $\mu$ g P ml<sup>-1</sup> were prepared by pipetting respectively 2, 4, 6, 8, and 10 ml of standard solution (200  $\mu$ g P ml<sup>-1</sup>) into 100 ml volumetric flask and made to volume with distilled water.

Calculation:

P content (%) =  $\__C \] C \] df \] 100$ 

where:

C =concentration of P (µg ml<sup>-1</sup>) as read from the standard curve df

= dilution factor

# 3.6 Determination of nutrient concentration in rock phosphate fertilizer

The rock phosphate fertilizer blend used in this study was characterized by determining its total phosphorus, citric acid soluble P, potassium, calcium and magnesium contents.

# 3.6.1 Determination of total phosphorus, potassium, calcium and magnesium

The total phosphorus content was determined following wet digestion of the sample in concentrated HNO<sub>3</sub>-60% HClO<sub>4</sub> mixture. The fertilizer was first homogenised by grinding and 0.5 g portion of it was weighed into a 50 ml volumetric flask. Ten millilitres of the 0.53*M* HNO<sub>3</sub> and 60% HClO<sub>4</sub> mixture were added and the flask was swirled to mix its contents. The flask was placed on a hot plate inside a fume hood and heated to a temperature of 80 °C. The temperature was further raised to 150 °C and heating was continued until the production of red NO<sub>2</sub> fumes ceased. The contents were heated till the volume was reduced to about 3 ml and became colourless, but was not allowed to dry. The digest was allowed to cool and made to volume (50 ml) with distilled water after which it was filtered through Whatman No

42 filter paper.

Using a pipette, 5 ml aliquot of the filtrate was taken into a 25 ml flask and 10 ml colouring reagent (ammonium paramolybdate) was added followed by a pinch of ascorbic acid. After mixing well, the mixture was then allowed to stand for 15 minutes to develop a blue colour. The colour was measured using a Spectrum Lab 23A (Lemfield Medical, England) spectrophotometer at a wavelength of 660 nm. The total phosphorus content of the sample was extrapolated from a standard curve. Calcium and magnesium contents of the filtrate were determined using Atomic Absorption Spectrometer while potassium was estimated using Flame Photometer.

# 3.6.2 Determination of citric acid soluble phosphorus

Citric acid soluble P content of the rock phosphate fertilizer blend was determined by shaking the fertilizer in 2% citric acid solution. Ten grams of ground fertilizer sample was weighed into an extraction bottle and 200 ml of 2% citric acid solution was added. The bottle was placed on a Stuart (United Kingdom) reciprocal shaker and shaken for
10 minutes, after which it was filtered through Whatman No. 42 filter paper. A 1ml aliquot of the filtrate was pipetted into a 100 ml flask and made to volume with distilled water for colour development and reading of the absorbance on a spectrophotometer for determination of extractable P content of the fertilizer sample as described in section 3.6.1 above. Calculations : DDf Total P (%) Absorbance 0.033 10,000 Emission Total K (%) 0.494 10,000 Total Ca/Mg (%) Absorption<sup>DDf</sup> 10,000 where, Df = Dilution factor 10,000 =Conversion factor from ppm to %

0.033 and 0.494 = Gradient of a straight line from a standard curve for P and K, respectively on spectrophotometer.

#### 3.7.1 Source of planting materials and bradyrhizobia inoculants

Both soybean and cowpea seeds as well as the commercial inoculants were obtained from Savanna Agricultural Research Institute. The inoculants were imported for N2 Africa dissemination activities. Soybean (Jenguma) and cowpea (Songotra) were used as test crops for this study. Jenguma is an improved (non - shattering) medium maturing variety (105 - 110 days maturity) with potential yield of 2.5 t ha<sup>-1</sup> and takes 45 days to attain 50% flowering (Dugje *et al.*, 2009). Songotra is an erect variety with moderate resistance to *Striga gesnerioides*. It has a potential yield of 3 tons ha<sup>-1</sup> and takes 69 -75 days to reach full maturity (Fosu *et al.*, 2012).

#### **3.7.2 Land preparation**

The field was ploughed, harrowed and ridged using a farm tractor. Ridges were made at an inter row spacing of 0.75 m and 0.60 m apart for soybean and cowpea, respectively. The field was sprayed with Pendimethalin (400 EC, 400g L<sup>-1</sup>), a pre emergence herbicide at a dosage of 3 Litres active ingredient ha<sup>-1</sup> to suppress weed growth. Subsequently, weeding was done manually using hand hoe as and when necessary.

#### 3.7.3 Field experimental design and treatments used

The experiments at all the study sites were laid out in a randomized complete block design with split-plot arrangement of the treatments which were replicated three times. Main and sub plots measured 4.5 m  $\times$  15 m and 3 m  $\times$  4.5 m, respectively. The treatments imposed at the various study sites are as shown below:

#### 3.7.3.1 Treatments for experiment on the Gleyic Lixisol at Bontanga

#### Main plots (Inoculation status)

T<sub>1</sub>= Inoculated (+ Rh), T<sub>2</sub>= Uninoculated (- Rh)

#### **Sub- plots (Fertilizer)**

 $T_0 = 0 \text{ kg } P_2 O_5 \text{ ha}^{-1}$  (Control)

 $T_1$  = Rock phosphate fertilizer blend (RPFB) 34.35 kg  $P_2O_5$  ha<sup>-1</sup>

 $T_2$  = Rock phosphate fertilizer blend (RPFB) 68.70 kg  $P_2O_5$  ha<sup>-1</sup>

 $T_3$ = Triple superphosphate (TSP) 34.35 kg  $P_2O_5$  ha<sup>-1</sup>

T<sub>4</sub>= Triple superphosphate (TSP) 68.70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>

3.7.3.2 Treatments for experiment on the Ferric Luvisol at Nyankpala Main plots (Inoculation)

 $T_1$ = Inoculated (+ Rh)

T<sub>2</sub>= Uninoculated (- Rh)

Sub - plots (Fertilizer)

 $T_o = 0 \text{ kg } P_2 O_5 \text{ ha}^{-1}$  (Control)

 $T_1 = Rock phosphate fertilizer blend (RPFB) 68.7 kg P_2O_5 ha^{-1}$ 

 $T_2$  = Triple superphosphate (TSP) 68.70 kg  $P_2O_5$  ha<sup>-1</sup> + 25 kg N ha<sup>-1</sup>

 $T_3$  = Triple superphosphate (TSP) 68.70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>

 $T_4$  = Rock phosphate fertilizer blend (RPFB) 68.70 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> + 25 kg N ha<sup>-1</sup> TSP

fertilizer was included in the treatments as a positive control.

#### 3.7.4 Seed inoculation, planting and pest control

Soybean and cowpea seeds were inoculated with peat-based commercial inoculant containing *Bradyrhizobium japonicum* strain 532c before planting. The inoculant was applied at a rate of 5 g kg<sup>-1</sup> of seed. The seeds were first moistened with water and the inoculant was applied by continuously stirring the seeds and the added inoculant until the seeds were uniformly coated with the inoculant after which the seeds were air-dried under a shade for 15 minutes. Plots with uninoculated soybean and cowpea seeds were planted first before the inoculated plots to avoid contamination. The seedlings were thinned at 2 weeks after planting to one seedling per hill at 5 cm apart within row for soybean and two seedlings per hill for cowpea at

20 cm apart within row spacing. The band method of fertilizer application was used. To prevent insect pest attack, two low – doses (100 ml in 15 litres of water) of Lambda Cyhalothrin (Karate 2.5 EC) insecticide were sprayed on both crops at flowering and at pod formation.

#### 3.7.5 Data collection

Data on nodulation (nodule number and dry weight), shoot and root dry matter yield, grain yield, shoot and grain N and P uptake were collected for both soybean and cowpea at both study sites.

#### 3.7.5.1 Nodulation

Ten plants used for the assessment of nodulation at 50% flowering growth stage were harvested from an area of  $0.375m^2$  (i.e.  $0.75 \text{ m} \times 0.50 \text{ m}$ ) by cutting the stem at about

5 cm above ground level. The roots were carefully dug out to a depth of 30 cm using a shovel. The roots together with detached nodules collected from the soil were placed in polyethylene bags and labelled accordingly. The roots were then washed thoroughly on a 1 mm sieve mesh under running tap water to remove adhered soil. The nodules were removed and blotted dry using a paper napkin. The nodules were then counted and oven dried at 65  $^{\circ}$ C to a constant weight after which nodule numbers and oven-dry weights were recorded.

#### 3.7.5.2 Shoot and root dry matter yield

At 50% flowering growth stage, 10 plants each of both soybean and cowpea were randomly selected from the row next to the boarder rows on each plot and harvested by cutting the stem with secateurs at 5 cm above the soil surface, bulked and sent to the laboratory. The samples were oven dried at 65 °C to a constant weight and the dry matter weight determined using an electronic balance. The below ground part of the plant was carefully dug out to a depth of 30 cm onto a plastic sheet using a shovel. The roots were carefully separated from the soil particles, washed and oven dried to constant weight at 65 °C and their respective weights recorded as root dry matter yield.

#### 3.7.5.3 Grain yield

At physiological maturity, grain yield of both soybean and cowpea were harvested from an area measuring 3.0 m<sup>2</sup> (i.e.  $1.5 \times 2.0$  m) within each plot. For soybean, all plants in the designated area from each plot were harvested and placed in sacks while only the pods were taken from the plants for cowpea. The pods after further air drying were threshed manually and were then winnowed to separate the seeds from the debris. The grains were air dried to 12% moisture content and the dry weights recorded. The dry weights were used to estimate the grain yield per hectare as described by Okogun *et al.* (2005).

Grain yield (kg ha-1) = 10000\_\_\_\_\_Harvestm<sup>2</sup>  $\Box$ areayield(m(2kg))

#### 3.7.5.4 Total nitrogen and phosphorus in shoot and grain

Nitrogen accumulation in plant shoots at 50% flowering and in harvested grain at physiological maturity was determined for both soybean and cowpea. Total nitrogen content of shoot and grain was determined by using the Kjeldahl method as described in section 3.3.2. N and P uptake in shoot and grain were calculated by multiplying the N and P concentrations of the shoot and grain by their respective shoot and grain dry weights.

#### 3.7.5.5 Phosphorus use efficiency indices

The beneficial effects of the different P fertilizers applied were evaluated by calculating the following P use efficiency indices as described by Mosier *et al.* 

(2004). Phosphorus use efficiency (PUE) (kg kg<sup>-1</sup>) Yield

Phosphorusapplied

Yield

Phosphorus utilization efficiency (PUtE) (kg kg<sup>-1</sup>) = \_\_\_\_\_

Phosphorus uptake

Phosphorus uptake efficiency (PUpE) (%)

Puptake = \_\_\_\_\_0100 Phosphorus applied

#### **3.8 Economic analysis**

The value - cost ratio analysis was used to assess the economic profitability of using the various inputs for soybean and cowpea production. Data used for the value - cost ratio determination was collected from farmers and agro-input dealers.

Calculation:

 $VCR = \frac{(Y \Box Yc)}{X}$  adopted from (Nziguheba *et al.*, 2010),

where:

Y = monetary value of the crop harvest from intervention (treated) plots

 $Y_c$  = monetary value of the crop harvested from control plots

X = monetary cost of inputs (fertilizers and seeds)

#### 3.9 Statistical analysis

Data from the field experiments were subjected to multivariate analysis of variance (MANOVA) to determine the effect of inoculation and phosphorus fertilizer on soybean and cowpea yield variables using GenStat version 11.1 (V.S.N, 2008) statistical package. Multiple pair-wise comparison and separation of treatment means were done using Fisher's protected Least Significant Difference (LSD) test at 5% level of significance.

#### **CHAPTER FOUR**

#### 4.0 RESULTS

#### 4.1 Selected physical and chemical properties of soils at the experimental sites

Data on the selected physical and chemical properties of soils at the experimental sites is presented in Table 4.1. The results indicate that the soils are low in available phosphorus, total nitrogen, nitrate-N as well as exchangeable calcium and magnesium based on soil testing interpretation manual by Landon (2014). The soils, however, had low to moderate available potassium and organic carbon content. The soils are predominantly sandy loam in texture, moderately acidic and suitable for soybean and cowpea cultivation.

#### 4.2 Indigenous soil rhizobia populations at the study sites

Results of MPN counts of the indigenous rhizobia population of soils at the study sites are presented in Table 4.1. Soils from the two experimental sites varied in their indigenous rhizobia populations for both soybean and cowpea. At all the study sites, the native cowpea rhizobia population was higher than that of soybean.

Table 4.1 Selected properties of soils at the experimental sites

#### **Experimental sites**

**Soil Property** 

Bontanga

Nyankpala

pH (1: 2.5 H <sub>2</sub> O)	$^{1}5.50 \pm 0.03$	$5.90\pm0.02$
Organic carbon (%)	1.17 ±0.01	$0.38\pm0.01$
Total nitrogen (%)	0.07 ±0.02	$0.04\pm0.01$
Exchangeable Ca (cmol <sub>c</sub> kg <sup>-1</sup> )	$2.00\pm0.15$	$2.13\pm0.01$
Exchangeable Mg (cmol <sub>c</sub> kg <sup>-1</sup> )	0.67 ± 0.10	$1.04\pm0.03$
Exchangeable K (cmol <sub>c</sub> kg <sup>-1</sup> )	$0.13 \pm 0.01$	$0.09 \pm 0.02$
ECEC (cmol <sub>c</sub> kg <sup>-1</sup> )	$3.38 \pm 0.02$	$3.80\pm0.25$
Available P (mg kg <sup>-1</sup> )	$5.22 \pm 0.44$	$7.74\pm0.51$
NO <sub>3</sub> -N (mg kg <sup>-1</sup> )	5.56 ± 0.23	$3.16\pm0.39$
Sand (%)	68.6	73.44
Silt (%)	22.20	10.00
Clay (%)	9.20	15.56
Textural Class <sup>b</sup> MPN	Sandy Loam	Sandy Loam
Count (Cells g <sup>-1</sup> soil)	<sup>c</sup> cowpea : $14.64 \times 10^2$	$6.98 \times 10^2$
	<sup>d</sup> soybean : $5.71 \times 10^1$	$2.15 \times 10^{1}$
Soil Classification (FAO, 1998)	Gleyic Lixisol	Ferric Luvisol

## 4.3 Nutrient characterization of rock phosphate fertilizer blend

Results of laboratory assessment of the concentration of some major nutrients in the rock phosphate fertilizer blend are presented in Table 4.2. The fertilizer blend contained lower concentrations of the various nutrients than that indicated on the

<sup>&</sup>lt;sup>1</sup> Means of triplicate samples ± standard error; <sup>b</sup>Most Probable Number of indigenous soil rhizobia; <sup>c</sup>cowpea as trap-host; <sup>d</sup>soybean as trap-host

product. Total phosphorus and potassium concentrations were at variance with those on the product by  $5.50 \pm 0.64\%$  and  $10.40 \pm 0.66\%$ , respectively. Calcium and magnesium concentrations were also lower by  $5.30 \pm 1.45\%$  and  $22.50 \pm 0.29\%$ , respectively compared to that indicated on the product.

Nutrient	Concentration on product (%)	Concentration from laboratory testing (%)
Total phosphorus	18	*17.00 ± 0.64
Potassium	13	$11.64 \pm 0.66$
Citric acid soluble P	ND	$1.53 \pm 0.41$
Calcium	31	$29.33 \pm 1.45$
Magnesium	2	$1.55 \pm 0.29$

Table 4.2 Nutrient concentration of rock phosphate fertilizer blend

\*Values are means of triplicate samples ± standard error; ND = not determined



#### Months



#### Fig. 4.1 Monthly rainfall distribution at Nyankpala in 2013

Fig. 4.2 Synchronized daily and cumulative rainfall distribution with growth of

soybean at Nyankpala 4.4 Effect of rock phosphate fertilizer blend and rhizobial inoculation on yield of soybean and cowpea on a Gleyic Lixisol at Bontanga

4.4.1 Phosphorus fertilizer and rhizobial inoculation effects on soybean

Results of a two-way MANOVA for phosphorus fertilizer and rhizobial inoculation effects on soybean nodule number, nodule dry weight, shoot dry matter yield, grain yield, shoot N uptake, shoot P uptake, grain N uptake and grain P uptake are presented in Table 4.3. The results indicate that rhizobial inoculation and phosphorus fertilizer had significant effects on soybean. All the variables assessed were significantly influenced by phosphorus fertilizer application and rhizobial inoculation with Wilk's Lambda values of 0.003 (p < 0.001) and 0.034 (p < 0.001), respectively, indicating that 99.7 and 96.6% of the variation in these variables could be attributed to variability in the levels of phosphorus fertilizer and rhizobial inoculation.

# 4.4.2 Effect of phosphorus fertilizer and rhizobial inoculation on soybean nodulation

Results of the effect of phosphorus fertilizers and rhizobial inoculation on soybean nodulation at 50% flowering (7 weeks after planting) representing the early reproductive growth stage (R2) are presented in Table 4.4. There were significant differences in nodule dry weight for both phosphorus fertilizer types and rhizobial inoculation treatments. The application of TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> gave the highest nodule dry weight (115 mg plant<sup>-1</sup>), while the least (49.80 mg plant<sup>-1</sup>) was obtained from the control. The application of TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> significantly (P < 0.001) increased soybean nodule dry weight by 132% over the control, while increases of

115 and 25% were obtained over those of RPFB 34.35 kg P2O5 and RPFB 68.7 kg

#### P<sub>2</sub>O<sub>5</sub> treatments, respectively.

Rhizobial inoculation increased soybean nodule dry weight significantly (P < 0.05) by 88% over the uninoculated control. There was a significant interaction between phosphorus fertilizer application and rhizobial inoculation for soybean nodule dry weight, where inoculation significantly (P < 0.001) increased soybean nodule dry weight for all the phosphorus fertilizer treatments except RPFB 34.35 kg P<sub>2</sub>O<sub>5</sub> and the control. The interactive effect of phosphorus fertilizer application and inoculation on soybean nodule dry weight is presented in Appendix 7.

