CONTRIBUTION OF RHIZOBIUM AND PHOSPHORUS FERTILIZER TO BIOLOGICAL NITROGEN FIXATION AND GRAIN YIELD OF SOYBEAN IN THE TOLON DISTRICT

BY

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DECLARATION

I hereby declare that this thesis has been fully written and compiled by me with references to specific authors duly acknowledged and that it is neither in part nor whole been presented for another certificate in this university or elsewhere.

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ABSTRACT

A field experiment was conducted in the Tolon district to increased soybean production by identifying efficient strategy for optimizing Biological Nitrogen Fixation (BNF) in soybean cultivars. The experiment was a split-split plot design with three replications and interactions tested were inoculation rates (0, 50 and 100% inoculation) as the main plot factor, soybean variety (Jenguma, Anidaso, Quarshie) as the sub-plot factor and phosphorus rate (0, 22.5 and 45 kg P_2O_5/ha) as the sub-sub plot factor. Each replication consisted of 27 treatment combinations and maize was added as a reference crop. The soybean seeds were sown at a spacing of 50 cm between rows and 10 cm between plants**.** Data collected include plant height, nodule number and dry weight, shoot dry weight, pods number and dry weight, grain yield and 100-seed weight. The Total Nitrogen Difference method was used in determining the amount of N_2 fixed. The results showed that inoculation of soybean with legumefix only increases nitrogen concentration of soybean by 0.13 %. The results also revealed that Jenguma, Quarshie and Anidaso varieties were similar in height, nodule number and dry weight, shoot dry weight, grain yield as well as the amount N_2 fixed. However, there was significant varietal difference in soybean with respect to pod number and yield, 100-seed weight, nitrogen concentration and phosphorus uptake efficiency. The amount of N_2 fixed by soybean ranged between 52.3-71 kg / ha. Soybean - N derived from the atmosphere (% Ndfa) ranged between 42.51 and 49.89 percent. The percentage of N_2 derived from the atmosphere strongly and significantly ($p < 0.1$) correlated with the yield components of soybean. Nitrogen concentration significantly increase by 0.21 and 0.44 % with the application of 22.5 and 45 kg P_20_5 / ha, respectively over the unfertilized control. Phosphorus application of 22.5 and 45 kg P_2O_5 / ha had 35.41 and 33.85 %, respectively yield increase over the unfertilized control. Also, phosphorus application of 22.5 and 45 kg P_2O_5 / ha increased the amount of N_2 fixed by 49.39 and 69.82 %, respectively over the control. Moreover, phosphorus application of 22.5 and 45 kg P_2O_5 / ha increased the % Ndfa of 34.42 and 48.98 %, respectively over the unfertilized control. The interactions among these treatments did not significantly influence all the parameters measured except for the nitrogen concentration and the phosphorus uptake efficiency where there was significant difference between soybean variety and the phosphorus rate. Therefore, it was concluded that inoculation of soybean with legumefix did not increase nodulation and BNF of the three soybean varieties and that phosphorus application to soybean increases growth and yield.

TABLE OF CONTENT

CONTENT **PAGE**

CHAPTER FIVE 41

DISCUSSION 41

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

CHAPTER ONE

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a legume plant belonging to the botanical family *leguminosae*. It is an economically important leguminous crop worldwide and also the most important legume in Ghana (Plahar, 2006). According to Dugje *et al*. (2009), soybean is more protein-rich than any of the common vegetable or legume food sources in Africa. It is a source of edible oil, 20-25 % and of 42-45 % protein contents (Alam *et al*., 2009). Soybean is a promising pulse crop proposed for the alleviation of the acute shortage of protein and oil worldwide (Mahamood *et al*., 2009). It used as a good source of unsaturated fatty acids, minerals (Ca and P) and vitamins A, B, C and D (Alam *et al*., 2009). Soybean was introduced in Ghana in 1910 (Plahar, 2006) and was used by local farmers in the northern sector. The northern parts of the country lead soybean production in Ghana. Mean acreage under soybean cultivation per farmer in the northern part of Ghana was 3.4 acres in 2006 with individual farm size holdings ranging from 0.5 acre to 80 acres. In 2006, the southern sector production was still comparatively at the rudimentary stages except for Ejura Farms of about 300 acres and a few satellite farmers (Plahar, 2006). The average soybean yield for northern Ghana (Northern, Upper West and Upper East regions) was about 1.5 t / ha on the farmers' field compared to that of USA which was 4.6 t / ha (Lawson *et al*., 2008). The significant increase in the grain yield of soybean in U.S.A. has been partly attributed to the development and improvement of cultural practices. In spite of the great potentials of the crop, soybean production is still inadequate owing to low yields, resulting in a wide gap between what is currently produced and what is needed. As a way of improving production level, one of the major areas to consider is the development of high yielding and improved varieties and development of improved cultural management practices.

Nitrogen is the most abundant element on the earth and about 78 % of the earth's atmosphere is nitrogen gas. There are hundreds of tons of nitrogen over every hectare of land surface. Despite the abundance of nitrogen in the atmosphere, plants are unable to use it directly because it is present in an inert form (N_2) and the nitrogen in the soil is lost through microbial dinitrification, soil erosion, leaching, chemical volatilization, removal of nitrogen containing crop residues from land. Nitrogen is therefore most limiting plant nutrient for crop production in West Africa (Sangakara *et al.*, 2003). Most legumes, through symbiosis with rhizobia have the ability to reduce N_2 through biological nitrogen fixation (BNF) into a form usable for growth. The amount of nitrogen fixed varies according to the legume species and variety. Within a species the amount of nitrogen fixed is directly related to (dry matter) yield. Most grain legumes including soybean can obtain between 50 and 80 % of their nitrogen concentration requirements through biological fixation, but some, like fababean will fix up to 90 % (Solomon *et al.*, 2012).

Currently, BNF contribution on majority of small holder farms rarely exceeds 5 kg N / ha / year with nitrogen fixing legumes in Ghana (Mapfumo, 2001). A measure of more than 240 kg N / ha of fixed N_2 in soybean in southern Africa on small holder farms with associated grain yield of more than 3.5 t / ha has been recorded (Gillar, 2001). This shows that the potential rates of BNF in soybean are not only limited by the efficiency of legume rhizobium symbiosis. Factors such as the environment and management could also limit the efficiency. These factors may include temperature, rainfall, soil or fertilizer nutrients like nitrogen and phosphorus, soil pH, etc. The establishment of an effective and efficient BNF depends on optimizing all of the components (i.e. legume rhizobium, management and environment) together (Giller, 2001). An understanding of the effects of these management factors on the symbiosis may assist in the selection of P-efficient soybean genotypes that will enhance symbiotic nitrogen fixation and soybean yield.

Phosphorus is an essential ingredient for Rhizobia bacteria to carry out BNF processes. Inadequate P restricts root growth, the process of photosynthesis, translocation of sugars and other such functions which directly influence N fixation by legume plants. The phosphorus serves as an energy source during the physiological processes taking place in the plant (Anonymous, 1999).

The study was therefore conducted based on the hypothesis that:

i. The use of Rhizobium inoculants increases nodulation and BNF of soybean
ii. Phosphorus fertilizer application increases growth and grain yield of soybean
iii. Rhizobium inoculation and phosphorus fertilizer application increases phosphorus uptake efficiency

The general objective of this study was however: to increase soybean production by identifying efficient strategies that will optimize biological nitrogen fixation in soybean

Specific objectives were:

i. To evaluate the effect of legumefix inoculation and P fertilizer application rate on nodulation and BNF of three soybean varieties.
ii. To evaluate the effect of legumefix inoculation and P application rate on growth and yield of three soybean varieties.
iii. To determine the effect of legumefix inoculation, P application rate on P uptake efficiency of three soybean varieties.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biological nitrogen fixation

2.1.1 Mechanism for BNF

The biological conversion of N_2 to ammonia performed by rhizobia bacteria is highly energy consuming. N_2 is reduced to NH_3 under consumption of ATP and redox equivalents, and is associated with the formation of H_2 as a byproduct ($N_2 + 8\ H + 8\ e- + 16\ ATP \rightarrow 2\ NH_3 + H_2 + 16\ ADP + 16\ Pi$). The enzyme that catalyzes the reaction is called nitrogenase and consists of the dinitrogenase reductase protein (Fe protein) and the dinitrogenase (MoFe protein) which actually catalyzes the reduction of N_2. The formation of hydrogen gas is always accompanied by formation of ammonia and is a wasteful process. Therefore, some micro-organisms possess hydrogenases that recover this otherwise lost form of energy.

Three major strategies of N_2 fixation can be differentiated in terrestrial ecosystems, symbiotic, non-symbiotic or associative, and free-living N_2 fixation. Symbiotic Introduction systems contribute with approximate 70 %, while non-symbiotic systems contribute with approximate 30 % (Peoples and Craswell, 1992). The contribution of free-living diazotrophs is very small, because the majority of these microorganisms are heterotrophic bacteria being subjected to substrate limitation (Marschner, 1995). The terrestrial input (natural origin and human activities) of N from BNF accounts for approximately 240–280 t N / year (Galloway, 1998), this amount is much higher compared to the 85 t N / year consumed by nitrogenous fertilizers all over the world in 2002 (FAO, 2008)

2.1.2 Symbiotic N_2 fixation

Bacteria of the genus *Rhizobium* and their relatives form important symbiotic interactions with leguminous plants. Depending on the plant species infection by the microsymbiont may occur on developing root hairs, at the junction of lateral roots or at the base of the stem. The first step in host plant infection is the release of phenolic compounds (flavonoids) by the roots, acting as a signal for rhizobia to stimulate chemotaxis and the expression of bacterial nodulation genes. *Nod*-gene induction is required for the production of lectins (*nod*-factors) and the attachment of the bacteria onto root hairs. The next steps are an invasion of the rhizobia through the plant infection thread and the development of the nodule meristem. Inside the dividing nodule rhizobia cells are packed into symbiosomes and transformed into bacteroids.

The transformation of the bacteroids is accompanied by the synthesis of hemoglobin, nitrogenase and other enzymes required for N_2 fixation (Rolfe and Gresshoff, 1988). To perform N_2 fixation, bacteroids need to obtain sources of carbon and energy from the plant. These are dicarboxylic acids, such as malate and succinate. It has been widely accepted that, in return, bacteroids simply provide the plant with ammonium or ammonia, which diffuses across the peribacteroid membrane and is assimilated into amino acids in the plant cytosol of the nodular tissue. The form in which N is exported from roots to shoots depends on the type of nodules, the indeterminate ones exporting amides (*aspargine, glutamine*), whereas tropical legumes, which form determinate nodules, export ureides, such as allantoine or citrulline (Mylona *et al.,* 1993). Actinomycetes of the genus *Frankia*, establish a N_2-fixing symbiosis in root nodules of a large number of non-leguminous woody dicotyledonous plants (e.g. members of the *Elaeagnaceae, Rhamnaceae*) (Clawson *et al.,* 1998) and with trees (e.g. of the genera *Casuarina* and *Alnus*) (Nazaret *et al.,* 1991). Another N_2-fixing symbiosis is found in cyanobacteria *Nostoc* living on Gunnera plant species or *Anabena* with the fern *Azolla anabaena* (Bergman *et al*., 1992).

2.1.3 Factors influencing nitrogen fixation in legumes

Establishment of effective N_2 fixing symbioses between legumes and their N_2 fixing bacteria is dependent upon many environmental factors, and can be greatly influenced by farm management practices (Peoples *et al.*, 1995). There are several environmental factors affecting BNF. The severe environmental conditions such as salinity, unfavourable soil pH, nutrient deficiency, mineral toxicity, extreme temperature conditions, low or extremely high levels of soil moisture, inadequate photosynthates, and disease conditions can affect the plant growth and development. As a result, even the persistent rhizobium strains will not be able to perform root infection and N fixation in their full capacity (Panchali, 2011).

The moisture stress can adversely affect the nodule functions. The drought conditions can reduce nodule weight and nitrogenase activity. After exposure to the moisture stress for 10 days, the nodule cell wall starts to degrade resulting in senescence of bacteroids (Ramos *et al*., 2003). The accumulation of Na^+ reduces the plant growth, nodule formation, and symbiotic N fixation capacity under salinity conditions (Sousssi *et al*., 1998; Kouas *et al.,* 2010). High salt level can directly affect the early interaction between the rhizobium legumes in nodule formation (Singleton and Bohlool, 1984). The plant nitrogenase activity reduces dramatically as a result of formation of ineffective nodules at high temperature (40 ^{o} C) (Hungria and Franco, 1993).

