INFLUENCE OF P SOURCES AND RHIZOBIUM INOCULATION ON GROWTH, NODULATION, N & P UPTAKE AND YIELD OF THREE SOYBEAN GENOTYPES IN TANCHERA SOIL SERIES OF THE NORTHERN GUINEA SAVANNAH ZONE OF GHANA.

BY

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DECLARATION

I hereby declare that, this thesis herein presented for a master of philosophy (M.Phil.) degree in the School of agriculture, is being self-investigated work. It has therefore not been presented either in part or in whole for another degree elsewhere. Except for references to other authors' works, sources of information are duly cited and acknowledged.

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DEDICATION

I dedicate this thesis to God Almighty. My late mother Ms. Gladys Som and the late Prof. Seth K. Akyea Danso all of blessed memory. May their souls rest in everlasting peace. Amen.

The thesis is also dedicated to Prof. E. Owusu-Bennoah and Dr. J. Futse for their special interest they showed to help me come this far. And also to all my family members especially Eliel K. K. Fia, Albert Elikem Fia, Peace Kumah and Georgina Fuvi. God richly bless you all.

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ABSTRACT

Soybean (*Glycine max* L) constitutes an important grain legume crop in semi-arid areas of sub Saharan Africa. The crop yields are usually low and falling relatively due to low P levels of the soil and the inability of the crop to fix nitrogen. Genotypes that make use of P applied to the soil and fix nitrogen could represent a key step in improving the productivity of soybean in the savannah zone. The objective of the study was to evaluate the effect of two P sources and Rhizobium inoculation on the growth, nodulation, N and P uptake and the yield performance of three soybean genotypes in Tachera soil series from the Northern Guinea savannah zone of Ghana. The P sources were Triple superphosphate (TSP) and Morocco phosphate rock (MPR) while the three genotypes were Jenguma (TGX1448-2E), TGX1904-6F and TGX1955-4F (new genotypes). The field experiment was carried out at Tili in the Bawku West District in the Upper East region during the 2015 cropping season. The experiment was repeated in the greenhouse at the School of Agriculture, University of Ghana, Legon. The field experiment was laid out in a split-split plot design with four replications and the greenhouse experiment was conducted using completely randomize design. The soybean genotypes constituted the main plot, the two sources applied at the rate of 30 kg P/ha and a control as sub-plots and rhizobium inoculation entries were sub-sub-plots. Parameters measured were indigenous rhizobia population (IRP) counts, plant height, nodule number, nodule dry weight and effective nodule number, yield and yield components, and nitrogen (N) and phosphorus (P) uptake and concentration in the shoot and in the grain. The results of the Most Probable Number (MPN) showed that Jenguma the local genotype contained 3.1×10^2 cells g^{-1} soil while the new genotypes contained similar indigenous rhizobia of 1.7×10^2 cells g^{-1} soil in

TGX1904-6F and TGX1955-4F respectively at start of the experiment. The results indicate TGX1955-4F>TGX1904-6F>Jenguma in terms of growth, yield and uptake of phosphorus. The grain yield ranged between 694 - 1033 kg/ha in the field and 2.31 - 2.35 g/pot in the greenhouse respectively. Grain yield and harvest index correlated positively with plant height and nodule dry weight in the field experiment. Genotypes showed similarity in nodule number and effective nodule number, but varied in nodule dry weight.

Rhizobium inoculation did not have positive effects on yield and yield components in this study but increased nutrient (N & P) uptake and concentration. Application of TSP had significant pod yield of 1265 kg/ha compared to that of MPR and the control which was positively correlated to plant height in the field experiment. Nitrogen and P uptake was higher in TSP than in MPR and control. The P uptake trend followed TSP (49.93 kg P/ha) > MPR (32.05 kg P/ha) > C (29.58 kg P/ha). P sources interacted with rhizobium inoculation to significantly influence most of the parameters measured in both experiments. The Tanchera soil has high potential for soybean production with considerable grain and biomass yield. These two soybean genotypes (Jenguma and TGX1955-4F) that have thrived well in the low P soil with the potential of taking up their phosphorus from the readily available P source (TSP). More P was taken up from TSP than MPR. These two gynotypes TGX1955-4F and Jenguma performed better in terms of yield, nutrient concentration and uptake than TGX1904-6F; but overall TGX1955-4F could be recommended to farmers in northern Guinea savannah zone of Ghana.

TABLE OF CONTENTS

DECLARATION I

DEDICATION III

ACKNOWLEDGMENTS IV

ABSTRACT V

TABLE OF CONTENTS VIVII

LIST OF TABLES VIIXII

LIST OF FIGURES XII

CHAPTER ONE 1

1.0 INTRODUCTION 1

CHAPTER TWO 5

2.0 LITERATURE REVIEW 5

2.1 GRAIN LEGUMES 5

2.2 SOYBEAN CROP 5

2.3 MECHANISM FOR BIOLOGICAL NITROGEN FIXATION 6

2.4 SYMBIOTIC N_2 FIXATION 7

2.5 FACTORS AFFECTING NODULATION 9

2.5.1 Soil moisture content 10

2.5.2 Soil acidity (pH) 111

2.5.3 High soil nitrogen content 112

2.5.4 Temperature 122

2.5.5 Salinity 143

2.6 PHOSPHORUS 154

2.6.1 Phosphorus Status in Ghanaian Surface Soils 176

2.6.2 Use of PRs in West Africa 188

2.6.3 Indigenous Phosphate deposits in some parts of the world 21
2.6.4 Phosphate rock deposits in Africa 22
2.6.5 Morocco Phosphate rock 22
2.7 FACTORS AFFECTING PR DISSOLUTION IN SOIL 23
2.7.1 Influence of phosphate rock properties 24
2.7.2 Soil properties 25
2.7.3 Soil acidity 27
2.7.4 Cation exchange capacity, and exchangeable calcium and magnesium 27
2.7.5 Effect of Soil Organic Matter 28
2.7.6 Climate conditions 29
2.7.7 Crop species 30
2.7.8 Management practices 331
CHAPTER THREE 34
3.0 MATERIALS AND METHODS 34
3.1 INTRODUCTION 34
3.1.1 SITE CHARACTERISTICS 34
3.1.2 The study area 34
3.1.3 Soil sampling 35
3.1.4 Field Preparation and Experimental Layout 38
3.2 GREENHOUSE EXPERIMENT 38
3.3 FIELD AND GREENHOUSE DATA COLLECTION 39
3.3.1 Phenological measurements and Shoot Biomass 39
3.3.2 Yield component 40
3.4 CHEMICAL ANALYSIS OF PLANT MATERIAL 41
3.4.1 Digestion of Plant Material 41
3.4.2 Determination of Total Plant Nitrogen 41
3.4.3 Determination of Total Plant Phosphorus 42
3.5.0 DATA ANALYSIS 43

3.5.1 Laboratory analyses of soil samples 43
3.5. 2 Physical analysis 43
3.5.3 Chemical Analysis 45
3.5.4 Biological Analysis 47
CHAPTER FOUR 49
4.0 RESULTS 49
4.1 PHYSICO-CHEMICAL CHARACTERISTICS OF THE SOIL 52
4.2 NODULATION POTENTIAL OF THE THREE SOYBEAN GENOTYPES IN THE TANCHERA SOIL. 53
4.3 EFFECT OF GENOTYPE, P SOURCES AND RHIZOBIA INOCULATION ON PLANT GROWTH, YIELD AND YIELD COMPONENTS IN THE GREENHOUSE 55
4.4 INTERACTION EFFECT OF THE P SOURCES AND RHIZOBIUM INOCULATION ON PLANT GROWTH AND YIELD IN THE GREENHOUSE 58
4.5 EFFECT OF GENOTYPE, P SOURCES AND RHIZOBIA INOCULATION ON NUTRIENT CONCENTRATION AND UPTAKE IN THE GREENHOUSE. 60
4.6 INTERACTION EFFECT BETWEEN P SOURCES AND RHIZOBIUM INOCULATION ON NUTRIENT CONCENTRATION AND UPTAKE IN THE GREENHOUSE 63
4.7 EFFECT OF SOYBEAN GENOTYPE, P SOURCES AND RHIZOBIA INOCULATION ON GROWTH, NODULATION AND GRAIN YIELD (FIELD EXPERIMENT). 65
4.8 INTERACTION EFFECTS OF P SOURCE AND RHIZOBIUM INOCULATION ON PLANT GROWTH, NODULATION AND YIELD IN THE FIELD EXPERIMENT. 68
4.9 INFLUENCE OF GENOTYPE, P SOURCES AND RHIZOBIA INOCULATION ON NITROGEN AND PHOSPHORUS ACCUMULATION IN THE FIELD EXPERIMENTS. 70

4.10 INTERACTION EFFECTS OF THE P SOURCES AND RHIZOBIUM INOCULATION ON NUTRIENT CONCENTRATION AND UPTAKE IN THE FIELD EXPERIMENT. 73

CHAPTER FIVE 75

5.0 DISCUSSION 75

5.1 SOIL CHARACTERISATION 75

5.2 NODULATION POTENTIAL OF THE THREE SOYBEAN GENOTYPES IN THE TANCHERA SOIL. 76

5.3 RESPONSE OF SOYBEAN GENOTYPES TO GROWTH, NODULATION AND GRAIN YIELD... 77

5.4 EFFECT OF P SOURCES ON PLANT GROWTH, NODULATION AND GRAIN YIELD. 81

5.5 EFFECT OF RHIZOBIUM INOCULATION ON PLANT GROWTH, NODULATION AND YIELD. 83

5.6 INTERACTION EFFECTS BETWEEN THE P SOURCES & INOCULATION ON PLANT GROWTH, NODULATION CAPACITY AND GRAIN YIELD. 84

5.7 NUTRIENT CONCENTRATION AND UPTAKE IN THE THREE SOYBEAN GENOTYPES. 85

5.8 EFFECT OF P SOURCES ON N &P CONCENTRATIONS AND THEIR UPTAKE IN SHOOT. 86

5.9 EFFECT OF RHIZOBIA INOCULATION ON NUTRIENT CONCENTRATION AND UPTAKE OF N & P. 87

5.10 INTERACTION EFFECTS BETWEEN P SOURCES AND RHIZOBIUM INOCULATION ON NUTRIENT CONCENTRATION AND UPTAKE IN BOTH EXPERIMENT. 88

5.11 EFFECT OF GENOTYPE, P SOURCES AND RHIZOBIUM INOCULATION ON TOTAL NITROGEN AND PHOSPHORUS UPTAKE IN GRAIN. 88

6.0 CONCLUSIONS 90

SUGGESTIONS/RECOMMENDATIONS 92

REFERENCES 93

APPENDICES 110

LIST OF FIGURES

Fig. 3.1 Location of farm territory and soil sampled……..34

Fig. 3.2 Annual mean rainfall of the study area Bawku west district ……35

LIST OF TABLES

1. **4.1** Some physico-Chemical properties of the topsoil (0-30 cm) of the Tanchera soil series……..44
2. **4.2a** Initial Soil Rhizobia population …….....45
3. **4.2b** Residual Rhizobia population…….....45

Greenhouse Result Tables

4. **4.3** Effect of genotype, rhizobia inoculation and P sources on growth, and grain yield of soybean…….....48
5. **4.4** Interaction effects between P sources and Rhizobium Inoculation on plant growth, and yield of soybean…….49
6. **4.5** Effect of genotype, rhizobia inoculation and P sources on nutrient concentration and uptake of soybean……51
7. **4.6** Interaction effects of the P sources and Rhizobium Inoculation on nutrient concentration and uptakes …….52

Field Result Tables

8. **4.7** Effect of genotype, rhizobia inoculation and P sources on growth, nodulation, yield and yield components of soybean…….55
9. **4.8** Interaction effects between P sources and Inoculation on plant growth, nodulation, yield and yield components ……56
10. **4.9** Effect of genotype, P sources and rhizobia inoculation on nutrient concentration and uptake.....57
11. **4.10** Interaction effects between the P sources and Inoculation on nutrient concentration and uptake in the three soybean genotypes……...59
12. **4.11** Effects of genotypes, P sources and Rhizobium Inoculation on Total nitrogen and Phosphorus content in the grain (Both Experiments) …….....60

CHAPTER ONE

1.0 INTRODUCTION

Soybean (*Glycine max* L) is a leguminous vegetable of the pea family that plays significant role in the provision of food security and livelihood of many rural dwellers in sub-Saharan Africa. It is a key source of high-protein products (Dugje *et al*., 2009) containing approximately 42% to 45% protein. The oil content (20%-25%) is more than any of the commonly known vegetables or legume food sources in the world (Alam *et al*., 2009). Soybean represents a major source of dietary protein in the diets of local farmers in most parts of sub-Saharan Africa and in the world. Apart from the nutritional role played by soybean, it also improves soil fertility through atmospheric nitrogen fixation. It can also improve soil fertility through green manuring. Soybean is presently cultivated worldwide under varying climatic conditions (Appunu *et al*., 2008).