Term	Df	Wilk's Lambda	Rao F	n.d.f	d.d.f	F.prob
P fertilizer (P)	4	0.00323	4.92	32	42	0.000
Inoculation (I)	1	0.03406	38.99	8	11	0.000
$(\mathbf{P} \times \mathbf{I})$	4	0.02504	2.26	32	42	0.007
			1 A A			

Table 4.3 MANOVA for phosphorus fertilizer and rhizobial inoculation effects on sovbean

n.d.f = numerator degree of freedom; d.d.f = denominator degree of freedom



of phosphorus fertilizer and

Table 4.4 E

soybean

inoculation on

nodulation on a C	Heyic Lixisol at Bontanga	
Phosphorus	Number*	Nodule dry weight
fertilizer	of nodules plant <sup>-1</sup>	(mg plant <sup>-1</sup> )
rate (P)		Т
RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>		53.80
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	9	92.20
TSP 34.35 kg P <sub>2</sub> O <sub>5</sub>	13	114.80
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	6	115.50
0 kg P <sub>2</sub> O <sub>5</sub> (Control)	5	49.80
	0.001	0.001
F pr.	< 0.001	< 0.001
LSD (5%)	1.69	19.79
CV (%)	17.80	19.00
Inoculation (I)		2
Inoculated (+ Rh)	10	111.30
Uninoculated (- Rh)	6	59.10
	1222	
F pr	0.009	0.002
LSD (5%)	1.70	9.31
CV (%)	6.20	14.40
F pr ( $P \times I$ )	0.001	< 0.001

\*Values are means of triplicate sample of 10 plants

#### 4.4.3 Shoot dry matter and grain yield

Table 4.5 shows results of the effect of phosphorus fertilizer and rhizobial inoculation on soybean shoot dry matter at 50% flowering and grain yield on the Gleyic Lixisol at Bontanga. Application of TSP at 68.70 kg  $P_2O_5$  ha<sup>-1</sup> resulted in a significantly (P < 0.001) higher shoot dry matter yield than all other treatments, representing 69, 48, 64 and 84% increase over the RPFB 34.35 kg  $P_2O_5$ , RPFB 68.70 kg  $P_2O_5$ , TSP 34.35 kg  $P_2O_5$  and control, respectively. The RPFB 68.70 kg  $P_2O_5$  treatment gave 25% increase in shoot dry matter yield over the control. The increase in shoot dry matter yield over the control from application of the phosphorus fertilizers was in the order: RPFB 68.70 kg  $P_2O_5 < TSP$  34.35 kg  $P_2O_5 < RPFB$  34.35 kg  $P_2O_5 < TSP$  68.70 kg  $P_2O_5$ .

Grain yield of soybean resulting from the application of phosphorus fertilizer and rhizobia inoculants ranged from a minimum of 265 kg ha<sup>-1</sup> to a maximum of 1,535 kg ha<sup>-1</sup> with an average yield of 760 kg ha<sup>-1</sup>. Application of phosphorus fertilizer and rhizobia inoculation resulted in significant differences in soybean grain yield. The application of phosphorus fertilizers significantly (P < 0.001) increased soybean grain yield relative to the control, while rhizobial inoculation did not. Application of RPFB 34.35 kg P<sub>2</sub>O<sub>5</sub> gave an increase in grain yield of 37% over the control compared to increases of 16, 18 and 22% from TSP 68.70 kg P<sub>2</sub>O<sub>5</sub>, RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub> and TSP 34.35 kg P<sub>2</sub>O<sub>5</sub> treatments, respectively. There was a significant interaction between phosphorus fertilizer application and inoculation, where inoculation decreased soybean grain yield with application of phosphorus fertilizer contrary to the expectation of this study. The interactive effect of phosphorus fertilizer and rhizobial inoculation on grain yield of soybean is presented in

Appendix 9.

ffect		rhizo	bial soybean		
Table 4.5 E   of phosphorus fertilizer a		fertilizer and	d inoculation on		
shoo	ot dry matter and	grain yield on a Glo	eyic Lixisol at Bontanga		
Phosphorus	fertilizer	Shoot dry	Grain yield		
Γł	ite (P)	matter yield	(kg ha <sup>-1</sup> )		
	12	(kg ha <sup>-1</sup> )	CT		
RPFB 34.35 kg	g P <sub>2</sub> O <sub>5</sub>	1581	877		
RPFB 68.70 kg	$g P_2 O_5$	1808	756		
TSP 34.35 kg I	$P_2O_5$	1623	784		
TSP 68.70 kg I	P <sub>2</sub> O <sub>5</sub>	2667	744		
0 kg P <sub>2</sub> O <sub>5</sub> (Con	ntrol)	1449	641		
F pr.		< 0.001	< 0.001		
LS <mark>D (5%)</mark>		235.70	90.10		
CV (%)	at	10.50	9.70		
Inoculation (I		2 73			
Inoculated (+ F	Rh)	1367	439		
Uninoculated (	- Rh)	2285	1082		
		- 1			
F pr	1	0.069	0.029		
LSD (5%)	10	NS	485.40		
CV (%)	2R	17.00	18.50		
F pr ( $P \times I$ )	14.	< 0.001	0.002		

NS= Not Significant at  $P \le 0.05$ 

#### 4.4.4 Nitrogen and phosphorus uptake in soybean shoot and grain

There were significant differences in shoot N and P uptake at 50% flowering as indicated in Table 4.6. Shoot N uptake was highest (75 kg ha<sup>-1</sup>) with application of TSP 68.70 kg  $P_2O_5$ , representing a significant increase of 103 and 52% over the control and TSP 34.35 kg  $P_2O_5$ , respectively. There was no significant difference in shoot N uptake between RPFB 68.70 kg  $P_2O_5$  and the control.

Soybean shoot P uptake was also highest (5.01 kg ha<sup>-1</sup>) with application of TSP 68.70 kg  $P_2O_5$ . This represented a significant increase in shoot P uptake of 127% over the control compared to 43% gain from the RPFB 68.70 kg  $P_2O_5$  treatment.

Rhizobial inoculation significantly decreased soybean shoot N uptake over the uninoculated control but the decrease in shoot P uptake was not significant.

Results of the effect of phosphorus fertilizer and rhizobial inoculation on soybean grain N and P uptake at Bontanga are presented in Table 4.7. The results indicate significant differences in grain N uptake with the application of the phosphorus fertilizers. Application of RPFB 34.35 kg P<sub>2</sub>O<sub>5</sub> gave the highest (66.66 kg ha<sup>-1</sup>) grain N uptake which was significantly higher than that from RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub>, TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> and the control. Rhizobial inoculation also had a significant interactive effect on grain N uptake with phosphorus fertilizer application (Appendix 10). There was no significant (P = 0.126) differences in grain P uptake for all the phosphorus fertilizer and rhizobial inoculation treatments imposed, neither was there a significant interaction.

#### Table 4.6 Eof phosphorus fertilizer andinoculation on

ffect	rhizobial	soybean					
shoot N and P uptake on a Gleyic Lixisol at Bontanga							
Phosphorus	PhosphorusShoot N uptakeShoot P uptake						
fertilizer rate (P)	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )					
RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	49.20	3.06					
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	54.20	3.15					
TSP 34.35 kg P <sub>2</sub> O <sub>5</sub>	49.40	3.28					
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	75.00	5.01					
0 kg P <sub>2</sub> O <sub>5</sub> (Control)	36.90	2.21					
For	0.033	< 0.001					
$\Gamma$ pr.	0.055	< 0.001					
LSD (5%)	22.48	0.95					
CV (%)	9.20	22.70					
32CA	REC	257					
Inoculation (I)	2	Z					
Inoculated (+ Rh)	34.10	2.47					
Uninoculated (- Rh)	71.80	4.22					
	1111						
F pr	0.011	0.275					
LSD (5%)	17.07	NS					
CV (%)	25.00	18.30					
F pr. (P × I)	0.400	0.070					
	and the second s						

 $NS = Not significant at P \le 0.05$ 



grain N and P uptake on a Gleyic Lixisol at Bontanga					
Phosphorus	Grain N uptake	Grain P uptake			
fertilizer rate (P)	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )			
RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	66.66	4.16			
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	56.00	3.77			
TSP 34.35 kg P <sub>2</sub> O <sub>5</sub>	59.50	3.35			
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	56.90	3.46			
0 kg P <sub>2</sub> O <sub>5</sub> (Control)	49.10	2.99			
F pr.	0.004	0.126			
LSD (5%)	7.84	NS			
CV (%)	11.10	21.20			
Inoculation (I)		125			
Inoculated (+ Rh)	33.50	1.96			
Uninoculated (- Rh)	81.80	5.21			
F pr	0.028	0.087			
LSD (5%)	35.25	NS			
CV (%)	17.40	24.40			
F pr (P × I)	0.004	0.272			

NS= Not significant at  $P \le 0.05$ 

# 4.4.5 Phosphorus fertilizer and rhizobial inoculation effects on P use efficiency indices of soybean on a Gleyic Lixisol at Bontanga

Results of a two-way MANOVA on the effect of phosphorus fertilizer and rhizobial inoculation on soybean P use efficiency (PUE), P utilization efficiency (PUE) and P uptake efficiency (PUpE) on the Gleyic Lixisol at Bontanga are presented in Table 4.8. The results indicate that rhizobial inoculation and phosphorus fertilizer had significant effects on soybean P use efficiency indices. All the P use efficiency indices were significantly affected by phosphorus fertilizer application and rhizobial inoculation with Wilk's Lambda values of 0.008 (P < 0.001) and 0.022 (P < 0.001), respectively. These indicate that 99.2 and 97.8% of the variation in the P use efficiency indices could be attributed to variability in the levels of phosphorus fertilizer and rhizobial inoculation, respectively.

Table 4.8 MANOVA for phosphorus fertilizer and rhizobial inoculation effects

on P us <mark>e efficiency indices of soybean</mark>						
Term	Df	Wilk's Lambda	Rao F	n.d.f	d.d.f	F.prob
P fertilizer (P)	3	0.00879	6.19	18	26	0.000
Inoculation (I)	1	0.02217	66.16	6	9	0.000
$(\mathbf{P} \times \mathbf{I})$	3	0.06230	2.40	18	26	0.020

n.d.f = numerator degree of freedom; d.d.f = denominator degree of freedom

## 4.4.6 Effect of phosphorus fertilizer and rhizobial inoculation on phosphorus use efficiency indices of soybean

Results of the effects of phosphorus fertilizer and rhizobial inoculation on phosphorus use efficiency indices of soybean on the Gleyic Lixisol at Bontanga are presented in Table 4.9. There were significant differences in P use efficiency (PUE) and P uptake efficiency (PUpE) of soybean with application of phosphorus fertilizers, while no significant differences were observed in phosphorus utilization efficiency (PUtE). For both phosphorus fertilizer sources, the lower P application rate of 34.35 kg P<sub>2</sub>O<sub>5</sub> significantly (P < 0.001) increased both PUE and PUpE over the corresponding higher application rate of 68.70 kg P<sub>2</sub>O<sub>5</sub>. Application of RPFB 34.35 kg P<sub>2</sub>O<sub>5</sub> significantly (P < 0.001) increased PUE and PUpE by 131 and 120%, respectively over the RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub>. Similarly, TSP 34.35 kg P<sub>2</sub>O<sub>5</sub> increased PUE and PUpE of soybean by 111 and 84%, respectively compared to the TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> treatment. Similar application rates from TSP and RPFB however, did not result in significant differences in both PUE and PUpE. Rhizobial inoculation caused a significant reduction in PUE of soybean over the uninoculated plants and interacted significantly with phosphorus fertilizer application (Appendix 11).

Rhizobial inoculation did not have any significant effect on both PUpE and PUtE of soybean, neither was there a significant interaction between inoculation and phosphorus fertilizer application for these P use efficiency indices.

NO

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Phosphorus			
fertilizer	PUE	PUtE	PUpE
rate (P)	KN	(kg kg <sup>-1</sup> )	(%)
RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	58.46	227.00	27.70
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	25.20	200.60	12.60
TSP 34.35 kg P <sub>2</sub> O <sub>5</sub>	52.29	<mark>26</mark> 0.50	22.30
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	24.80	220.20	12.10
	And and the second seco		
F pr.	< 0.001	0.217	< 0.001
LSD (5%)	6.10	NS	6.97
CV (%)	12.10	20.40	24.40
	Ell	5/3	
Inoculation (I)	So.	1 3	57
Inoculated (+Rh)	24.08	237.90	10.60
Uninoculated (-Rh)	56.30	216.30	26.80
	~		
F pr	0.033	0.727	0.115
LSD (5%)	2 <mark>5.95</mark>	NS	NS
CV (%)	18.40	23.60	26.40
F pr ( $P \times I$ )	< 0.001	0.648	0.088

efficiency indices of soybean on a Gleyic Lixisol at Bontanga

 $\overline{NS}$  = Not significant at P  $\leq$  0.05, PUE = Phosphorus use efficiency, PUtE = Phosphorus utilization efficiency, PUpE = Phosphorus uptake efficiency.

## 4.4.7 Phosphorus fertilizer and rhizobial inoculation effects on cowpea

Results of a two-way MANOVA on the effects of phosphorus fertilizer and rhizobial inoculation on cowpea nodule number and dry weight, shoot dry matter yield, grain yield, shoot N uptake, shoot P uptake, grain N uptake, and grain P uptake on the Gleyic Lixisol at Bontanga are presented in Table 4.10. The results show that rhizobial inoculation and phosphorus fertilizer treatments had significant interactive effects on cowpea yield variables. Phosphorus fertilizer application and rhizobial inoculation significantly affected all cowpea yield variables with Wilk's Lambda values of 0.0001 (P < 0.001) and 0.0162 (P < 0.001), respectively. This means that 99.99 and 98.38% of the variation in the measured variables could be attributed to variability in the levels of phosphorus fertilizer and rhizobial inoculation, respectively.

on	cowpea	5		51		2
Term	Df	Wilk's Lambda	Rao F	n.d.f	d.d.f	F.prob
P fertilizer (P)	4	0.000162	12.73	32	42	0.000
Inoculation (I)	1	0.0162 <mark>02</mark>	83.49	8	11	0.000
(P × I)	4	0.000259	11.06	32	42	0.000

Table 4.10 MANOVA for phosphorus fertilizer and rhizobial inoculation effects

**4.4.8 Effect of phosphorus fertilizer and rhizobial inoculation on cowpea nodulation** Results of the effects of rhizobial inoculation and phosphorus fertilizer application on cowpea nodulation on a Gleyic Lixisol at Bontanga are presented in Table 4.11. Application of the phosphorus fertilizers resulted in significant differences in cowpea nodule dry weight. The RPFB 34.35 kg  $P_2O_5$  gave the highest (121 mg plant<sup>-1</sup>) nodule dry weight, representing 32, 10, and 24% increase over RPFB 68.70 kg  $P_2O_5$ , TSP 34.35 kg  $P_2O_5$ , and control treatments, respectively.

Rhizobial inoculation however, decreased cowpea nodule dry weight significantly by 10% compared to the uninoculated control. There was a significant interactive effect of rhizobial inoculation and application of RPFB 34.35 kg  $P_2O_5$ , RPFB 68.70  $P_2O_5$  and TSP 34.35 kg  $P_2O_5$  treatments on cowpea nodule dry weight (Appendix 12).



Table 4.11 Effect of phosphorus fertilizer and rhizobial inoculation on cowpea

nodulation on a Gleyic Lixisol at Bontanga						
Phosphorus	*Number	Nodule dry weight				
fertilizer	of nodules plant	(mg plant <sup>-1</sup> )				
rate (P)	2 5	BAD				
RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	SAGE NO	121.20				
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	4	91.70				
TSP 34.35 kg P <sub>2</sub> O <sub>5</sub>	6	100.10				
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	7	111.00				

0 kg P <sub>2</sub> O <sub>5</sub> (Control)	4	97.70
F pr.	< 0.001	< 0.001
LSD (5%)	0.84	8.32
CV (%)	13.00	6.50
		CT
<b>Inoculation</b> (I)	KINU	21
Inoculated (+ Rh)	5	98.60
Uninoculated (- Rh)	6	110.10
	Nº M	
F pr	0.145	0.023
LSD (5%)	NS	7.59
CV (%)	10.30	12.10
F pr (P × I)	0.003	< 0.001

\*Values are means of triplicate sample of 10 plants

4.4.9 Effect of phosphorus fertilizer and rhizobial inoculation on cowpea shoot dry

#### matter and grain yield

The results in Table 4.12 indicate that there were significant (P < 0.001) differences in cowpea shoot dry matter and grain yield for the phosphorus fertilizer and rhizobial inoculation treatments imposed. The TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> gave the highest (1204 kg ha<sup>-1</sup>) shoot dry matter yield which was significantly (P < 0.001) higher than that obtained from TSP 34.35 kg P<sub>2</sub>O<sub>5</sub> and RPFB 34.35 kg P<sub>2</sub>O<sub>5</sub> treatments. There were no significant differences in shoot dry matter yield between similar levels of 34.35 kg P<sub>2</sub>O<sub>5</sub> and 68.70 kg P<sub>2</sub>O<sub>5</sub> from both rock phosphate and triple superphosphate fertilizer sources. There were significant increases in shoot dry matter yield over the control in both higher (68.70 kg P<sub>2</sub>O<sub>5</sub>) and lower (34.35 kg P<sub>2</sub>O<sub>5</sub>) application rates of phosphorus

from both fertilizer sources. TSP  $68.70 \text{ kg P}_2\text{O}_5$  treatment significantly increased shoot dry matter yield by 81% over the control, relative to 67% increase from RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub>.

Cowpea shoot dry matter yield responded negatively to rhizobial inoculation with a 34% reduction in shoot dry matter yield over the uninoculated control. Among all the phosphorus fertilizer treatments, only RPFB 68.70 kg  $P_2O_5$  increased cowpea grain yield significantly by 70% over the control.

Contrary to the expectation of this study however, rhizobial inoculation decreased grain yield significantly by 35% over the uninoculated control. There was also a significant interactive effect of phosphorus fertilizer and rhizobial inoculation on shoot dry matter and grain yield of cowpea as indicated in Appendices 13 and 14, respectively.

cowpea dry matter and grain yield on a Gleyic Lixisol at Bontanga					
Phosphorus	Shoot dry	Grain			
fertilizer rate (P)	matter yield	yield			
	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )			
RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	879	709			
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	1108	1027			
TSP 34.35 kg P <sub>2</sub> O <sub>5</sub>	865	626			
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	1204	649			
0 kg P <sub>2</sub> O <sub>5</sub> (Control)	665	605			

Table 4.12 Effect of phosphorus fertilizer and rhizobial inoculation on

F pr.	< 0.001	0.001
LSD (5%)	148.80	187.30
CV (%)	12.90	21.20
Inoculation (I)		
Inoculated (+Rh)	753	572
Uninoculated (- Rh)	1136	874
F pr.	0.023	0.049
LSD (5%)	256.60	299.00
CV (%)	7.70	11.80
F pr. ( $P \times I$ )	< 0.001	< 0.001

#### 4.4.10 Nitrogen and phosphorus uptake in cowpea shoot and grain

Table 4.13 shows the effect of phosphorus fertilizer and rhizobial inoculation on N and P uptake in cowpea shoot and grain on the Gleyic Lixisol at Bontanga. Application of RPFB 34.35 kg P<sub>2</sub>O<sub>5</sub> gave the highest (16.90 kg ha<sup>-1</sup>) shoot N uptake which was significantly (P < 0.001) higher than that of RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub> and the control. The RPFB 34.35 kg P<sub>2</sub>O<sub>5</sub> also increased shoot N uptake by 104% over the control compared to 69% by RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub>. Contrary, TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> and control treatments. There were no significant differences in shoot N uptake between TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> and RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub> treatments. However, RPFB 34.35 kg P<sub>2</sub>O<sub>5</sub> gave a 51% increase in shoot N uptake over the TSP 34.35 kg P<sub>2</sub>O<sub>5</sub> treatment.