Rhizobial colonization in the legume rhizosphere can be reduced by the extreme soil pH. Nitrogen fixation can be inhibited by low soil pH (van Jaarsveld, 2002). The characteristics of highly acidic soils ($pH < 4$) are low level of phosphorous, calcium, and molybdenum along with aluminum and manganese toxicity, which affects both plant and the rhizobia. As a result, under low soil pH conditions, nodulation and N fixation are more severely affected than the plant growth. Highly alkaline ($pH > 8$) soils tend to be high in sodium (Na^+), chloride (Cl^-), bicarbonate (HCO_3^-) and borate (BO_3^-) which reduce the N fixation (Bordeleau and Prevost, 1994).

In addition to environmental factors, agricultural management factors influence percentage of N_2 derived from the atmosphere (% Ndfa) as well. Management factors which include inoculation, P-fertilization, choice of variety and plant density affect the plant growth and development (Roner and Franke, 2012). The need for inoculation depends on the presence of compatible rhizobia in the soil and their effectiveness. If a legume is promiscuous, rhizobium strains with which it can form effective nodules are often present, and then it will rarely respond to inoculation (e.g. cowpea or groundnut). In grain legumes, a response to inoculation is most commonly seen in soybean. Many varieties are highly specific and do not always nodulate with indigenous rhizobia in Africa (Giller, 2001).

Also, some varieties are more specific than others, or are better adapted to local environmental circumstances. In general, long duration, indeterminate species fix more N_2 due to their longer period of growth than determinate, short-duration varieties. Phosphorus fertilization improves nodulation and plant growth where P is limiting (Roner and Franke, 2012). Legumes in intercropping often show a higher percentage of nitrogen from N_2 fixation than legumes in a mono-cropping, since cereals like maize or sorghum, grown as main crops, has a high N demand. With less N available in the soil, legumes in intercropping rely more on N_2 fixation (Vesterager *et al.*, 2008; Rusinamhodzi *et al.*, 2006). Higher plant population density show either positive for percentage of nitrogen from N_2 fixation due to increased competition for soil N, or negative as a result of competition for other nutrients and moisture (Naab *et al*., 2009; Makoi *et al*., 2009).

2.1.4 Methods of estimating BNF

To improve the fixation efficiency and determine its contribution to an agricultural system, accurate measurement of symbiotic biological N fixation in legumes is important. Biological nitrogen fixation can be quantified using several methods. The choice of a particular method depends on the type and site of the experiment, the available resources, the species and the system in question. Some of these methods include: i) the acetylene reduction assay (ARA), (ii) the xylem sap analysis, iii) the total nitrogen difference (TND) methods, and (iv) the ^{15}N isotope methods. All of these methods have limitations and so to obtain accurate measurements of N fixation in the field, the limitations of each method must be recognised to reduce their effect on the calculation of symbiotic activity.

2.1.4.1 Acetylene reduction assay (ARA)

The Acetylene reduction assay methods developed from observations in the 1980s that the N_2 fixing enzyme, nitrogenase, catalyzed the reduction of acetylene (C_2H_2) to ethylene (C_2H_4) (Ngome, 2006). Since that time, ARA technique accounts for many estimates of BNF in legumes (Danso, *et al*., 1992). The amount of ethylene produced by incubating excised nodules, decapitated roots or whole plants in an atmosphere containing acetylene has sometimes been converted into total N_2 fixed by multiplying with a conversion factor of three (Danso *et al.*, 1992).

Nowadays, ARA is largely limited to quantitative studies due to the following: (i) it requires interpolation between single, short-term measurements to obtain time-integrated measurements, (ii) the conversion factor of three does not apply in all cases and large errors are likely to arise, and (iii) because it is very difficult to recover all nodules under field conditions for comprehensive assessment of BNF (Ngome, 2006).

The ARA is the most widely used method because of its simplicity and low cost, but it is questionable because the product ethylene can inhibit the activity of the nitrogenase by 50 % after 30 minutes (Olga-Cristina and Cornella, 2009). Moreover, it does not indicate if the fixed N is incorporated into the plant (Olga-Cristina and Cornella, 2009) and therefore can only be used as an indirect method not providing absolute values.

2.1.4.2 Xylem sap analysis

The products of BNF (mainly glutamate and ureides) are transported to other parts of the plant through the xylem whereas N absorbed from the soil is either transported directly to the shoots as NO_3 or reduced to amides before long-distance transport. Many legumes transport most of their fixed N in the form of ureides (Giller and Wilson, 1991). The proportion of N in the xylem sap as ureides is directly proportional to amount of N fixed (Herridge *et al.*, 1996).

In legumes that do not transport fixed N as ureides, BNF can only be correlated to the proportion of amide N in the xylem sap (Ngome, 2006). Xylem sap analysis has a major disadvantage as the relationship between the composition of the sap and the rate of BNF must be calibrated against an independent method of measurement (Herridge *et al.*, 1996). However, the method is reliable and seems to agree with ^{15}N isotope methods.

2.1.4.3 Nitrogen Balance and Nitrogen Difference Method

The simplest form of the N balance method is to grow an N-fixing crop and an adjacent non-N-fixing (reference) crop and assume the difference in plant tissue N at harvest between the two crops is the quantity of N fixed. Although this method is inexpensive and easy to carry out in a field setting, it has been shown to be highly inaccurate and unreliable in that it largely under- or over-estimates the effect of soil N in the system (McCauley, 2011).

A recommended alternative to the N balance method is the N difference (ND) method in which available soil N levels under both the legume and the reference crop are taken into account. By incorporating the soil N component, soil N transformations over the growing season and respective differences in N uptake from the two crops can be assessed, assuming soil N transformations and losses are equal between the crops. Another assumption of the N Difference method is that root N between the two crops is similar. Since it is not practical to effectively harvest roots from field plants, shoot N and root N ratios are assumed to be similar between the crops. This assumption is difficult to validate in field settings and root N, including losses of root N to the soil, may represent a large pool of fixed N that is ignored in N fixation estimates (McCauley, 2011). This method is particularly complicated when dealing with intercropped legumes because intercrop competition may affect the ability of the legume and non-legume fixing reference crops to access soil N (Giller, 2001). It is recommended for sandy or low-N soils, because increasing soil N also increases the error in BNF estimates (Danso *et al.,* 1992).

2.1.4.5 ^{15}N Isotope Methods and Natural abundance

These methods can be advantageous over the N balance methods as they provide a yield-independent estimate of N fixation (Chalk *et al.*, 2000). Both ^{15}N-isotopic methods rely on the naturally-occurring ^{15}N abundance in the atmosphere to quantify N fixation. Atmospheric ^{15}N concentrations are a uniform 0.3663 atom % globally, but because of N transformation processes that preferentially discriminate for or against ^{15}N, soil and biological material tend to have ^{15}N concentrations different from that of atmosphere (McCauley, 2011). The change in ^{15}N concentrations with respect to atmospheric ^{15}N is expressed as δ^{15}N in parts per thousand (‰); hence, the δ^{15}N of atmospheric N is 0 ‰. Many soils become enriched in ^{15}N over time due to microbial discrimination against the heavier ^{15}N isotope in favor of the lighter ^{14}N isotope.

The degree of soil ^{15}N enrichment in a field can vary considerably and is influenced by a number of physical and biochemical factors (Hauggaard-Nielsen *et al.*, 2010). The Isotope Dilution (ID) method can partially adjust for this variability by adding a known quantity of plant available ^{15}N to the system, usually with ^{15}N-labeled fertilizer, and including a non-N-fixing reference crop. The ID method was most widely used in the 1970s-1990s prior to advancements in isotopic mass spectrometry in the 1980s that led to development of the natural abundance method (Unkovich *et al*. 2008). The ID method may still be favorable for soils where the δ^{15}N of plant-available soil N is less than 2 ‰ or where high-precision mass spectrometry analysis is not available.

The natural abundance method quantifies N fixation by calculating the difference between a non-N fixing reference plant and an N-fixing legume that is obtaining N from both the soil and atmosphere, after accounting for isotopic fractionation between

^{14}N and ^{15}N in the aboveground shoot of the legume (the β value). The first assumption of the NA method is that the legume and reference plant are accessing the same pools of soil N. This assumption requires that the two plants are grown near one another and also that the two plants have similar stature and rooting morphology. A second assumption is that there is either no discrimination or identical discrimination between ^{14}N and ^{15}N in the plants' uptake and metabolism of N, or any discrimination has been accounted for (McCauley, 2011).

2.2 Effects of nitrogen application on growth and yield

Like most annual legumes, soybean can provide part of its own N requirement through symbiotic N_2 fixation when the plants are inoculated. It has been reported that rhizobial inoculation alone is not enough for obtaining high yields of legumes because of poor nodulation and nitrogenase activity (Sosulski and Buchan, 1978). These authors concluded that annual legumes may require a high level of plant N fertility to achieve maximum yield. Indigenous populations of rhizobia for legumes may be present in tropical soils, but these indigenous populations may be ineffective for inducing N_2 fixation under semi-arid environments (Kucey and Hynes, 1989).

Application of starter N at an early vegetative growth stage or flowering can increase the pod yield and crop biomass by 44 and 16 %, respectively. The proportion of the plant N derived from the N fixation is highest when N is applied at the pod filing stage where the plant N demand is high (Panchali, 2011). It has been reported that the use of urea $\{(NH_2)_2\ CO\}$ or ammonium nitrate (NH_4NO_3) as the starter N fertilizer at rates of 8, 16, and 24 kg / ha promoted the early plant biomass and plant N compared to the no N treatment. Further, the soybean grain yield increased by 16 % at the N rate of 16 kg / ha over the control treatment with no improvement either in seed protein or oil content (Osborne and Riedell, 2006).

In a study conducted to identify the effects of time and method of application and the source of N on soybean plant growth, grain yield, protein, and oil content at 12 sites. Schmitt *et al*. (2001) concluded that in-season application of N fertilizer did not increase the soybean grain yield or the oil content. The soybean grain yield, protein, oil and fiber content did not increase with the fertilizer nitrogen rates of 45 and 90 kg / ha (urea / slow release N) application at early reproductive stage (Barker and Sawyer, 2005). The early application at (V2 / R1) growth stage of Nitrogen as a top dressing at a rate of 25 kg / ha promoted the soybean plant total biomass and the N accumulation during the seed filling stage (R5) boosted the grain yield (Gan *et al*., 2003). Top dressing N at the seed filling stage (R3 / R5) could not improve the plant total biomass, N accumulation and the grain yield (Panchali, 2011; Gan *et al*., 2003). Also, top-dressing N at R1 and R3 stages drastically reduced the soybean nodulation, whereas at V1 stage there was an optimistic effect, which increased the soybean nodulation (Gan *et al*., 2003).

2.3 Response of soybean to rhizobial Inoculation

High yielding soybean plants require a lot of nitrogen and it is estimated that BNF can cover 60 to 70 % of the nitrogen requirement of the plant (Herridge *et al*., 2008). According to Salvagiotti *et al.* (2008), an average of about 50 to 60 % of nitrogen required by soybean plants is provided by BNF. Soybean responds most strongly to inoculation when they are introduced into new areas where soils lack appropriate rhizobia (van Kessel and Hartley, 2000). There is presumably a yield advantage to crop inoculation in soils with inadequate inorganic N supply. However, the yield response to inoculation was highly variable and affected by inherent field variability, and by differences in environmental and edaphic conditions (van Kessel and Hartley, 2000). Musyoki *et al*. (2003) observed that rhizobial inoculation increase nodulation in all agro ecologies and average grain yield in two agro ecologies. Responses were however largest when control yields ranged between 0.5-1.0 t / ha and when soil N content varied between 0.05 and 0.15 % N.

Most soils usually lack effective indigenous strains of *Bradyrhizobium japonicum* unless soybean is grown on them for at least five or more years. Introduced strains may disappear completely without repeated inoculation or genetic exchange may dilute the beneficial capacities of introduced strains over time though it has also been reported that strains are highly adaptable to new environments (Lindstrom, 2010). Hiltbold *et al*. (1985) reported that numbers of *Bradyrhizobium japonicum* in 52 Iowa fields were correlated with whether soybeans had been grown at the site within the previous 13 years. They concluded that inoculation of seeds with relevant strains of bacteria before sowing was important especially in areas where legume crops were going to be grown for the first time on the land.

Legume response to inoculation largely depends on the number of rhizobia already established in the soil, the availability of soil nitrogen, and the management practice (Thies *et al*., 1991). Response to inoculation remains highly site specific and depends on factors beyond the effectiveness and competitiveness of the strain (s) used and host cultivar (s) seeded. Though N_2 fixation in grain legumes has focused on selection of superior rhizobial strains; however, significant variation in strain effectiveness have been observed in different trials (Choudhry, 2012). Even the most effective rhizobia-host plant symbiosis will fix little N_2 if the soil nitrogen is sufficient to meet the nitrogen demand of the crop. Indeed, a less effective rhizobia-host plant symbiosis may well fix more nitrogen when the demand of nitrogen by the host is increased by management practices and adequate nutrient availability. Mengel (1994) concluded that nitrogenase activity is a flexible process that adjusts to the nitrogen demand of the host. The amount of N_2 fixed becomes much more dependent on the demand of nitrogen by the host plant than on the intrinsic capacity of the rhizobia to fix nitrogen. As a result, management practices that increase nitrogen demand will likely be a more effective means of increasing the amount of N_2 fixed by grain legumes compared to attempting to improve the effectiveness of the rhizobia-host plant symbiosis (Choudhry, 2012).