Despite all these benefits, soybean production still faces numerous challenges in Africa at large and Ghana in particular. Some of the major factors responsible for low yields of soybean include: the deficiency or imbalance of nutrients needed by the crop, poor crop establishment, use of poor quality seeds, lack of effective nodulation and inherently low soil fertility levels (Lawson & Quainoo, 2008). Other constraints include lack of agricultural input such as certified seeds, commercial inoculants, phosphorus fertilizers and weak extension services (Rusuke *et al*., 2013).

In Ghana, soybean is mostly cultivated in the three northern regions and the northern part of Volta region. However, the soils of these regions are very low in phosphorus (P) due to iron pan concretion and other macronutrients which are needed for the optimal growth and establishment of the crop. The importance of P has been well documented on soybean

growth, nodulation and yield, and N_2-fixation process by Giller and Cadisch, (1995); O' Hara *et al*., (2002); and Jemo *et al*., (2006). The growth of legume and its N_2-fixation are generally limited by nutrient deficiencies caused by poor supply of available P (Toomsan, *et al*., 1995, Giller, 2001 and Jemo *et al*., 2008). To correct P deficiency and increase its content in soils of the savanna zone, P fertilizers such as superphosphates are often used. However, these P soluble fertilizers are either not readily available for purchase or are expensive and therefore its usage is not attractive to the local farmers. This has caused the farmers in Ghana, to apply little or no mineral P fertilizers to their crops, leading to poor crop establishment and low yields. Meanwhile, there are indigenous phosphate rocks (Togo Phosphate rock, Morocco phosphate rock, and Tilemsi) which are available in large deposits in SSA that could serve as cheaper alternative P fertilizers to improve soil fertility, yield index and enhance Biological Nitrogen Fixation (BNF) of soybean.

Despite the increasing knowledge of the benefits of rhizobium inoculation to soybean production, the smallholder farmers in these northern regions do not inoculate their crop but depend solely on the presence of inefficient, low populated indigenous strains for the nodulation of their leguminous crops. According to Peoples, *et al*., (1995), effective N_2-fixing symbioses is established between legumes and their N_2-fixing bacteria (rhizobia) and this depends on the sufficient number of effective rhizobia in the soil. Inoculation with rhizobia is best performed on soils which contain small and inefficient indigenous rhizobial population (Araujo *et al*., 1994). Fortunately, technology exists which allows efficient rhizobia strains to be incorporated into soil through inoculation and, this technology has been successfully practiced on a large scale for many years in many countries, (Unkovich, *et al*., 2008). Therefore, there is the need to consider the introduction

of an appropriate rhizobium strain inoculants to soybean grown on P deficient soils of the three northern regions of Ghana. Earlier workers have shown the importance of rhizobium inoculation of legumes particularly in areas where it had not been grown before (Bruno *et al*., 2003; Naeem *et al*., 2004, Achakzai, 2007).

Currently there is a rising demand for soybean in Ghana both as a source of feed for livestock, and industries and human consumption. But current world production stands at 136, 653 MT against the demand of 182, 083 MT (USAID, 2015). Soybean yields in Ghana hardly exceed 1.0 MT/ha, although yields could be as high as 2.5 MT/ha (SARI, 2006; Giller *et al*., 2013). One way to achieve improved yield of soybean in northern Ghana is to select the right genotype with drought and heat tolerance characteristics which could attain high stability in yield. A popular soybean genotype TGX1448-2E (Jenguma) released to the smallholder farmers in the northern Ghana is a late maturing genotype (110-115) but has the ability to tolerate and withstand shattering. However, with shortening of the growing season as a result of change in climate conditions in the region, the yield of this genotype has begun to decline. In search for early or medium maturing varieties to replace this late maturing variety currently being cultivated by farmers, two new soybean genotypes namely TGX1904-6F and TGX1955-4F considered to be medium maturing genotypes with 105-110 days and 104-115 days respectively, have been developed for the three northern regions by the International Institute of Tropical Agriculture (IITA) under the N_2 Africa project. Much work has not been done on the performance of these two genotypes as compared with the local genotype on low P soils as found in the northern Guinea savanna zone. The identification of the most efficient genotype capable of utilizing P from sparingly P source and also capable of producing increased yields will represent a

key step in improving the productivity of soybean in the Northern Guinea savanna zone of Ghana.

\The objectives of the study were to investigate the effect of two P sources and inoculation on the growth, nodulation, and yield of three soybean genotypes and determine the most efficient genotype among the three soybean genotypes capable of exploiting P from the two P sources in a typical soil series (Tanchera soil series) of the Northern Guinea savanna zone of Ghana.

CHAPTER TWO

2. 2.0 LITERATURE REVIEW

2.1 Grain legumes

Grain legumes are plants in the family Fabaceae or leguminosae. They are generally grown mainly for their seed, and for human and animal consumption. They also serve as source of green manure. Example of well-known legumes include peas, beans, alfalfa, clover, lupins, lentils, peanuts, soybean and carob. Legumes are notable for their ability to fix nitrogen symbiotically through the help of bacteria in their root structure called root nodules and thereby enhance soil fertility through crop rotation (Mannetje *et al*., 1980, Norman, 1982) and when incorporated into the soil. Therefore legumes have been used in agricultural production since the earliest of civilizations. For example, under good moisture conditions, annual legumes can improve significantly the nitrogen supply for subsequent crops because they are able to fix large quantities of nitrogen. However, the short growth period, small and shallow root system possessed by grain legumes limits the quantity of nitrogen fixed by grain legumes and also the effect they have on the physical conditions of soil.

Legumes are known to use soil available nitrogen rather than to fix atmospheric nitrogen when available N in the soil is high. So the amount of nitrogen fixed by the legume is significantly reduced by high levels of soil available nitrogen. However, soils low in nitrogen will inhibit the growth and seeding of these legumes therefore a low rate of starter nitrogen will improve the growth of legume seedling prior to the establishment and full functioning of nodules.

2.2 Soybean crop

The most economically important and widely cultivated grain legume crop that has accounted for about 30% of processed vegetable oil in the world is soybean (*Glycine max* L.). It is also used to produce bio-diesel fuel in some parts of the world (Graham & Vance, 2003).

Soybean is grown in diverse environment such as in the tropical, subtropical and temperate climates and considered to be among the most important sources of food for men and feed for animals. It is also considered as the single most nature's adaptable crop that supplies a lot of oil and protein in both tropical and temperate environments.

Soybean has served as a main source of food in the eastern Asia and other parts of the world since it has been domesticated in the northeast China in the 11^{th} century B. C (Hymowitz, 1970 and Harold *et al*, 1992). Since then it has become significant forage crop considered the next most valued crop in U.S. that is exceeded only by maize (Anon.., 1999).

In most African countries smallholder farming communities, soybean is considered as new crop but has gradually gained popularity as a result of its increasing demand for livestock and industries (Sanginga *et al*., 2002, Jemo, *et al*, 2007). Soybean was introduced into Ghana in 1910 and was used by local farmers in the northern Ghana (Plahar, 2006).

2.3 Mechanism for Biological Nitrogen Fixation

Biological nitrogen fixation is a process by which nitrogen in the earth's atmosphere is converted into ammonia NH_3 or other molecules available to living organisms. It also occurs naturally in the by means of NO production through lighting. The biological nitrogen fixation is accomplished by metalloenzymes called nitrogenases.

N_2 fixation consists of three major strategies in the terrestrial ecosystems that can be differentiated into free-living, non-symbiotic or associative, and symbiotic N_2 fixation. According to Peoples and Craswell, (1992) Symbiotic Introduction systems is a system that contributes approximate 70% to N_2 fixation while about 30 % from non-symbiotic systems. The contribution derived from free-living diazotrophs to N_2 fixation is very small, since most of these micro-organisms are heterotrophic bacteria, and are being endangered to substrate constraint (Marschner, 1995). The amount of N contributed by terrestrial is about 240–280 t N / year which is far higher than what is obtained from nitrogenous fertilizers (85 t N / year) in the world in 2002 (FAO, 2008).

High energy is consumed during the process of converting N_2 to ammonia performed biologically by rhizobia bacteria. The consumption of ATP, and redox equivalents by explanation redox equivalent refers to any number of chemical species that transfer equivalent of one electron in redox reactions., occurs during the reduction of N_2 to NH_3 and is followed by the formation of H_2 as a byproduct ($N_2 + 8\ H + 8\ e^- + 16\ ATP \rightarrow 2\ NH_3 + H_2 + 16\ ADP + 16\ Pi$). Reactions consisting of dinitrogenase (MoFe protein) and dinitrogenase reductase protein (Fe protein) are catalyzed by enzyme called nitrogenase that really catalyzes the reduction of N_2 during the process. Hydrogen gas formation is always accompanied by the formation of ammonia which is considered as an inefficient process. A number of micro-organisms which have hydrogenases recover the process else energy would be lost.

2.4 Symbiotic N_2 Fixation

Legume-*Rhizobium* symbiosis is a close association involving bacteria of the genus Rhizobium or Bradyrhizobium. This association is very important in that, it results in the fixation and utilization by plants of large amounts of nitrogen. The symbiotic relationship is formed between these bacteria and the target leguminous plant, and they both benefit (Danso, 1985; Giller, 2001). The micro-symbiont infection may arise on the emerging root hairs, at the joint of lateral roots or at the base of the stem depending on the plant species (Howard & Rees, 1996). The association is started by the rhizobia by infecting the root of the legume and forming root nodules anywhere on the root and biological nitrogen fixation occurs via the activity of a bacterial enzyme, known as "Nitrogenase" (Masson-Boivin *et al*., 2009).

The host plant infection involves steps that release phenolic compounds (flavonoids) through the roots, which is an indicator for the rhizobia to stir up chemotaxis and express bacterial nodulation genes (Danso *et al*., 1992). The formation of lectins (*nod*-factors) requires *Nod*-gene induction and the connection of the bacteria on to the root hairs. Other phases involve the rhizobia attacking the plant through infection thread and the growth of the nodule meristem. Cells of rhizobia are then packed into symbiosomes and are converted into bacteroids inside the dividing nodule.

The synthesis of hemoglobin and nitrogenase accompanies the transformation of the bacteroids and other enzymes essential for N_2 fixation. In the process, carbon and energy sources are obtained by bacterial from the plant in order to complete N_2 fixation. These sources of carbon and energy are found in the dicarboxylic acids, such as succinate and

malate forms. Research has found that, in return, ammonium or ammonia are simply supplied to the plant by bacteroids (Rees *et al*. 1996) that circulate across the peri-bacteroid membrane and is then converted into amino acids in the plant cytosol of the nodular tissue (Burris, 1974 and Burns, 1975). There is N exportation from the roots to shoots in the form that depends on the type of nodules. Determinate nodules formed by tropical legumes transfer ureides, such as allantoine or citrulline (Mylona *et al.,* 1993), and the indeterminate ones export amides (*asparagine, glutamine*). Actinomycetes of the genus *Frankia*, form N_2-fixing symbiotic relationship in the root nodules of various non-leguminous woody dicotyledonous plants (e.g. members of the *Elaeagnaceae*, *Rhamnaceae*) (Clawson *et al.,* 1998) and with trees (e.g. of the genera *Alnus* and *Casuarina*) (Nazaret *et al.,* 1991). Other N_2-fixing symbiosis can be achieved through *Anabena* with the fern *Azolla anabaena* or cyanobacteria *Nostoc* that lives on Gunnera plant species (Bergman *et al*., 1992).

2.5 Factors affecting nodulation

It is reported that, grain legumes are capable of fixing nitrogen biologically within the range of 15-210 kg N/ha annually in Africa and thus feature greatly into the cropping systems of subsistence farmers (Dakora & Keya, 1997). Though, there are several environmental and nutritional factors affecting BNF. Several environmental factors determines the formation of effective relationship between legumes and their N_2 fixing bacteria and this relationship may greatly be affected by farm management practices (Peoples *et al.,* 1995). Plant growth and development can be affected by extreme environmental conditions for example nutrient deficiency, mineral toxicity, salinity, extreme temperature conditions, inadequate photosynthates, unfavourable soil pH, low or excessive soil moisture, and disease

conditions. Many researchers have reviewed the effects of those factors on the symbiotic nitrogen fixation in leguminous plants (Sprent, 1976; Vincent, 1980; Haque & Jutzi, 1984).