Shoot N uptake responded negatively to rhizobia inoculant application where inoculation decreased shoot N significantly (P < 0.05) by 38%. There was a significant  $\frac{70}{70}$ 

(P < 0.001) interactive effect of rhizobial inoculation and phosphorus fertilizer application on shoot N uptake (Appendix 15).

Application of the phosphorus fertilizers resulted in increases in shoot P uptake over the control. Shoot P uptake was highest (4.57 kg P ha<sup>-1</sup>) with application of TSP 68.70 kg P<sub>2</sub>O<sub>5</sub>, which was significantly higher than the control. TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> significantly increased shoot P uptake by 182% over the control, compared to increases of 80, 70 and 79% from TSP 34.35 kg P<sub>2</sub>O<sub>5</sub>, RPFB 68.7 kg P<sub>2</sub>O<sub>5</sub> and RPFB 34.35 kg P<sub>2</sub>O<sub>5</sub> treatments, respectively.

Rhizobial inoculation gave a significant interactive effect with phosphorus fertilizer, where application of RPFB 68.7 kg  $P_2O_5$  and TSP 68.7 kg  $P_2O_5$  decreased shoot P uptake (Appendix 16).

Contrary to the trend observed in grain N uptake, application of phosphorus fertilizer and rhizobia inoculants resulted in significant differences in grain P uptake. TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> gave the highest (4.78 kg ha<sup>-1</sup>) grain P uptake representing 176% increase over the control compared to gains of 16, 65 and 90% from TSP 34.35 kg P<sub>2</sub>O<sub>5</sub>, RPFB 68.7 kg P<sub>2</sub>O<sub>5</sub>, and RPFB 34.35 kg P<sub>2</sub>O<sub>5</sub> treatments, respectively. TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> also increased grain P uptake by 138 and 68% relative to TSP 34.35 kg P<sub>2</sub>O<sub>5</sub> and RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub>, respectively. The RPFB 34.35 kg P<sub>2</sub>O<sub>5</sub> treatment increased P uptake by 63% over TSP 34.35 kg P<sub>2</sub>O<sub>5</sub>, which was statistically significant at P = 0.05.

There was a significant interactive effect of rhizobial inoculation and phosphorus fertilizer application on cowpea grain P uptake (Appendix 17).

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Table 4.13 Effect of phosphorus fertilizer and rhizobial inoculation on N and P

uptake Phosphorus	in cowpea shoot	and grain on a (	Heyic Lixisol at	Bontanga
fertilizer rate (P)	N uptake	P uptake	N uptake	P uptake
	Æ,	kg ha <sup>-1</sup>	AP	7
RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	16.90	2.90	47.30	3.28
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	14.01	2.75	39.00	2.85
TSP 34.35 kg P <sub>2</sub> O <sub>5</sub>	11.13	2.91	34.20	2.01
TSP 68.7 <mark>0 kg P<sub>2</sub>O5</mark>	13.03	4.57	43.10	4.78
0 kg P <sub>2</sub> O <sub>5</sub> (Control)	8.27	1.62	38.10	1.73
F pr.	< 0.001	< 0.001	0.304	< 0.001
LSD (5%)	1.25	2.59	NS	0.59
CV (%)	8.0	25.00	24.60	16.60

Inoculation (I)					
Inoculated (+Rh)	9.67	2.38	36.00	2.01	
Uninoculated (-Rh)	15.67	3.51	44.70	3.85	
F pr.	0.005	0.201	0.768	0.004	
LSD (5%)	1.87	NS	NS	0.49	
CV (%)	4.20	26.30	20.20	4.80	
F pr. ( $P \times I$ )	< 0.001	0.009	0.471	< 0.001	

 $NS = Non significant at P \le 0.05$ 

#### 4.4.11 Phosphorus fertilizer and rhizobial inoculation effects on phosphorus use efficiency

#### indices of cowpea on a Gleyic Lixisol at Bontanga

Results of a two-way MANOVA on the effects of phosphorus fertilizer and rhizobial inoculation on P use efficiency, P uptake efficiency and P utilization of cowpea on the Gleyic Lixisol at Bontanga are presented in Table 4.14. The results show that rhizobial inoculation and phosphorus fertilizer had significant effects on cowpea P use efficiency indices. Phosphorus fertilizer application and rhizobial inoculation had a significant influence all the P use efficiency indices with Wilk's Lambda values of 0.00358 (P < 0.001) and 0.08331 (P < 0.001), respectively. This indicates that 99.6 and 91.7% of the variation in the measured indices could be attributed to the variability in the levels of phosphorus fertilizer and rhizobial inoculation, respectively.

Table 4.14 MANOVA for	phosphorus fertilizer a	and rhizobial inoculat	ion effects
-----------------------	-------------------------	------------------------	-------------

on phosphorus use efficiency indices of cowpea						
Term	Df	Wilk's Lambda	Rao F	n.d.f	d.d.f	F.prob
P fertilizer (	(P) 3	0.00358	9.12	18	26	0.000

Inoculation (I)	1	0.08331	16.50	6	9	0.000
$(\mathbf{P} \times \mathbf{I})$	3	0.00254	10.48	18	26	0.000

n.d.f = numerator degree of freedom; d.d.f = denominator degree of freedom

4.4.12 Effect of phosphorus fertilizer and rhizobial inoculation on phosphorus use

#### efficiency indices of cowpea

Results of the effects of phosphorus fertilizer and rhizobial inoculation on phosphorus use efficiency indices of cowpea on the Gleyic Lixisol at Bontanga are presented in Table 4.15. There were significant differences in all P use efficiency indices among the phosphorus fertilizer treatments imposed. For both TSP and RP fertilizer sources, the lower P application rate of 34.35 kg P<sub>2</sub>O<sub>5</sub> significantly (P < 0.001) increased P use efficiency and P uptake efficiency over the higher application rate of 68.70 kg P<sub>2</sub>O<sub>5</sub>. RPFB 34.35 kg P<sub>2</sub>O<sub>5</sub> increased P use efficiency by 38% over

RPFB 68.70 kg  $P_2O_5$ , while TSP 34.35 kg  $P_2O_5$  gave an increase of 93% over the TSP 68.70 kg  $P_2O_5$  treatment. Application of RPFB 68.70 kg  $P_2O_5$  significantly increased P use efficiency by 58% relative to TSP 68.70 kg  $P_2O_5$ , while no significant difference in P use efficiency was observed between TSP 34.35 kg  $P_2O_5$  and RPFB 34.35 kg  $P_2O_5$  treatments.

RPFB 68.70 kg  $P_2O_5$  increased P utilization efficiency by 55 and 113% over RPFB 34.35 kg  $P_2O_5$  and TSP 68.70, respectively, while TSP 34.35 kg  $P_2O_5$  gave increases of 43 and 98% over RPFB 34.35 kg  $P_2O_5$  and TSP 68.70 kg  $P_2O_5$ , respectively. Application of RPFB 34.35 kg  $P_2O_5$  increased P uptake efficiency by 130 and 38% over RPFB 68.70 kg  $P_2O_5$  and TSP 68.70 kg  $P_2O_5$  treatments, respectively.

Rhizobial inoculation significantly decreased both P use efficiency and P uptake efficiency, while P utilization efficiency was not significantly (P = 0.156) increased by inoculation. There was a significant interaction between phosphorus fertilizer and rhizobial inoculation for all P use efficiency indices of cowpea (Appendices 18, 19 and 20).

 Table 4.15 Effect of phosphorus fertilizer and rhizobial inoculation on P use

 efficiency indices of cowpea on a Gleyic Lixisol at Bontanga

Sec.

Phosphorus		4	
fertilizer	PUE	PUtE	PUpE
rate (P) -	( <b>kg</b>	kg <sup>-1</sup> )	(%)
RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	47.30	226	21.90
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	34.20	350	9.50
TSP 34.35 kg P <sub>2</sub> O <sub>5</sub>	41.70	324	13.40
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	21.60	164	15.92
F pr.	< 0.001	< 0.001	< 0.001
LS <mark>D (5%)</mark>	7.93	74.20	3.01
CV (%)	17.40	22.00	15.70
Inoculation (I)	W JEAN	NO	St.
Inoculated (+Rh)	30.00	287	10.43
Uninoculated (-Rh)	42.50	245	19.92

F pr.	0.030	0.156	0.005
LSD (5%)	9.54	NS	2.82
CV (%)	7.50	8.80	5.30
F pr $(P \times I)$	0.002	0.013	0.007

PUE = Phosphorus use efficiency, PUtE = Phosphorus utilization efficiency, PUpE = Phosphorus uptake efficiency

#### 4.5 Effect of phosphorus fertilizer and rhizobial inoculation on soybean

Table 4.16 shows results of a two-way MANOVA on the effects of phosphorus fertilizer and rhizobial inoculation on soybean nodule number, nodule dry weight, shoot biomass yield, grain yield, shoot N uptake, shoot P uptake, grain N uptake and grain P uptake on the Ferric Luvisol at Nyankpala.

Rhizobial inoculation and phosphorus fertilizer had significant effects on soybean yield variables. All the variables were significantly affected by phosphorus fertilizer application and rhizobial inoculation with Wilk's Lambda values of 0.00032 (P < 0.001) and 0.04551 (P < 0.001), respectively indicating that 99.97 and 95.45% of the variation in the measured variables could be attributed to the variability in the levels of phosphorus fertilizer and rhizobial inoculation, respectively.

on se	oybean	H		-		
Term	Df	Wilk's Lambda	Rao F	n.d.f	d.d.f	F.prob
P fertilizer (P)	4	0.00032	6.59	32	36	0.000

Table 4.16 MANOVA for phosphorus fertilizer and rhizobial inoculation effects

Inoculation (I)	1	0.04551	18.88	8	9	0.000
$(P \times I)$	4	0.00159	4.02	32	36	0.000

n.d.f = numerator degree of freedom; d.d.f = denominator degree of freedom **4.6 Effect of phosphorus fertilizer and rhizobial inoculation on yield of soybean on** 

a Ferric Luvisol at Nyankpala

## 4.6.1 Effect of phosphorus fertilizer and rhizobial inoculation on soybean nodulation

The data in Table 4.17 shows the effects of phosphorus fertilizer and rhizobia inoculants application on soybean nodule number and dry weight at 50% flowering growth stage. Application of the phosphorus fertilizer resulted in significant differences in soybean nodule dry weight. TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> gave the highest (156.40 mg plant<sup>-1</sup>) nodule dry weight representing a significant increase of 131% and 62% in nodule dry weight over the control and TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> + 25 kg N treatments, respectively. Nodule dry weight in RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub> treated plots was also significantly (P < 0.05) higher than that of the control but not the RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub> + 25 kg N significantly increased nodule dry weight by 122% over that of the control while an increase of 82% was obtained from the application of RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub> Surprisingly, soybean nodule dry weight responded negatively to rhizobial inoculation, as it resulted in 26% decrease in nodule dry weight compared to the uninoculated control. There was also a significant interactive effect of rhizobial inoculation and phosphorus fertilizer application on nodule dry weight of soybean (Appendix 22).

Phosphorus	*Number	Nodule dry
fertilizer rate (P)	of nodules	plant <sup>-1</sup> weight
12	NTET	(mg plant <sup>-1</sup> )
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	20	123.30
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub> + 25 kg N	20	96.40
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	19	156.40
RPFB 68.7 kg P <sub>2</sub> O <sub>5</sub> + 25 kg N	17	150.70
0 kg P <sub>2</sub> O <sub>5</sub> (Control)	13	67.70
F pr.	< 0.001	0.005
LSD (5%)	2.82	47.57
CV (%)	12.90	23.70
Inoculation (I)	2 7 3	
Inoculated (+Rh)	15	101.50
Uninoculated (- Rh)	20	136.30
F pr.	0.098	0.045
LSD	NS	33.02
CV (%)	11.60	7.90
F pr. ( P × I)	< 0.013	0.003

 Table 4.17 Effect of phosphorus fertilizer and rhizobial inoculation on soybean

nodulation on a Ferric Luvisol at Nyankpala

NS = Not significant at  $P \le 0.05$ , \* Values represent mean of triplicate sample of 10 plants.

## 4.6.2 Effect of phosphorus fertilizer and rhizobial inoculation on soybean dry matter and grain yield

Both soybean shoot and root dry matter recorded significant differences from application of the phosphorus fertilizers on the Ferric Luvisol at Nyankpala (Table 4.18). The application of TSP 68.70 kg  $P_2O_5 + 25$  kg N resulted in the highest shoot dry matter yield (3,127 kg ha<sup>-1</sup>) which was significantly higher (P < 0.05) than that of the control (2,479 kg ha<sup>-1</sup>) and the other treatments. TSP 68.70 kg  $P_2O_5 + 25$  kg N also increased shoot dry matter yield by 15% over the RPFB 68.70 kg  $P_2O_5 + 25$  kg N treatment.

The application of TSP 68.70 kg  $P_2O_5 + 25$  kg N significantly increased root dry matter yield by 16.05 and 17.95% over the TSP 68.70 kg  $P_2O_5$  and control, respectively. There was no significant difference in root dry matter yield between TSP 68.70 kg  $P_2O_5 + 25$  kg N and RPFB 68.70 kg  $P_2O_5 + 25$  kg N while RPFB 68.70 kg  $P_2O_5 + 25$ kg N increased root dry matter yield by 46.27 and 20.47% over that of RPFB 68.70 kg  $P_2O_5$  and control, respectively.

TSP 68.70 kg  $P_2O_5 + 25$  kg N gave the highest mean grain yield of 2,214 kg ha<sup>-1</sup> representing a significant increase of 74% over the control compared to 9% increase from RPFB 68.70 kg  $P_2O_5 + 25$  kg N . Application of TSP 68.70 kg  $P_2O_5 + 25$  kg N increased grain yield by 74 and 60% over TSP 68.70 kg  $P_2O_5$  and RPFB 68.70 kg  $P_2O_5$  + 25 kg N treatments, respectively. There were no significant differences in grain yield among TSP 68.70 kg  $P_2O_5$ , RPFB 68.70 kg  $P_2O_5 + 25$  kg N, RPFB 68.70 kg  $P_2O_5$  and control treatments.
There was a significant interaction between rhizobial inoculation and phosphorus fertilizer application for shoot and root dry matter yields of soybean as indicated in Appendices 23 and 24, respectively, where application of RPFB 68.70 kg  $P_2O_5 + 25$ kg N significantly increased shoot and root dry matter yields of soybean. Root dry matter yield positively correlated with grain yield of soybean with 57% of the variation in grain yield attributed to variation in root dry matter yield (Fig.4.3)



Fig. 4.3 Relationship between grain yield and root dry matter yield of soybean Table 4.18 Effect of phosphorus fertilizer and rhizobial inoculation on dry

matter and grain y <mark>ield of soybean on a F</mark> erric Luvisol at Nyankpala						
Phosphorus	Shoot dry	Root dry	Grain			
fertilizer	weight	weight	yield			
rate (P)	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )			
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	1969	281.60	1628			
TSP 68.70 kg $P_2O_5 + 25$ kg N	3127	403.30	2214			
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	2642	347.60	1274			

RPFB 68.70 k $P_2O_5 + 25$ kg N	2723	411.90	1383
0 kg P <sub>2</sub> O <sub>5</sub> (Control)	2479	341.90	1271
F pr	< 0.001	< 0.001	0.006
LSD (5%)	244	32.29	513.60
CV (%)	7.70	7.40	23.00
Inoculation (I)	× 1 × 1 ×		
Inoculated (+ Rh)	2549	362.40	1452.00
Uninoculated (- Rh)	2626	352.10	1656.00
F pr	0.357	0.230	0.604
LSD (5%)	NS	NS	NS
CV (%)	3.10	2.10	26.40
F pr. ( $P \times I$ )	< 0.001	< 0.001	0.145

#### NS = Not significant at $P \le 0.05$

# 4.6.3 Nitrogen and phosphorus uptake in soybean shoot and grain

Effect of phosphorus fertilizer application on N and P uptake in soybean shoot and grain on the Ferric Luvisol at Nyankpala are presented in Table 4.19. Shoot N uptake was highest (57.80 kg ha<sup>-1</sup>) in TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> + 25 kg N amended plots and was significantly (P < 0.05) higher than that of RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub> but not the other treatments. TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> + 25 kg N increased shoot N by 52% over that of RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub>.

There were significant differences (P < 0.05) in soybean grain N uptake for the phosphorus fertilizer treatments applied. The application of TSP 68.70 kg  $P_2O_5 + 25$ 

kg N resulted in a significant increase of 78% in grain N uptake over that of the control while RPFB 68.70 kg  $P_2O_5 + 25$  kg N rather decreased grain N uptake by 7% over that of the control but this was not significant at P = 0.05. Furthermore, TSP 68.70 kg  $P_2O_5 + 25$  kg N increased grain N uptake by 77 and 90% over the TSP 68.70 kg  $P_2O_5$  and RPFB 68.70 kg  $P_2O_5 + 25$  kg N treatments, respectively. Soybean shoot N uptake positively correlated with grain (Fig. 4.4) and root dry matter (Fig. 4.5) yields.

The TSP 68.70 kg  $P_2O_5 + 25$  kg N treatment gave a significantly higher (P < 0.05) shoot P uptake than all the other treatments. The application of TSP 68.70 kg  $P_2O_5 + 25$  kg N resulted in 67% increase in shoot P uptake over the control relative to 40% from TSP 68.70 kg  $P_2O_5$ . Shoot P uptake resulting from the application of RPFB 68.70 kg  $P_2O_5 + 25$  kg N was significantly higher than RPFB 68.70 kg  $P_2O_5$  by 41%, while TSP 68.70 kg  $P_2O_5$  significantly increased shoot P uptake by 74% over RPFB 68.70 kg  $P_2O_5$ . There was a positive correlation between soybean shoot P uptake and root dry matter yield (Fig. 4.6).