Studies have demonstrated positive response of rhizobia inoculation on nodulation, dry matter yield and grain yield. Dorivar *et al.* (2009) reported that the application of rhizobia inoculation increased soybean grain yield by an average of 130 kg / ha and that plant dry matter, N concentration, N accumulation and grain N were also increase in soybean with soybean seed inoculation. Also, a study conducted on three legume (ground nut) cultivar in a sandy loamy soil observed that rhizobia inoculation increased the amount of N_2 fixed by 46 % over the uninoculated control.

Cultivar variation affects levels of nitrogen fixation in many legume crop species, and in some crops particular combinations of strain and cultivar have been shown to be especially efficient at fixing nitrogen. There were varying reports on the interaction between variety and strain in soybean. Thao *et al.* (2002) found a significant interaction between variety and strain on nitrogen fixation parameters whereas Munyinda *et al.* (1988) reported a non-significant interaction

2.4 Response of soybean to phosphorus fertilizer application

Phosphorus plays a major role in many plant processes such as storage and transfer of energy, stimulation of root growth, flowering, fruiting and seed formation, nodule development and N_2 fixation (Mclaren and Cameron, 1996). As a result, phosphorus application on legumes can also increase leaf area, yield of tops, roots and grain;

nitrogen concentration in tops and grain; number and weight of nodules on roots; and increased acetylene reduction rate of the nodules (Yahiya *et al.,* 1995).

The N_2 fixation process in legumes is sensitive to P deficiency and this deficiency leads to reduced nodule mass and decreased ureide production (Sinclair and Vadez, 2002). Nodules are strong sink of P and nodule P concentration normally exceeds that of roots and shoots (Drevon and Hartwig, 1997). Therefore, nodule number, and dry weight can be increased by treating P deficient soils with fertilizer P (Cassman *et al.,* 1981). However, Bremer and Mulvany (1982) found that P application of 45 kg P / ha increased dry matter (12.25 kg / ha) and grain yield (1.0- 2 t / ha) but did not affect N_2 fixation indicating that the legume host was more responsive to P application than the rhizobia.

Phosphorus fertilizer application and its management under low available soil P status are of importance in attaining high yields in soybean. Phosphorus availability has been noted to affect the functioning of the BNF system (McLaughlin *et al*., 1990; Chein *et al*., 1993). Among essential nutrients elements, influence of phosphorus on symbiotic nitrogen fixation in legume plants has received considerable attention. Tsevetkova and Georgiev (2003) reported that P deficiency treatments in soybean decreased the whole plant fresh and dry mass, nodule weight, number and function. Similarly, significant increases in soybean growth, 100-grain weight and grain yield (43-54 kg / ha) in response to added levels of 90 and 100 kg P_2O_5 / ha was reported by several workers (Taj *et al*., 1986; Jamro *et al*., 1990).

Differential growth has been reported among soybean genotypes in low-P soils and several mechanisms have also been proposed to explain the ability of plants to tolerate soils with low P levels (Abdelgadir, 1998). Although the mechanisms exhibited by soybean genotypes in adapting to low availability of P are not well understood, the identification and use of P-efficient genotypes with the ability to utilize sparingly soluble soil or fertilizer P may alleviate P deficiency in the short term (Gaume *et al*., 2001).

Soybean response to phosphorus fertilizer application depends on the crop, environment and management factors. Different rate of phosphorus application have been recommended to increase the growth, grain yield and yield components of soybean. Experiment on soybean fertilization using a locally adapted low yielding Malayan variety recommended 30 kg P_2O_5 / ha and 20 to 60 kg N / ha application for optimum yield (2.0 t / ha). In a research involving high yielding cultivars Ogoke, (2004) reports that yield increase of 15 kg / ha for 40 kg P /ha and 35 kg / ha for 80 kg P / ha. Haradagatti *et al.* (1996) and Nimje and Potkile (1998) reported soybean seed yield increased with increasing P rate up to 80 and 125 kg P_2O_5 / ha respectively. Similarly, Fageria *et al*. (1995) had reported that large quantities of P fertilizer (90 kg P / ha) may be required for successful soybean production.

Soil factors such as low level of P aggravate pod abortion thereby reducing yield in soybean (Chiezey, 2001). It has also been observed elsewhere that soybean crop response to P is dependent on soil available P (Mallarino and Reuben, 2005), while Ferguson *et al*. (2006) have also reported that P application is not likely to increase seed yield at soil P concentration above 12 ppm. Phosphorus fertilizer application along with Rhizobium inoculants influenced nodulation and N fixation of legume crops (Bhuiyan *et al.*, 2008). Hoque and Haq (1994) also observed similar trend when they treated several legumes with Rhizobium and phosphorus and found an increase in the number of nodules and maximum growth features.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was carried out on-station in the Savanna Agricultural Research Institute at Nyankpala in the Northern Region of Ghana from June to December 2012. Nyankpala is located about 16 km west of Tamale and lies on the latitudes N 09^o 24’ 15.9” and longitude W 01^o 00’ 12.1’ of the interior Guinea Savanna agro-ecological zone of Ghana. Rainfall in the Interior Savannah zone comes in one peak, which starts in April - May and builds up slowly to a height in August-September and declines sharply in October-November. The total precipitation is about 1,100 mm per annum, with a range from about 800 mm to about 1,500 mm. Average ambient temperatures are high year round (about 28°C) but the *harmattan* months of December and January are characterised by minimum temperatures that may fall to 13°C at night, while March and April may experience 40°C in the early afternoon. The soil name is the Fluvic luvisols classified as the Tingoli series and the vegetation type is a tree Savannah (SARI, 2008).

3.2 Soil sampling, preparation and analysis

Soil samples were taken before the field was ploughed. Five soil cores were taken at the depths of 0–15 cm from each replication and bulked to obtain three samples. Soil samples were air-dried, ground and sieved through a 1 mm mesh before using it to analyse for some selected physical and chemical properties using standard laboratory procedures. The particle size analysis was done by using the hydrometer method as outlined by Anderson and Ingram (1993). Soil pH was measured in the supernatant suspension of 1: 2.5 soils and water mixture by using a pH meter. Soil organic carbon was determined by using Walkley and Black method (Walkley and Black, 1934). Whilst total nitrogen of the soil was determine by the Kjeldahl method (Bremner and Mulvaney, 1982). Available phosphorus was determined by the Bray 1 method (Bray and Kurtz, 1945). Cation Exchange Capacity (CEC) was determined by leaching the soil with neutral 1 N ammonium acetate (FAO, 2008).

3.3 Soil Characteristics

The physical and chemical analyses of the soils have shown in Table 4.1 that the experimental site is loamy sand in texture and neutral in reaction (pH range 5.5-7.0) with very low Organic carbon (< 20 g / kg), Total nitrogen (< 1 g / kg), Exchange cations (< 5 c mol (+) / kg), effective cation exchange capacity (< 5 c mol (+) / kg) and available P (< 10 mg / kg soil) according to Landon (1991).

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

Parameter	**Test Value**
Sand (g / kg)	855.00
Silt (g / kg)	83.00
Clay (g / kg)	62.00
Texture	Loamy sand
pH (1:2.5; H_2O)	6.70
Organic carbon (g / kg)	11.00
Total N (g / kg)	0.50
Available P (mg / kg)	2.20
Exchange Cations (c mol (+) / kg)	
Ca	1.50
Mg	0.70
K	0.08
Na	0.06
Exchangeable acidity (c mol (+) / kg)	0.73
Effective cation exchange capacity (c mol (+) / kg)	3.07

3.4 Experimental design and treatments

The experiment was designed in a split-split plot with three replications and interactions tested were inoculation rate (0, 50 and 100 % inoculation) as the main plot, soybean variety (Jenguma, Anidaso, Quarshie) as the sub-plot and phosphorus rate (0, 22.5 and 45 kg P_2O_5 / ha) as the sub-sub plot. The plot size was 3 m x 4 m and spacing between plots and blocks were 1 m and 2 m, respectively. For the purpose of assessing BNF, non-fixing reference crop (maize) was added to the treatment combinations.

Phosphorus was applied as triple super phosphate to individual sub-sub plots at the rate of either 22.5 or 45 kg P_2O_5 / ha but not to the reference crop plots. However, Muriate of Potash (MOP) was applied to all experimental plots at 30 kg K / ha. The fertilizers were deposited in holes 5 cm away from the plants and covered with the soil at two weeks after sowing.

3.5 Seeds inoculation

Seeds were inoculated with legume fix inoculants using the Slurry method outlined by Woomer *et al.* (1994). The seeds of Jenguma, Quarshie and Anidaso varieties were each divided into three bowls with each one weighing 1 kg. The inoculum was then poured over the 1 kg seeds of each soybean variety in the bowl after sprinkling water over the seeds. The seeds and inoculum in the bowl were mixed carefully until seeds were coated with black film of inoculants and allowed to dry for a few minutes after which they were planted on the ridges. The full recommended rate of inoculation (100 % inoculation) was done at the rate of 5 g of legume fix inoculant per 1 kg of seed just before planting while the half recommended inoculation rate was done by mixing 2.5 g of legume fix inoculant with 1 kg of the seeds.

3.6 Land preparation and planting

The field which was cropped with maize in the previous year was ploughed and harrowed by a tractor and later manually ridged according to the planting distance of 50 cm x 10 cm. Three soybean varieties, namely Jenguma, Anidaso and Quarshie were sown on the ridges at the said spacing. These varieties were obtained from the Savanna Agricultural Research Institute at Nyankpala. This experiment was carried out under rain-fed conditions. Manual weeding was done at two weeks after emergence and three weeks after the first weeding.

Both the Jenguma and Quarshie varieties were released by CSIR-SARI in 2000. The Jenguma variety is resistant to pod shattering and has the potential grain yield of 2.5 t / ha. It is a medium maturing variety (105-110 days) and takes 45 days to flower at 50 percent. It has a mean hundred seed weight of 13.6 g. It has better qualities (higher grain and fodder yields, higher BNF potential, better control of Striga than local varieties in Ghana) than other local varieties in Ghana. The Quarshie variety is also moderately resistant to pod shattering and has a potential grain yield of 2.0 t / ha. It is also a medium maturing variety (100-105 days) and its days-to-50 % flowering is between 42 and 43 days. It has a mean hundred seed weight of 13.4 g.

The Anidaso variety was released in 1992 by CSIR- Crop Research Institute (MoFA and CSIR, 2005) at Kumasi. The seed are small with mean 100-seed dry weight of 13.0 g, rounded and yellow in colour and matures in 110 days. Anidaso has the potential grain yield of 1.2 -1.8 t / ha.

3.7 Determination of plant total nitrogen

To determine the plant total N concentration, the plant samples were acid digested and distilled by using the Kjeldahl distillation method (Bremner and Mulvaney, 1982).

The digestion block or unit was used to digest the soybean plant samples. Ground plant tissue samples of 2 g were weighed into a digestion tube and 10 ml of concentrated sulphuric (H_2SO_4) acid was added and the tube was placed on a preheated (300 ^{o} C) digestion block for 40 minutes in the fume hood. The digestion tubes were then removed from the digestion block and cooled for 5 minutes. Two ml of 30 % hydrogen peroxide (H_2O_2) was mixed with the partially digested tissues sample and kept on the digestion block for another 15 minutes. Again, the sample was removed from the digestion block and cooled for 5 minutes, after which 2 ml of H_2O_2 was added. This step was repeated until the digestion solution was colourless. Once the process was completed, the digested plant tissues were kept under the fume hood for 30 minutes after which the solution was transferred into a 50 ml volumetric flask and volumerized by using distilled water. The diluted digestion solution was used for the total N analysis, as described below.

Ten millilitres of the digested solution were transferred into a distillation tube and mixed with 10 ml of 40 % (10 N) sodium hydroxide (NaOH) solution. This mixture was then distilled until its volume of the receiving flask, which contained 5 ml of boric acid (H_3BO_3) and 5 drops of mixed indicator (methyl red and green bromocresol), doubled. Finally, the distillate was titrated against 0.01 N HCl until the colour became pinkish grey. The total plant N (% N) was calculated.

Calculation

$$\% N = \frac{14\ x\ (A - B)\ x\ N\ x\ 100}{1000\ x\ 0.2}$$

Where A = volume (ml) of HCl used for sample titration, B = volume (ml) of H Cl used for blanck titration, 0.2 = Dillution factor = $(2\ g\ x\ 10\ ml)/100$

3.8 Measurement of crop variables

3.8.1 Plant height

Five plants from each plot were randomly selected and tagged for height measurements which were taken eight Weeks after Planting (8 WAP) with a measuring tape. Height was taken from the ground level to the topmost point, and the average for each plot was calculated.