2.5.1 Soil moisture content

The growth of soil macro and micro-organisms is greatly influenced by soil moisture/water via the processes of mass flow, diffusion, and nutrient content in the soil. Soil texture plays an important role because it determines the amount of water a particular soil can hold within the pore space of the soil; thus soils with large pores and pore spaces hold less water. Hence, soil aggregates with lesser inner pores such as clayey loam, loamy sand etc, are more suitable environments for the growth of rhizobia as well as other soil microbes (Turco & Sadowsky, 1995).

Generally, the growth and establishment of rhizosphere micro-organisms like rhizobia are directly affected by supply of soil water due to decreasing activity beneath the critical tolerance limits. Soil water also indirectly affects plant by causing fluctuation in plant growth, root exudates and then root architecture.

Little or no nodulation of legumes in tropical soils might probably be attributed to low rhizobial population levels in dry seasons. Therefore the effect of soil moisture/water on micro-organisms, plant growth, vigor and nodulation, must not be taken for granted. Rhizobia have diverse ways in which they adapt to osmotic pressure, largely through the accumulation of organic and inorganic solutes in the intracellular. The *R. meliloti* is a typical example of rhizobium that overcomes osmotic stress that inhibits growth through the accumulating compatible solutes such as K^+, trehalose, glycine, betaine, glutamate, dipeptide, N-acetyl glutminyl glutamine amide, proline and the proline betaine (Boscari 2002). Some of these compatible solutes could provide the organisms either with carbon

or nitrogen source for their growth; to avoid degradation throughout osmotic stress their catabolism might be controlled.

2.5.2 Soil Acidity (pH)

Soil acidity has been known to induce nitrogen deficiency in legumes when they solely depend on symbiotic N_2 fixation. Toxicity of aluminium and manganese as well as calcium and phosphorus deficiency in acid soils impedes rhizobium growth and root infection which leads to symbiotic failure (Keyser & Munns, 1979; Negi *et al*., 2006; Bambara & Ndakidemi, 2010). Colonization of rhizobia around the root zone of the legume may decrease by extreme soil pH and thereby reducing amount of nitrogen fixed (van Jaarsveld, 2002; Munns, 1979). It has also been identified that, nodulation and nitrogen fixation by legume rhizobium symbiosis was impaired when grown on acid soils (Munns *et al*., 1981; Anetor & Akinnade, 2006; Mohammadi *et al*., 2012). Some of the characteristics of highly acidic soils (pH < 4) that affects both plant and rhizobia are low calcium, molybdenum, and phosphorous status alongside manganese and aluminum toxicity. Unlike plant growth, nodulation and N_2 fixation are adversely affected in low soil pH conditions. Acid soils are normally weathered and leached and thus deficient in a range of nutrients (Salisbury and Ross, 1982). Phosphorus and molybdenum deficiencies are common in acid soils as they are bound into forms not available for uptake, whilst other nutrients (e.g. zinc, iron) are unavailable when the pH is very high (Salisbury and Ross, 1982).

2.5.3 High soil nitrogen content

The variability in soils in Africa affects nodulation of legumes and their nitrogen fixation owing to poor N level in the soil (People & Herridge, 1990). Soils with high nitrogen

content prevents the ability of the rhizobia to form nodules and fix atmospheric N. This seems to be a way probably to prevent the misuse of photosynthate on assisting a nitrogen–fixing symbiosis once it is unnecessary when there is abundant soil available N (Hirsch, 1996). Several studies have revealed that the presence of N in soil causes reduction in nitrogen fixation in cowpea, groundnut, soybean and common bean (Eaglesham, 1982; Ssali & Keya, 1986). While the application of large amounts of nitrogen fertilizer has been reported to impede N_2 fixation, (Bekere & Hailemariam, 2012) found out that low levels of nutrient stimulate early growth of legumes and increase N_2 fixation. The supply of N from N-fixation to meet plant demands might not be enough at early stages of plant growth that is at the time the symbiosis is emerging. Furthermore, as the crop reaches the pod filling stage, there is high requirement by photosynthetic processes that can easily result in nodule senescence. Nodulation might be small in fields where soybeans had not been previously grown. Most of the essential nutrients for plants growth or bacteria play specific roles in nodulation and N_2-fixation. Deficiencies in these nutrients or other essential nutrients for the growth of bacteria or plants could cause reductions in their population and sizes of nodules formed and in the amount of N_2-fixed (Giller and Wilson, 1991).

2.5.4 Temperature

The Rhizobium legumes symbiosis is greatly affected by temperature. High soil temperature could kill a lot of the bacteria in the surface layers of the soil, even though some rhizobia could withstand periods at 70°C in dry soil (Marshall, 1964). Survival of bacteria in soils at high temperatures appear to be improved by the presence of clay particles and soil organic matter (Giller and Wilson, 1991), but many of the soils where high temperatures are experienced are sandy.

Bacterial infection and their ability to fix N_2 in many legume plants, including soybean could be affected by high root temperatures (Munevar and Wollum, 1982), (Possingham *et al*., 1965). Extreme heat can hinder nodulation, and the process of BNF could be inhibited when nodulation did not take place, even though the root nodules could be insulated from the highest temperatures by the soil (Day *et al*., 1978). The growth and N_2-fixation of legume species is determined widely by optimum temperatures and reflect their environmental adaptation. Critical temperatures necessary for N_2- fixation in various legume are; clover and pea 30°C and peanut, soybean, and cowpea range between 35 and 40°C. Ideal temperature range for nodulation in common beans is between 25 and 30°C but hindered by temperatures between 30 and 33°C of the root (Hernandez-Armenta *et al*., 1989; Pankhurst and Sprent, 1976; Piha and Munns, 1987). Also high temperature (40 ^{o}C) reduces plant nitrogenase activity dramatically which could result in the formation of ineffective nodules (Hungria and Franco, 1993).

However, early growth of plants is inhibited by cool temperature which also leads to late emergence of nodules and hence decreases the amounts of N_2-fixation (Giller and Wilson, 1991). Temperature has also been identified to be a key determining factor of soybean yield. An increase in daytime temperature from 18 ^{o} C to 26 ^{o} C throughout the whole growing period resulted in improved number of seed and yield (Sionit *et al*., 1987). According to Gibson and Mullen (1996) an increase in temperature from 20 or 30^{o} C to 30 or 35^{o} C (day/night) caused a decrease in seed number and size. It was also been forecasted that a 0.8 ^{o} C increase in temperature could result in 2.4% decrease in the yield of soybean in the southern part of USA, the present growing season temperature of 26.7 ^{o} C). However, soybean yield increased with the same temperature rise in the Midwestern USA

approximately by 1.7% (Hatfield *et al*., 2011). Yield of soybean therefore is highly dependent on temperature impacts.

2.5.5 Salinity and Agricultural management practices

Under salinity conditions, accumulation of sodium (Na^+) is known to reduce the physiological growth of plant, its ability to nodulate, and symbiotically fix N (Sousssi *et al*., 1998; Kouas *et al.,* 2010). The initial interaction between the legumes and the rhizobium during nodulation could be directly affected by high salt levels (Singleton and Bohlool, 1984). According to Bordeleau and Prevost, (1994), very high alkaline soils with pH greater than 8 tend to be high sodium (Na^+), chloride (Cl^-), bicarbonate (HCO_3^-) and borate (BO_3^-) that decrease N fixation.

Besides the aforementioned environmental factors, agricultural management practices would significantly influence the amount of N_2 derived from the atmosphere. Management practices such as type of crop variety inoculated, Phosphorus-fertilization, and plant density affect the performance of the plant (Roner and Franke, 2012). Inoculation can only be effective if there exists in the soil the right rhizobia strain which are very effective. In grain legumes, soybean is seen most common to response to inoculation. However, several legume species are greatly specific with indigenous rhizobia and do not nodulate always in Africa (Giller, 2001). In addition, some species are highly specific and well adapted to local environmental conditions. Grain legumes are classified as long duration, indeterminate species that largely fix more N_2 because of the longer time they take to grow than determinate and short-duration types.

In P limiting soil, fertilization with phosphorus enhances the growth and nodulation of the legume plant (Roner and Franke, 2012). Soils with less available N, in intercropping, legumes relie highly on N_2 fixation (Rusinamhodzi *et al*., 2006; Vesterager *et al*., 2008).

2.6 Phosphorus

Phosphorus (P) is a crucial plant nutrient that has spread extensively in nature. Phosphorus, nitrogen (N) and potassium (K) are important elements for plant and animal life. Phosphorus is a limited, nonrenewable global resource, causing its efficient use very important and its deficiency in soils severely restricts crop yields. Phosphorus is an important element in plant bioenergetics. During photosynthesis, phosphorus which is part of ATP, is required for the conversion of light energy to chemical energy (ATP). In phosphorylation process, P is used to alter the activity of some enzymes and also for cell signaling. Phosphorus is important for plant growth and flower/seed formation since many plant biomolecules could use ATP for the biosynthesis.

DNA, RNA, and phospholipids are product made up from Pphosphate esters. P is most common in the form of polyprotic phosphoric acid (H_3PO_4) in soil which is insoluble, but it is taken up most readily in the monovalent and divalent form of $H_2PO_4^-$ and HPO_4^{2-} by plants at nearly neutral pH 6-7 where each represents 50 % of total P. $H_2PO_4^-$ is about hundred percent (100%) of entire P in solution at the pH of 4-6 (Black, 1968). Also at pH of 8, $H_2PO_4^-$ represents only 20 % and HPO_4^{2-} represents 80 % of entire P. Under most environmental conditions it is the limiting element because of its small concentration in soil and high demand by plants and microorganisms. Phosphorus uptake by plants can increase when there is mutualism with mycorrhiza. Phosphorus shortage in plants is

characterized by an intense purple coloration in leaves. Marschner, (1993), has indicated that the amount of P supply at the reproductive growth phase controls the division of photosynthates among the source, leaves and also the reproductive organs, the result being crucial for N-fixing legumes.

Studies have shown that, if a plant is experiencing high phosphorus deficiency during early growth there is general stunting of the plant which tends to show an abnormal discoloration. The plants are generally dark bluish-green in color with leaves and stem becoming purplish. Since phosphorus is a highly mobile nutrient in plants, older leaves will show the first signs of deficiency and it may be translocated from older tissues to actively growing areas (McBride, 1994). Applying a high content of phosphorus fertilizer to perennial crops will be useful and may help with successful root formation. Adequate soil phosphorus (P) is crucial for optimal crop yields. Phosphorus also allows a plant to promote root growth, store and transfer energy, flower and fruit development, and enable early maturity. Conversely, most of it is in insoluble compounds and are not always accessible to plants owing to the physico-chemical characteristics of P in most soils. The P-soluble compounds are immobile, very highly reactive, and low solubility indices. P cycling in soils involves an important processes such as mineralization and immobilization of organic P compounds.

Research has revealed that, 0.2µg/ml P was enough for ultimate growth that is, low P content in the soil solution generally, is sufficient for normal plant growth (FAO, 1984 and Barber 1995). One of the opportunities to improve soil fertility constraints for sustainable agriculture in West African's savanna is to develop soil nutrient management technologies from a sufficient supply and viable share of inorganic and organic fertilizers.

2.6.1 Phosphorus Status in Ghanaian Surface Soils

Generally, the nutrient that limits crop production most in Ghanaian soils is phosphorus (P) particularly in acid soils and the challenge is more severe when the applied P is converted to unavailable forms owing to reactions with crystalline and noncrystalline oxides of Fe and Al (sesquioxides) (Nye 1952; Parfitt, 1979, Mokwunye *et al*., 1986; Warren, 1992; Owusu-Bennoah *et al*., 1997; Abekoe M. K., Sahrawat K. L. and Diatta S. 2001). The average available value, (Bray I) was below 9 mg P kg^{-1} soil in 48 top soils from different ecological zones in Ghana (Acquaye and Oteng, 1972). It has also been reported by Owusu-Bennoah and Acquaye (1989) that, the total P values of four modal forest soils ranges from 54 mg P kg^{-1} soil to 243 mg P kg^{-1} soil. Nyamekye (1987) reported phosphorus status for Tingoli soil series of the Nyankpala Agricultural station as 3.5 mg P kg^{-1} (Bray 1) and Kanabo *et al*., (1978) also reported a value of less than 10 mg P kg^{-1} in some soils of northern Ghana. Similarly, majority of soils studied in Nigeria had available P values ranging from traces to 12 mg P kg^{-1} (Uzu *et al.,* 1975). The low P in Ghanaian soils is attributed to the low content of mineral apatite [$Ca_3 (PO_4)_2$] in the parent rocks and also the extreme period of weathering intensity which the rocks had been exposed to, long-term anthropogenic mismanagement through imbalance between nutrient inputs and exports, and P loss by soil erosion (Nye and Bertheux, 1957). P availability is limited in many tropical soils and can be attributed to severe P fixation or retention, which is particularly strong in soils of low P status (Fairhurst, 1999).