The TSP 68.70 kg  $P_2O_5 + 25$  kg N treatment gave a significantly higher (P < 0.05) grain P uptake than the other treatments. Also, TSP 68.70 kg  $P_2O_5 + 25$  kg N resulted in the highest grain P uptake of 11.03 kg P ha<sup>-1</sup> representing 120% increase over the control, while an increase of 49% was obtained for TSP 68.70 kg  $P_2O_5$ . The RPFB 68.70 kg  $P_2O_5$  and RPFB 68.70 kg  $P_2O_5 + 25$  kg N treatments gave increases of 52 and 32%, respectively over the control. Moreover, application of TSP 68.70 kg  $P_2O_5 + 25$  kg N increased grain P uptake by 47% relative to TSP 68.70 kg  $P_2O_5$ .

Rhizobia inoculation did not significantly affect both N and P uptake in soybean shoot and grain, neither was there any significant interaction between phosphorus fertilzer and inoculants application (Table 4.19).



Fig. 4.4 Relationship between grain yield and shoot N uptake of soybean

uptake in soybean shoot and grain on a Ferric Luvisol at Nyankpala						
Phosphorus	Sho	ot	Grain			
fertilizer	N uptake	P uptake	N uptake	P uptake		
rate (P)	SANE	kg ha <sup>-1</sup>	<b>x</b>			
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	38.00	2.84	106.70	7.65		
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub> + 25 kg N	57.80	5.90	150.00	11.03		

Table 4.19 Effect of phosphorus fertilizer and rhizobial inoculation on N and P

TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	53.00	4.94	84.80	7.49
RPFB 68.70 kg $P_2O_5 + 25$ kg N	52.70	4.00	78.80	6.62
0 kg P2O5(Control)	47.70	3.54	84.30	5.02
12	NI	LIC	- The second sec	
F pr.	0.016	< 0.001	0.017	0.001
LSD (5%)	10.96	0.81	43.28	2.40
CV (%)	18.00	15.50	21.30	25.90
Inoculation (I)	11	127		
Inoculated (+Rh)	47.20	4.32	95.60	7.48
Uninoculated (-Rh)	52.50	4.17	106.30	7.65
C CE	R		FF	7
F pr.	0.283	0.531	0.624	0.919
LSD (5%)	NS	NS	NS	NS
CV (%)	9.00	6.00	22.60	24.30
F pr. $(P \times I)$	0.318	0.025	0.391	0.083
EL A	2		13	E/
$NS = Not significant at P \le 0.05$		2	and the	
W	2		-	
	SAN	E RUS		



Fig. 4.5 Relationship between soybean root dry matter yield and shoot N uptake



# Fig. 4.6 Relationship between soybean root dry matter yield and shoot P uptake

# 4.6.4 Effect of phosphorus fertilizer and rhizobial inoculation on phosphorus use

#### efficiency indices of soybean

Table 4.20 shows results of a two-way MANOVA on the effects of phosphorus fertilizer and rhizobial inoculation on soybean P use efficiency, P utilization efficiency and P uptake efficiency on a Ferric Luvisol at Nyankpala. The results indicate that rhizobial inoculation and phosphorus fertilizer had no significant effect on soybean P use efficiency indices with Wilk's Lambda values of 0.3080 (P =

0.072) and 0.8342 (P = 0.520), respectively. The Wilk's Lambda values indicate that 69.2 and 16.6% of the variation in the P use efficiency indices could be explained by variability in the levels of phosphorus fertilizer and rhizobial inoculation, respectively.

Results from the univariate ANOVA also indicate that, imposition of phosphorus fertilizer and rhizobial inoculation treatments did not result in significant differences in P use and P utilization efficiencies of soybean on the Ferric Luvisol at Nyankpala (Table 4.21). Phosphorus fertilizer, however, gave a significant effect (P < 0.05) on soybean P uptake efficiency while inoculation did not. The TSP 68.70 kg  $P_2O_5 + 25$  kg N treatment significantly increased P uptake efficiency by 67 and 47% over the RPFB 68.70 kg  $P_2O_5 + 25$  kg N and TSP 68.70 kg  $P_2O_5$  treatments, respectively. The application of TSP 68.70 kg  $P_2O_5$  and RPFB 68.70 kg  $P_2O_5$  did not result in significant differences in P uptake efficiency.

## Table 4.20 MANOVA for phosphorus fertilizer and rhizobial inoculation effects

WJ SANE NO

Term	Df	Wilk's Lambda	Rao F	n.d.f	d.d.f	F.prob
P fertilizer (P)	3	0.3080	2.03	18	26	0.072
Inoculation (I)	1	0.8342	0.79	6	9	0.520
$(\mathbf{P} \times \mathbf{I})$	3	0.5518	0.90	18	26	0.536

## on P use efficiency indices of soybean on a Ferric Luvisol at

Nyankpala

n.d.f. = numerator degree of freedom; d.d.f. = denominator degree of freedom

# 4.6.5 Effect of phosphorus fertilizer and rhizobial inoculation on cowpea

Results of a two-way MANOVA on phosphorus fertilizer and rhizobial inoculation effects on cowpea shoot dry matter yield, root dry matter yield, grain yield, shoot N uptake, shoot P uptake, grain N uptake and grain P uptake on the Ferric Luvisol at Nyankpala are presented in Table 4.22. The results indicate that phosphorus fertilizer had significant effect on the cowpea (Wilk's Lambda = 0.0253, P < 0.05). Rhizobial inoculation did not have a significant effect on the cowpea variables assessed (Wilk's Lambda = 0.3579, P = 0.083). Phosphorus fertilizer and rhizobial inoculation also had no significant interactive effect on the cowpea variables (Wilk's lamba = 0.0985, P = 0.329).

# Table 4.21 Effect of phosphorus fertilizer and rhizobial inoculation on

phosphorus use efficiency indices of soybean on a Ferric Luvisol at

# Nyankpala

Phosphorus	fertilizer PUE	PUtE PU	JpE rate (P)
(kg	kg <sup>-1</sup> ) (%)		
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	54.30	215.70	25.50
TSP 68.70 kg $P_2O_5 + 25$ k	ag N 59.40	194.40	36.80
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	42.50	169.70	25.00
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub> + 25	kg N 46.10	220.20	22.10
F pr.	0.135	0.062	0.003
LSD (5%)	NS	NS	6.99
CV (%)	23.40	15.80	20.30
Inoculation (I)	$\sim$		
Inoculated (+Rh)	54.30	191.80	28.40
Uninoculated (-Rh)	47.10	208.20	26.20
75	22	22	7
F pr.	0.357	0.589	0.761
LSD (5%)	NS	NS	NS
CV (%)	14.20	15.80	23.80
F pr (P × I)	0.063	<mark>0</mark> .734	0.149
The	A. C.	and the second s	55/

NS = Not significant at  $P \le 0.05$ , PUE = Phosphorus use efficiency, PUtE = Phosphorus utilization efficiency, PUpE = Phosphorus uptake efficiency

Table 4.22 MANOVA for Phosphorus fertilizer and rhizobial inoculation effects

on cowpea

Term	Df	Wilk's	Rao F	n.d.f	d.d.f	F.prob
		Lambda				

P fertilizer (P)	4	0.0253	2.25	28	42	0.007
Inoculation (I)	1	0.3579	2.47	7	11	0.083
$(\mathbf{P} \times \mathbf{I})$	4	0.0985	1.15	28	42	0.329

n.d.f = numerator degree of freedom; d.d.f = denominator degree of freedom

# 4.6.6 Effect of phosphorus fertilizer and rhizobial inoculation on cowpea shoot dry matter and grain yield

Application of the phosphorus fertilizers gave significant differences in cowpea shoot dry matter yield on the Ferric Luvisol at Nyankpala (Table 4.23). TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> + 25 kg N gave the highest shoot dry matter yield (2,603 kg ha<sup>-1</sup>) which was significantly higher than those of RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub> + 25 kg N, TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> and control treatments. The application of TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> + 25 kg N increased cowpea shoot dry matter yield by 72% over the control, compared to gains of 34.34 and 32% from RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub> + 25 kg N and TSP 68.70 kg P<sub>2</sub>O<sub>5</sub>, respectively. The TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> + 25 kg N treatment also increased shoot dry matter yield by 28 and 30% over the RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub> + 25 kg N and TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> treatments, respectively. There was no significant difference in shoot dry matter yield between the RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub> and the control. However, RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub> + 25 kg N increased shoot dry matter yield by 37% over that of the control.

There were significant differences in cowpea root dry matter yield as a result of the application of the phosphorus fertilizers (Table 4.23). As observed in shoot dry matter yield, TSP 68.70 kg  $P_2O_5 + 25$  kg N gave the highest root dry matter yield (207 kg ha<sup>-</sup>

<sup>1</sup>) representing 44% increase over the control relative to 16% increase obtained by TSP 68.70 kg  $P_2O_5$ . Also, the RPFB 68.70 kg  $P_2O_5 + 25$  kg N and RPFB 68.70 kg  $P_2O_5$  treatments gave an increase in root dry matter yield of 29 and 8%, respectively over the control. Both TSP fertilizer treatments significantly increased cowpea shoot to root ratio over the control while that of the RPFB did not.

TSP 68.70 kg  $P_2O_5 + 25$  kg N gave the highest grain yield (1,189 kg ha<sup>-1</sup>), representing 53% increase over the control while TSP 68.70 kg  $P_2O_5$  gave a corresponding increase of 36%. RPFB 68.70 kg  $P_2O_5 + 25$  kg N also increased grain yield significantly by 51% over the control compared to 11% increase from RPFB 68.70 kg  $P_2O_5$  which was not statistically significant at P = 0.05.

Rhizobia inoculant application did not significantly affect shoot and root dry matter yields, shoot to root ratio and grain yield of cowpea. Also, there was no significant interactive effect of phosphorus fertilizer and rhizobial inoculation on the yield variables assessed.



Table 4.23 Effect of phosphorus fertilizer and rhizobial inoculation on drymatter and grain yield of cowpea on a Ferric Luvisol at Nyankpala

Phosphorus	Dry matter yield	Shoot to	Grain root	
fertilizer	Shoo	t Roo	ot	yield

		<sup>-1</sup> )	. rat	io (kg ha <sup>-1</sup> )
rate (P)(	(kg ha			
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	1483	154.60	9.60	860
TSP 68.70 kg $P_2O_5 + 25$ kg N	N 2603	206.50	12.60	1189
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	2003	166.20	12.05	1061
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub> + 25 kg	N 2034	185.40	10.97	1171
0 kg P <sub>2</sub> O <sub>5</sub> (Control)	1514	143.20	10.57	778
F pr	< 0.001	0.011	0.007	0.003
LSD (5%)	437.90	34.84	1.58	224
CV (%)	18.60	16.60	11.50	18.10
Inoculation (I)		5		
Inoculated (+ Rh)	1984	169.20	11.76	895
Uninoculated (- Rh)	1870	173.10	10.72	1129
TR	Str X	335	R	
F pr	0.680	0.899	0.128	0.148
LSD (5%)	*NS	NS	NS	NS
CV (%)	15.20	17.70	4.50	12.30
F pr. $(P \times I)$	0.264	0.780	0.212	0.060
AP.	>	Sab	2	

NS = Not significant at  $P \le 0.05$ 

# 4.6.7 Nitrogen and phosphorus uptake in cowpea shoot and grain

Table 4.24 shows results of the effects of phosphorus fertilizer with low N input and rhizobial inoculation on N and P uptake in cowpea shoot dry matter and grain. The TSP 68.70 kg  $P_2O_5 + 25$  kg N treatment gave the highest shoot N uptake (78.20 kg N

ha<sup>-1</sup>) which was significantly (P < 0.05) higher than those of all other treatments. TSP 68.70 kg  $P_2O_5 + 25$  kg N increased shoot N significantly by 83% over that of the control, compared to 41% increase from TSP 68.70 kg  $P_2O_5$ . Although there was a 38% increase in shoot N uptake over the control from the application of RPFB 68.70 kg  $P_2O_5 + 25$  kg N, the increase in shoot N uptake was not statistically significant at P = 0.05. Moreover, TSP 68.70 kg  $P_2O_5 + 25$  kg N increased shoot N uptake by 32% over RPFB 68.70 kg  $P_2O_5 + 25$  kg N.

There were significant differences in grain N uptake as a result of the application of both TSP and RPFB in combination with N fertilizer. Grain N uptake was highest (47.10 kg N ha<sup>-1</sup>) in RPFB 68.70 kg  $P_2O_5 + 25$  kg N treatment representing a 44% increase over the control compared to the 6% increase from TSP 68.7 kg  $P_2O_5$ , which was not statistically significant. TSP 68.70 kg  $P_2O_5 + 25$  kg N also increased grain N uptake by 40% over that of the control. There were no significant differences in grain N uptake among the RPFB 68.70 kg  $P_2O_5$ , TSP 68.70 kg  $P_2O_5$  and control treatments. Cowpea root dry matter yield positively correlated with shoot N uptake (Fig. 4.7) and shoot P uptake (Fig. 4.8).

Table 4.24 Effect of phosphorus fertilizer and rhizobial inoculation on N and P

uptake in cowpea shoot and grain on a Ferric Luvisol at Nyankpala					
Phosphorus	<b>Shoot</b> Grain				
fertilizer	N uptake	P uptake	N uptake	P uptake	
rates (P)		kg ha	.1		
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	44.10	4.43	35.50	3.27	

TSP 68.70 kg $P_2O_5 + 25$ kg N	78.20	8.47	45.90	4.93
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	60.40	7.16	34.70	4.28
RPFB 68.70 kg $P_2O_5 + 25$ kg N	59.10	5.41	47.10	4.74
0 kg P <sub>2</sub> O <sub>5</sub> (Control)	42.70	3.97	32.80	2.72
F pr.	0.002	< 0.001	0.009	0.002
LSD (5%)	16.67	1.55	9.10	1.11
CV (%)	24.00	21.50	19.00	22.80
	N I			
Inoculation (I)	20	13		
Inoculated (+Rh)	58.50	6.03	34.60	3.19
Uninoculated (-Rh)	55.20	5.74	43.80	4.78
F pr.	0.780	0.527	0.028	0.153
LSD (5%)	NS	NS	6.88	NS
CV (%)	22.10	7.90	5.0	20.60
F pr. $(P \times I)$	0.225	0.28	0.014	0.122

NS = Not significant at  $P \le 0.05$ 

Application of the different phosphorus fertilizers resulted in significant differences in cowpea shoot P uptake. Sole and combined application of TSP and N gave higher shoot P uptake than RPFB. Cowpea shoot biomass P was highest (8.47 kg P ha<sup>-1</sup>) in TSP 68.70 kg  $P_2O_5 + 25$  kg N amended plots and this was significantly (P < 0.05) higher than P uptake in RPFB 68.70 kg  $P_2O_5 + 25$  kg N amended plots. The TSP 68.70 kg  $P_2O_5 + 25$  kg N treatment gave 113% increase in shoot P uptake over that of the control compared to the 36% increase from RPFB 68.70 kg  $P_2O_5 + 25$  kg N treatment

plots. The TSP 68.70 kg  $P_2O_5$  and RPFB 68.70 kg  $P_2O_5$  treatments gave 80 and 12% increases in shoot P uptake, respectively over that of the control.

Grain P uptake was however, highest (4.93 kg P ha<sup>-1</sup>) with application of TSP 68.70 kg  $P_2O_5 + 25$  kg N representing 81% increase over that of the control while the control gave the least grain P uptake of 2.72 kg P ha<sup>-1</sup>. TSP 68.70 kg  $P_2O_5$  also increased grain P uptake by 57% over that of the control compared to 20% increase from RPFB 68.70 kg  $P_2O_5$  which was not significant at P = 0.05.



Fig. 4.7 Relationship between cowpea root dry matter yield and shoot N uptake



Root dry matter yield (kg ha<sup>-1</sup>)

Fig. 4.8 Relationship between cowpea root dry matter yield and shoot P uptake 4.6.8 Phosphorus fertilizer and rhizobial inoculation effects on P use efficiency indices of cowpea on a Ferric Luvisol at Nyankpala

Table 4.25 shows the results of a two-way MANOVA on the effects of phosphorus fertilizer and rhizobial inoculation on P use efficiency, P utilization efficiency and P uptake efficiency of cowpea on a Ferric Luvisol at Nyankpala. The results indicate that phosphorus fertilizer had significant effect on P use efficiency indices (Wilk's Lambda = 0.3769, P < 0.05) while rhizobial inoculation did not (Wilk's Lambda = 0.4695, P = 0.159). Phosphorus fertilizer and rhizobial inoculation did not have significant interactive effect on the P use efficiency indices of cowpea (Wilk's lamba = 0.3067, P = 0.070).



## on P use efficiency indices of cowpea

Term	Df	Wilk's Lambda	Rao F	n.d.f	d.d.f	F.prob
P fertilizer (P)	3	0.3769	1.61	18	26	0.024
Inoculation (I)	1	0.4695	4.52	6	9	0.159
$(P \times I)$	3	0.3067	2.04	18	26	0.070

n.d.f = numerator degree of freedom; d.d.f = denominator degree of freedom

# 4.6.9 Effect of phosphorus fertilizer and rhizobial inoculation on P use efficiency indices

#### of cowpea

Application of the phosphorus fertilizers resulted in significant differences in P use efficiency and P uptake efficiency of cowpea on the Ferric Luvisol at Nyankpala, while no such differences were observed in P utilization efficiency (Table 4.26). Application of RPFB 68.70 kg  $P_2O_5 + 25$  kg N significantly increased P use efficiency by 36% over RPFB 68.70 kg  $P_2O_5$  while no such difference was observed between TSP 68.70 kg  $P_2O_5 + 25$  kg N and TSP 68.70 kg  $P_2O_5$  treatments. However, TSP 68.70 kg  $P_2O_5$  gave an increase of 23% in P use efficiency over RPFB 68.70 kg  $P_2O_5$ .

Similarly, application of RPFB 68.70 kg  $P_2O_5 + 25$  kg N significantly increased P uptake efficiency by 45% over RPFB 68.70 kg  $P_2O_5$ , while no such difference was observed between TSP 68.70 kg  $P_2O_5 + 25$  kg N and TSP 68.70 kg  $P_2O_5$  treatments.

The TSP 68.70 kg  $P_2O_5$  treatment however, increased P uptake efficiency by 31% over the RPFB 68.70 kg  $P_2O_5$ .