3.8.2 Nodule number per plant and nodule dry weight

At 8 WAP, ten plants from the two middle rows were randomly selected and gently dug out. The plants were then washed through a fine sieve with water to remove soil particles and organic debris. The number of nodules on each plant was then determined and the average nodules per plant calculated. The nodules were oven dried at 65 ^{o} C for 24 hours. The dry nodules were then weighed and nodule dry weight recorded

3.8.3 Determination of shoot dry weight

Ten plants were randomly selected from the two boarder rows on each side of the treatment plot and cut at the ground level for shoot dry matter determination at 8 WAP. Total fresh shoot weight was measured using an electronic balance. Plant materials were then put in brown envelopes and oven dried at 65 ^{o}C for 72 hours. The dry materials were weighed and shoot dry weight recorded.

3.8.4 Pod number per plant and pod yield

Ten plants were randomly selected (every 5th plant was picked in the two middle rows) from the net harvest area and their pods counted to obtain the number of pods per plant. The pods from the ten plants were then added to the pods from the respective net plot and weighed to obtain the pod weight in grams. This was then extrapolated to obtain total pod yield per hectare basis.

3.8.5 Grain yield and mean hundred seed weight

After threshing the pods harvested in the harvest area of each treatment plot, the grains were weighed on an electronic balance. Hundred seeds from each treatment were randomly picked and weighed. This was replicated three times and the average 100-seed weight determined.

3.9 Estimation of N_2 fixed

The technique used to estimate N fixation was the Total Nitrogen Difference (TND) method. This was done by comparing total nitrogen of the legume with that of a non-legume (Murray *et al*., 2008). The amount of N fixed was calculated by subtracting total nitrogen of the reference crop (maize) from that of the legume (soybean), and the difference value is assumed as N derived by BNF (N_2 fixed).

Thus, N_2 fixed = Total N in legume -Total N in reference crop

$$\text{where Total N in plants} = \frac{(\text{Dry matter weight } \left(\frac{\text{kg}}{\text{ha}}\right) \text{X } \% \text{ N in plants})}{100}$$

$$\% \text{ Ndfa} = \frac{[\text{ Total N in legume -Total N in reference crop}]}{\text{Total N in legume}} \text{ x } 100$$

Where % Ndfa is the percentage of N_2 derived from the atmosphere

3.10 Phosphorus Uptake efficiency

Phosphorus uptake efficiency (PUE) was estimated using an equation by Khair *et al.* (2002).

$$\text{PUE} = 100 \text{ x} \left[\text{P in fertilized plants - P in control plants } \left(\frac{\text{kg}}{\text{ha}}\right)\right] \Big/ \text{P applied } \left(\frac{\text{kg P2O5}}{\text{ha}}\right)$$

3.11 Statistical analysis

All data collected were subjected to statistical analysis using Genstat Discovery editions 10. Nodule count was transform before the analysis. The analysis of variance procedure treatments was followed to determine difference in means among treatments. All treatment means were compared using the Least Significant Difference (LSD) at 5 % level of significance. A correlation analysis between yield and yield components was also carried out.

CHAPTER FOUR

RESULTS

4.1 Growth parameters

4.1.1 Plant height

The application of inoculants and variety had no significant ($p > 0.05$) effect on plant height at 8 WAP (Table 4.1). However, phosphorus application significantly ($p < 0.05$) affected the height of soybean. Table 4.2 also revealed that P application of 22.5 and 45 kg P_2O_5 / ha significantly increase plant height by 2.76 and 6.87 %, respectively over the control (0 kg P_2O_5 / ha). The interactions had no significant effect ($p > 0.05$) with respect to plant height of soybean (Table 4.1).

Table 4.1: Effects of inoculation rate, variety and phosphorus rate on plant height of soybean

Treatment	Plant height (cm)
Inoculation rate (%)	
0	69.99
50	69.79
100	69.24
Pr (I)	0.816
Lsd (0.05)	3.26
Variety	
Jenguma	69.34
Quarshie	69.13
Anidaso	70.55
Pr (V)	0.443
Lsd (0.05)	2.53
Phosphorus rate (kg P_2O_5 / ha)	
0	67.38
22.5	69.29
45	72.35
Pr (P)	<.001
Lsd (0.05)	1.05
Pr (I x V)	0.950
Pr (I x P)	0.851
Pr (V x P)	0.304
Pr (I x V x P)	0.371
CV (%)	6.94

4.1.2 Nodule formation and development

Table 4.2: Effects of inoculation rate, variety and phosphorus rate on nodulation and nodule development of soybean

Treatment	Nodule number (No / plant)	Nodule dry weight (mg / plant)
Inoculation rate (%)		
0	1.37	2233
50	1.35	1904
100	1.34	2049
Pr (I)	0.891	0.672
Lsd (0.05)	0.18	978.2
Variety		
Jenguma	1.38	2237
Quarshie	1.42	2289
Anidaso	1.26	1659
Pr (V)	0.106	0.109
Lsd (0.05)	0.16	658.4
Phosphorus rate (kg P_2O_5 / ha)		
0	1.19	686
22.5	1.35	2304
45	1.52	3196
Pr (P)	<.001	<.001
Lsd (0.05)	0.05	1186.4
Pr (I x V)	0.956	0.944
Pr (I x P)	0.719	0.417
Pr (V x P)	0.896	0.451
Pr (I x V x P)	0.103	0.250
CV (%)	7.0	23.4

The results presented in Table 4. 2 above show that inoculation and variety had no significant ($p > 0.05$) on nodule number and dry weight. However, there was significant ($p < 0.05$) effect on nodule number and dry weight of soybean when phosphorus was applied (Table 4.3). The application of 45 kg P_2O_5 / ha significantly increase nodule number in soybean relative to 22.5 kg P_2O_5 / ha. Phosphorus application of 22.5 and 45 kg P_2O_5 / ha significantly increase number of nodules by 11.85 and 21.71 %, respectively over the control (0 kg P_2O_5 / ha). There was no significant difference between phosphorus application of 22.5 and 45 kg P_2O_5 / ha with respect to nodule dry weight. The interactions had no significant effect ($p < 0.05$) on nodule number and dry weight (Table 4.2).

4.1.3 Shoot dry weight

Result of the shoot dry weight is presented in Table 4.3. Shoot dry weight was not significantly ($p > 0.05$) affected by inoculation rate and variety. However, shoot dry weight of phosphorus applied soybean without inoculation was significant ($p < 0.05$). Phosphorus application of 22.5 and 45 kg P_2O_5 / ha significantly increase shoot dry weight by 35.58 and 53.58 %, respectively over the control (0 kg P_2O_5 / ha). There was also significant ($p > 0.05$) difference between phosphorus application of 22.5 and 45 kg P_2O_5 / ha on shoot dry weight. The interactions had no significant effect ($p < 0.05$) on shoot dry weight (Table 4.3).

Table 4.3: Effects of inoculation rate, variety and phosphorus rate on shoot dry weight of soybean

Treatment	Shoot dry weight (kg / ha)
Inoculation rate (%)	
0	5604
50	4984
100	5089
Pr (I)	0.269
Lsd (0.05)	956.7
Variety	
Jenguma	5198
Quarshie	5647
Anidaso	4833
Pr (V)	0.595
Lsd (0.05)	1704.8
Phosphorus rate (kg P_2O_5 / ha)	
0	3331
22.5	5171
45	7176
Pr (P)	<.001
Lsd (0.05)	646.5
Pr (I x V)	0.762
Pr (I x P)	0.835
Pr (V x P)	0.184
Pr (I x V x P)	0.383
CV (%)	22.4

4.2 Yield components

4.2.1 Pod number per plant and pod yield

Results of number of pods per plant and pod yield of soybean are presented in Table 4.4. Inoculation rate did not significantly ($p > 0.05$) affect pod number and pod yield. However, varietal difference and phosphorus rate significantly ($p < 0.05$) affected number of pod and pod yield. The Quarshie variety produced mean pod number of 66.1, and this was statistically ($P<0.05$) higher than the mean pod number of the Anidaso variety but statistically similar to the Jenguma variety. Also, mean pod yield produced by the Jenguma variety was 2840 kg / ha and this was statistically higher than the mean pod yield of the Quarshie and Anidaso varieties. Moreover, phosphorus application significantly ($p < 0.05$) affected both pod number and yield of soybean. Phosphorus application of 22.5 and 45 kg P_2O_5 / ha increased pod number by 33.39 and 47.87 %, respectively over the control (0 kg P_2O_5 / ha). Also, phosphorus application of 22.5 and 45 kg P_2O_5 / ha increased pod yield by 31.78 and 39.98 %, respectively over the control (0 kg P_2O_5 / ha). No significant ($p > 0.05$) difference was observed among the interactions with respect to pod number and yield of soybean (Table 4.4).

Table 4.4: Effects of inoculation rate, variety and phosphorus rate on pod number and yield of soybean

Treatment	Pod number (No / plant)	Pod yield (kg / ha)
Inoculation rate (%)		
0	55.6	2447
50	57.9	2599
100	59.3	2643
Pr (I)	0.310	0.554
Lsd (0.05)	5.83	486.6
Variety		
Jenguma	60.5	2840
Quarshie	66.1	2513
Anidaso	46.2	2336
Pr (V)	0.018	0.011
Lsd (0.05)	13.3	305.4
Phosphorus rate (kg P_2O_5/ha)		
0	39.1	1861
22.5	58.7	2728
45	75.0	3100
Pr (P)	<.001	<.001
Lsd (0.05)		336.9
	7.32	
Pr (I x V)	0.874	0.178
Pr (I x P)	0.916	0.598
Pr (V x P)	0.337	0.885
Pr (I x V x P	0.595	0.975
CV (%)	23.0	23.8

4.2.2 Grain yield and hundred (100) seed weight

Table 4.5: Effects of inoculation rate, variety and phosphorus rate on grain yield and 100- seed weight of soybean

Treatment	Grain yield (kg / ha)	100 - seed weight (g)
Inoculation rate (%)		
0	1545	12.1
50	1646	11.8
100	1815	12.1
Pr (I)	0.260	0.389
Lsd (0.05)	385.60	0.58
Variety		
Jenguma	1881	12.7
Quarshie	1560	12.6
Anidaso	1566	10.8
Pr (V)	0.078	<.001
Lsd (0.05)	318.10	0.58
Phosphorus rate (kg P_2O_5 / ha)		
0	1233	11.5
22.5	1909	12.2
45	1864	12.36
Pr (P)	<.001	0.002
Lsd (0.05)	258.80	0.49
Pr (I x V)	0.265	0.993
Pr (I x P)	0.901	0.195
Pr (V x P)	0.267	0.653
Pr (I x V x P)	0.402	0.908
CV (%)	28.10	7.40

Table 4.5 above shows the results of hundred (100) seed weight and grain yield. Both grain yield and hundred (100) seed weight were not significantly ($p > 0.05$) affected by inoculation rate. Varietal differences were observed to be significant ($p < 0.05$) for only hundred (100) seed weight and not for grain yield. The Jenguma variety recorded mean hundred (100) seed weight of 12.57 g and this was statistically higher than the mean hundred (100) seed weight of the Anidaso variety but statistically similar to the mean hundred (100) seed weight of the Quarshie variety. Phosphorus rate significantly ($p < 0.05$) increased both grain yield and hundred (100) seed weight. Statistically, phosphorus application of 22.5 and 45 kg P_2O_5 / ha to soybean was

similar with respect to grain yield and hundred (100) seed weight but both were significantly higher than that of the control (0 kg P_2O_5 / ha). Interactions had no significant effect ($p > 0.05$) on grain yield and hundred (100) seed weight of soybean (Table 4.5).

4.3 Estimation of N_2 fixed

4.3.1 Nitrogen concentration in soybean

The application of inoculation, variety and phosphorus had a significant effect on nitrogen concentration of soybean (Table 4.6). Inoculation rate of 50 and 100% increased the nitrogen concentration of soybean by 0.09 and 0.12 %, respectively over the uninoculated control. However, there was no significant difference between inoculation rate of 50 and 100 percent. Also, phosphorus application of 22.5 and 45 kg P_2O_5 / ha increased nitrogen concentration of soybean by 0.22 and 0.44 %, respectively over the control (0 kg P_2O_5 / ha). There was significant difference between phosphorus application of 22.5 and 45 kg P_2O_5 / ha. No significant difference was observed among the interactions with respect to nitrogen concentration of soybean (Table 4.6).