Phosphate ions are chemically unstable in soil solution, and reacts mainly with aluminium (Al-P), iron (Fe-P) and calcium (Ca-P) into insoluble and more stable compounds (Bolland *et al*., 2004 and Mullins, 2009). Sorption influences the ability of most soils to release

phosphorus (P) into soil solution for the uptake by plant. Although P sorption is high in all soils, their ability to maintain P may vary (Idris and Ahmed, 2012, Paini *et al*., 1999 and Mnkeni *et al*., 2008). Also Lilienfein et al, (2004) found out that inorganic phosphate (PO_4^{-3}) was more strongly sorbed than dissolved organic phosphorus. It was suggested by Lilienfein et al 2004, that dissolved organic P was therefore more susceptible to leaching than PO_4^{-3}.

Also Nye and Bertheux (1957), determined that, the total P content of 67 top soils from high-grass savanna zone of Ghana averaged 134 mg P kg^{-1}. However, differences in P content also existed among the soils of the three main agro-ecological zones, namely, Savannah, Forest /Savannah intergrade and the Forest.

2.6.2 Use of Phosphate rocks (PRs) in West Africa

African continent is richly endowed with mineral resources. The US Geological Survey (USGS) has ranked Africa as the leading or second most largest reserve worldwide for mineral resources such as cobalt used to make alloys and batteries, bauxite which is the main source of aluminium, industrial diamonds needed to cut hard steel, vermiculite a component in fireproof material and phosphate rock a key ingredient in fertilizers.

In West Africa, the first investigation of the use of PR for annual maintenance fertilization after initial basal dressings of PR from Senegal was investigated in experiments at Saria and Farako Ba in Burkina Faso. Yields of sorghum, cotton and groundnut were improved with PR addition. However, in the first year of application, TSP was superior to PR. This advantage disappeared during the second year at Saria. The initial response of North

Carolina PR in greenhouse trials using an Alfisol and an Ultisol was conducted in Nigeria, by Juo and Kang (1978).

Under similar pH conditions, both phosphate rocks performed better in the Alfisol than in the Ultisol. This shows how significantly, PR were needed in higher quantities for the Ultisol. Using Togo PR, Mokwunye (1979) revealed that the Togo PR was 63% as effective as Single Super Phosphate (SSP) in the first year of application in an experiment conducted in the Nigerian Savannah environment. Continued applications of PR for two additional years raised the P fertility level and by the third year, the mean yield of the phosphate rock plots was 96% as effective as that of the SSP treatments which were also reapplied.

In Ghana for instance, Togo PR and 50% partially acidulated Togo phosphate rock (PAPR-50) were investigated by Owusu-Bennoah and Acquaye (1996) on their initial and residual response in greenhouse trials in a concretionary soil (Alfisol) in which maize was used for the test. The results produced from the experiment showed that the relative agronomic effectiveness (as compared with SSP) of PAPR - 50 was 58% while that of Togo PR was only 23% in increasing growth of the crop. The residual effect of either PAPR-50 or PR was found to be insignificant when compared with SSP on biomass yield and nutrient uptake and this showed that the apatitic P was not very effective relative to SSP in the soils used. The experimental results corresponds with the work done by (Bruce and Marquette, 1977; IFDC, 1985); in Togo with maize on Alfisols which also confirmed the finding of (Mokwunye and Pinto-Toyi, 1991) that superphosphate and the phosphate rock acidulated to 50%, gave higher yields than finely ground untreated Togo phosphate rock; that the former are similar in effectiveness. Several experimental results that compared some of the

West Africa phosphate rocks Togo PR; Kodjari, (Burkina Faso); Tilemsi (Mali) and Tahoua (Niger,) were also summarized by Truong *et al.* (1978). Their conclusion was that Tahoua and Tilemsi only can be directly applied. These results were confirmed by experiments conducted at three sites in Mali by Thibout *et al.*, (1980), in which Tilemsi PR and Taiba PR from Senegal were compared. The conclusion was that Tilemsi PR was reactive enough to be used for direct application. In Gambia, at Sapu Tilemsi PR was also evaluated in trials using maize and groundnut. Though that year (1984) was a very dry year, the finely ground Tilemsi PR showed 74 and 92% as efficient as SSP for maize and groundnut correspondingly. Gikonyo *et al*., 2010, reported that more P leached from TSP than GPR treatments when triple superphosphate (TSP) was compared to Gafsa phosphate rock (GPR).

Phosphate rock has been identified as the core source of phosphorous which is crucial to the life of every living thing. A common term that refer to rock containing high concentration of phosphate minerals is phosphate rock. PR is mainly mined to produce chemical fertilizers for agriculture purposes. Phosphorite is a marine sedimentary deposit which is the most common source of phosphate rock. A different source of PR is guano, a buildup of bird or bat excrement. The apatite group, $Ca_5(FCl,OH)(PO_4)_3$, is classified as the commonest phosphate minerals with the key minerals being collophane, dahlite and francolite.

2.6.3 Indigenous Phosphate Deposits in some places of the world

Deposits of phosphate rock occur in several parts of the world especially in Asia, Africa and Latin America (Menon *et al.*, 1991).

Country	Phosphate Rock (PR)
Bolivia	Capinota
Brazil	Araxa, Patos de Mina, Tapira.
Burundi	Matongo
Canada	Martison
Chile	Bahia Inglesa
China	Kaiyang
Colombia	Pesca, Huila, Media Luna, Sardinata
Indian	Mussories
Israel	Arad
Jordon	El-Hassa
Mexico	Baja California
Peru	Bayovar PR, Sechura
South Africa	Phalaborwa
Tunisia	Gasfa
Uganda	Sukulu Hills
USA	North Carolina, Tennessee, Central Florida
Zambia	Chilembure
Zimbabwe	Dorowa

Source: Chien and Menon (1993b).

2.6.4 Phosphate rock deposits in Africa

Country	% P_2O_5	Phosphate Rocks (PR)
Burkina Faso	25 - 27	Kodjari
Mali	29.5	Tilemsi
Morocco	30	Morocco
Niger	32	Tahoua
Senegal	35 - 38	Taiba, Thies
Togo	35 - 38	Hahotoe & Kpogame

Source: Johnson (1995).

2.6.5 Morocco Phosphate rock

In Morocco for instance, phosphate rock is more important compared to other minerals and accounted for the majority of the country's mining output value. It is revealed that, Morocco sits on about one-half of the phosphate reserves in the world (Minigreveiw.com, 2011) and produces 14% of the world's output of phosphate rock (Jasinki 2012, Miller 2012a). Morocco was ranked as the world's third producer of phosphates after China and the United States (Arab Greek Chamber, 2011). Phosphates is one of the major resources of the Moroccan economy. It however, has about 26.65 – 32.05% P_2O_5, 44.02 – 52.01% CaO, 2.24 % MgO and 8.29% CO_2. Its solubility was tested in conventional reagents in percent of total P2O5 as ranges from 16.7- 26.0% in Neutral Ammonium Citrate, 29.9-38.4% in Citric acid and 56.5- 60.4% in Formic acid (Chien, 1977)

2.7 Factors Affecting PR Dissolution in Soil

The efficacy of PR-resources differs and is usually affected in several ways by factors such as PR properties, soil characteristics, plant effects and crop species, effect of climate and management practices that determine the dissolution of PR materials in soils (Khasawneh and Doll, 1978; Bolan *et al.,* 1990). The effectiveness of PR determines the degree to which the uptake rate of P needed for plant growth is conserved by the rate of dissolution of the PR (Sale and Mokwunye, 1993). This review focused on each of these factors.

2.7.1 Influence of phosphate rock properties

Solubility and chemical reactivity are the most important properties of PR that are measured conventionally using 2% citric acid or 2% formic acid and 1N neutral ammonium citrate, (Chien and Hammond, 1978; Chien, 1993) that is relative to its agronomic effectiveness. These however, depends on the mineralogical and chemical composition, effect of apatite crystallinity, effect of free carbonate (calcite, dolomite) and cementing of apatite with silica and gypsum in the mixture of the PR and effect of monocalcium phosphate (Chien, 1993). Generally, the higher the calcium content of the phosphate rocks in the crystal structure, the higher the solubility and therefore suitability for direct application.

Hence the extent to which PR releases phosphorus (P) when it is applied to the soils is controlled by the level of isomorphic substitution of carbonate for phosphate inside the lattice of the apatite in the rock (McClellan and Van Kanwenberg, 1992, Smith and Lehr, 1966). The most abundant mineral in phosphate rocks is calcium carbonate. The dissolution of calcium increases its pH and concentration on the phosphate mineral surface because

the solubility of calcium carbonate is higher than other chemically reactive phosphate minerals (Silverman *et al*., 1952). Therefore, if there exists a significant amount of free carbonates, it can however minimize the solubility of the apatite (Anderson *et al*., 1985; Robinson *et al*., 1992). This contributes to the fact that apatite are less soluble than free carbonates more resulting to calcium common-ion effect which reduces the apatite solubility.

Work done by Chien and Hammond, (1978), on seven PRs, in which chemical extractions was used to measure the degree of PRs reactivity and then calculated by X-ray diffraction (absolute citrate solubility), revealed that, in neutral ammonium citrate, the solubility of P remained fairly constant that is with the first and second extractions for each of the PRs except Huila PR from Colombia. However, in the second extraction, the citrate-soluble P of this PR improved significantly from 0.4% to 1.5%. The Huila PR has about 10% $CaCO_3$ which in the first extraction improved from 1.4% to 4.4% in the second extraction.

Chien, 1993, found that, the intermixed (cemented) of silica and apatite has reduced the chemical solubility of PR significantly. Thus using the absolute citrate solubility (X-ray diffraction method) Pesca PR from Colombia for instance is considered to be highly soluble than Tennessee PR from USA. Nevertheless, the chemical solubilities of Pesca PR from Colombia when measured were found to be smaller than that of Tennessee PR. Also the apatite in Pesca PR, as scanning electron microscope revealed is intermixed with siliceous minerals in such a manner that makes the apatite less available to be attacked by the chemical solution or by the soil solution (Chien *et al*., 1978). However, with Tapira PR from Brazil the citrate solubility was only 1.9%, whereas that of Lumphum PR was 7.2%.

It was contended that, the dissolution process of PR is a reaction that takes place on the surface of the PR particles, hence grinding would improve upon the exposed surface area of the PR to the soils, reduce the particle size and consequently enhances the degree with which P is unconfined from the PR (Barrow, 1990). Barrow (1990), however, cautioned that grinding PR finely into powder does not substitute for its reactivity. That is by reducing particles of PR to a size below 100 mesh (150 μm) usually by grinding is not necessary because finer particles of PR would not greatly improve its efficiency (Khasawneh and Doll, 1978; Rogers *et al*., 1953).

The PR solubility has been reported to relate effectively with crop response (Engelsted *et al*., 1974; Chien and Van Kauwenberg, 1992), Chien *et al*., (1990b) has also noted that, it was hard to relate crop growth to water soluble P fertilizers and PRs due to the interactions between fertilizer and soil. Chien *et al*., (1990b), have therefore indicated that, the relative agronomic effectiveness (RAE) of P fertilizers would be difficult to evaluate based on their solubility.

2.7.2 Soil Properties

One of the qualifications of PR to be effective agronomically is that, the PR has to dissolve and also be accessible to plants. Soils low in pH (less than 5.5), low P fertility levels, low solution concentration of Ca ions and high organic-matter are some of the soil properties that can cause the dissolution of PR for direct application (Chien and Menon, 1993a). The best indicator of the agronomic performance of PR is its solubility that is usually measured with 2% citric acid, or 2% formic acid and neutral ammonium citrate in the laboratory. The solubility of PR shows the mineralogical and chemical characteristics of the exact P

minerals. The main mineral in most PR sources is apatite, which however differs extensively in physical, crystallographic, and chemical properties. The solubility of PR mostly rises with smaller particle size. Nonetheless, the agronomic efficiency of ground and unground extremely reactive PR sources does not directly follow the solubility pattern. For instance, comparing unground reactive PR (-35 mesh; 0.5 mm) to ground PR (-100 mesh; 0.15 mm), the solubility of the unground is less than that of the former but their agronomic effectiveness may be highly comparable under field and greenhouse conditions (Chien and Friesen, 1992). Therefore it is not enough to relate the solubility and the agronomic effectiveness of various PR sources only based on their particle-size distribution (Smalberger *et al*., 2006).