Application of rhizobia inoculant also did not significantly affect any of the phosphorus use efficiency indices. An interactive effect between rhizobial inoculation and RPFB 68.70 kg  $P_2O_5 + 25$  kg N treatment was however, observed for P use efficiency of cowpea as rhizobial inoculation significantly decreased cowpea P use efficiency (Appendix 27).



efficiency indices of cowpea on a Ferric Luvisol at Nyankpala					
Phosphorus	fertilizer	PUE	PUtE		
PUpE	2 X	1	2		
rate (P)	" he	(kg kg <sup>-1</sup> )	(%)		
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	28.70	267.20	10.90		
TSP 68.70 kg $P_2O_5 + 25$ kg N	39.60	260.60	16.43		
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	35.40	256.60	14.28		
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub> + 25 kg N	39.00	251.20	15.79		
W		NO			
F pr.	0.011	0.921	0.013		
LSD (5%)	6.49	NS	3.24		
CV (%)	14.50	15.80	18.00		

Table 4.26 Effect of phosphorus fertilizer and rhizobial inoculation on P use

# **Inoculation** (I)

Inoculated (+Rh)	31.60	284.00	11.46	
Uninoculated (-Rh)	39.70	233.80	17.23	
F pr.	0.212	0.251	0.184	
LSD (5%)	NS	NS	NS	
CV (%)	15.30	14.80	20.40	
F pr ( $P \times I$ )	0.016	0.335	0.046	

NS = Not significant at  $P \le 0.05$ , PUE = Phosphorus use efficiency, PUtE = Phosphorus utilization efficiency, PUpE = Phosphorus uptake efficiency.

# 4.7 Economic profitability of N and P fertilizer application for soybean and cowpea

# production on a Ferric Luvisol at Nyankpala

The economic benefit of application of phosphorus and N fertilizers for soybean and cowpea production on the Ferric Luvisol at Nyankpala was assessed using value - cost ratio analysis. Effect of nitrogen and phosphorus fertilizer application on the monetary value of soybean and cowpea yields is presented in Table 4.27 while that on the input cost of production is indicated in Table 4.28. The TSP 68.70 kg  $P_2O_5 + 25$  kg N treatment gave the highest soybean crop value of GHC 2,546.10 while the least value of GHC 1,465.10 was obtained from TSP 68.70 kg  $P_2O_5 + 25$  kg N and

RPFB 68.70 kg  $P_2O_5$  gave the highest and lowest crop values of GHC 2,496.90 and GHC 1,806, respectively.

The data in Table 4.28 indicates that the input cost for soybean was highest (GHC 752.30) with application of RPFB 68.70 kg  $P_2O_5 + 25$  kg N while TSP 68.70 kg  $P_2O_5$ 

gave the least (GHC 423.92) input cost. Similarly, the total input cost for cowpea was highest (GHC 656.87) with application of RPFB 68.70 kg  $P_2O_5 + 25$  kg N while TSP 68.70 kg  $P_2O_5$  gave the least (GHC 385.02) total input cost.

Results of the value-cost ratio analysis of phosphorus and nitrogen fertilizer application for soybean and cowpea production at Nyankpala are presented in Figures 4.9 and 4.10, respectively. The results indicate that none of the fertilizer treatments imposed gave the economically viable VCR threshold (VCR  $\ge$  2). The TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> + 25 kg N treatment however, was relatively the most profitable input (VCR = 1.77) for soybean production (Fig. 4.9) while TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> was the least profitable (VCR = 0.01).

Table 4.27 Effect of phosphorus and nitrogen fertilizer application on the

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monetary value of soybean and cowpea					
Phosphorus fertilizer rate	Crop yield	No. of	Price of	Value of	
	(kg ha <sup>-1</sup> )	Bags	100 kg	crop	
Soybean	8-3	(100 kg	bag	(GH ¢)	
	5 7	bag <sup>-1</sup> )	(GH C )		
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	1628	16.28	115.00	1872.20	
TSP 68.70 kg $P_2O_5 + 25$ kg N	2214	22.14	115.00	2546.10	
TSP 6 <mark>8.70 kg P</mark> 2 O5	1274	12.74	115.00	146 <mark>5.</mark> 10	
PRPFB 68.70 kg P2 O5 +25 kg N	1383	13.83	115.00	1590.45	
40,		<	"an	/	
Cowpea	SANE	NO	1		
RPFB 68.70 kg P2 O5	860	8.60	210.00	1806.00	
TSP 68.70 kg $P_2O_5 + 25$ kg N	1189	11.89	210.00	2496.90	
T RPFB $68.70 \text{ kg } P_2 \text{ O}_5$	1061	10.61	210.00	2228.10	

For cowpea, TSP 68.70 kg  $P_2O_5$  and TSP 68.70 kg  $P_2O_5 + 25$  kg N were the most profitable inputs with VCR values of 1.54 and 1.64, respectively (Fig 4.10). The RPFB 68.70 kg  $P_2O_5 + 25$  kg N treatment however gave a VCR of 1.26 representing a breakeven point with 26% return on investment.



Phosphorus fertilizer rate	Cost of seeds ha- 1 (GH C)	Cost of fertilizers/ labour (GH C )	Total input cost (GH C)
Soybean	Tr 11		
<sup>a</sup> RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	80.00	486.62	566.62
<sup>b</sup> TSP 68.70 kg P <sub>2</sub> O <sub>5</sub> + 25 kg <sup>c</sup> SOA	80.00	529.61	609.61
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	80.00	343.92	423.92
RPFB + 68.70 kg P <sub>2</sub> O <sub>5</sub> + 25 kg SOA	80.00	672.30	752.30
Cowpea	2 SANE	NO	
<sup>d</sup> RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	42.00	476.22	518.22
$^{\mathrm{e}}\mathrm{TSP}$ 68.70 kg P <sub>2</sub> O <sub>5</sub> + 25 kg $^{\mathrm{f}}\mathrm{SOA}$	42.00	481.67	523.67
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	42.00	334.02	376.02

Table 4.28 Input cost for soybean and cowpea production at Nyankpala

656.87

<sup>a</sup>Quantity of RPFB applied =  $381.62 \text{ kg ha}^{-1}$  at GH C50 per 50 kg bag of fertilizer

<sup>b</sup>Quantity of TSP applied = 149.33 kg ha<sup>-1</sup> at GH  $\mathbb{C}$  80 per 50 kg bag of fertilizer

<sup>c</sup>Quantity of Sulphate of Ammonia (SOA) applied = 119.03 kg ha<sup>-1</sup> at GH C78.00 per 50 kg bag of fertilizer.

<sup>d</sup>Quantity of RPFB applied = 356.22 kg ha<sup>-1</sup> at GH  $\mathbb{C}$  50 per 50 kg bag of fertilizer

<sup>e</sup>Quantity of TSP applied = 139.39 kg ha<sup>-1</sup> at GH C 80 per 50 kg bag of fertilizer

<sup>f</sup>Quantity of Sulphate of Ammonia (SOA) applied = 88.88 kg ha<sup>-1</sup> at GH C 78.00 per 50 kg bag of fertilizer



# Fig. 4.9 Value - cost ratio of fertilizer input for soybean



Fig. 4.10 Value - cost ratio of fertilizer input for cowpea EVT-Economic viability threshold

# **CHAPTER FIVE**

#### **5.0 DISCUSSION**

**5.1 Effect of rhizobial inoculation on nodulation and grain yield of soybean and cowpea** Rhizobial inoculation significantly increased soybean nodule number and dry weight on the Gleyic Lixisol at Bontanga (Table 4.4). The positive response of soybean to rhizobial inoculation corroborates other researchers who reported increases in nodulation of soybean after inoculation (Aliyu *et al.*, 2013; Kumaga and Etu-Bonde, 2004). Kumaga and Ofori (2004) also reported an increase in nodulation after inoculation of both promiscuous and non-promiscuous soybean varieties and attributed the increment to the high competitive ability of the bradyrhizobia inoculant used. Similar findings were reported by Okereke *et al.* (2000) for promiscuous soybean grown in the moist savanna of West Africa.

The positive response of soybean to inoculation on the Gleyic Lixisol could be attributed to the fact that the bradyrhizobia strains in the inoculants were more competitive than the indigenous soybean rhizobia with a population of  $5.71 \times 10^1$  g<sup>-1</sup> of soil. Thies *et al.* (1991) reported that positive response to rhizobial inoculation and the ability of an introduced strain to compete with and overcome the indigenous rhizobia is inversely related to the number of indigenous rhizobia.

On the Ferric Luvisol at Nyankpala, however, soybean nodule dry weight decreased significantly with inoculation while nodule number was not significantly affected, although the soil had a relatively low  $(2.15 \times 10^1 \text{ g}^{-1} \text{ of soil})$  indigenous rhizobia population. The lack of significant response in nodule dry weight of soybean to rhizobial inoculation on the Ferric Luvisol at Nyankpala is similar to the findings of Chemining'wa *et al.* (2007) and Okogun *et al.* (2005) who found no significant increases in nodule dry weight following rhizobial inoculation.

Several reasons have been cited for the lack of nodulation response to inoculation in grain legumes including poor inoculant viability, adequate soil mineral N, incompatibility of the inoculants strain with specific grain legume or the presence of highly competitive native rhizobia (Chemining'wa *et al.*, 2007). Failure of soybean to respond to inoculation could be due to the high ( $6.98 \times 10^2$  g<sup>-1</sup> of soil) indigenous cowpea rhizobia population and the promiscuous nature of the variety.

There are varied opinions about the threshold size of population of resident rhizobia below which inoculation is likely to contribute to nodulation. Howieson and Ballard (2004) reported that legumes will respond to inoculation where the rhizobia community is less than 100 rhizobia cells  $g^{-1}$  soil. Herridge (2008), however, proposed

a slightly higher threshold value of 300 rhizobia cells  $g^{-1}$  soil below which inoculation is likely to benefit the farmer. The high indigenous cowpea rhizobia population might have prevented full establishment of the inoculants strains in the soil at normal inoculation rates. Moreover, Abaidoo *et al.* (2007) reported that it was unlikely for soybean to respond to inoculation due to the difficulty in establishing inoculated strains in soils containing substantial indigenous rhizobia populations. It is very likely that the introduced inoculants strains could not survive the competition for nodule occupancy from the native rhizobia due to their relatively high population.

The lack of response to rhizobial inoculation on the Ferric Luvisol could also have been due to poor inoculant viability as at the time it was being applied. This is because the inoculant used in this study had bradyrhizobia population of  $6.13 \times 10^6 \text{g}^{-1}$  as at the time of its application on the Gleyic Lixisol.

This population, though was lower than the recommended ( $10^8$  cells g<sup>-1</sup>) for high quality inoculants (Vivas-Marfisi, 2013), it was however, within the  $10^5$ - $10^6$  cells g<sup>-1</sup> minimum threshold to yield a positive inoculation response as reported by Hiltbold *et al.* (1980). This might have accounted for the positive nodulation response of soybean to inoculation on the Gleyic Lixisol. On the contrary, soybean failed to respond positively to inoculation on the Ferric Lusvisol. This could be due to a further reduction in the number of viable bradyrhizobia cells in the inoculants as storage of the product experienced frequent interruptions in electricity supply. Moreover, Catroux *et al.* (2001) asserted that the number of viable bacteria present to initiate the infection process was very critical for the successful establishment of rhizobia in commercial inoculants.

Brockwell *et al.* (1987) reported also of the possibility of a reduction in the population of introduced strains in an inoculant arising from mortalities following its introduction into the soil thus causing a reduction in the optimum numbers required for symbiosis. The failure of rhiozobial inoculation to yield significant responses could also have arisen from reduced population of introduced rhizobia as a result of the 3-week period of drought that occurred between 22 and 44 days after planting (Fig. 4.2). Rhizobia survival and activity in the soil is dependent on their distribution among microhabitats and soil moisture dynamics (Orchard and Cook, 1983). Moreover, studies by Hunt *et al.* (1981) attributed lack of soybean's response to rhizobia inoculation to low soil water content. Studies by Brockwell *et al.* (1989) also highlighted a strong linear relationship between rhizosphere population of rhizobia, nodulation, plant growth, and yield.

Soybean plants were nodulated by the indigenous rhizobia population on both soil types at Bontanga and Nyankpala, which clearly confirmed the promiscuous nature of the soybean variety (Jenguma) used in this study. Promiscuous soybean varieties have reduced nodulation specificity and therefore could effectively be nodulated by native bradyrhizobium strain populations as reported by Abaidoo *et al.* (2007). The significant increase in soybean nodule dry weight following rhizobial inoculation on the Gleyic Lixisol at Bontanga suggests that the soybean variety responded positively to inoculation in spite of its promiscuity. This could be attributed to the relatively low indigenous soybean rhizobia population at the study site. According to Fening and Danso (2002), there is much variability in the effectiveness and population of indigenous bradyrhizobia in a given locality. This therefore makes it necessary for promiscuous varieties to be inoculated with foreign strains of bradyrhizobia depending on the indigenous rhizobia population and their effectiveness in the locality (Okereke

*et al.*, 2000) as well as the variety's degree of promiscuity (Sanginga *et al.*, 1999). The nodulation response of soybean on the Gleyic Lixisol at

Bontanga is in agreement with studies by Okereke and Eaglesham (1993) and Sanginga et al. (1996) who observed increases in nodulation after inoculation with corresponding increases in grain yield. Contrary to these findings, however, the results of this study indicate that the increase in nodulation following inoculation on the Gleyic Lixisol at Bontanga was not translated into grain yield (Table 4.5). The failure of rhizobial inoculation to increase soybean grain yield in spite of a significant increase in nodulation could be attributed to a limitation in the crop's yield potential caused by stressful edaphic factors such as insufficient soil moisture during both the vegetative and reproductive stages of growth as experienced in this study (Fig. 4.2). Under such conditions, the plant's N demand is reduced to such an extent that it could easily be met by available soil N or through nitrogen fixation by less effective soil rhizobia (Singleton and Tavares, 1986). Although application of rhizobia inoculant on the Glevic Lixisol at Bontanga resulted in 79% increase in nodule dry weight, this increase was not up to the threshold that could elicit significant differences in biomass dry weight and grain yield through N<sub>2</sub> fixation. Many researchers including Singleton and Tavares (1986) and Sarkodie-Addo et al. (2006) have reported that higher nodulation does not always translate into higher nitrogen fixation and grain yield. Singleton and Tavares (1986) reported that inoculated plants must have 2.5 times more nodule number and weight than uninoculated plants before a corresponding increase in grain yield could be achieved, but this was not the case in this study.

Grain yield response of soybean to rhizobial inoculation on the Gleyic Lixisol (Table 4.5) and Ferric Luvisol (Table 4.18) is contrary to that of previous studies by Ahiabor *et al.* (2014) and Rechiatu *et al.* (2015), who reported of significant increases in

soybean grain yield after inoculation of soybean in the Northern savanna zones of Ghana. The differences in inoculation response of this study and that from previous studies could be as a result of differences in environmental conditions, crop variety and the type of inoculant used as legume response to rhizobial inoculation is highly unpredictable but variable and site specific (Date, 2000). Moreover, studies by Chemining'wa et al. (2007) confirmed that rhizobia inoculant application does not always give the desired results. The reduction in grain yield of inoculated soybean and cowpea could also be due to a reduction in plant population caused by preinoculation treatment of the seeds. Moistening of the seeds prior to inoculants application might have adversely affected seed germination and crop establishment leading to a reduction in plant population; as grain yield and other grain yielddependent variables from the inoculated treatments were in most cases lower than those from the uninoculated ones. (Appendix 10 - 27).

Table 4.11 indicates that cowpea responded negatively to rhizobial inoculation with significant reduction in nodule dry weight. Similar results were obtained by Mathu *et al.* (2012) who showed no response of cowpea to rhizobial inoculation. According to Danso and Owiredu (1988) and Thies et al. (1995), the inability of rhizobial inoculation to increase nodulation and yield of legumes is to some extent due to the high indigenous rhizobia population size found in tropical soils. The MPN count from the Gleyic Lixisol at Bontanga indicated high indigenous cowpea rhizobia population of  $14.64 \times 10^2$ . Studies by Thies *et al.* (1991) and Brockwell *et al.* (1995), however, revealed that soils with rhizobia population. This, therefore, confirms that the high resident rhizobia population might have contributed greatly to failure of cowpea to respond to rhizobial inoculation. According to Brockwell *et al.* (1995), indigenous

rhizobia population size is a major significant factor that determines inoculant strains' establishment in soils. Several reports have demonstrated that, cowpea as a promiscuous legume, does not normally respond to inoculation due to the fact that it is capable of being nodulated by Bradyrhizobia strains which are present in most tropical soils (Ahmad et al., 1981). Inoculant strains introduced to soils with high indigenous rhizobia population of variable effectiveness always have a low proportion of them occupying the nodules which constitute a major barrier to increased nitrogen input through atmospheric nitrogen fixation (McInnes and Haq, 2003). The significantly high nodule number and dry weight observed in the uninoculated plants on the Gleyic Lixisol at Bontanga is an indication of the presence of effective indigenous cowpea rhizobia. In spite of the fact that cowpea is a promiscuous legume, there is the possibility of it responding to rhizobial inoculation in soils with high numbers of already established rhizobia. This is the case when the strains used are competitive and highly effective in N<sub>2</sub> fixation as reported by Hungria *et al.* (1998). Also, studies by Fening (1999) confirmed the possibility of a positive response of cowpea to inoculation with indigenous cowpea bradyrhizobia isolates in soils with high native rhizobia population in Ghana.

# 5.2 Effect of phosphorus fertilizer on soybean nodulation

Phosphorus fertilizer application significantly increased soybean nodulation on the Gleyic Lixisol at Bontanga (Table 4.4) and the Ferric Luvisol at Nyankpala (Table 4.17). Nodulation response of soybean to increased phosphorus application is similar to the findings of Bekere and Hailemariam (2012) and Devi *et al.* (2012) who observed significant increases in soybean nodule dry weight with increasing levels of soil phosphorus. The nodulation response to increasing phosphorus supply can be

attributed to the significant role that phosphorus plays in the legume nodulation process. Phosphorus is an essential nutrient required by legumes for optimum growth and nitrogen fixation as it has been found to play a specific role in nodule initiation, growth and function (Ankomah *et al.*, 1996). Carsky *et al.* (2001) indicated that nodulation in legumes can be limited by phosphorus deficiency while phosphorus fertilizer application can ameliorate this deficiency. According to Israel (1993), phosphorus deficiency in soybean has been found to have a more direct impact on nodule functioning. Moreover, specific nodule activity presents higher ATP requirements for the function of nitrogenase (Ribet and Drevon, 1996). Also, P needs for signal transduction and nodule development are directly dependent on P supply and as a result contribute to high P requirement by nodulated legumes (AlNiemi *et al.*, 1997; Tang *et al.*, 2001).

The positive nodulation response of soybean to phosphorus fertilization could be attributed to an increased nitrogenase activity as P fertilization has been cited as the cause of increased nitrogenase activity, nodule number and plant N accumulation of legume species dependent on  $N_2$  fixation (Olivera *et al.*, 2004).