Table 4.6: Effects of inoculation rate, variety and phosphorus rate on nitrogen concentration of soybean

Treatment	Nitrogen concentration (%)
Inoculation rate (%)	
0	1.991
50	2.081
100	2.120
Pr (I)	0.052
Lsd (0.05)	5.880
Variety	
Jenguma	2.010
Quarshie	1.970
Anidaso	2.213
Pr (V)	<.001
Lsd (0.05)	0.102
Phosphorus (kg P_20_5/ha)	
0	1.847
22.5	2.063
45	2.282
Pr (P)	<.001
Lsd (0.05)	0.089
Pr (I x V)	0.397
Pr (I x P)	0.523
Pr (V x P)	0.130
Pr (I x V x P)	0.829
CV (%)	7.900

4.3.2 Amount of N_2 fixed and Percentage of N_2 derived from the atmosphere (% Ndfa)

The amount of N_2 fixed and the % Ndfa in soybean was not significantly ($p > 0.05$) affected by inoculation rate and variety (Table 4.7). However, phosphorus rate significantly ($p < 0.05$) affected the amount of N_2 fixed and the % Ndfa. Statistically, there were differences between phosphorus application of 22.5 and 45 kg P_2O_5 / ha with respect to the amount of N_2 fixed and the % Ndfa. The amount of N_2 fixed in soybean was significantly ($p < 0.05$) increased by 49.39 and 71.90 %, with phosphorus application of 22.5 and 45 kg P_2O_5 / ha respectively over the control (0 kg P_2O_5 / ha). The % Ndfa increased with the application of 22.5 and 45 kg P_2O_5 /

ha by 34.43 and 48.98 %, respectively over the control (0 kg P_2O_5 / ha). No significance difference ($p > 0.05$) was observed among the interactions with respect to the amount of N_2 fixed and the % Ndfa (Table 4.7).

Table 4.7: Effects of inoculation rate, variety and phosphorus rate on amount of N_2 fixed and percentage of N_2 derived from the atmosphere (% Ndfa)

Treatment	Amount of N_2 fixed (kg / ha)	% Ndfa
Inoculation rate (%)		
0	59.4	45.17
50	59.4	45.09
100	63.6	46.08
Pr (I)	0.132	0.579
Lsd (0.05)	5.08	2.723
Variety		
Jenguma	59.1	43.94
Quarshie	71.0	49.89
Anidaso	52.3	42.51
Pr (V)	0.228	0.147
Lsd (0.05)	22.58	8.033
Phosphorus rate (kg P_2O_5 /ha)		
0	29.0	30.40
22.5	57.3	46.36
45	96.1	59.59
Pr (P)	<.001	<.001
Lsd (0.05)	9.74	3.416
Pr (I x V)	0.697	0.533
Pr (I x P)	0.711	0.900
Pr (V x P)	0.238	0.102
Pr (I x V x P)	0.919	0.548
CV (%)	29.0	13.6

4.4 Estimation of phosphorus uptake efficiency

4.4.1 Phosphorus concentration in soybean

Table 4.8: Effects of inoculation rate, variety and phosphorus rate on phosphorus concentration of soybean

Treatment	Phosphorus concentration (%)
Inoculation rate (%)	
0	0.280
50	0.226
100	0.231
Pr (I)	0.313
Lsd (0.05)	0.091
Variety	
Jenguma	0.257
Quarshie	0.231
Anidaso	0.249
Pr (V)	0.387
Lsd (0.05)	4.360
Phosphorus rate (kg P_2O_5 / ha)	
0	0.182
22.5	0.244
45	0.311
Pr (P)	<.001
Lsd (0.05)	0.017
Pr (I x V)	0.555
Pr (I x P)	0.512
Pr (V x P)	0.022
Pr (I x V x P)	0.614
CV (%)	12.30

Phosphorus concentration in soybean was not significantly ($p > 0.05$) affected by inoculation rate and variety (Table 4.8). Phosphorus application contributed significantly ($p < 0.05$) to phosphorus concentration of soybean. Apart from variety and phosphorus rate, none of the interactions had a significant ($p > 0.05$) effect on phosphorus concentration in soybean (Table 4.8). The combination of variety and phosphorus application significantly ($p < 0.05$) increased P concentration of soybean in each variety (Figure 4.1). There was significant difference between phosphorus application of 22.5 and 45 kg P_2O_5 / ha to the Quarshie and Anidaso variety while Jenguma was statistically similar.

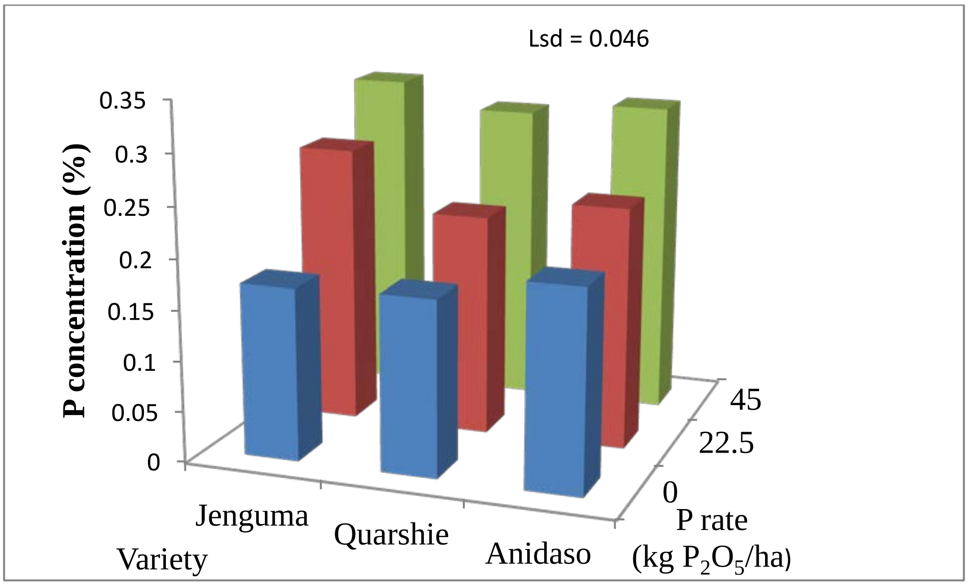

Figure 4.1: Interaction effect of variety and phosphorus application rate on phosphorus concentration of soybean

4.4.2 Phosphorus uptake efficiency

Table 4.9: Effects of inoculation rate, variety and phosphorus rate on phosphorus uptake efficiency

Treatment	Phosphorus uptake efficiency (%)
Inoculation rate (%)	
0	16.67
50	21.11
100	17.78
Pr (I)	0.503
Lsd (0.05)	10.022
Variety	
Jenguma	23.70
Quarshie	13.33
Anidaso	18.52
Pr (V)	0.003
Lsd (0.05)	5.040
Phosphorus rate (kg P_2O_5 / ha)	
0	-
22.5	33.33
45	22.22
Pr (P)	<.001
Lsd (0.05)	2.743
Pr (I x V)	0.536
Pr (I x P)	0.202
Pr (V x P)	<.001
Pr (I x V x P)	0.697
CV (%)	26.8

Results of the effects of inoculation rate, variety and phosphorus rate on phosphorus uptake efficiency are presented in Table 4.9 above. No significant difference ($p > 0.05$) was observed in inoculation rate with respect to phosphorus uptake efficiency. However, significant differences ($p < 0.05$) were observed in soybean variety and phosphorus application. The Jenguma variety had phosphorus uptake efficiency of 23.70 %, and this was statistically higher than that of the Quarshie and Anidaso varieties which were statistically similar. There was significant difference between phosphorus application of 22.5 and 45 kg P_2O_5 / ha with respect to phosphorus uptake efficiency.

Interaction effect of variety and phosphorus rate on phosphorus uptake efficiency of soybean is presented in Figure 4.1. Phosphorus uptake efficiency was significantly affected ($p < 0.05$) by the interaction of variety and phosphorus rate. The Jenguma variety had mean phosphorus uptake efficiency of 41.11% when phosphorus was applied, and this was statistically higher than that of the Quarshie and Anidaso varieties. However, there was no significant effect ($p > 0.05$) among the other interactions with respect to phosphorus uptake efficiency (Table 4.9).

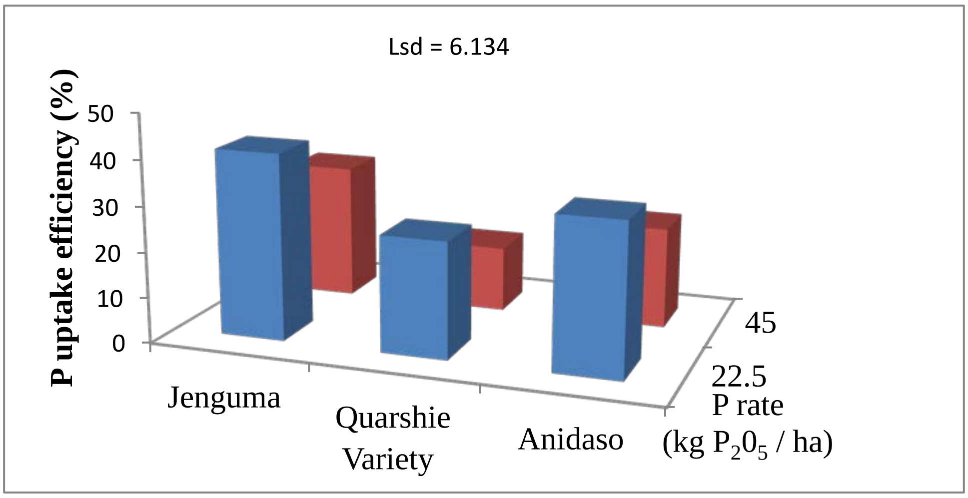

Figure 4.2: Interaction effect of variety and phosphorus application rate on phosphorus uptake efficiency of soybean

4.5 Relationship between yield components and nutrient supply

The results of the correlation matrix for pod number per plant, pod yield, grain yield, nitrogen concentration (N conc.), phosphorus concentration (P conc.), phosphorus uptake efficiency (PUPE), N_2 fixed and percentage of N_2 derived from the atmosphere (% Ndfa) are presented in Table 4.10.

All the variables have positive and significant ($p < 0.1$) relationship with percentage of N_2 derived from the atmosphere. Pod number, pod yield, grain yield, P concentration and amount of N_2 fixed were strongly and highly correlated ($p < 0.1$) with percentage of N_2 derived from the atmosphere. Correlation analysis showed positive and highly significant ($p < 0.1$) relationships of pod yield with grain yield. Pod number also showed a positive and significant ($p < 0.01$) relationship with the grain yield. All variable have positive and significant relationship with each other except nitrogen concentration and 100-seed weight, nitrogen concentration and pod number, phosphorus uptake efficiency and 100-seed weight which was not significant ($p > 0.05$).

Table 4.10: Correlation coefficient of selected parameters

Variables	Pod number	Pod yield	Grain yield	100- seed weight	N conc.	P conc.	PUPE	N_2 fixed	Ndfa
pod number	1.000								
Pod yield (kg / ha)	0.378^{***}	1.000							
Grain yield (kg/ha)	0.291**	0.598***	1.000						
100-seed weight (g)	0.514***	0.299**	0.271^{**}	1.000					
N conc. (kg/ha)	-0.149	0.362^{***}	0.249*	-0.206	1.000				
P conc. (kg / ha)	0.499^{***}	0.394^{***}	0.325**	-0.118	0.317^{**}	1.000			
PUPE (%)	0.418***	0.575***	0.496***	-0.183	0.382^{***}	0.444^{***}	1.000		
N_2 fixed (kg / ha)	O.710***	0.569***	0.268*	0.332**	0.343^{***}	0.497^{***}	0.444***	1.000	
% Ndfa	0.634***	0.595***	0.318**	0.318**	0.452^{***}	0.482^{***}	0.516***	0.939^{***}	1.000

(-) not significant, (*) significant at $p < 0.05$, (**) significant at $p < 0.01$, (***) significant at $p < 0.001$.

CHAPTER FIVE

DISCUSSION

5.1 Effect of inoculation rate, variety and phosphorus rate on growth and nodulation of soybean

5.1.1 Plant height

Inoculated soybean plants were virtually of the same height with that of the uninoculated control at 8 WAP with reference to the means and this suggest that inoculation had no influence on plant height. Plants grown with high amount of phosphorus application (45 kg P_2O_5 / ha) were taller (72.35 cm) than plants grown with low amount of phosphorus (69.29 cm). Within either rate of phosphorus application differences in leaf color were more visible than plant height. This finding is in agreement with reports of Bekere *et al.* (2012) that phosphorus application of 60, 120 and 180 mg P / kg significantly influenced soybean height under controlled environment.

There was no significant difference in plant height at 8 WAP among the three soybean varieties but Anidaso showed early growth. Numerically, Jenguma emerge early but did not have the highest height. The tallest plants were produced by the Anidaso variety among the soybean varieties. The Jenguma and Quarshie varieties produced similar heights. Talaka *et al.* (2013) worked on the growth performance of five soybean varieties under rain-fed condition reported significant difference ($p = 0.01$) among varieties on plant height at 6 WAP while there was no significant difference on plant height at 8 WAP.