2.7.3 Soil acidity

Among the soil properties, pH is another property which can impact the agronomic effectiveness of PR greatly. According to Chien, (2003), comparing the relative agronomic effectiveness (RAE) of a highly reactive Gafsa PR (Tunisia) to TSP was 100%, and increases when soil pH is low in 15 soils with different properties. Though, 56% of variability of RAE in this study was only explained by soil pH alone. It is also possible to explain 74% of variability of RAE by considering the clay content that is related to soil pH buffering capacity and cation ion exchange capacity (Chien, 2003). The agronomic effectiveness of PR would decrease sharply as soil pH rises above 5.5 since pH is a measure of acidity. Thus, the dissolution of PR or agronomic value decreases above this pH except with an effective crop species (Bolan and Hedley, 1990).

Also this carbonate apatite dissolution is driving by pH, which is the neutralization reaction between soil hydrogen-ions and the PO_4 -ions, CO_3-ions, F-ions and OH-ions that results

from dissolution, that are neutralized to H_2PO_4-ions, H_2CO_3 , HF and H_2O respectively (Chien, 1993). Thermodynamic thoughts show that these reactions happen naturally as the apatite dissolves (Chien, 1977). Various laboratory and field observations ascertain that low soil pH (Bolan, 1989, Kanabo and Gilkes, 1987)), with a high level of soil acid generation and also a high level of buffer capacity (Apthorp *et al*., 1987) all contribute to enhance PR dissolution.

Reports have shown that, the relative efficacy of non-granulated North Carolina PR was four times higher in a more acidic soil (pH 4.2) than for a high pH (6.2) soil (Vijay Kumor *et al*., 1993). An experimental trials using one soil sample adjusted to several pH levels show that the phosphate rock dissolution has linearly increased with declining pH (Kanabo and Gilkes, 1987). In a study to show the effect of soil pH on the efficacy of different PRs compared to triple super phosphate (TSP.), Engelstad *et al*., (1974) reported that soil pH has slight influence on rice response to TSP; nevertheless, the efficiency of the PRs highly dependent on soil pH.

2.7.4 Cation exchange capacity, and exchangeable calcium and magnesium

As PR dissolution continuous, it is essential that, some key products of the reaction, such as Ca ions, be eliminated or that would be favoured by soil conditions that maintain low concentrations of Ca and P in the soil solution according to the mass action law (Barnes and Kamprath, 1975).

Soils with high content of exchangeable Ca can therefore delay the dissolution of PR. Most tropical acid soils have relatively low exchangeable Ca hence provide favourable conditions for PR application (Chien and Menon, 1993a). Cation exchange capacity (CEC) that is closely correlated to soil texture and soil Ca is another soil property which influences

PR dissolution. Khasawneh and Doll (1978). In a trial to assess the impact of leaching on the PR materials dissolution, Hanafi and Syers (1993) reported that, the highest and the lowest dissolution happened in a sandy clay loam and clay soils, respectively. It was concluded that the greater permeability of the sandy clay loam than the clayey soil resulted in constant leaching of the product of PR dissolution, Ca^{2+} and H_2PO_4, from the site of reaction. This maintained sinks for Ca^{2+} and H_2PO_4 and thereby promoted dissolution. Kanabo and Gilkes (1988) suggested that sandy soils containing low CEC does not give a sink for Ca-ions released from PR and thus, slow down the dissolution of the PR, and this may end in a decrease in agronomic effectiveness.

Also soils with high exchangeable magnesium (Mg) can improve PR dissolution (Perrott, 2003). Mg is held more strongly by soils than Ca, so the adsorption of Ca released on dissolution of PR can be blocked once Mg is present at the exchange sites of the soil and thus enables its elimination from the fertilizer system of the soil. This will be better dissolution effect on PR. The exchangeable Ca and Mg will always be small in soils with low pH <5.5 that is, (low base saturation) and the solution concentrations of these ions will be low.

2.7.5 Effect of Soil Organic Matter

Soil organic matter is another property known to greatly enhance the dissolution of PR (Johnson, 1954b, Drake, 1964, Chien *et al*., 1990b). Upon hydrolysis some types of soil organic matter, may supply some anions such as citrate or organic functional groups and oxalate that can hold Ca^{2+} ions and thence lower the activity of Ca^{2+} in soil solution. Also fractions of organic matter (fulvic and humic) form complexes with Ca^{2+} which can decrease Ca^{2+} concentration in soil solution, therefore leading to improved PR dissolution

(Schnitzer and Skinner yr). This in turn provides a driving force for further dissolution of PR. Chien, (2003) reported the positive effect of soil organic matter on increasing the agronomic effectiveness of PR.

Formation of a chemical complex between soil organic matter and Ca^{2+} ions enhanced PR dissolution is suggested to be the mechanism. The mechanism may also explain the beneficial effect of farmyard manures on increasing P availability from PR (Guzman *et al*., 1980; Villarroel and Augstburger, 1984).

2.7.6 Climate conditions

Dry soils will not dissolve PRs therefore rainfall is important for PR dissolution (Sale, 1990). Increased rainfall or irrigation increases PR dissolution by increasing soil water (Weil *et al*., 1994). Also, as soil moisture content is decreased, PR-dissolution declines and this was confirmed in incubation studies (Gregg *et al.*, 1978). Research conducted by Bambey, (1957) in Senegal showed that the fertilizer uptake efficiency of PR increased with increasing rainfall. The dissolution of PR improve plant development and P uptake as a result of adequate water supply. In a series of trials over a range of annual rainfall between 500 and 1300 mm, to ascertain the yield increases of groundnuts over control showed a highly significant linear correlation with the mean annual rainfall for the first two (2) years following basal fertilization.

Nonetheless, the rainfall requirement depends on soil properties (Bolan, 1997; Sale *et al*., 1997b). PR dissolution is promoted by high leaching indices which facilitate the elimination of the products of dissolution away from the surface of the PR particles.

PR dissolution in soil has been found not to be affected significantly by temperature. Temperature within a range of 5-35^{o} C has no influence on the solubility of PRs as reported

by Chien *et al.*, (1980b) and hence, on its agronomic effectiveness. The implication is that temperature has less effect on the available P released from PRs in the tropical soils as compared with P released from straight fertilizers (Hammond *et al.,* 1986). In relation to soluble P forms, this could tend to enhance the effectiveness of PRs in warm environments. Such result would depend on the degree to which P sorption reactions were effective in the soils as the rate of P sorption by soil is not markedly affected by temperature (Barrow, 1983).

2.7.7 Crop type

The effectiveness of PR as a nutrient source differs with the crop species. Generally, the Relative Agronomic Efficiency (RAE) of PRs with respect to water-soluble P sources (such as SSP and TSP) would be higher for long-term or perennial crops (example tea, oil palms, rubber pastures, fruits, perennial pasture) than for shorter-term or annual food crops (example groundnut, maize, rice, millet) (Chien *et al.,* 1990b). In Asia phosphate rock has been used widely for many tree crops, including oil palm, tea and rubber. The differences among crop species to use PR depends on the plant rhizosphere acidification (Van Ray and Van Diest, 1979; Bekele *et al.* 1993).

The results of using six different plant species such as maize, wheat, buckwheat, soybean, molasses grass, and paspalum grass have revealed that, Gafsa PR (Tunisia) was comparable to TSP for buckwheat, which produced much lower rhizosphere pH than the other plant types (Van Ray and Van Diest 1979).

Mostly rape (canola) is identified to be efficient in utilizing PR among the crop species. Root exudation of organic acids is known to contribute to PR dissolution. Legume on the other hand, are particularly suitable for utilizing PRs. Legumes are known to effectively

cause the dissolution of PR and they absorb the dissolution products because of their demand for Ca and the acidifying effect of nitrogen (N) fixation in the soil near the root rhizosphere system (Ankomah *et al*., 1995; Kamh *et al*., 1999).

Phosphorus from the PR at a faster rate by plant which have greater density of root in the soil surface layers where PR is easily located since there would be a greater possibility of the root encountering localized high concentration of soluble P adjacent to the PR. Chien *et al*., (2003) later observed that the RAE of nine PR sources improved from 0% to 88% for rape grown on an alkaline soil (pH 7.8) as the 2% citric acid solubility of PR increased from 2.1% to 13.1% P_2O_5.

2.7.8 Management practices

The management practices that highly affect the agronomic effectiveness of PRs are: time of application, rate of application, placement of the PR material and lime application (Chien and Menon, 1995).

The most effective way to achieve PR easily released into soil solution is to broadcast it onto the soil, followed by uniformly incorporation into the soil surface. This practice allows greater chances of PR to react well with soil and therefore leads to the reduction of interaction between PR particles. However, band application of PR (Khasawneh and Doll, 1978; Sale and Mokwunye 1993; Chien and Menon, 1995) and granulation of fine particles (Chien, 2003) is less effective and not recommended since it restricts the interaction of PR particles with the soil, causing dissolution to reduce.

Another important management practice that influences PR dissolution is the time of application. Research has demonstrated that, the low effectiveness of PR was enhanced when applied directly on acid soils earlier before planting (Hammond *et al*., 1986b).

Also agronomic effectiveness of PR was enhanced when PR was applied in advance of flooding. However, the effectiveness would seriously reduce when applied at flooding or after flooding. The expectation was that, when PR is applied early, it would allow some time dissolution to occur. Too early application of PR to high P –sorbing soils, the agronomic effectiveness would be reduced (Hammond *et al*., 1986b).

Addition of lime to soil is practiced commonly to correct soil acidity by raising the soil pH and decreasing Al toxicity. However, when pH is raised above neutrality by introducing additional Ca from the liming could be unfavorable to PR dissolution. Liming practices should therefore balance the need to improve the Al toxicity with reducing PR dissolution (Chien and Friesen, 1992). It is however, suggested that liming to raise soil pH be limited to a range of pH 5.2 to 5.5 in order to improve the agronomic effectiveness of PR.

The application of rock phosphate can be effective in increasing crop production. The rock phosphate enhances the replenishment of N through biological fixation, and also in the maintenance or improving soil chemical properties and soil fertility (Sahrawat *et al.,* 2001).

From the review it can be concluded that works have been done showing how Phosphorus as well as rhizobium inoculants are essentially needed in legume production to establish growth and increase yield of soybean legume. However, the soils in the tropical regions have very low levels of macronutrient especially P which is needed most as source of energy ATP during photosynthesis.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Introduction

In this chapter the basic analytical techniques used, the greenhouse and the field experiments were described.

3.1.1 Site Characteristics

3.1.2 The study area

The experiment was carried out at two different places, in the field and greenhouse using the same soil. The field experiment was carried out at Tilli (Bawku West District) (Fig 3.1) in the Northern Guinea savannah zone of Ghana during the 2015 farming season and the greenhouse study was conducted in the School of Agriculture, University of Ghana. The field is located within Latitude 10º 53'78 N and Longitude 000º 33'49 W with elevation of 193 m above sea level. The area receives an annual rainfall ranging between 800 and 1100 mm, considered enough for a single farming season. The rainy season starts from May to September with peak in August/September and a long dry season from October to April. The annual rainfall during the year 2015 of the study is shown in Fig 3.2. Temperatures are usually high, averaging 34^0 C. The maximum temperature could rise as high as 42^0 C and the minimum as low as 16^0 C. The low temperatures are experienced from December to late February. The soil used for both experiments is Tanchera series classified by Asiamah and Adjei-agyapong (2001) as Ferric Lixisol.