Soybean nodule dry weight response to TSP fertilizer at Bontanga was significantly higher than the rock phosphate based fertilizer formulation. This could be attributed to the low reactivity and low water solubility of the rock phosphate in the fertilizer formulation (Sanchez, 2002)

Togo rock phosphate is a fluorine deficient francolite with substitution of its phosphate by carbonate (Agyin-Birikorang *et al.*, 2007). An assessment of the mineral composition of Togo rock phosphate by Agyin-Birikorang *et al.* (2007) indicated that, it has a low  $CO_2$  index which corresponds to a low degree of carbonate substitution. It has also been identified as having a large crystal size (Chien and Menon, 1995 b) with low P solubility of 0.20 and 0.93g kg<sup>-1</sup> in water and neutral ammonium citrate, respectively. Numerous studies, both in the laboratory and greenhouse have confirmed the low reactivity of Togo rock phosphate (Asomaning *et al.*, 2006; Owusu-Bennoah *et al.*, 2002). Laboratory determination of citric acid soluble P content of the rock phosphate in the fertilizer blend confirmed its low solubility in citric acid with citric acid soluble P content of 1.53% (Table 4.2).

# 5.3 Effect of phosphorus fertilizer on shoot dry matter and grain yield of soybean and cowpea on a Gleyic Lixisol at Bontanga

#### 5.3.1 Shoot dry matter yield

Soybean and cowpea shoot dry matter yields responded positively to increasing levels of P from both TSP and RPFB application on the Gleyic Lixisol at Bontanga (Tables 4.5 and 4.12). The TSP fertilizer gave a higher shoot dry matter yield response in soybean (Table 4.5) than the RPFB blend. The response of soybean shoot dry matter yield to P fertilizer application confirms the findings of Israel (1993) and Kwari (2005) who observed significant increases in whole plant growth of soybean in response to increased soil P supply. The positive response of soybean shoot dry matter yield to increasing phosphorus fertilizer levels was expected since initial soil analysis showed that available phosphorus at the Bontanga experimental site was low (5.22 mg P kg<sup>-1</sup>) (Table 4.1) based on the classification by Landon (2014). Grain legumes require phosphorus in large amounts for growth and nitrogen fixation and it has been found to promote biomass yield and nodulation in a number of leguminous crops (Gentili and Huss\_Danell, 2003). The increase in soybean shoot dry matter yield to TSP fertilizer application can be attributed to key plant physiological processes such as root development, photosynthesis, nitrogen fixation, flower and seed formation, fruiting and crop quality improvement which are influenced by phosphorus nutrition as indicated by Brady and Weil (2002). Moreover, several energy transformation processes and biochemical reactions such as nitrogen fixation are controlled by phosphorus acquisition as phosphorus deficiency in legumes has been found to have reduced leaf expansion and shoot development with a corresponding reduction in photosynthetic area and the utilization of carbohydrates (Ahloowalia *et al.*, 1995). The increased dry matter yield with application of P fertilizer could also be due to adequate supply of P especially from the TSP fertilizer which resulted in an increased plant growth through increases in number of branches per plant and leaf area. Jennifer (2000) observed that an increase in the concentration of soil P resulted in an increase in dry matter accumulation and total leaf area in soybean. An increase in leaf area will also result in an increase in photosynthetic area as well as the number of pods per plant which have been found to be highly correlated with the accumulation of dry matter and yield (Turuko and Mohammed, 2014).

Table 4.5 shows that soybean shoot dry matter yield was significantly influenced by the sources of phosphorus fertilizer with soybean responding better to TSP fertilizer than the RPFB blend. This can be attributed to the fact that TSP fertilizer has a higher water solubility of about 95% (Dalshad *et al.*, 2013). Phosphorus from TSP is, therefore, much readily available for plant uptake. Therefore, the lack of response of soybean to RPFB application can be attributed to the low water solubility of the phosphate rock in this fertilizer. Baligar and Bennett (1986 a) attributed the ineffectiveness of rock phosphate to its low water solubility. However, the effectiveness of rock phosphate as a source of P has been reported to be influenced by other factors such as specific soil and moisture conditions with site specific crop responses (Vanlauwe *et al.*, 2000 b). Moreover, studies by Bado (1998) showed that rock phosphate sometimes fails to dissolve when applied for the first time especially when it is not treated to improve on its solubility. In a green house study, Asomaning *et al.* (2006) showed that untreated Togo rock phosphate was less soluble than TSP and partially acidulated rock phosphate as it resulted in the lowest dry matter yield of maize. The heterogeneous nature of the fertilizer may also have played a role in its poor solubility as it does not provide a uniform surface area for its dissolution. Application of such fertilizer with heterogeneous texture results in segregation of the nutrients and this does not ensure equal distribution of nutrients in quantities of the

fertilizer applied.

#### 5.3.2 Grain yield

Contrary to the trend observed in soybean shoot dry matter yield, grain yield of soybean did not respond significantly to increasing phosphorus fertilizer application as the increases observed in the shoot dry matter yield with TSP application were not translated into grain yield (Table 4.5). Grain yield response of both soybean and cowpea to phosphorus fertilizer application at on the Gleyic Lixisol at Bontanga was generally low compared to their potential yield of 2,500 and 3,000 kg ha<sup>-1</sup>, respectively. Average grain yields of soybean (760 kg ha<sup>-1</sup>) and cowpea (798 kg ha<sup>-1</sup>) were only 30.4 and 26.6% of their potential yields, respectively. The inability of soybean to translate accumulated biomass into grain yield could be due to the fact that nitrogen fixation and nitrate assimilation processes were compromised as a result of insufficient soil moisture during the late vegetative and early reproductive stages of growth as there was interruption in irrigation water supply for 12 days due to repair works on the lateral canal supplying water to the field. Serraj *et al.* (1999) stated that, in legumes drought stress has a drastic effect on N<sub>2</sub> fixation and N<sub>2</sub> fixation rates are more sensitive to soil moisture stress than other plant physiological processes such as

photosynthesis, transpiration and leaf growth rate. Nodulation and  $N_2$  fixation response of soybean to soil moisture stress also depend on the growth stage of the plant. According to Pena-Cabriales and Castellanos (1993), soil moisture stress occurring during the vegetative growth stage has been reported to be more detrimental to nodulation and nitrogen fixation than when it occurred at the reproductive stages where there is little chance of recovery from water stress.

# 5.4 Effect of phosphorus and N fertilizer application on dry matter and grain yield of soybean and cowpea on a Ferric Luvisol at Nyankpala

## 5.4.1 Shoot dry matter yield

The data in Tables 4.18 and 4.23 indicate that both soybean and cowpea shoot dry matter yields on the Ferric Luvisol at Nyankpala were significantly higher with TSP application than that of RP fertilizer. Shoot dry matter yield response of soybean to TSP application was, however, higher than that of the control only when it was combined with 25 kg N compared to the RPFB treatments. The increased response of soybean shoot dry matter yield to application of TSP 68.70 kg  $P_2O_5 + 25$  kg N could be due to the fact that both nitrogen and phosphorus were limiting plant growth as evidenced in the low initial soil available P and total N levels at Nyankpala (Table 4.1). The response of soybean shoot and root dry matter yield to N application is similar to the findings of Mrkovacki et al. (2008), who reported increased growth in soybean at lower (30 kg N ha<sup>-1</sup>) dose of N fertilization while an increase in N fertilization reduced growth of soybean as reported by Diep et al. (2002). Jemo et al. (2006) also reported that application of mineral N and P increased shoot dry matter yield of grain legumes. This finding, however, contradicts that of Tahir et al. (2009) who indicated that shoot and root dry matter yield of soybean did not respond significantly to N fertilizer application when no phosphorus and rhizobium inoculants

were applied. The response of both shoot and root dry weights to N application could be due to N deficiency which limited plant growth at the initial stages prior to the onset of nitrogen fixation. Peoples and Craswell (1992) indicated that N benefits are generally higher if N is a limiting factor or deficient in soil as a result of high N demand of high yielding cultivars (Dashti *et al.*, 1998).

It is very likely that the low (3.16 mg kg<sup>-1</sup>) soil NO<sub>3</sub><sup>-</sup> - N level could not meet the crop N demand during the "nitrogen hunger" period before the onset of nitrogen fixation. Nodule growth and the symbiotic association between the legume and bradyrhizobia are known to be controlled by P and N demand of the plant (Hellsten and Huss-Danell, 2000). Under such conditions, application of nitrogen at low rates is able to stimulate seedling growth and early nodulation such that both N<sub>2</sub> fixation and yields are enhanced as reported by Hardarson and Atkins (2003).

Soybean and cowpea shoot dry matter yield response to phosphorus from TSP was higher than that from RP fertilizer because TSP is much more soluble in water than rock phosphate. This makes P in TSP more readily available for plant uptake, enhanced root development and nodulation than P in RP. Similar results were obtained by Saidou *et al.* (2012) that both grain and fodder yield responses of cowpea to phosphorus as single super phosphate (SSP) were higher than those from rock phosphate fertilizer.

#### 5.4.2 Grain yield

Soybean and cowpea grain yields on the Ferric Luvisol at Nyankpala were significantly influenced by the different phosphorus fertilizers as indicated in Tables 4.18 and 4.23, respectively. The application of phosphorus fertilizer treatments gave soybean grain yield of 1,554 kg ha<sup>-1</sup> on the average, which is about 60% of the potential yield of the crop. The application of TSP fertilizer + 25 kg N significantly increased grain yield by 74% representing 86% of potential yield of soybean.

Grain yield response of soybean to combined application of N and P corroborates the results of Tahir *et al.* (2009) who reported a significant increase of 57% in soybean grain yield when 25 kg N ha<sup>-1</sup> was combined with P. It, however, contradicts the findings of Chemining' wa *et al.* (2007) that application of 26 kg N ha<sup>-1</sup> had no significant effect on grain yield of six grain legumes. There have been contradictory reports in N fertilization studies which do not provide clear evidence as to the need for N fertilization to complement the N supply from nitrogen fixation to achieve yields close to potential levels (Salvagiotti *et al.*, 2008). According to Yinbo *et al.* (1997), seed yield of soybean only rarely could be enhanced by low rates of N application. Studies by van Kessel and Hartley (2000) also indicate that low rates of N application was likely to be effective bradyrhizobia. This assertion cannot be wholly true since other factors such as moisture and other nutrient deficiencies influence crop grain yield as well.

Soybean meets its high N requirement mainly through biological nitrogen fixation and mineral soil or fertilizer N. According to Streeter (1985), one major constraint of soybean in terms of increasing N uptake has to do with the antagonism between concentration of nitrate in the soil solution and the nitrogen fixation process. This is normally the case in the absence of other abiotic stresses such as soil moisture, pH or temperature that reduce BNF activity (Purcell *et al.*, 2004). In this study, the case of N fertilizer application antagonising the process of nodule formation could not have occurred since application of N rather increased soybean nodulation with application
of phosphorus from TSP and RPFB (Table 4.17). Similar results were obtained by Chemining'wa *et al.* (2012) who reported that application of 30 kg N ha<sup>-1</sup> enhanced nodule biomass of garden pea. Although the average yield of soybean at Nyankpala was low (1554 kg ha<sup>-1</sup>) compared to the crop's yield potential of 2500 kg ha<sup>-1</sup>, it was higher than the average of 800 kg ha<sup>-1</sup> obtained by most farmers who seldom apply fertilizer.

The reduction in soybean grain yield could be due to the dry spells the crop experienced during both the vegetative (22 to 43 days after sowing) and late reproductive stages (85 days after sowing) of growth (Fig 4.2). Soybean requires more water for its growth and development with the peak demand for water occurring at flowering and early pod formation. According to Mundstock and Thomas (2005), drought stress during these critical period of growth results in abortion of flowers and pods which ultimately leads to low seed production and yield. In soybean, maximum nitrogen fixation occurs between the late reproductive phenological stages (R3) i.e. pod formation and (R5), the beginning of grain - filling (Zapata et al., 1987). Moreover, soil moisture stress has been reported to reduce nodulation and nitrogen fixation (Ladrera *et al.*, 2007) especially when it occurs at the reproductive stages of growth where there is very little chance of recovery from moisture stress (Pena-Cabriales and Castellanos, 1993). During this period, any deficit in crop N demand and supply through nitrogen fixation must be met by the uptake of N from other sources. Studies by Yinbo et al. (1997) indicated that soybean grain yield could be further increased by 20% when supplementary fertilizer

N is strategically applied during pod filling stage, when the crop's N demand is at its peak.

Translocation of fixed nitrogen and other nutrients into grain may have been impaired as the relationship between shoot N uptake and grain yield indicated that shoot N uptake was positively and significantly correlated with grain yield (Fig 4.4).

The reduction in grain yield could also be as a result of a reduction in N supply during grain - filling stage due to reduction in nitrogen fixation activity between (R5) and the beginning of maturity (R7) stages of crop development (Zapata *et al.*, 1987).

Studies in which there were no significant increases in grain yield following N fertilizer application attributed it to the substitution of N from BNF with that from fertilizer N. There could also be translocation of N from vegetative reserves in cases where there is reduction in the rate of N<sub>2</sub> fixation due to applied fertilizer nitrogen (Herridge *et al.*, 1984). In this study, the response of soybean to N application could be attributed to the inability of the nitrogen fixation apparatus to meet the N demand of soybean during the latter stages of the crop's growth. During this period there is a reduction in nitrogen fixation and as such the crop needs to make up the N deficit by remobilizing N accumulated in leaf biomass into grain with the resultant effect of reducing the photosynthetic capacity of the leaves thereby limiting the crop's potential yield.

In this study, grain yield response of soybean to N fertilizer application corroborates the findings of Salvagiotti *et al.* (2008), who asserted that yield response of soybean to N fertilizer application was much dependent on the yield potential of the production environment as well as biotic and abiotic constraints that reduce crop growth and crop N demand.

The performance of cowpea with TSP fertilizer application was significantly higher than that of sole RPFB and the control. Yield from RPFB application was statistically different from the control only when N was applied (Table 4.23). The application of RPFB in combination with N significantly increased cowpea grain yield compared to that without N fertilizer. Grain yield response of cowpea to TSP fertilizer is similar to the findings of Arkoful *et al.* (2015) who reported highest cowpea grain yield of cowpea from application of 60 kg  $P_2O_5$  as TSP. The increase in grain yield with application of TSP could be attributed to the readily available P from TSP compared to RPFB which is sparingly soluble in water. Moreover, P is a critical nutrient for cowpea growth and yield as it is known to stimulate some processes such as nodule initiation and formation and the efficiency of legumerhizobium symbiosis (Nkaa *et al.*, 2014). Grain yield response of cowpea to N fertilizer is contrary to the finding of Chemining'wa *et al.* (2007) who reported no significant increase in grain yield of cowpea after application of 26 kg N as starterN. They probably did not get the desired effect of starter N application since the initial soil N content (0.2 g kg<sup>-1</sup> of soil) was high enough for plant growth during the

'nitrogen hunger' period prior to the onset of N<sub>2</sub>-fixation process. In this study, the response of cowpea to N fertilizer application could be attributed to the low (3.16 mg kg<sup>-1</sup> NO<sub>3</sub><sup>-</sup> N) content of the soil. The yield response of cowpea to RPFB 68.70 kg P<sub>2</sub>O<sub>5</sub> + 25 kg N could also be due to the high shoot and root biomass accumulation which resulted in better P uptake and translocation of shoot N into grain.

# 5.5 Effect of phosphorus fertilizer and N application on N and P uptake in soybean and cowpea

## 5.5.1 Shoot N and P uptake in soybean and cowpea

Application of all the phosphorus fertilizer treatments did not significantly increase soybean shoot N uptake relative to the control. The TSP 68.70 kg  $P_2O_5$  however,

increased soybean shoot N by 39.5% relative to the RPFB 68.70 kg  $P_2O_5$  (Table 4.19). Soybean shoot N response to TSP 68.70 kg  $P_2O_5$  fertilizer application is similar to the findings of Shu-Jie *et al.* (2007) who reported an increase in N concentration in root and shoot with a good correlation between shoot N and P supply.

Cowpea shoot N was also increased by 41 and 83% relative to the control as a result of the application of TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> and TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> + 25 kg N treatments, respectively. Shoot N response of cowpea to combined application of N and P could have been due to the increase in shoot to root ratio with increased P supply (Table 4.23). The application of starter N fertilizer also prevented the onset of an early-season N- deficiency which could have delayed early crop growth as well as the development of an efficient nodulation system as reported by Hungria *et al.* (2005a). The increased response of shoot N uptake to starter N application could be explained by the fact that applied N was the major source of N for development due to the low (3.16 mg kg<sup>-1</sup> NO<sub>3</sub><sup>-</sup> N) level of the Ferric Luvisol at Nyanpkala.

For cowpea, application of TSP and starter N could also have resulted in root proliferation for increased nodulation and higher nitrogen fixation as root dry matter yield was significantly correlated ( $R^2$ = 0.66) with shoot N uptake (Fig 4.7). Although studies have shown that high levels of inorganic nitrate N do have a suppressive effect on legume nodulation (Chemining'wa and Vessey, 2006), application of moderate dose of N in soils with low mineral N content has been found to stimulate seedling growth such that both N<sub>2</sub> fixation and grain yield are enhanced (Hardarson and Atkins, 2003). Legume plants require inorganic N during the "nitrogen hunger" period for nodule development, shoot and root growth before the onset of N<sub>2</sub>-fixation process (Hansen, 1994). Application of TSP 68.70 kg  $P_2O_5$  + 25kg N increased soybean and cowpea shoot P uptake by 66 and 113%, respectively over the control compared to 13 and 36% from the application of RPFB 68.70 kg  $P_2O_5 + 25$  kg N. The increased shoot biomass P uptake as a result of P additions from sole and combined application of P from TSP and starter-N fertilizer could be due to the more readily available P in TSP fertilizer treatments than the RPFB. This finding is in agreement with Olivera et al. (2004) who reported that P application to legumes resulted in an increase in plant biomass and shoot P content due to increased rate of nitrogen fixation. Tahir et al. (2009) also reported highest P uptake in shoot biomass of soybean when P was combined with 25 kg N ha<sup>-1</sup> as starter N. Application of 25 kg N could have resulted in an increase in plant vigour and greater root proliferation which promoted better uptake of nutrients as root dry matter yield was significantly correlated with shoot P uptake with 62% of the total variation in shoot P uptake being explained by variation in root dry matter yield (Fig 4.6). Moreover, some studies have shown that the most important trait for P acquisition and efficiency in legumes has more to do with root morphology and architecture, root symbiosis and exudates (Cheng et al., 2008; Wang et al., 2004).