None of the Interactions had any influence on soybean height. No significant difference between treatments due to the interaction of variety and biofertilizer in a green house on plant height has also been reported by Uddin *et al*. (2008).

5.1.2 Nodule number

The application of the inoculant did not affect nodulation, indicating N was not only limiting nutrient at this site. Other nutrients such soil pH factors, low phosphorus and molybdenum can affect inoculation response. Also, native rhizobia can prevent the rhizobia introduced in the inoculant from forming nodules on the crop. In other cases, the native rhizobia can fix as much nitrogen as the plant needs, making inoculation unnecessary. The significant positive effects of high rates of inoculation (high numbers of rhizobia per seed) have been demonstrated (Roughley *et al.*, 1993; Date, 2001). High numbers of viable cells on the seed or applied to the soil are an important criterion for good nodulation but this was not always evident in these experiments. Contrary to this finding, larger response to inoculation and higher number of nodules per plant in comparison to un-inoculated treatments in a field that has no soybean cropping history was reported by other workers (Tahir *et al*., 2009; Okereke *et al.*, 2004; Bekere *et al.*, 2012). Similar works have suggested that inoculation does not always elicit or enhance nodulation. Okogun *et al.* (2004) reported that improve soybean varieties (TGx 1448-2E) did not respond to inoculation in terms of nodule production in the Nigeria’s moist savanna zone.

Effective nodulation is essential for a functioning legume/*Rhizobium* symbiosis. Plants most susceptible to infection and capable of producing effective nodules should have greater potential to fix more atmospheric N. However, this assumption often depends on other factors such as the environment, crop management, choice of micro and macro symbiont and the ability of the plant to support high rates of N fixation

(Kellman, 2008). The results of this experiment showed no variability in nodulation between the three soybean varieties and among the *Rhizobium* strain used. This suggests no differences in compatibility between the soybean varieties and the *Rhizobium* strain used.

Legume has a high internal phosphorus requirement for their symbiotic nitrogen fixation. Singleton *et al*. (1984) reported that, in addition to the nodule formation, deficiency of phosphorus in legume also markedly affects the development of effective nodules and the nodule leghaemoglobin content. It therefore suggests that the presence of high phosphorus in soils as in the soils of experimental field may be beneficial to nodule nitrogen fixation through the prevention of the phosphorus concentration in the plants at the later growth stage. The highest nodule number was obtained when phosphorus was applied at the rate of 45 kg P_2O_5 / ha whilst the lowest was the control (0 kg P_2O_5 / ha). Nodule number increases with increasing phosphorus application rate.

Neither the combined application of P and inoculation nor P and variety had any significant effect on nodule number. This suggests that neither combination would result in increase in nodule number under similar conditions. This observation affirms the report of Hakoomat *et al.* (2004) who reported that the interaction between inoculated seed and phosphorus fertilizer on nodule number in chickpea were found to be non-significant. In contrast to this finding positive interaction effect and response of legume host and rhizobium strain in *Vicia faba* for nodulation has been reported by Mytton and Skot (1993).

5.1.3 Nodule dry weight

The failure of inoculation to increase nodule dry weight as it has occurred in the nodule number suggest that nodule dry weight could have a positive correlation with nodule number. This means that if inoculation had increase nodule number, it would have resulted in increased in nodule dry weight. Similar observation was reported by Sarkodie-Addo *et al.* (2006). Indeed Giller (2001) reported that the ability to form nodules is not enough to obtain an effective nitrogen fixation symbiosis

Variety influence on nodule dry weight was not significant. The greatest mean nodule dry weight was produced by the Quarshie variety (2289 mg) followed closely by the Jenguma variety (2237 mg) and the Anidaso variey (1659 mg) respectively. This result is in line with the findings of Solomon *et al.* (2012) on inoculation of soybean varieties on the Nitisol of Bako.

The fact that phosphorus was able to increase nodule dry weight underlines the influence phosphorus has on nodule development through its basic functions as an energy source. The lowest and the highest nodule dry weight was produced when soybean was supplied with the control (0 kg P_2O_5 / ha) and 45 kg P_2O_5 / ha respectively. The result is in line with the findings of Ogoke *et al.* (2006) on N_2 fixation by soybean in Nigeria’s moist savanna. But contradict the findings of Bekere *et al*. (2012) and Bekere and Hailemariam (2012). These authors observed that phosphorus application levels without inoculation did not influence nodule dry weight.

5.1.4 Shoot dry weight

The fact that inoculation was not able to significantly influence nodulation reflected in the shoot dry weight. The ineffectiveness of the inoculation may have been caused by the presence of indigenous *Bradyrhizobia* that were more competitive than the strain used in the experiment. Similarly, Bekere *et al*. (2012) found no significant difference for shoot dry matter with seed inoculation. On the contrary, Solomon *et al.* (2012) reported that the main effect of inoculation of rhizobium strains showed significant difference with respect to dry matter production at mid-flowering.

Similar shoot dry weight was produced by Jenguma, Quarshie and Anidaso varieties at 8 WAP. This shows that, the varieties studied had equal growth and shoot dry weight potential. This is because the conditions of growth were similar, so producing similar dry matter attest to the fact that, the growth potentials were similar in these varieties. Shoot dry weight shown by varieties of the same crop under similar growth conditions is indication of similar potential (Salisbury and Ross, 1992).

Shoot dry weight increased significantly with increasing phosphorus fertilizer rate. This indicated that omission of P from optimum nutrition of soybean dramatically reduced shoot dry matter yield of soybean. This agreed with the reports of other workers (Bekere *et al*., 2012; Bekere and Hailemariam, 2012). Neither variety and inoculation combination nor phosphorus application had influence on shoot dry weight. No significant variety and strain interaction with respect to shoot dry weight was also reported by Solomon *et al*. (2012) of soybean varieties on Nitisols of Bako.

5.2 Effect of inoculation rate, variety and phosphorus rate on yield components of soybean

5.2.1 Pod number

The productive potential of soybean is ultimately determined by number of pods per plant which is a main yield component. Pod number per plant was greater in the inoculated (50 and 100 % inoculation) than the uninoculated control. The mean pod number increased with an increase in inoculation rate and this was not significant. This result is in disagreement with reports of Bhuiyan *et al*. (2008) and Malik *et al*. (2006) who concluded that pod number of mung bean and soybean significantly increased by inoculating with *Bradyrhizobium.* Similar to this result, Shahid *et al*., 2009 reported that seed inoculation of soybean produced more pods per plant than uninoculated control.

The Quarshie variety produced the highest number of pods. This was significantly ($P < 0.05$) higher than that produced by Jenguma. The varietal effect between Quarshie and Jenguma, and Anidaso respectively was not significant. Genetic factors of the varieties might have contributed to the significant effect in pod yield, with Quarshie being the best performer and Anidaso the least. This corroborates with the report of Bouquet (1998) that, genotype selection is one of most important factors for increasing pod yield in soybean.

Phosphorus rate significantly affected number of pods per plant in soybean. Phosphorus application of 22.5 and 45 kg P_2O_5 / ha produced higher number of pods than that of the control (0 kg P_2O_5 / ha). Relative to 22.5 kg P_2O_5 / ha, supplying soybean with 45 kg P_2O_5 / ha produced more number of pods per plant. This result supports the finding of Mohan and Rao (1997) that higher number of pods was produced when higher doses of phosphorus were applied. The results revealed increased in the number of pods with increased phosphorus rate application. This result is similar to what was reported by Bekere *et al.* (2012) after supplying soybean with 20, 40 and 60 kg P_20_5 / ha.

5.2.2 Pod yield

At inoculation rate, the highest pod yield (2643 kg / ha) was obtained with the full recommended *Bradyrhizobium* inoculation rate (100 % inoculation) which was statistically similar to the pod yield of the half recommended rates (50 % inoculation). Pod yield was greater in the inoculated (50 and 100% inoculation) than the un-inoculated control which produced the lowest pod yield. Inoculation of soybean with the half and full recommended rate gave 5.68 % and 7.85 % respectively pod yield increase over the uninoculated control and this was not significant. However there was no evidence of yield reduction as pod yield increased with increased inoculation rate. In contrast to this result, Idris *et al.* (1988) reported that inoculation significantly increased pot yield by 7 %.

Soybean variety played a significant role on pod yield of soybean. Jenguma produced pod yield of 2840 kg / ha which was significantly greater than Quarshie (2513 kg / ha) and Anidaso (2336 kg / ha). There was no statistical difference between pod yields of Quarshie and Anidaso. Therefore Jenguma performed better than Quarshie and Anidaso on the pod yield. This indicates that varietal difference play a role in soybean pod yield. The result is in line with Mahamood *et al.* (2009) that pod yield was not significantly different ($p > 0.05$) among soybean varieties.

Phosphorus rate significantly affected pod yield of soybean. The highest pod yield was obtained from 45 kg P_2O_5 / ha treatment and this was significantly ($p < 0.05$) higher than 22.5 kg P_2O_5 / ha. This result is in line with Mahamood *et al*. (2009) who reported that phosphorus application to soybean significantly affected both pod yield and grain yield.

5.2.3 Grain yield

The greatest grain yield of 1815 kg/ha (1.8 t/ha) was obtained in full recommended rate (100% inoculation) treatment. The lack of grain yield response to inoculation can be attributed to the fact that inoculation did not increase nodulation and N_2 fixation indicating an abundance of effective soybean rhizobia at this location. Other factors may include cultivar and strain interaction and drought. Graham, (1992) reported that drought affects symbiosis between host and rhizobia and this influences rhizobial survival in the soil, the host or the process of nodulation and grain yield. Lack of significant effect on yield improvement by inoculation has also been reported by other workers (Chemining' wa *et al.*, 2004; Otieno *et al.*, 2007). The non-significant effect of inoculation was however in disagreement with reports of other workers (Sable *et al.*, 1998; Shahid *et al.,* 2009).

Soybean grain yield increased with the application of phosphorus fertilizer and this was significant ($p < 0.05$). Maximum grain yield (1909 kg / ha) was given by 22.5 kg P_2O_5 / ha, followed by 45 kg P_2O_5 / ha which gave seed yield of 1864 kg / ha. Minimum seed yield 1233 kg/ha was observed in the uninoculated control. Soybean has a relatively high requirement for phosphorus and yield and seed quality can be enhanced by phosphorus fertilizer in soils testing low in phosphorus (Shahid *et al.,* 2009). Bakere and Hailemariam, (2012) also reported that seed yield of soybean increased with phosphorus fertilizer application of 20, 40 and 60 kg P_2O_5 / ha.

Soybean varieties produce statistically similar amount of seed yield. Jenguma variety produced the highest seed yield of 1881 kg / ha. Quarshie and Anidaso variety produce grain yield of 1560 and 1566 kg / ha respectively. The grain yield obtained in this study is lower compared with the potential yield reported of these varieties. The result agrees with the work done by Alam *et al*. (2009) and Rahman *et al*. (2012) who reported varietal difference in rice and soybean respectively.

5.2.4 Hundred (100) seed weight

Hundred (100) seed weight is also an important yield contributing component. It reflects the magnitude of seed development which ultimately reflects the final yield of a crop. There was no significant ($p > 0.05$) increase of hundred seed weight for both the half and full recommended rates (50 and 100 % inoculation) over the uninoculated control. This might be due to the effectiveness and usually the high competitiveness of the indigenous rhizobia at the experimental site. The result is in line with the work of Bekere and Hailemariam (2012) on nitrogen fixation attributes and yield of soybean.

There was varietal difference in soybean and this was significant ($p<0.05$). Jenguma variety had the highest hundred (100) seed weight of 12.67 g followed closely by Quarshie (12.57 g) while Anidaso produced the lowest seed weight of 10.79 g. The Jenguma variety produced the greatest seed weight and was also observed to be more pronounce at the early growth stage. Seed weight has been noted to affect seedling vigour and it was reported that genotypes with heavier seeds produced more vigorous seedlings, and the relationship between seed weight and seedling vigor was more pronounced in the earlier growth stage (Qiu and Mosjidis, 1993). Similarly, thousand (100) seed weight was found to be affected significantly ($p \leq 0.01$) by the main effect of variety (Solomon *et al*., 2012). Contrarily, Alam *et al*. (2009) reported that soybean varieties did not show any significant response on 100-grain weight.

Neither inoculation and phosphorus nor the other interactions had any influence on the hundred (100) seed weight. Contrarily, Interactive effect of the factors under study

(I x P) on 1000-seed weight was significantly reported (Shahid *et al*., 2009; Sharma and Namdeo, 1999).