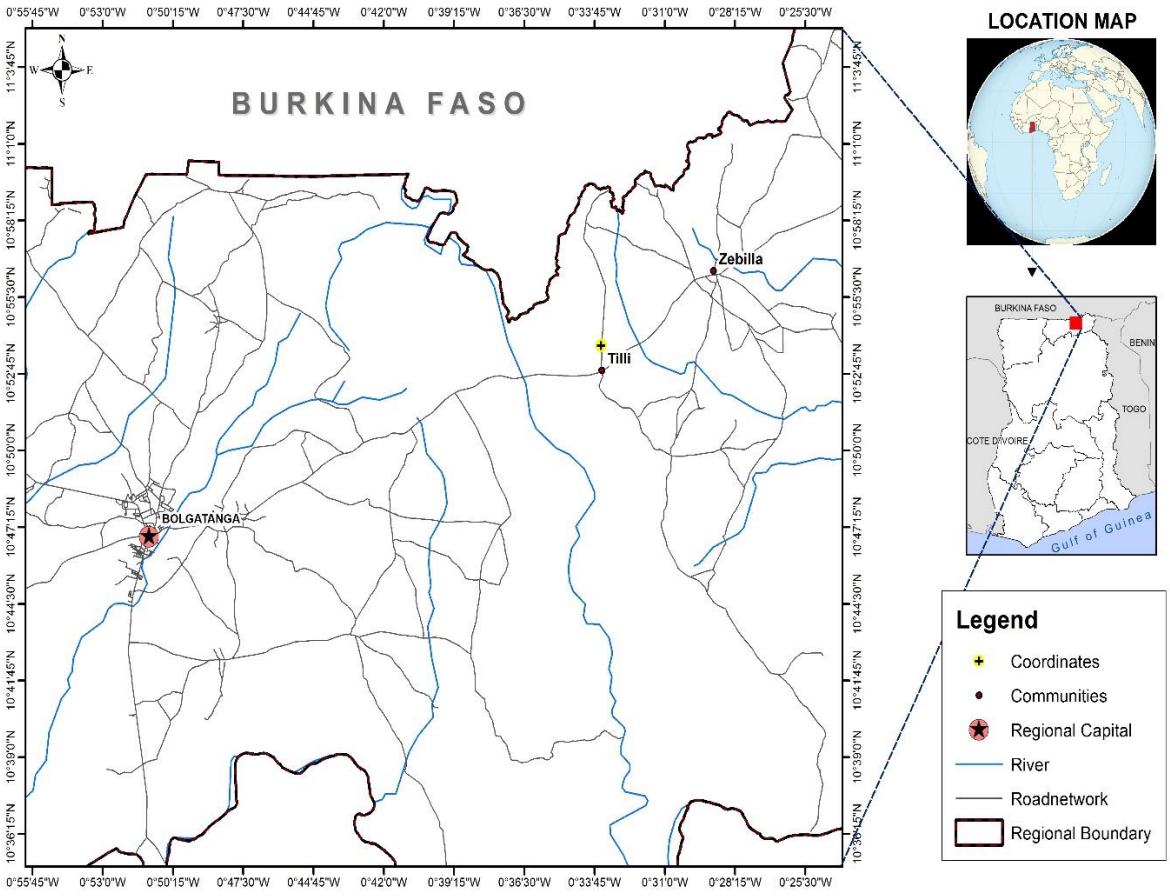
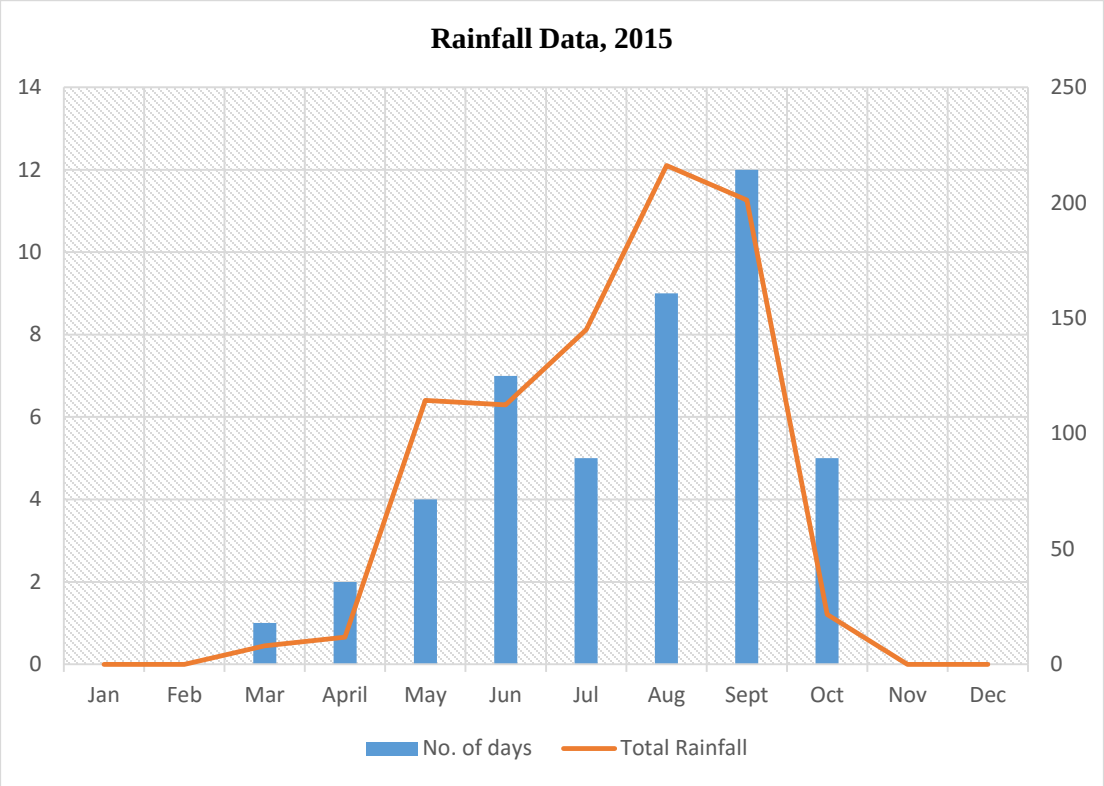


Fig. 3. 1. Location of farm territory and soil sampled

Fig. 3. 2. Annual mean rainfall of the study area Bawku West District.



3.1.3 Soil Sampling

Soil sampling was carried out at the start of the experiment at 0-30 cm depth using an auger for initial soil characterization. A composite sample was made from 20 samples collected randomly from different parts of the plot thoroughly mixed, sub-sampled, air-dried, crushed, and sieved through a 2 mm sieve to remove twigs, plant roots and ironstone. The sieved soil samples were brought to the laboratory and stored for physico-chemical analyses and greenhouse studies. Fresh soil was also taken before planting and after harvest for the estimation of both initial and residual rhizobia population using the Most Probable Number (MPN) method (Vincent, 1970).

3.1.4 Field Preparation and Experimental Layout

The experimental plot was previously cultivated to maize. The plot was ploughed and harrowed with hoe after which the layout was done. The experiment was conducted in a split-split-plot design. The main plots were made up of three Soybean genotypes while sub-plots were the two P sources (Triple Super Phosphate (46% P_2O_5), Morocco Rock Phosphate (MPR, 30% P_2O_5) and no P control and sub-sub-plots were with or without rhizobium inoculation. There were four replications giving a total of (3 sources of P fertilizers applied at 30 kg P/ha and 2 rates of inoculation (6 treatments) x 3 Varieties x 4 Reps) 72 experimental units. The soybean genotypes used were TGX 1904-6F (104 -114 days maturity period), and TGX 1955-4F (105-110 days maturity period) developed by IITA, Ibadan and Jenguma (110-115 days maturity period) the local genotype developed by the Savanna Agricultural Research Institute and usually cultivated by the farmers in the northern regions of Ghana. Thus all the three genotypes are medium maturing genotypes.

The main-plot size was 5 m × 5 m with an alley of 2 m between main plots and 1 m between sub-plots. The sub-plot consisted of 10 rows each 5 m long with 50 cm spacing between the rows and 10 cm spacing within a row at 3 seeds per stand which was later thinned to 2 seedlings per stand three weeks after planting (WAP). All the fertilizer inputs were applied at planting in furrows 10 cm away from the planting line. In addition, the soybean genotypes were either inoculated with or without rhizobium inoculant (Nodumax). The inoculant (Nodumax) contained 10^{10} cells g^{-1} of *Bradyrhizobium japonicum* strain USDA 110 developed at the International Institute of Tropical Agriculture, Ibadan, Nigeria.

The soybean seeds were moistened with Gum Arabica solution in a basin and the inoculant was added at the rate of 7g per kg of seeds. The mixture was stirred thoroughly and

uniformly with wooden spatula until even coating was attained. The seeds were then spread on a sack under a shade and allowed to air dry for 30 min to enable the inoculant to stick well enough onto the surface of the seeds before planting. The treated seeds were sown early in the morning to avoid its exposure to direct sun rays that might affect the quality of the inoculant. With these treatments the uninoculated seeds were sown before the inoculated ones but on same day to avoid contamination. Weed control was done twice manually using hoe. All cultural practices recommended for growing soybean were applied equally to all plots. The crops were grown for a maximum of 110 days.

3.2 Greenhouse Experiment

The experimental design for the greenhouse was completely randomized design (CRD) involving the same soil series used for the field experiment. The same treatment combinations of soybean genotypes, rhizobium inoculation rate and the two P sources, TSP (46% P_2O_5) and Morocco Phosphate Rock (MPR, 30% P_2O_5) at the application rate of 30 kg P/ha which is equivalent to 258 mg P (MPR) and (TSP) 168 mg P/2 .5 kg soil and a control treatment. The experiment was replicated four times, giving a total of (18 treatments x 4 Reps) 72 pots. Equal amount (2.5 kg) of the soil was weighed carefully into plastic pots and the P fertilizers (Morocco rock phosphate and Triple super phosphate) were added at the rate of 258 mg P and 168 mg P/ 2.5 kg soil respectively. To ensure uniform addition of the P sources to the pots, the soil in each pot was transferred into a large plastic

basin and the weighed amount of the P source was added, thoroughly mixed and returned to the respective pots.

Three seeds of each soybean genotype were sown in each pot and distilled water added to bring the soils to 60% field capacity. The pots were arranged on greenhouse benches in randomized complete design (RCD). To minimize uneven environmental effects within the greenhouse the pots were rotated weekly. The plants were thinned to two per pot, three days after germination to ensure adequacy of nutrients other than P. The plants were sprayed against white flies and leave miners two weeks after germination. The plants were grown for 110 days. The plants were immediately weighed after cutting them at the soil surface for fresh weight. The plants were dried in an oven at 70° C to a constant weight. The dried matter were then ground to pass through a 1 mm sieve and later stored in small plastic bags for subsequent P and N analyses.

3.3 Field and Greenhouse Data collection

3.1 Phenological measurements and Shoot Biomass

The phenological changes of the soybean crops were determined. These changes included plant height at 10 WAP and nodulation capacities. Ten plants were randomly selected from each plot as representative plants for their nodulation capacities and height at 10 WAP; the plant height was measured from the ground level to the apex of the plant using graduated measuring pole both in the greenhouse and field and the average was calculated for each plot/pot. The root zone of the representative plants were gently cut and washed on 2 mm mesh sieve under tap water. The nodules removed were weighed freshly, counted and oven-dried at 65 ^{o}C for 48 h to determine their dry weights. Data on nodulation were not taken

for the greenhouse experiment due to limited replication of pots. However, all other data taken for the field study were taken for the greenhouse study.

3.3.2 Yield Component

Harvesting was done 53 days after planting (DAP) when all the plants have attained physiological maturity. Physiological maturity was considered to have taken place when 95% of the plants had turned golden yellow (Tukamuhabawa, *et al*., 2002) and 75% of the plants had their pods filled with seeds and hardened (Masumba, 1984). At the late pod filling stage, the plants within an area of 4 m × 3 m = 12 m^2 (6 rows of 4 m length) were harvested from the 25 m^2 plot leaving 50 cm from the outer ends of the plots. The plants were cut using a sharp knife at about 5 cm above the soil level.

Ten plants were randomly picked after harvest from the sampled plants to determine their podding capacities. All the pods were removed from these 10 plants, counted and the average number of pods per plot was calculated. Shoot biomass from each plot was bulked and weighed with a digital scale after which the pods were removed, shelled, and weight of fresh and dry seeds were recorded. . The fresh weight of the seeds were subtracted from initial weight (whole plant with pods) to obtain the actual weight of shoot biomass/plot. The dry weight was used to estimate grain yield and harvest Index. Seed yield per hectare was determined by threshing the harvested plants from the harvested area (4 m x 3 m) central twelve square meter of each plot. The resulting weights, in kilograms per harvest area, were then extrapolated to kilogram per hectare basis to obtain the average seed yield per hectare.

For the greenhouse, at harvest all the whole plants with pods per pot were weighed after which the pods from the plants were detached, counted, shelled and weighed. The fresh

weight of the seeds were subtracted from initial weight (whole plant with pods) to obtain the actual weight of shoot biomass/pot. The shoot biomass for both experiments were then oven-dried at 80^{o}C for 48 h to a constant weight and their weights were also recorded after which they were ground into powder for plant analysis.