According to Sanginga (1992), at low soil P levels, symbiotic nitrogen fixation tends to have a higher P requirement than plant growth depending upon plant genotype. Grain legumes differ in the mechanisms they employ to acquire P under phosphate limiting conditions in the soil (Graham and Vance, 2000). These mechanisms include acidification of the rhizosphere through exudation of acid phosphatase, changes in root architecture, enhanced P transport and mycorrhizal symbiosis as reported by Vance *et al.* (2003).

## 5.5.2 Grain N and P uptake in soybean and cowpea

The application of TSP 68.70 kg  $P_2O_5 + 25$  kg N significantly increased soybean grain N and P uptake by 78 and 120%, respectively over that of the control compared to 40 and 32% increase from RPFB 68.70 kg  $P_2O_5$ . Table 4.19 shows that soybean grain N and P uptake response to P addition were maximised when combined with starter N fertilizer. This result is similar to the findings of Yoseph and Worku (2014) who reported a 35% increase in grain N uptake with an increase in N fertilizer application from 0 to 46 kg N ha<sup>-1</sup>. Studies by Hossain *et al.* (2007) indicated that applied N influenced the N content of seed while Tahir *et al.* (2009) reported significant increases in both grain N and P uptake by soybean when P application was combined with 25 kg N ha<sup>-1</sup>.

Soybean as an oil seed crop has high N requirement which the crop is able to meet through the assimilation of soil mineral N and symbiotic nitrogen fixation. Although most studies on the effect of N fertilizer application on soybean have indicated that N fertilizer application increased crop growth but resulted in reduced nitrogen fixation via a reduction in the number, weight and activity of the nodules (Starling *et al.*, 1998), this effect has been found to be largely dependent on a number of factors including the amount of N applied, soil type and climatic conditions. Moreover, studies by Ray *et al.* (2006) have also shown that application of a reduced quantity of N as starter fertilizer improved soybean crop growth and N uptake.

The increase in soybean grain N uptake using starter N fertilizer could be attributed to enhanced crop growth resulting from increased nitrogen fixation as N fertilizer fulfilled the immediate plant N need at the early stages of growth. Moreover, nitrogen fixation has been found to be positively correlated ( $R^2 = 0.62$ , P < 0.001) with N uptake in soybean (Salvagiotti, 2008). Furthermore, soybean grain yield was significantly correlated ( $R^2 = 0.64$ ) with shoot N uptake, as shoot N uptake could explain as much as 64% of the variation in grain yield (Fig. 4.4).

Phosphorus supply has been found to enhance N accumulation at both flowering and late pod filling stage in soybean (Taylor and Philadelphia, 2006). The supply of P increased P uptake in grain probably due to improved root development and nodulation since soybean root biomass significantly correlated with shoot P uptake ( $R^2 = 0.62$ , Fig. 4.6). The well-developed rooting system could have resulted in better absorption of water and nutrients for efficient translocation. Increasing P levels was also reported to have increased P uptake by pigeon pea due to higher amount of biomass and greater P accumulation by the plant (Kumar and Kushwaha, 2006).

Grain N and P uptake in cowpea were also increased significantly by starter N application and phosphorus fertilizers. Grain N and P uptake as a result of the application of RPFB 68.70 kg  $P_2O_5$ + 25 kg N were, however, comparable with those of TSP 68.70 kg  $P_2O_5$ + 25 kg N (Table 4.24). The performance of cowpea with RPFB regarding grain P uptkae confirms the findings of Krasilnikoff *et al.* (2002) who reported cowpea's ability to solubilise P from sparingly soluble P sources such as RP through exudation of organic acids.

# 5.5.3 Effect of phosphorus fertilizer on P use efficiency of soybean and cowpea

The data in Tables 4.9 and 4.15 indicate that P use efficiency (PUE) and P uptake efficiency (PUpE) of soybean and cowpea increased significantly with low rates  $(34.35 \text{ kg P}_2\text{O}_5)$  of P applied from both fertilizers. Both P use efficiency indices were, however, not significantly influenced by the type of P fertilizer applied. The increased response of soybean PUE and PUpE to low amounts of applied P confirms the

assertion by Mosier *et al.* (2004) that in the short term, all P efficiency indices increase with decreasing amounts of fertilizer applied even to levels that are below the optimum recommended rates. The increase in these efficiency indices at low P rates could be due to the fact that where more P is applied, much of the applied P is likely to be tied up and rendered unavailable for plant uptake in the soil through fixation by the formation of complexes with Al and Fe in acidic soils (Gyaneshwar *et al.*, 2002).

Phosphorus use efficiency of soybean and cowpea were not influenced by the type of P fertilizer because this parameter is mostly genetically controlled with special plant traits for P acquisition and utilization. Moreover, genotypic variations in root system of legumes has been associated with substantial variation in P uptake and utilization. Phosphorus uptake efficiency (PUpE) of soybean at Nyankpala was significantly influenced by P fertilizer source, where PUpE as a result of the application of TSP  $68.70 \text{ kg } P_2O_5 + 25 \text{ kg N}$  was higher than that of RPFB  $68.70 \text{ kg } P_2O_5 + 25 \text{ kg N}$ . This could be attributed to the more readily available P from TSP fertilizer than RPFB fertilizer due to the slow release nature of the latter to the soil. Application of starter N may also have contributed to improved seedling growth and root proliferation for better absorption of P as evidenced by the good correlation ( $R^2 = 0.62$ ) between soybean root dry matter and shoot P uptake (Fig. 4.6). Given the fact that P is immobile in the soil, a well-developed rooting system is necessary for effective P uptake. Studies by Prochnow et al. (2004) indicate that the presence of water soluble fertilizer P is able to increase soil P uptake by roots as a result of fertilizer P - induced improvement in plant root development.

For cowpea, application of RPFB 68.70 kg  $P_2O_5 + 25$  kg N increased both PUE and PUpE relative to RPFB 68.70 kg  $P_2O_5$  while both indices were not significantly

increased by the application of TSP 68.70  $P_2O_5+$  25kg N (Table 4.26). Plant species and genotypes have several adaptive mechanisms to respond to soil P deficiency.

According to Saidou *et al.* (2012), a major index for assessing the response of legume cultivars to soils with low P content is the shoot to root ratio. The data in Table 4.23 indicates that cowpea significantly increased its shoot to root ratio in response to increasing P supply on the Ferric Luvisol at Nyankpala. The increase in cowpea shoot to root ratio with P fertilizer application also supports the observation by Anghioni and Barber (1980) regarding how shoot to root ratio is influenced by P application. A corresponding increase in shoot to root ratio with increasing P supply has been explained as a mechanism adopted by certain crops to efficiently use added P in producing dry matter (Saidou *et al.*, 2012). Under abundant P supply, the plant does not need to develop extensive root system for P absorption and as such uses more of the readily available P in the production of shoot biomass. Moreover, cowpea has been identified as a P efficient legume crop through its ability to mobilize P from sparingly soluble P pools in the soil (Krasilnikoff *et al.*, 2002).

# 5.6 Economic profitability of nitrogen and phosphorus fertilizer application for soybean and cowpea production on a Ferric Luvisol at Nyankpala

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Value - cost ratio analysis following application of the phosphorus fertilizers and starter -N indicated that none of the treatments imposed was economically viable for soybean and cowpea production as none of them gave a VCR  $\ge$  2. The application of TSP 68.70 kg P<sub>2</sub>O<sub>5</sub> + 25 kg N however, gave a VCR of 1.77 for soybean which indicated a break-even point with 77% return on investment (Fig. 4.9). For cowpea, application of TSP 68.7 kg  $P_2O_5 + 25$  kg N and TSP 68.7 kg  $P_2O_5$  were the most profitable inputs with VCR of 1.65 and 1.54, representing 65 and 54% return on investment, respectively (Fig. 4.10). Dogbe *et al.* (2013), in assessing the economics of soybean production in Saboba and Chereponi districts of northern Ghana reported that soybean production was not profitable in Chereponi for both male and female farmers. This was mainly due to the low (less than 10 %) level of fertilizer patronage by the farmers. They however reported some level of profitability for male farmers in Saboba although none of these farmers used fertilizers at higher rates.

Legume production in northern Ghana is constrained by many factors including low adoption of improved soybean technologies such as the use of inorganic fertilizers, rhizobia inoculants, row planting and other good management practices (Mbanya, 2011). Production constraints including lack of rights over land, low prices, poor marketing of produce, inadequate access to credit facilities, bad weather, low yield and poor storage facilities among others affect the profitability of production.

Application of TSP 68.70 kg  $P_2O_5$  fertilizer and 25 kg N as starter N gave a VCR of 1.77 as a result of the 74% increase in grain yield (2,214 kg ha<sup>-1</sup>) from this treatment relative to (1,271 kg ha<sup>-1</sup>) from the control, while the average yield of soybean at Nyankpala was 1,554 kg ha<sup>-1</sup> which is above the average yield of 800 kg ha<sup>-1</sup> (SRID, 2012) obtained by most smallholder farmers who do not apply fertilizer. Application of TSP 68.70 kg  $P_2O_5 + 25$  kg N gave a VCR of 1.77 which although was less than 2, provides a 77% return on investment for smallholder farmers.

Tables 4.27 and 4.28 show that TSP 68.70 kg  $P_2O_5 + 25$  kg N gave a relatively high soybean crop value of GHC 2,546 with a low total input cost of GHC 609 compared

to a crop value and total input cost of GHC 1,590 and GHC 752, respectively for RPFB 68.70 kg  $P_2O_5 + 25$  kg N. Although the price of 50 kg bag of TSP was higher (GHC 80.00) than that of RPFB (GHC 50.00), RPFB had a higher fertilizer input cost than TSP. This was primarily due to the fact that for a particular rate of P application, one needed to apply more of the RPFB as it contained 18%  $P_2O_5$  compared to 46% in TSP.

One major advantage with the use of rock phosphate fertilizers by resource – poor farmers in Africa has to do with its low price relative to that of imported fertilizers (Chien and Menon, 1995 a). Most farmers are, however discouraged from using this resource due to the limited success in its use. Moreover, direct application of this fertilizer requires that it is applied at higher rates due to its lower solubility and low P content thereby increasing the cost of transportation (Omamo, 1998).

Results from this study indicated that, combined application of TSP fertilizer and starter N proved to be a more profitable intervention for soybean and cowpea production in the short term than RPFB and starter N. Sole application of 68.70 kg  $P_2O_5$  TSP gave a VCR of 1.54 for cowpea production which represented a breakeven situation with 54% return on investment compared to VCR of 0.33 from application of RPFB 68.70 kg  $P_2O_5$  which represented a situation where the farmer gets only 33% of the total amount invested. This confirms the importance of the need for farmers to adopt the practice of starter N fertilizer application to increase grain yield and their income levels as well. There is the need for fertilizer manufacturers to improve on P availability from rock phosphate - based fertilizer blends through further milling to homogenize its texture for easy dissolution and partial acidulation for maximum benefits in the short term, as farmers' decision to use such fertilizers is determined by

its cost-effectiveness. Farmers could also exploit the more cost-effective option of coapplication of RP fertilizers with on-farm organic fertilizers such as farm yard manure to improve their effectiveness.

#### **CHAPTER SIX**

# 6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

#### 6.1 Summary

Grain legume production in northern Ghana is limited by low soil fertility as a result of deficiencies in some essential soil macronutrients including phosphorus and nitrogen. The rising cost of water soluble P fertilizers has necessitated the search for other less expensive and readily available P fertilizer sources to increase crop production. A rock phosphate fertilizer blend (Yara legume), purported to be a balanced crop nutrient formulation to correct P and other soil nutrient deficiencies for legume production was used in this study. The main aim of this study, therefore, was to evaluate yield response of soybean and cowpea to rhizobial inoculation and rock phosphate fertilizer blend application and its economic implication to farmers in northern Ghana. A preliminary assessment of the indigenous soil rhizobia population at the study sites was done by conducting MPN counts of soil rhizobia using the plant infection method.

Field experiments were conducted during the 2012 and 2013 cropping seasons to assess the influence of rock phosphate fertilizer blend and rhizobial inoculation on nodulation, dry matter and grain yields of soybean and cowpea. The effect of rock phosphate fertilizer and low N fertilizer application on N and P uptake and P use efficiency in soybean and cowpea were also evaluated. Value-cost ratio analysis was used to determine the short term profitability of rock phosphate fertilizer blend and N fertilizer application for soybean and cowpea production.

Although application of rhizobial inoculants significantly increased soybean nodule dry weight on the Gleyic Lixisol at Bontanga by 79%, this increase was not translated into grain yield. On the contrary, cowpea nodulation did not respond positively to rhizobial inoculation.

Soybean nodulation on the Gleyic Lixisol was significantly influenced by the type of phosphorus fertilizer applied; as nodule dry weight for soybean obtained from the application of TSP 34.35 and 68.70 kg P<sub>2</sub>O<sub>5</sub> were significantly higher than that from RPFB 34.35 and 68.70 kg P<sub>2</sub>O<sub>5</sub>. The application of RPFB 34.35 kg P<sub>2</sub>O<sub>5</sub> and TSP 68.70 kg P<sub>2</sub>O<sub>5</sub>, however, resulted in significantly higher nodule dry weight of cowpea than that of the control.

The application of TSP 68.70 kg  $P_2O_5$  fertilizer significantly increased soybean shoot dry matter yield on the Gleyic Lixisol by 84% relative to the control while RPFB 68.70 kg  $P_2O_5$  gave an increase of 25%. Moreover, TSP 68.70 kg  $P_2O_5$  treatment increased shoot dry matter yield of soybean by 47% relative to the RPFB 68.70 kg  $P_2O_5$ treatment. Cowpea shoot dry matter yield also increased by 81 and 67% with application of TSP and RPFB treatments, respectively relative to the control, while no such differences were observed between TSP and RPFB treatments.

The RPFB 34.35 kg  $P_2O_5$  treatment increased soybean grain yield on the Gleyic Lixisol at Bontanga by 38% relative to the control, while 22% increase was obtained from the application TSP 34.35 kg  $P_2O_5$ . The application of TSP 68.70 kg  $P_2O_5 + 25$  kg N and RPFB 68.70 kg  $P_2O_5 + 25$  kg N increased soybean grain yield on the Ferric Luvisol at

Nyankpala by 74 and 9%, respectively compared to the control. Similarly, the TSP 68.70 kg  $P_2O_5 + 25$  kg N and RPFB 68.70 kg  $P_2O_5 + 25$  kg N treatments increased grain yield of cowpea by 53 and 51%, respectively over the control.

Sole application of TSP 68.70 kg  $P_2O_5$  gave an increase of 36% in grain yield over the control compared to 11% gain from the RPFB 68.70 kg  $P_2O_5$  treatment.

The TSP 68.70 kg  $P_2O_5 + 25$  kg N treatment significantly increased soybean grain N and P uptake by 78 and 120%, respectively over the control compared to 40 and 32% increase from RPFB 68.70 kg  $P_2O_5$ . Grain N and P uptake in cowpea were also increased by 40 and 81%, respectively over the control following application of TSP 68.70 kg  $P_2O_5 + 25$  kg N. The RPFB 68.70 kg  $P_2O_5 + 25$  kg N treatment gave an increase in grain N and P uptake of 44 and 74%, respectively over the control. The application of phosphorus fertilizers without nitrogen did not result in significant increase in grain N uptake of cowpea but the TSP 68.70 kg  $P_2O_5$  treatment increased grain P uptake by 30% relative to the RPFB 68.7 kg  $P_2O_5$  treatment.

Phosphorus use efficiency (PUE) and P uptake efficiency (PUpE) of soybean and cowpea on the Gleyic Lixisol at Bontanga increased significantly with low rates of P (34.35 kg P<sub>2</sub>O<sub>5</sub>) applied from both fertilizer sources. The P use efficiency indices were however, not significantly influenced by the type of P fertilizer applied.

Phosphorus uptake efficiency of soybean on the Ferric Luvisol at Nyankpala was significantly influenced by the type of P fertilizer applied, as P uptake efficiency resulting from application of TSP 68.70 kg  $P_2O_5 + 25$  kg N was higher than that of RPFB 68.70 kg  $P_2O_5 + 25$  kg N treatment.

For cowpea, application of RPFB 68.70 kg  $P_2O_5 + 25$  kg N increased both PUE and PUpE relative to RPFB 68.70 kg  $P_2O_5$  while both indices were not significantly increased by the application of TSP 68.70  $P_2O_5 + 25$ kg N.

Value - cost ratio analysis indicated that though TSP 68.7 kg  $P_2O_5 + 25$  kg N did not give the desired VCR of  $\geq 2$ , a VCR of 1.77 is a promising treatment combination for soybean production than RPFB 68.70 kg  $P_2O_5 + 25$  kg N fertilizer. The application of TSP 68.70 kg  $P_2O_5 + 25$  kg N and TSP 68.70 kg  $P_2O_5$  were the most profitable inputs for cowpea production on the Ferric Luvisol at Nyankpala.

### **6.2** Conclusions

Based on analyses and interpretation of data on the variables used in evaluating the yield of soybean and cowpea as influenced by rhizobial inoculation and rock phosphate fertilizer blend application, the following conclusions were made:

i. application of TSP 68.70 kg  $P_2O_5$  and RPFB 68.70 kg  $P_2O_5$  better enhanced nodulation, biomass and grain yields of soybean on the Gleyic Lixisol than rhizobial inoculation, while RPFB 68.70 kg  $P_2O_5$  was more effective in enhancing grain yield of cowpea than TSP 68.70 kg  $P_2O_5$ .

ii. application of TSP 68.70 kg  $P_2O_5 + 25$  kg N and RPFB 68.70 kg  $P_2O_5 + 25$  kg N in low N environments is necessary to stimulate higher N and P uptake and P use efficiency in soybean and cowpea than sole application of these fertilizers.

iii. in the short term, application of TSP 68.70 kg  $P_2O_5 + 25$  kg N and TSP 68.70 kg  $P_2O_5$  will be cost effective for soybean and cowpea production on the Ferric Luvisol at Nyankpala relative to RPFB 68.70 kg  $P_2O_5 + 25$  kg N and RPFB 68.70 kg  $P_2O_5$ .

## **6.3 Recommendations**

Results from this study have demonstrated the potential of the different phosphorus fertilizers and nitrogen application in improving nodulation, biomass and grain yields of soybean and cowpea. The study was, however, limited in the estimation of the effect of these treatments on the amount of nitrogen fixed by cowpea and soybean and the residual effects of rock phosphate fertilizer application. It is therefore recommended that:

i. further studies be carried out to quantify nitrogen fixation in soybean and cowpea as influenced by the different phosphorus fertilizer treatments in these environments, with the aim of estimating savings in inorganic nitrogen fertilizer imports by Government.

ii. the residual effect of rock phosphate fertilizer blend application on soybean and cowpea be determined; against the background that application of rock phosphate has a positive residual effect on crop yields for several continuous cropping seasons.