5.3 Effect of inoculation rate, variety and phosphorus rate on nitrogen concentration, N_2 fixed and percentage of N_2 derived from the atmosphere

5.3.1 Nitrogen concentration in Soybean

In grain legumes, plant % N is highly sensitive to early growth conditions of the plant which affect nodule formation and efficiency (Kellman, 2008). In addition, it is also influenced by the ratio of seed to other plant components (Kellman, 2008). In this work, plant % N was measured on composite plant samples which could have influenced any differences in N_2 fixation. The incorporation of the seed could have affected the final plant % N, as N is transferred from other plant parts to the seed at the start of seed filling (Green wood *et al*., 1991). The fact that inoculated plants had the highest plant N % concentration implies a positive effect of nodulation on plant % N. Hence the ability of the rhizobia to establish a symbiosis had an impact on plant % N. A recent report by Tejera *et al*. (2005) working with bean showed a positive significant correlation between nodule number and shoot dry weight and nodule number and N % confirming the importance of symbiosis in N accumulation in legumes.

The Anidaso variety accumulated more N by flowering time as compared to the Jenguma and Quarshie. The variety treatments affected the N content at flowering. The highest N content was found with the Anidaso variety and the N content obtained with the Jenguma variety was significantly lower than the N content obtained in the Anidaso variety, while the Quarshie variety and Jenguma variety was statistically similar. This finding agrees with the reports of Solomon *et al*. (2012) that varietal differences exist in soybean with respect to nitrogen concentration.

Addition of P tended to enhance N concentration at flowering. Plant N concentration significantly increased with increasing in phosphorus application rate. The highest plant N was when soybean was supplied with 45 kg P_2O_5 / ha. This increase may be due to supply of phosphorus that seems important for Rhizobium to fix relatively more nitrogen from soil, which resulted in increased plant growth and N uptake by root and then to shoots. Phosphorus plays a vital role in physiological and developmental process in plant life and favorable effect of this important nutrient might have accelerated the growth process that increases N uptake in plants (Fatima *et al*., 2007). Enhance flows of nutrients to crops will obviously be require in sustainable crop production. This finding is contrarily to what Bekere *et al*. (2012) reported when they applied 20, 40 and 60 kg P_2O_5 / ha to soybean.

Interactions of inoculation and variety did not increase nitrogen concentration in soybean. This result was different from those of Teye *et al.* (2010) who reported significant interaction of rhizobial strains with varieties in straw nitrogen in pea.

5.3.2 Amount of N_2 fixed and Percentage of N_2 Derived from the Atmosphere (% Ndfa)

Inoculation rate did not significantly increase the amount of N_2 fixed and the percentage of N_2 derived from the atmosphere (% Ndfa). The mean amount of N_2 fixed and % Ndfa for the 100% inoculation was higher than that of the 50 % inoculation and the uninoculated control. The 50 % inoculation was virtually the same as the uninoculated control with respect to the mean amount of N_2 fixed and % Ndfa. This nevertheless indicates higher numbers of rhizobia can effectively increase the amount of N_2 fixed and % Ndfa. The lack of inoculation response to the amount of N_2 fixed and % Ndfa might be due to the inability of the inoculation to outcompete the indigenous rhizobia for the nodulation of soybean. Increase in nodulation has been reported to increase N_2 fixation in soybean (Ruark, 2009). This observation is contrary to the report that the percentage of nitrogen derived from the atmosphere (% Ndfa) in *Phaseolus vulgaris L.* was increased significantly with *Rhizobium* inoculation in all organs (roots, shoots, pods and whole plant) both in the glass house and field experiments compared with the un-inoculated control treatment (Bambara, 2009).

There was no significant varietal difference in soybean on the amount of N_2 fixed and % Ndfa. The non-significant difference of variety on amount of N_2 fixed and % Ndfa supports the facts that soybean maturity date affects the amount of N_2 fixed in soybean. N_2 fixation has been reported to increase with increasing crop duration (Ogoke *et al*., 2006). This is because longer growth duration allows for a longer period of N_2-fixation in the nodules. Increased crop duration in the field means a longer period of nodule activity. The soybean varieties used in this study belong to the same maturity class (i.e. medium maturing varieties). This might explain why the difference in amount of N_2 fixed was not significant among the three varieties.

An estimate of 26-188 kg N / ha in the tropics have been made while soybean could fix 15-162 kg N / ha (Giller and Wilson, 1991; Larue et al., 1981). Based on the experimental site used, amount of N_2 fixed was in the range of 52.1 to 71 kg N / ha in the soybean varieties studied. This is higher than the 41-50 kg N / ha reported by Yusuf et al. (2006). However, the estimate from the work was lower than 61-109 kg N / ha that Ogoke *et al.* (2006) reported in the soybean varieties studied. The soybean varieties used by Ogoke *et al.* (2006) were short, medium and late maturing varieties while medium maturity class (soybean varieties) was used in this study. On the average, soybean N_2 derived from the atmosphere in this study was 45 % of the total above ground N against the 70-90 % from previous studies (especially Ogoke *et al*., 2006).

The amount of N_2 fixed and percentage of N_2 derived from the atmosphere increase with increasing phosphorus rate. The maximum amount and percentage of N_2 fixed were 96.1 kg / ha and 59.59 %, respectively when phosphorus was applied at the rate of 45 kg P_2O_5 / ha. There were significant difference between the amount and percentage of N_2 fixed between the phosphorus application of 22.5 and 45 kg P_2O_5 / ha. This indicates that P deficiency does not only limit plant growth, it can also limit symbiotic N_2 fixation as the latter has been noted to have a higher P requirement for optimal functioning than either plant growth or nitrate assimilation**.**

The combination of inputs (interactions) failed to increase the amount of N_2 fixed and % Ndfa. This finding is in contrast to what Asghar *et al*. (2000) reported that a specific combination of soybean genotype with rhizobium strains resulting in many fold increase in the amount of N_2 fixed and grain yield harvested.

5.4 Effect of inoculation rate, variety and phosphorus rate on phosphorus concentration and phosphorus uptake efficiency of soybean

5.4.1 Phosphorus concentration in soybean

Numerically, inoculation of soybean decreased phosphorus concentration (by 0.24 and 0.28 %, for 50 and 100 % inoculation respectively) in the plant with the uninoculated control accumulating the highest P than the inoculated treatments. This observation affirms Basu *et al*. (2008) report that P concentration in soybean decreases by 0.1 % the same trend as N and K with respect to P concentration when soybean was inoculated.

Nutrient uptake and concentration in plants growing in soils depends on the configuration and growth rate of the root system, the nutrient uptake characteristics per unit of root, and the nutrient supply characteristics of the soil. The fact that varietal differences were not observed with respect to P accumulation suggests that the roots system was the same of these three soybean varieties because the soil characteristics were the same. Numerically, P concentration for Jenguma variety was 0.26 % and this was higher than the Quarshie variety (0.23 %) and the Anidaso variety (0.25 %). The Quarshie variety gave lower P concentration while the Jenguma variety accumulated the highest than that of the Quarshie variety and the Anidaso variety.

The highest P (0.31 %) was accumulated when soybean was supplied with 45 kg P_2O_5 / ha while the minimum of 0.18 % was accumulated in the control (0 kg P_2O_5 / ha). Supplying soybean with 45 kg P_2O_5 / ha resulted in an increased in P concentration due to supply of nutrients and well developed root system resulting in better absorption of water and nutrient. Increase P concentration in the plant is favourable to nodulation and biological nitrogen fixation in legumes. The significant increase in P concentration with increasing P application rate suggests the quantities of P that is required by the plant. This means that higher amount of P application (45 kg P_2O_5 or more) is required by the plant to carry out processes such as photosynthesis, translocation of sugars and other functions which directly influence N fixation.

Among the interactions, variety and phosphorus application rate proved to be the best combination of inputs with respect to P concentration of soybean. The soybean varieties increase with increasing phosphorus application rate. The Jenguma and the

Quarshie variety accumulated more P (0.32 %) and the least (0.29 %) when 45 kg P_2O_5 / ha was applied.

5.4.2 Phosphorus uptake efficiency

Inoculation rate of 50 % gave the highest phosphorus uptake efficiency of 21.11% followed by 100% inoculation which also recorded 17.78 % and this was not significant. The uninoculated control recorded the least phosphorus uptake efficiency of 16.67 %. This indicates that inoculation beyond the 50 and 100% inoculation produced phosphorus uptake efficiency of 4.44 and 1.11 % respectively, over the uninoculation control. This observation is in line with the report of Khair *et al*. (2002) that inoculation slightly increases (0.01 %) phosphorus uptake efficiency in soybean.

The Jenguma variety was identified as the most P-efficient variety among the three varieties while the Quarshie variety was the least P-efficient variety. The observation of varietal differences in the three soybean varieties suggest that differences in Phosphorus uptake efficiency may occur among plant species or genotypes due to the ability of the root system to acquire P from the soil and accumulate it in the shoots (Rao *et al.*, 1997). Soybean varieties are known to differ in their ability to grow under low-P conditions (Neue, 1991). The more P efficient varieties may have internal and/ or external mechanisms that allow greater soil P extraction and grain yield. An understanding of the internal and external P efficiencies of modern soybean varieties is very important in selection of varieties adaptable to P deficient conditions.

Phosphorus uptake efficiency was more (33.33 %) when soybean was applied with 22.5 kg P_2O_5 / ha than 45 kg P_2O_5 / ha (22.22 %). This indicates that increasing rate of phosphorus application increases phosphorus uptake inefficiency. The efficiency of applied phosphorus to the soil reduces as a result of soil erosion, leaching and gaseous losses. The utilization of nutrients decreased as increasing the rate of nutrient application by the law of limiting factors (Hussein, 2009). This observation is similar to the work of Khair *et al.* (2002) who reported that P uptake efficiency decrease in rate of applied P in un-inoculated plots but increased in inoculated plots at NIFA. The efficiency of the applied P in this study was within the range of 22.22-33.33 %. This is higher than what was reported by Baligar and Bennet, (1986) that under tropical conditions, the efficiency of applied nutrient has been estimated to be less than 50 % N, 10-30 % for P and about 40 % for K.

Among the interactions, the application of P and variety was proved to be the best combination with respect to phosphorus uptake efficiency. The Jenguma variety was more efficient (41.11 %) than the Quarshie variety (25.56 %) when 22.5 kg P_20_5 / ha was applied. The Jenguma and Anidaso variety was the most and the least efficient variety respectively. This observation contradicts the work Daoui *et al.* (2012) on genotypic variation of phosphorus use efficiency among Moroccan fababean varieties reported no significant interaction of genotype and phosphorus (G $*$ P) on P uptake efficiency.

5.5 Relationship between yield components and nutrients supply

From the correlation analysis, it is observed that, all the variables selected for this analysis had significant ($p < 0.05$) positive correlation with each other except N concentration and pod number, N concentration and 100-seed weight, P concentration and 100-seed weight and also phosphorus uptake efficiency and 100-seed weight. These results showed that a rise in any of these variables would result in a corresponding increase in the other and vice versa (Table 4.12). However, a negative correlation was produced between nitrogen uptake and hundred seed weight indicating that an increase in nitrogen uptake would lead to a corresponding fall in hundred seed weight and vice versa (Table 4.12).

The results showed a strong and significant relationship between the entire variable selected for this analysis and percentage of N_2 derived from the atmosphere. This is an indication that, any increase of the above variables leads to corresponding increase in the percentage of N_2 derived from the atmosphere. Rosario *et al*. (1997) reported similar observation that, % Ndfa, the proportion of the nitrogen in the plant contributed by fixation, was highly correlated with all nodulation and BNF traits.

It is also of interest to note that the amount of nitrogen fixed was found to be correlated with the entire selected parameters from the matrices (Table 4.12). This means that any increase in nodulation, nitrogen uptake or % Ndfa as well as any improvement in one or more of the BNF-associated agronomic variables should be accompanied by a corresponding increment in the amount of N_2 fixed. This is also an indication that any of the above characters could appropriately be used as an index for biological nitrogen fixation potential.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

Results showed that inoculation of soybean with legume fix at the rate of 2.5 or 5 g / kg seed had no significant effect on plant height, nodulation, shoot dry weight, pod number, pod yield, grain yield, 100-seed weight, P concentration, P uptake efficiency, amount of N_2 fixed and percentage of N_2 derived from the atmosphere. However, legumefix inoculation at the rate of 5 g / kg significantly increased nitrogen concentration by 0.13 % of the soybean. The combined application of P fertilizer and inoculation had no significant effect on nodulation and BNF of soybean varieties. Hence, it is concluded that inoculation of soybean with legumefix on Fluvic luvisol does not affect nodulation and BNF of the three soybean varieties.