3.4 Chemical Analysis of Plant Material

3.4.1 Digestion of Plant Material

A weight of 0.1 gram of milled plant sample was taken into a 25 mL conical flask and 5 mL concentrated sulphuric acid (H_2SO_4) was added. The flask was spun intermittently for easy contact between the acid and the sample. The mixture was then allowed to dissolve overnight. Then each solution was heated for 1 h and Hydrogen peroxide (H_2O_2) was then added at drop wise until the solution became clear. The solutions were again allowed to cool and settle overnight after 50 mL distilled water was added and after which they were poured into a 100 mL flask. Aliquots of the extract were taken for nitrogen and phosphorus determinations.

3.4.2 Determination of Total Nitrogen in Plant Material

A volume of 5 mL aliquot from section 3.4.1 was poured into a Markhan distillation apparatus. Then to the aliquot 5 mL of 40% NaOH solution was added and the mixture distilled into 5 mL of 2% boric acid. A mixed indicator containing methyl red and methylene blue was dropped three times to the distillate in a 50 mL Erlenmeyer flask and then titrated against 0.01 M HCl acid solution (Bremner, 1965). The percentage nitrogen was calculated as:

$$\%N = \frac{Titre \times Molarity\ of\ HCl \times Vol.\ of\ extract \times 0.014}{Vol.\ of\ aliquot \times Weight\ of\ plant\ sample} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where 0.014 = miliequivalent weight of nitrogen

The plant N content (%N) and the shoot biomass were used to calculate the shoot total N (mg/plant) as:

Shoot Total N (mg/plant) = shoot dry weight (*g/plant*) $\times \frac{Shoot\,\%\,N}{100} \times 1000$ ………… (2)

3.4.3 Determination of Total Plant Phosphorus

An aliquot (5 mL) of the digest described in section 3.4.1 was taken into a 50 mL volumetric flask in four replicates. P-nitrophenol indicator was used to adjust the pH and it was neutralized with some few drops of 4 M NH_4OH up to the time the solution turned yellow colour. Eight (8 mL) of reagent B (prepared by dissolving 1.056 g of ascorbic acid in 200 mL of reagent A (12 g of ammonium molybdate + 0.2998 g of antimony potassium tartrate dissolved in 250 mL of distilled water). The dissolved reagents were added to 1000 mL of 2.5 M H_2SO_4 (148 mL conc. H_2SO_4), thoroughly mixed and top up to the 2000 mL (Watanabe & Olsen, 1965). The solutions were carefully shaken and allowed to stand for 15 min for the colour to develop. Depending on the concentration of phosphorus available in the soil the colour changed from yellow to blue in different shades. Distilled water and 8 mL of reagent B was used to prepare a blank solution. A 25 mg/L standard P solution was used to calibrate the spectrophotometer prepared in the same method as described. The blue colour (intensity) was measured using the Philips PU 8620 spectrophotometer at a wavelength of 712 nm. The P concentration in the plant tissue was calculated as:

% P = $\frac{R \times Vol.of\ extract}{Vol.\ of\ \ aliquot \times Weight\ of\ soil \times 10} \times 100$ ……………………………………… (3)

Where R is the spectrophotometer reading in mg/L

The dry matter yield (shoot biomass) and the shoot P content (%P) was used to calculate the shoot total P (P uptake) as:

Shoot Total P (mg/plant) = shoot dry weight (g) $\times \frac{shoot\ \%P}{100} \times 1000$ …… (4)

3.5.0 Data Analysis

Data collected from the two experiments at 10WAP for plant height, nodule number, nodule dry weight, and at harvest for pod number and weight per ten plants, plant biomass production, and grain weight were subjected to analysis of variance (ANOVA) using GENSTAT software version 9 at P 0.05 and the means were separated using Duncan test.

3.5.1 **Laboratory analyses of soil samples**

3.5. 2 Physical analyses

3.5.2.1 Soil Bulk Density

The bulk density determination of the soil was carried out using the core method by Blake (1965). After clearing the vegetation cover, core samples were taken randomly at the study site using a cylindrical metal core samplers ($5\ cm\ in\ diameter\ \times 5\ cm\ in\ height$). This was done by driving the core sampler into the soil deep enough to fill the volume of the core. It was then removed and trimmed carefully at both ends. The core samples of the soil were later transferred into moisture cans of a known weight (W_1) and oven dried at 105 ^{o}C for 48 h. The oven-dried weights of the soil and the moisture were noted as (W_2), after which the bulk density of the soil was calculated as mass of dried soil per the volume of soil. It was presumed that the volume of soil sample taken was the volume of the core samples and calculated as: $V = (cm^{-3}) = \pi r^2 h$, with **r** being the radius of the core sampler and **h** the vertical height of the core sampler.

Thus the bulk density (g/cm^3) = $\frac{W2-W1}{V}$ …… (5)

3.5.2.2 Particle size analysis

Particle size analysis was carried out by the method of Bouyoucos (1962). To 40 g sample of 2 mm air dried soil of the soil series was added a 100 mL of 0.01M calgon (sodium hexametaphosphate) solution. It was shaken on a mechanical shaker for 30 min.

The suspension was emptied into a 1000 mL graduated sedimentation cylinder. Water was added to make it to the litre mark. A plunger was lowered into the cylinder and the suspension stirred vigorously by moving the plunger up and down for about 5 times. Timing was started immediately with a stop watch after stirring and the first hydrometer reading taken 5 min from the time of mixing of the suspension. The second hydrometer reading was taken 5 h after stirring. The first and second hydrometer readings represent silt and clay and clay respectively .The sand fraction was obtained after decanting the suspension from the sedimentation cylinder into a 0.2mm sieve. The 0.2mm fraction (sand) was thoroughly washed under tap water on the sieve and then oven dried at 105^{0}C for 2 days and the dried weight recorded. The USDA textural triangle was used to determine the textural class of the soils. Blank hydrometer readings of sodium hexametaphosphate solution at 5min and 5 h were taken.

The percentages of the various soil separates were then determined as follows:

a) Silt (%) + Clay (%) = $\frac{Hydrometer\ reading\ at\ 5\ min}{Weight\ of\ soil\ (g)} \times 100$……(6a)

b) Clay (%) = $\frac{Hydrometer\ reading\ at\ 5\ h}{Weight\ of\ soil\ (g)} \times 100$……(6b)

c) Silt (%) = % (Clay + Silt) - % Clay… (6a – 6b)……(6c)

d) Sand (%) = $\frac{Weight\ of\ oven-dry\ sand\ retained\ on\ the\ 47\mu m\ seive}{Weight\ of\ soil\ (g)} \times 100$……..(6d)

3.5.3 Chemical Analyses

3.5.3.1 Soil pH

The pH of the soil sample was measured electrometrically, using a Pracitronic MV 8 8 pH glass electrometer in both distilled water and in 0.01M $CaCl_2$ solution at a ratio of soil: water (1:1) and soil: 0.01 M $CaCl_2$ solution (1:2). Twenty grammes (20 g) of the soil sample was weighed into a 50 mL beaker and 20 mL of distilled water was added. The soil-liquid suspension was stirred for 30 min. The suspension was allowed to stand for 30 min. to allow most of the suspended clay to settle out. The pH electrometer was then standardized using buffer solutions of pH 4.0 and 7.0. The standardized electrode was inserted into the suspension to measure the pH of the sample.

Also ten 10 g of soil was weighed into a 50 mL beaker and 20 mL of $CaCl_2$ was added and the above process was repeated for the measurement of pH in the 0.01M $CaCl_2$ solution.

3.5.3.2 Organic Carbon

The wet combustion method of Walkley and Black (1934) was used to determine the organic carbon content of the soil. Ten (10) ml of IN potassium dichromate ($K_2Cr_2O_7$) solution plus 20 ml of concentrated sulphuric acid (H_2SO_4) were added to 0.5 g sample of a 0.5 mm sieved soil in a 250 ml Erlemeyer- flask. To ensure full contact of the soil with the solution the flask was swirled carefully then after the solution was cooled for 30 min. Two hundred ml (200ml) of distilled water and 10 mL of orthophosphoric acid were also added. After the oxidation of the oxidizable organic material in the soil sample, the unreduced $K_2Cr_2O_7$ remaining in solution was titrated with 0.2 N ammonium ferrous

sulphate solution after adding 2 ml of barium diphenylamine sulphate indicator. The percent (%) organic carbon was calculated as:

$$\% \text{ C} = \frac{0.3\ (10 - \text{XM}) \times 1.33}{\text{W}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(8)}$$

Where: X = ml of Fe $(NH_4)_2(SO_4)_2$ required for the titration

0.3 =

M = Molarity of Fe $(NH_4)_2(SO_4)_2$

W= weight of soil sample (g)

3.5.3.3 Total nitrogen determination

The total nitrogen of the soil was determined using Kjedahl digestion method. A weight of 2 g of soil was taken into a 250 mL Kjedahl flask after which a tablet of digestion accelerator, selenium catalyst, was added and then followed by 5 ml concentrated H_2SO_4. The mixture was then digested until it became clear. The flask was cooled and the clear solution was transferred into a 100 ml volumetric flask using distilled watr and made up to the volume quantitatively. Five (5) ml aliquot of the digested solution was then taken into a Markhan distillation apparatus. Also to the aliquot was 5 ml of 40% NaOH solution added and the mixture distilled. The collection of the distillate was done in 5 ml of 2% boric acid and three (3) drops of a mixed indicator of methyl red and methylene blue were added to the distillate in 50 ml Erlenmeyer flask and then titrated against 0.01N HCL acid solution (Bremner, 1965). The percentage (%) nitrogen was calculated as:

$$\% \text{ N} = \frac{\text{Molarity of HCl} \times \text{titre Vol} \times 0.014 \times \text{Vol.of extractant}}{\text{weight of soil sample} \times \text{Vol.of aliquot}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(9)}$$

Where 0.014 = milliequivalent of nitrogen.

3.5.3.4 Total P Determination

Total P was determined by digesting 2 g of a 0.5 mm sieved soil with 25 ml of a mixture of concentration HNO_3 and 60% $HClO_4$ in ratio 2:3. The solution was heated on a digestion rack until the solution became colourless. The digest was cooled, diluted and filtered through a Whatman filter paper No.42 into 250 ml volumetric flask. The samples were analyzed for phosphorus using Murphy and Riley method (1962). The solutions were mixed thoroughly by shaking and allowed to stand for 15 min for the colour to stabilize (the colour changed to blue of different shades depending on the concentration of the P in each sample). The colour intensity was read using spectrophotometer at wavelength of 712 nm. P was calculated using the formula:

$$\text{P (mg/kg)} = \frac{(\text{Sp. reading} - \text{blank reading}) \times \text{Vol. extract}}{\text{weight of soil sample} \times \text{Vol.of aliquot}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (10)$$

3.5.3.5 Available P determination

Available Phosphorus of the soil was determined by using Bray 1 method (Bray and Kurtz 1945). Five grams (5 g) of soil sample was weighed into a centrifuge bottle and 50 ml of the Bray 1 solution (0.03 N NH_4^+ F+ 0.025 N HCl) was added. The suspension was shaken for 5 min on a mechanical shaker and thereafter made to stay overnight for the suspension to settle after which the suspension was filtered through a No.42 Whatman filter paper into a 100 ml volumetric flask and made up to the volume. Available phosphorus in the filtrate was determined using molybdate-ascorbic acid method of Watanabe and Olsen (1965) as follows: Ten (10) mL aliquots of the filtrate were taken into a 50 ml volumetric flask containing distilled water in duplicates. The pH was adjusted using P-nitrophenol indicator and neutralized with a few drops of 4 N NH_4OH until the solution turned yellow. The

solutions were diluted to 40 ml with distilled water after which 8 ml of a mixture of 12 g ammonium molybdate, 0.29 g antimony tartrate, 140 ml concentrated H_2SO_4 and 1.056 g of ascorbic acid (reagent B) were added. The solutions were mixed vigorously by shaking and allowed to stand for 15 min for the colour to stabilize (The colour changed to blue of varying shades depending on the concentration of the P in each sample).