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#### **APPENDICES**

#### **Appendix 1 Composition of Broughton and Dilworth N-free plant nutrient solution**

		A J AMES PROPERTY	
Stock		1111	
Solutions	Element	Form	g/l
E	Ca	C <mark>aCl2•2H2O 294.1</mark>	2
E	2 2		5/3/
2	P	KH <sub>2</sub> PO <sub>4</sub> 136.1	a ser
	1		10
3	Fe	Fe-citrate 6.7	
	Mg	MgSo <sub>4</sub> •7H <sub>2</sub> O	123.3
	Κ	$K_2SO_4$	87.0
	Mn	MnSO <sub>4</sub> •H <sub>2</sub> O	0.338
4	В	H <sub>3</sub> BO <sub>3</sub> 0.247	
		173	

Zn	$ZnSO_4 \bullet 7H_2O$	0.288
Cu	$CuSO_4 \bullet 5H_2O$	0.100
Co	$CoSO_4 \bullet 7H_2O$	0.056
Mo	Na <sub>2</sub> MoO <sub>2</sub> •2H <sub>2</sub> O	0.048

Source: Somasegaran and Hoben (1985)



Appendix 2 Most probable number count of cowpea bradyrhizobia on the Gleyic Lixisol



4223.2)

Appendix 3 Most probable number count of soybean bradyrhizobia on the Gleyic Lixisol

REPLICATIONS

	1	2	3	4	TOTAL
5-					
1	+	+	+	+	4
5-2	+	+	+	+	4
5-3	+	-	-	-	1
5-4	-		1-10	CT.	0
5-5	-			2	0
5-6	-	1.001.00	~ `	-	0
TOTAL					9

MPN Estimate = 57.1 cells g<sup>-1</sup> of soil , P = 0.05. Confidence interval = (19.8 – 164.7) Appendix 4 Most probable number count of cowpea bradyrhizobia on the Ferric Luvisol

	REPLICATIONS					
	1	2	3	4	TOTAL	
5-	5		1 and	1		
	- t		+	+	4	
5-2	+	2+10	+	3+7	4	
5-3	7+0	+	+	×+7	4	
5-4	+	+	100	227	2	
5-5	10	ality	3.0	+	1	
5-6	-		-	+	] 1	
TOTAL		2	22		16	
MPN Estimate	= 698.4 cells	s g <sup>-1</sup> of soil, P =	0.05. Confi	dence interva	al = (242.3 –	

2013.5)

Appendix 5 Most probable number count of soybean bradyrhizobia on the

Ferric Luvisol				
	REP	LICATIONS		
1	2	3	4	TOTAL

5-					
1	+	+	+	+	4
5-2	+	+	-	-	2
5-3	+	-	-	-	1
5-4	-	-	-	-	0
5-5	-	- 20 A	an ta s	_	0
5-6	-	IAN	- 3		0
TOTAL			U.		7

MPN Estimate = 21.5 cells  $g^{-1}$  of soil, P = 0.05. Confidence interval = (7.5 - 62.1)

Appendix 6 Most probable number count of bradyrhizobia cells in the commercial inoculant



176

10-10

#### TOTAL

MPN Estimate =  $6.13 \times 10^6$  cells g<sup>-1</sup> of soil, P = 0.05,

Confidence interval = 
$$(1.61 \times 10^6 - 2.33 \times 10^7)$$

## Appendix 7 Interactive effect of phosphorus fertilizer and rhizobial inoculation on soybean nodule dry weight on the Gleyic Lixisol

	Phosphorus fertilizer rate					
Inoculation	RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	TSP 34.35 kg P <sub>2</sub> O <sub>5</sub>	TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	0 kg P2O5 Control	
Inoculated	54.00	116.50	170.00	170.00	<u>46.00</u>	
(+ Rh)		EU	S/	J.	1	
Uninoculated	53.00	68.00	59.50	61.00	53.50	
(- Rh)		Yr.	1	2	\	

#### Appendix 8 Interactive effect of phosphorus fertilizer and rhizobial inoculation

#### on soybean shoot dry matter yield on the Gleyic Lixisol

	Phosphorus fertilizer rate				
Inoculation	RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	RPFB 68.70 TSP 34.35 kg $P_2O_5$ kg $P_2O_5$	TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	0 kg P2O5 Control	

Inoculated	1066	1159	1473	1877	1258
(+ Rh)					
Uninoculated	2096	2457	1773	3456	1640
(- Rh)					
	$F pr = \langle 0 \rangle$	0.001. LSD (	(5%) = 868.60.	CV (%) =10.50	
	$\mathbf{P} = \mathbf{v}$	0.001, LDD (	<i>570) =</i> 000.00, C		
		K	NU	ST	
			m		
F		E.	DV-	15	F
	-2	E.	y s	12	7
	11	Fr	2	1500	
		aus	2		
INT	-to-	<u>k</u>	$\leq$		New York
	COP.	R		5 BAD	
		103	ANE N		

#### Appendix

rhizobial inoculation

Inoculation	RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	Phos RPFB 68.70 P <sub>2</sub> O <sub>5</sub> kg P <sub>2</sub> O <sub>5</sub>	phorus fertilize TSP 34.35 kg	r rate TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	0 kg P <sub>2</sub> O <sub>5</sub> Control
Inoculated	469	361	558	476	333
(+ Rh) Uninoculated	1285	1151	1011	1012	948
(- Rh)		PT-	14		

# 9 Interactive effect of phosphorus fertilizer and on soybean grain yield on the Gleyic Lixisol

F pr = 0.002, LSD (5%) = 398.30, CV (%) = 18.50

Appendix 10 Interactive effect of phosphorus fertilizer and rhizobial inoculation

#### on soybean grain N uptake on the Gleyic Lixisol

	Phosphorus fertilizer rate					
Inoculation	RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	TSP 34.35 kg P <sub>2</sub> O <sub>5</sub>	TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	0 kg P2O5 Control	
Inoculated	35.50	26.10	42.60	37.90	25.30	
(+ Rh) Uninoculated (- Rh)	97.70	86.00	76.40	75.90	73.00	

F pr = 0.004, LSD (5%) = 27.85, CV (%) =11.10

#### Appendix

rhizobial inoculation

	Phosphorus fertilizer rate						
Inoculation	RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	TSP 34.35 kg P <sub>2</sub> O <sub>5</sub>	TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>			
Inoculated	31.24	12.03	37.18	15.85			
(+ Rh)		NO					
Uninoculated	85.68	38.37	67.40	33.75			
(- Rh)	5						
]	F pr $< 0.001$ , LSD (5%) = 20.56, CV (%) =12.10						

#### 11 Interactive effect of phosphorus fertilizer and on P use efficiency of soybean on the Gleyic Lixisol

Appendix 12 Interactive effect of phosphorus fertilizer and rhizobial inoculation

on nodule dry weight of cowpea on the Gleyic Lixisol

	Phosphorus fertilizer rate					
Inoculation	RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	TSP 34.35 kg P2O5	TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	0 kg P2O5 Control	
Inoculated	115.30	78.60	85.20	116.50	97.30	
(+ Rh)	40			St.		
Uninoculated	127.10	104.70	115.00	105.50	98.11	
(- Rh)	~	W J SAN	VE NO	3		

F pr < 0.001, LSD (5%) = 11.07, CV (%) = 16.5

#### rhizobial inoculation

### 13 Interactive effect of phosphorus fertilizer and on cowpea shoot dry matter yield on the Gleyic Lixisol Phosphorus fertilizer rate

Appendix

	Phosphorus fertilizer rate					
Inoculation	RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	RPFB 68.70 P <sub>2</sub> O <sub>5</sub> kg P <sub>2</sub> O <sub>5</sub>	TSP 34.35 kg	TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	0 kg P <sub>2</sub> O <sub>5</sub> Control	
Inoculated (+ Rh)	1145	663	696	652	609	
Uninoculated (- Rh)	614	1554	1033	1757	722	

F pr = < 0.001, LSD (5%) = 231.40, CV (%) = 12.90

Appendix 14 Interactive effect of phosphorus fertilizer and rhizobial inoculation

#### on grain yield of cowpea on the Gleyic Lixisol



F pr = < 0.001, LSD (5%) = 282.20, CV (%) = 21.20

#### Appendix

rhizobial inoculation

		Phos	phorus ferti	lizer rate	
Inoculation	RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	<b>RPFB</b> 68.70 kg P <sub>2</sub> O <sub>5</sub>	TSP 34.35 P <sub>2</sub> O <sub>5</sub>	5 kg TSP 68.70 P <sub>2</sub> O <sub>5</sub>	) kg 0 kg P <sub>2</sub> O <sub>5</sub> Control
Inoculated	13.61	8.23	9.47	9.63	7.42
(+ Rh) Uninoculated (- Rh)	20.18	19.80	12.80	16.44	9.13
	F pr = < 0	.001, LSD (5%	(6) = 1.84, C	V (%) = 8.00	

#### 15 Interactive effect of phosphorus fertilizer and on shoot N uptake of cowpea on the Gleyic Lixisol

#### Appendix 16 Interactive effect of phosphorus fertilizer and rhizobial inoculation

	on shoot P uptake of cowpea on the Gleyic Lixisol						
(	A	Phos	phorus fertiliz	er rate	)		
Inoculation	RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	TSP 34.3 kg P <sub>2</sub> O <sub>5</sub>	5 TSP 68.7 kg P <sub>2</sub> O <sub>5</sub>	0 0 kg P <sub>2</sub> O <sub>5</sub> Control		
Inoculated	3.43	1.70	2.34	3.19	1.26		
(+ Rh) Uninoculated (- Rh)	2.37	<b>3.80</b>	3.48	5.95	1.97		

### on shoot P uptake of cowpea on the Gleyic Lixisol

F pr = < 0.009, LSD (5%) = 1.99, CV (%) = 24.00

### AppendixInteractive effect of phosphorus fertilizer and rhizobial inoculation17 on grain P uptake of cowpea on the Gleyic Lixisol

		Pho	sphorus ferti	lizer rate			
Inoculation	RPFB	RPFB	TSP 34	.35 TSP 68	.700 kg P <sub>2</sub> O <sub>5</sub>		
	34.35 kg P <sub>2</sub> O <sub>5</sub>	68.70 kg P <sub>2</sub> O <sub>5</sub>	kg P <sub>2</sub> O <sub>5</sub>	kg P <sub>2</sub> O <sub>5</sub>	Control		
Inoculated	2.690	1.870	1.521	2.228	1.728		
(+ Rh)			2				
Uninoculated	3.882	3.828	2.498	7.321	1.739		
(- Rh)							
	F pr = $< 0.001$ , LSD (5%) = 0.781, CV (%) = 16.60						

Appendix 18 Interactive effect of phosphorus fertilizer and rhizobial inoculation

on grain P use efficiency of cowpea on the Gleyic Lixisol						
Y	Phosphorus fertilizer rate					
Inoculation	RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	TSP 34.35 kg P2O5	TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>		
Inoculated (+ Rh)	49.20	17.80	<mark>36.4</mark> 0	16.40		
Uninoculated (- Rh)	45.30	50.60	47.10	26.90		

F pr = <0.002, LSD (5%) = 10.70, CV (%) =17.40

#### Appendix Interactive effect of phosphorus fertilizer and rhizobial inoculation 19 on grain P uptake efficiency of cowpea on the Gleyic Lixisol

	Phosphorus fertilizer rate				
Inoculation	RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	TSP 34.35 kg P <sub>2</sub> O <sub>5</sub>	TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	
Inoculated	17.94	6.23	10.14	7.43	
(+ Rh) Uninoculated (- Rh)	25.88	12.76	16.65	24.40	

Appendix 20 Interactive effect of phosphorus fertilizer and rhizobial inoculation on grain P utilization efficiency of cowpea on the Gleyic Lixisol

7	Phosphorus fertilizer rate					
Inoculation	RPFB 34.35 kg P <sub>2</sub> O <sub>5</sub>	RPFB 68.70 kg P2O5	TSP 34.35 kg P2O5	TSP 68.70 kg P2O5		
Inoculated	275	286	365	222		
(+ Rh) Uninoculated	176	413	282	107		
(- Rh)	W J	SANE S	10			

F pr = <0.013, LSD (5%) = 98.40, CV (%) = 22.20

		Phos	phorus fertili	zer rate	
Inoculation	RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	TSP 68.70 kg P <sub>2</sub> O <sub>5</sub> + 25 kg N	TSP 68.7 kg P <sub>2</sub> O <sub>5</sub>	70 RPFB 68.70 kg P2O5 + 25 kg N	0 kg P <sub>2</sub> O <sub>5</sub> Control
				U	
Inoculated	20.90	18.15	15.50	12.85	9.63
(+ Rh)				Ğ.	
Uninoculated	19.47	22.77	22.25	22.03	15.60
(- Rh)		25			

### AppendixInteractive effect of phosphorus fertilizer and rhizobial inoculation21 on soybean nodulation on the Ferric Luvisol

F pr = 0.013, LSD (5%) = 5.65, CV (%) = 12.90

#### Appendix 22 Interactive effect of phosphorus fertilizer and rhizobial inoculation

#### on soybean nodule dry weight on the Ferric Luvisol

(	Phosphorus fertilizer rate					
Inoculation	RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	TSP 68.70 kg P <sub>2</sub> O <sub>5</sub> +	TSP 68 kg P <sub>2</sub> O <sub>5</sub>	8.70 RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub> +	0 kg P <sub>2</sub> O <sub>5</sub> Control	
12	6 2	25 kg N		25 kg N	5	
Inoculated (+ Rh)	157.50	65.50	124.6	81.60	78.40	
Uninoculated (- Rh)	89.00	127.4	188.30	219.8	56.90	

F pr =0.03, LSD (5%) = 61.86, CV (%) = 23.70

# AppendixInteractive effect of phosphorus fertilizer and rhizobial inoculation23 on shoot dry matter yield of soybean on the FerricLuvisol

	Phosphorus fertilizer rate					
Inoculation	RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	TSP 68.70 kg P <sub>2</sub> O <sub>5</sub> + 25 kg N	TSP 68.7 kg P <sub>2</sub> O <sub>5</sub>	70 RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub> +	0 kg P2O5 Control	
			2	25 kg N		
Inoculated	1712	2948	2351	3272	2464	
Uninoculated (- Rh)	2225	3305	2932	2175	2494	
	F pr < 0.0	01, LSD (5%) =	= 336.0, CV	(%) = 7.70		

on root dry	matter vield	d of sovbean	on the Ferric	Luvisol
on root ary	matter yier	a of Soybean	on the reffic	

	Phosphorus fertilizer rate				
Inoculation	RPFB 68.70 kg	TSP 68.70 kg P <sub>2</sub> O <sub>5</sub> +	TSP 68.70 P <sub>2</sub> O <sub>5</sub> 68.70	RPFB	kg 0 kg P2O5 Control
12	$P_2O_5$	25 kg N		kg P <sub>2</sub> O <sub>5</sub> -	take /
1	P		<	25 kg N	
Inoculated (+ Rh)	238.90	394.90	351.60	468.40	358.00
Uninoculated (- Rh)	324.30	411.60	343.60	355.30	325.90

F pr < 0.001, LSD (5%) = 42.41, CV (%) = 7.40

#### Phosphorus fertilizer rate Inoculation RPFB TSP 68.70 **TSP68.70 RPFB** 0 kg P<sub>2</sub>O<sub>5</sub> $kg P_2O_5 +$ kg P<sub>2</sub>O<sub>5</sub> 68.70 kg 68.70 kg Control 25 kg N $P_2O_5$ $P_2O_5 +$ 25 kg N Inoculated 2.63 5.98 4.31 4.58 4.10 (+ Rh) 5.82 Uninoculated 3.04 5.57 3.42 2.98 (- Rh)

#### Appendix Interactive effect of phosphorus fertilizer and rhizobial inoculation 25 on shoot P uptake of soybean on the Ferric Luvisol

F pr = 0.025, LSD (5%) = 1.10, CV (%) = 15.50

Appendix 26 Interactive effect of phosphorus fertilizer and rhizobial inoculation

on grain N uptake of cowpea on the Ferric Luvisor						
/	Phosphorus fertilizer treatments					
Inoculation Status	RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	TSP 68.70 kg P <sub>2</sub> O <sub>5</sub> + 25 kg N	TSP 68.7 kg P <sub>2</sub> O5	0 RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub> + 25 kg N	0 kg P <sub>2</sub> O <sub>5</sub> Control	
Inoculated	40.80	39.90	28.20	34.90	29.00	
(+ Rh) Uninoculated (- Rh)	30.30	51.90	41.30	59.30	36.50	

F pr = 0.014, LSD (5%) = 11.90, CV (%) = 19.00

	Phosphorus fertilizer rate				
Inoculation	RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	TSP 68.70 kg P <sub>2</sub> O <sub>5</sub> + 25 kg N	TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub> + 25 kg N	
Inoculated	31.10	36.50	28.10	30.90	
(+ Rh) Uninoculated (- Rh)	26.20	42.80	42.60	47.10	

AppendixInteractive effect of phosphorus fertilizer and rhizobial inoculation27 on grain P use efficiency of cowpea on the Ferric Luvisol

Appendix 28 Monthly cumulative rainfall distribution at Nyankpala in 2013



Value of Total input	Value	crop cost cos	t $(GH \notin)$
			(GH ¢) ratio*
Phosphorus fertilizer rate		Soybean	
0 kg P <sub>2</sub> O <sub>5</sub> (Control)	1461.65	ND	ND
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	1872.20	566.62	0.72
$TSP \ 68.70 \ kg \ P_2O_5 + 25 \ kg \ SOA$	2546.10	609.61	1.77
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	1465.10	423.92	0.01
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub> +25 kg SOA	1590.45	752.31	0.17
Phosphorus fertilizer rate		Cowpea	
0 kg P <sub>2</sub> O <sub>5</sub> (Control)	1633.80	ND	ND
RPFB 68.70 kg P <sub>2</sub> O <sub>5</sub>	1806.00	518.22	0.33
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub> + 25 kg SOA	2496.90	523.67	1.64
TSP 68.70 kg P <sub>2</sub> O <sub>5</sub>	2228.10	385.02	1.54
RPFB 68.70 kg $P_2 O_5 + 25$ kg SOA	2495.10	656.87	1.25

#### Appendix 29 Value - cost ratio analysis of phosphorus fertilizer rates

TSP = triple superphosphate, SOA= Sulphate of Ammonia, RPFB= Rock phosphate

fertilizer blend

\*Value cost ratio = \_\_\_\_\_ \_value of fertilized

crop□value of unfertilized crop

Total cost of inputs