The results also revealed that Jenguma, Quarshie and Anidaso varieties were similar in height, nodulation formation and development, shoot dry weight, grain yield, amount $_2$ fixed and the percentage of N_2 derived from the atmosphere. However these varieties differed significantly with respect to pods number, pod yield, 100-seed weight, nitrogen concentration and phosphorus uptake efficiency as Jenguma > Quarshie > Anidaso.The application of phosphorus fertilizer significantly increased all the parameters measured. For most of the parameters, supplying soybean with 45 kg P_2O_5 / ha increased growth and yield more than application of 22.5 kg P_2O_5 / ha. Phosphorus fertilizer application of 22.5 kg P_2O_5 / ha was more efficiently used by the crop than 45 kg P_2O_5 / ha applied. It is therefore concluded that P application increases growth and yield of the three soybean varieties and Jenguma is the most P-efficient variety.

6.2 RECOMMENDATIONS

The study revealed that phosphorus fertilizer application increased growth and yield of soybean. Phosphorus application rate of 45 kg P_2O_5 / ha significantly increased growth and yield more than the 22.5 kg P_2O_5 / ha. It is therefore recommended that farmers should adopted phosphorus application rate of 45 kg P_2O_5 / ha to increase soybean production in the Northern Region of Ghana.

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APPENDICES

APPENDIX 1

Analysis of variance

Variate: Plant_Height (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	420.356	210.178	11.27	
Rep.Ino stratum					
Ino	2	7.997	3.998	0.21	0.816
Residual	4	74.627	18.657	1.02	
Rep.Ino.V stratum					
V	2	31.785	15.893	0.87	0.443
Ino.V	4	12.266	3.066	0.17	0.950
Residual	12	218.535	18.211	5.01	
Rep.Ino.V.P stratum					
P	2	340.355	170.177	46.85	<.001
Ino.P	4	4.911	1.228	0.34	0.851
V.P	4	18.294	4.574	1.26	0.304
Ino.V.P	8	32.679	4.085	1.12	0.371
Residual	36	130.753	3.632		
Total	80	1292.557			

Analysis of variance

Variate: Nodule_Number_per_plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.470854	0.235427	4.31	
Rep.Ino stratum					
Ino	2	0.013008	0.006504	0.12	0.891
Residual	4	0.218652	0.054663	0.80	
Rep.Ino.V stratum					
V	2	0.374031	0.187015	2.73	0.106
Ino.V	4	0.043073	0.010768	0.16	0.956
Residual	12	0.823098	0.068591	7.72	

```
Rep.Ino.V.P stratum
P                           2    1.439835    0.719918     81.01   <.001
Ino.P                       4    0.018593    0.004648      0.52   0.719
V.P                         4    0.009585    0.002396      0.27   0.896
Ino.V.P                     8    0.130062    0.016258      1.83   0.103
Residual                   36    0.319908    0.008886

Total                      80    3.860699

Analysis of variance

Variate: Nodule_Dry_Weight_mg_plant

Source of variation      d.f.        s.s.        m.s.      v.r.   F pr.

Rep stratum                 2    3133830.    1566915.      0.94

Rep.Ino stratum
Ino                         2    1474052.     737026.      0.44   0.672
Residual                    4    6703363.    1675841.      1.36

Rep.Ino.V stratum
V                           2    6600052.    3300026.      2.68   0.109
Ino.V                       4     892474.     223119.      0.18   0.944
Residual                   12   14793052.    1232754.      5.30

Rep.Ino.V.P stratum
P                           2   87470496.   43735248.    187.89   <.001
Ino.P                       4     936474.     234119.      1.01   0.417
V.P                         4     876474.     219119.      0.94   0.451
Ino.V.P                     8    2518133.     314767.      1.35   0.250
Residual                   36    8379822.     232773.

Total                      80  133778222.

Analysis of variance

Variate: Shoot_dry_weight_kg_ha

Source of variation      d.f.        s.s.        m.s.      v.r.   F pr.

Rep stratum                 2  109241156.   54620578.     34.07

Rep.Ino stratum
Ino                         2    5949956.    2974978.      1.86   0.269
Residual                    4    6412444.    1603111.      0.19
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Rep.Ino.V stratum
V                              2     8962489.     4481244.     0.54   0.595
Ino.V                          4    15299111.     3824778.     0.46   0.762
Residual                      12    99181600.     8265133.     6.02

Rep.Ino.V.P stratum
P                              2   199648356.    99824178.    72.77   <.001
Ino.P                          4     1982044.      495511.     0.36   0.835
V.P                            4     9040711.     2260178.     1.65   0.184
Ino.V.P                        8    12120089.     1515011.     1.10   0.383
Residual                      36    49385600.     1371822.

Total                        80     517223556.

Analysis of variance

Variate: Pod number_no_plant
Source of variation         d.f.          s.s.         m.s.     v.r.   F pr.
Rep stratum                    2        4492.1       2246.0    37.75

Rep.Ino stratum
Ino                            2         189.7         94.8     1.59   0.310
Residual                       4         238.0         59.5     0.12

Rep.Ino.V stratum
V                              2        5693.2       2846.6     5.69   0.018
Ino.V                          4         596.2        149.1     0.30   0.874
Residual                      12        6007.9        500.7     2.85

Rep.Ino.V.P stratum
P                              2       17485.4       8742.7    49.74   <.001
Ino.P                          4         166.4         41.6     0.24   0.916
V.P                            4         827.8        206.9     1.18   0.337
Ino.V.P                        8        1144.2        143.0     0.81   0.595
Residual                      36        6328.1        175.8

Total                         80       43169.1

Analysis of variance
Variate: Pods_dry_weight
Source of variation         d.f.          s.s.         m.s.     v.r.   F pr.
Rep stratum                    2       955310.      477655.     1.15
Rep.Ino stratum
Ino                            2       569760.      284880.     0.69   0.554
Residual                       4      1659012.      414753.     1.56

Rep.Ino.V stratum
V                              2      3538783.     1769392.     6.67   0.011
Ino.V                          4      1998061.      499515.     1.88   0.178
Residual                      12      3183301.      265275.     0.71
```

Rep.Ino.V.P stratum					
P	2	21813388.	10906694.	29.28	<.001
Ino.P	4	1042032.	260508.	0.70	0.598
V.P	4	427274.	106818.	0.29	0.885
Ino.V.P	8	771625.	96453.	0.26	0.975
Residual	36	13411718.	372548.		
Total	80	49370264			

Analysis of variance

Variate: Grain_yield_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1564336.	782168.	3.00	
Rep.Ino stratum					
Ino	2	999626.	499813.	1.92	0.260
Residual	4	1041569.	260392.	0.90	
Rep.Ino.V stratum					
V	2	1826041.	913020.	3.17	0.078
Ino.V	4	1717806.	429452.	1.49	0.265
Residual	12	3453326.	287777.	1.31	
Rep.Ino.V.P stratum					
P	2	7710877.	3855438.	17.54	<.001
Ino.P	4	229844.	57461.	0.26	0.901
V.P	4	1194682.	298670.	1.36	0.267
Ino.V.P	8	1889807.	236226.	1.07	0.402
Residual	36	7912620.	219795.		
Total	80	29540532.			

Analysis of variance
Variate: 100_Seed_Weight_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.7356	0.3678	0.63	
Rep.Ino stratum					
Ino	2	1.4156	0.7078	1.21	0.389
Residual	4	2.3422	0.5856	0.61	
Rep.Ino.V stratum					
V	2	60.6252	30.3126	31.60	<.001
Ino.V	4	0.2193	0.0548	0.06	0.993
Residual	12	11.5111	0.9593	1.23	

Rep.Ino.V.P stratum					
P	2	11.4541	5.7270	7.35	0.002
Ino.P	4	4.9926	1.2481	1.60	0.195
V.P	4	1.9230	0.4807	0.62	0.653
Ino.V.P	8	2.5526	0.3191	0.41	0.908
Residual	36	28.0444	0.7790		
Total	80	125.8156			

Analysis of variance
Variate: Nitrogen_concentration_in_soybean _(%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.90472	0.45236	26.19	
Rep.Ino stratum					
Ino	2	0.23525	0.11763	6.81	0.052
Residual	4	0.06910	0.01727	0.58	
Rep.Ino.V stratum					
V	2	0.91534	0.45767	15.46	<.001
Ino.V	4	0.13121	0.03280	1.11	0.397
Residual	12	0.35527	0.02961	1.12	
Rep.Ino.V.P stratum					
P	2	2.56109	1.28054	48.34	<.001
Ino.P	4	0.08653	0.02163	0.82	0.523
V.P	4	0.20204	0.05051	1.91	0.130
Ino.V.P	8	0.11137	0.01392	0.53	0.829
Residual	36	0.95364	0.02649		
Total	80	6.52556			

Analysis of variance

Variate: Amount _of_ N_2_Fixed_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	154.4	77.2	1.71	
Rep.Ino stratum					
Ino	2	318.0	159.0	3.51	0.132
Residual	4	180.9	45.2	0.03	
Rep.Ino.V stratum					
V	2	4858.7	2429.3	1.68	0.228
Ino.V	4	3238.1	809.5	0.56	0.697
Residual	12	17399.4	1450.0	4.66	
Rep.Ino.V.P stratum					
P	2	61287.3	30643.6	98.38	<.001
Ino.P	4	666.9	166.7	0.54	0.711
V.P	4	1806.5	451.6	1.45	0.238
Ino.V.P	8	972.6	121.6	0.39	0.919

Residual	36	11213.0	311.5		
Total	80	102095.8			

Analysis of variance

Variate: % Ndfa

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	789.59	394.80	30.41	
Rep.Ino stratum					
Ino	2	16.32	8.16	0.63	0.579
Residual	4	51.93	12.98	0.07	
Rep.Ino.V stratum					
V	2	828.69	414.35	2.26	0.147
Ino.V	4	606.87	151.72	0.83	0.533
Residual	12	2202.06	183.50	4.79	
Rep.Ino.V.P stratum					
P	2	11535.90	5767.95	150.56	<.001
Ino.P	4	40.31	10.08	0.26	0.900
V.P	4	321.00	80.25	2.09	0.102
Ino.V.P	8	267.67	33.46	0.87	0.548
Residual	36	1379.15	38.31		
Total	80	18039.49			

Analysis of variance

Variate: P_concentation in soybean_(% P)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0309728	0.0154864	1.02	
Rep.Ino stratum					
Ino	2	0.0480691	0.0240346	1.58	0.313
Residual	4	0.0609901	0.0152475	3.23	
Rep.Ino.V stratum					
V	2	0.0097062	0.0048531	1.03	0.387
Ino.V	4	0.0148790	0.0037198	0.79	0.555
Residual	12	0.0566370	0.0047198	5.13	

Rep.Ino.V.P stratum					
P	2	0.2269136	0.1134568	123.27	<.001
Ino.P	4	0.0030716	0.0007679	0.83	0.512
V.P	4	0.0119901	0.0029975	3.26	0.022
Ino.V.P	8	0.0058247	0.0007281	0.79	0.614
Residual	36	0.0331333	0.0009204		
Total	80	0.5021877			

Analysis of variance

Variate: Phosphorus uptake efficiency (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	7.41	3.70	0.02	
Rep.Ino stratum					
Ino	2	288.89	144.44	0.82	0.503
Residual	4	703.70	175.93	2.44	
Rep.Ino.V stratum					
V	2	1451.85	725.93	10.05	0.003
Ino.V	4	237.04	59.26	0.82	0.536
Residual	12	866.67	72.22	2.93	
Rep.Ino.V.P stratum					
P	2	15555.56	7777.78	315.00	<.001
Ino.P	4	155.56	38.89	1.58	0.202
V.P	4	725.93	181.48	7.35	<.001
Ino.V.P	8	140.74	17.59	0.71	0.679
Residual	36	888.89	24.69		
Total	80	21022.22			

APPENDIX 2

```
Two-sided test of correlations different from zero

                                    Probabilities

   Number_of_pods_no_plant           < 0.001
           Pods_dry_weight           < 0.001        < 0.001
         Grain_yield_kg_ha           0.00841        < 0.001        < 0.001
      %100_Seed_Weight_g             < 0.001        0.00677        0.01446        < 0.001
           N_conc_in_plt_%           0.18386        < 0.001        0.02488        0.06527
               P_conc_%P             < 0.001        < 0.001        0.00309        0.29274
                  %FPUPE             < 0.001        < 0.001        < 0.001        0.10168
           N2_Fixed_kg_ha            < 0.001        < 0.001        0.01545        0.00249
                   %Ndfa             < 0.001        < 0.001        0.00387        0.00386
   Number_of_pods_no_plantPods_dry_weightGrain_yield_kg_ha%100_Seed_Weight_g


           N_conc_in_plt_%           < 0.001
               P_conc_%P             0.00390        < 0.001
                  %FPUPE             < 0.001        < 0.001        < 0.001
           N2_Fixed_kg_ha            0.00172        < 0.001        < 0.001        < 0.001
                   %Ndfa             < 0.001        < 0.001        < 0.001        < 0.001
                                N_conc_in_plt_%   P_conc_%P      %FPUPEN2_Fixed_kg_ha


                   %Ndfa             < 0.001
```