A blank solution was prepared with 8 ml of reagent B and distilled water. The spectrophotometer was calibrated using 25 mg L^{-1} standard P solution as done above. The intensity of the blue colour was measured using the Philips PU 8620 spectrophotometer at a wavelength of 712 nm. The P concentration was read using the spectrophotometer and calculated as follows:

$$\text{Mg P kg}^{-1}\text{ soil} = \frac{(Spectrophotometer\ reading - blank\ reading) \times vol.\ of\ extract}{Vol. of\ aliquot \times Sample\ weight\ (g)} \quad \ldots\ldots\ldots\ldots (11)$$

3.5.4 Biological Analysis

3.5.4.1 Most Probable Number Analysis

The Most Probable Number (MPN) plant infection assay (Vincent, 1070) was used to estimate the population of rhizobia in the soil that were capable of nodulating the test legume (Vincent, 1970). This was done using a modified Leonard jar assembly (Ferreira and Marques, 1992). The assembly consisted of a plastic cup tapered to a similar cup at the bottom. The cup with the rooting medium (acid-washed sand) was put into another plastic cup of similar shape containing the nutrient solution. The lower container of each Leonard jar was then filled with about 100 mL of N-free nutrient solution. Cotton wick connecting the upper and the lower units, was to supply the rooting medium with solution and the

whole assembly was thereafter autoclaved to get rid of microorganisms. Seventy percent (70%) alcohol and 0.1% mercuric chloric were used to surface sterilized the legume seeds for 3 min and then washed carefully in several changes of sterilized distilled water (Somasegaran and Hoben, 1994). Pre-germination of the seeds was conducted on moist filter paper in petri dishes just for the radicles to be about 1-2 cm long. The seedlings were then transplanted into each sterilized Leonard jar which contained autoclaved acid-washed sand using a pair of sterilized forceps. The forceps was used to make holes in planting medium of each jar. Seedlings with radicle length of about 1-2 cm were picked up and placed one per hole with the sterile pair of forceps, the seedlings were put in such a way that the radicle would be facing downwards. The holes were made deep enough to accommodate pre-germinated seeds at least 0.5 cm below the surface of the sand. The assemblies were then arranged randomly in a screen house. One milliliter (1 mL) of four-fold dilutions was made (that is ten grams of the soil sample were diluted in 90 mL of sterile distilled water) and the soil suspension was used to inoculate the seedling in each jar (Somasegaran and Hoben, 1994) with each dilution made in four replicates 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}. N-free nutrient solution was supplied to the plants when necessary. After 6 weeks of planting, each plant was evaluated for the presence of nodules and the most probable number of rhizobial cells per gram of soil per legume was calculated (Vincent, 1970). By summing up the nodulated units at each dilution level, the total number of nodulated units was obtained at harvest. The uninoculated controls were used to check for sterile conditions. The MPN was then calculated using the formula

: $$\mathrm{MPN} = \frac{\mathrm{m} \times \mathrm{d}}{\mathrm{v}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (12)$$

Where: m = most likely number from MPN table,
d = lowest dilution in the series

v = volume of aliquot used for inoculation.

CHAPTER FOUR

4.0 Results

4.1 Physico-chemical characteristics of the soil

Table 4.1 gives the physical and chemical characteristics of the soil used for both the field and greenhouse studies. The soil particle size analysis showed that the texture of the topsoil of Tanchera soil series was loamy sand. The mean sand content of Tanchera was 700 g/kg, while the silt and the clay content were of same value (150 g/kg). The soil analysis also showed that the Tanchera was moderately acidic with pH values of 5.55 and 5.05 in (1:1 soil: water) and (1:2 soil: 0.01M $CaCl_2$), respectively. The organic carbon and total nitrogen content in the surface soil were low. The organic carbon was (2.7 g/kg), while the total nitrogen was (0.5g/kg). Phosphorus availability as determined by Bray-1 was also low (1.12 mg P/kg) while that of total P soil was not high (18.28 mg P/kg). Generally, the soil contains no exchangeable acidity. The soil is classified as Ferric Lixisol by Asiamah and Adjei-Gyapong, (2001) according to FAO classification system.

Table 4.1 Some Physico-chemical properties of the topsoil (0-30 cm) of the Tanchera soil

Properties	Mean values
Physical	
Sand g/kg	700
Silt g/kg	150
Clay g/kg	150
Bulk Density (Mg/m^3)	1.70
Chemical	
pH (Water) (1:1)	5.55
pH (0.01M ($CaCl_2$) (1:2)	5.05
Total N g/kg	0.5
Organic Carbon g/kg	2.7
Total Phosphorus (mg/kg)	18.28
Available Phosphorus (mg/kg)	1.12
Exchangeable bases (cmol (+)/kg	
Ca^{2+}	2.81
Mg^{2+}	1.04
K^{+}	0.11
Na^{+}	0.07
Exchangeable acidity (cmol (+)/kg	
Al^{3+}	0.00
H^{+}	0.00
Cation Exchange Capacity	4.03

4.2 Nodulation potential of the three soybean genotypes in the Tanchera soil

The results of rhizobia population count in the experimental soil before (initial rhizobia population IRP) and after (residual rhizobia population RRP) in the field experiment with respect to the three soybean genotypes are shown in the Tables 4.2a and 4.2b respectively. In the initial count, higher indigenous rhizobia population was associated with Jenguma (the local genotype) than with the new genotypes namely TGX1904-6F and TGX1955-4F (Table 4.2a) this might be the fact that Jenguma has been cultivated in the Tanchera soil over the years. On the other hand the results of the residual rhizobia population (RRP) analyses (after harvest) showed that, there was an increase in rhizobia population over the control when rhizobium inoculant was added (Plus I = rhizobium inoculation only) (Table 4.2b). It was also observed that the performance of the three soybean genotypes in the TSP treated soil without inoculation produced higher indigenous rhizobia population than the control with and without inoculation (Table 4.2b), which occurred mostly in TGX1904-6F. There was however, a decrease in IRP when TSP and rhizobium inoculation (TSP + I) were applied to the three genotypes, and the rhizobia population obtained in this treatment was similar to what was obtained in the control without inoculation. Similarly, application of Morocco phosphate rock alone to the three genotypes increased the indigenous rhizobia population. The opposite results were obtained with the combination of MPR and inoculant (Table 4.2b). The estimated total rhizobia population associated with Jenguma and TGX1955-4F when a combination of MPR and the commercial inoculant was added was higher than TGX1904-6F. The performance of the genotypes in the MPR treated soil without inoculation was TGX1955-4F > Jenguma >TGX1904-6F while, their performance with TSP was TGX1904-6F > TGX1955-4F = Jenguma. The interaction

effect between rhizobium inoculation and the P sources on the rhizobia population associated with the genotypes was Jenguma > TGX1955-4F > TGX1904-6F (Table 4.2b).

Table 4.2a: Initial Rhizobia Population of the three soybean genotypes in the Tanchera soil (before the field experiment).

Genotype	JENGUMA	TGX1904-6F	TGX 1955-4F
IRP (cells g^{-1} of soil)	3.1×10^2	1.7×10^2	1.7×10^2

❖ IRP – Indigenous Rhizobia Population.

Table 4.2b: Residual Rhizobia Population (RRP*)* after the field experiment.

IRP (cells g^{-1} of soil)

Genotype	Control No I	Control Plus I	TSP No I	TSP Plus I	MPR No I	MPR Plus I
JENGUMA	6.8×10^1	1.24×10^2	2.36×10^2	1.24×10^2	4.0×10^2	2.36×10^2
TGX 1904-6F	4×10^1	6.8×10^1	4.0×10^2	4.0×10^1	1.24×10^2	2.32×10^1
TGX 1955-4F	6.8×10^1	2.36×10^2	2.36×10^2	6.8×10^1	7.2×10^2	6.8×10^1

❖ No I = without inoculation; Plus I = with inoculation; TSP = Triple Superphosphate; MPR = Morocco Phosphate Rock.

PART A: Greenhouse Results

4.3 Effect of genotype, P sources and rhizobia inoculation on plant growth, yield and yield components in the greenhouse trial.

Significant differences existed among the soybean genotypes for plant growth, biomass and yield components (Table 4.3). Plant height was significantly ($p<0.05$) higher in TGX1904-6F than Jenguma but statistically similar to TGX1955-4F. Jenguma produced the higher dry matter yield than the other two genotypes which did not significantly ($p<0.05$) differ from each other. Genotypic differences were observed with respect to pod number per pot and the trend was similar to the results obtained with dry matter yield. Although TGX1955-4F numerically recorded less pods, its pods appeared to be quite heavy and were comparable to those of Jenguma than TGX1904-6F. TGX1955-4F and Jenguma had similar grain yield and were significantly higher than TGX1904-6F. The performance trend was TGX1955-4F/Jenguma >TGX19004-6F. The highest harvest index was obtained with TGX1955-4F.

Growth response of the soybean genotypes to rhizobium inoculant was significant but there was no significant ($p<0.05$) effect on dry matter yield, pod number and grain yield (Table 4.3). Inoculated crop grow taller than the uninoculated crop, although the application of rhizobium inoculant had effect on dry matter yield, the effect was statistically not significant. Harvest index was significantly ($p<0.05$) higher in the uninoculated treatment than the inoculated treatment (Table 4.3).

Sources of P significantly ($p<0.05$) influenced plant height, dry matter yield and HI per pot. Plants that received TSP grew taller and had highest biomass per pot. Control treatment

produced highest pod number per pot, but it was not significantly different from that of TSP and MPR. The pod weights were quite heavier in TSP than MPR and control but statistically, the results were similar. Correspondingly, Triple super phosphate (TSP) and Morocco (MPR) produced higher grain yield than the control, but the yields between the P source (TSP and MPR) were similar and not significant ($p<0.05$). The results of HI for the P source in the greenhouse experiment was similar to what was obtained in the field experiment.

Table 4.3: Effect of genotype, rhizobia inoculation and P sources on growth, and yield of soybean (Greenhouse).

	Plant Height (cm/pot) 10WAP	Dry Matter (g/pot)	Pod No/pot	Pod wt. (g/pot)	Grain yield (g/pot)	Harvest Index
Genotype						
JENGUMA	41**b**	4.77**a**	12**a**	3.28**a**	2.31**a**	0.29**b**
TGX1904-6F	44**a**	4.35**b**	10**b**	2.88**b**	2.01**b**	0.28**b**
TGX1955-4F	42**ab**	4.10**b**	10**b**	3.19**a**	2.35**a**	0.33**a**
LSD (p< 0.05)	2.32	0.32	1.01	0.27	0.22	0.03
CV (%)	9.5	12.4	16.7	14.9	17	14.8
Rhizobium inoculation						
Uninoculation	41**b**	4.33**a**	11**a**	3.27**a**	2.30**a**	0.32**a**
Inoculation	43**a**	4.48**a**	10**b**	2.97**b**	2.14**a**	0.29**b**
LSD (p<0.05)	2	0.26	0.82	0.22	0.18	0.02
CV (%)	9.5	12.4	16.7	14.9	17	14.8
P sources						
Control	38**c**	3.79**c**	11**a**	3.07**a**	2.09**a**	0.32**a**
TSP	46**a**	5.15**a**	10**a**	3.15**a**	2.27**a**	0.27**b**
MRP	42**b**	4.29**b**	10**a**	3.14**a**	2.30**a**	0.31**a**
LSD (p< 0.05)	2.32	0.32	1.01	0.27	0.22	0.03
CV (%)	9.5	12.4	16.7	14.9	17	14.8

Means with the same letter are not statistically significant (p<0.05). TSP = Triple super phosphate and MRP = Morocco rock phosphate.

4.4 Interaction effect of the P sources and Rhizobium inoculation on plant growth and yield in the greenhouse experiment.

The results on the effect of interaction between P sources and rhizobium inoculation on growth and yields of soybean are presented in Table 4.4. The results indicate that, the interaction between P sources and rhizobium inoculation did not significantly ($p<0.05$) affect plant height. P sources however, significantly ($p<0.05$) affected the shoot dry matter yield and grain yield and HI. The application of rhizobium inoculant to TSP (P_1I_1) led to significant increase in the dry matter yield over MPR plus inoculant (P_2I_1) and rhizobium inoculation alone (P_0I_1). However, Morocco rock phosphate plus rhizobium inoculation resulted in a significantly ($p<0.05$) higher dry matter yield than the application of rhizobium inoculation alone (P_0I_1) and control without inoculation (P_0I_0). Grain yield and harvest index (HI) were higher in MPR without Inoculation (P_2I_0) and rhizobium Inoculation alone (P_0I_1) than TSP + Inoculant (P_1I_1) but the differences were statistically not different. The other interactions (Genotype x P sources, Genotype x Rhizobium Inoculation, and Genotype x P sources x Rhizobium Inoculation) had no significant ($p>0.05$) effect on the above parameters measured. The interactions can be obtained at the appendix.

Table 4.4: Interaction effects between the P sources & Rhizobium Inoculation on Plant growth, and yield component (Greenhouse Experiment).

Treatment	Plant height (cm) 10WAP	Dry matter (g/pot)	Pod No.	Pod wt. (g/pot)	Grain Yield (g/pot)	HI
P_oI_o	37**a**	2.27**c**	11**a**	3.12**a**	2.02**b**	0.37**a**
P_oI_1	40**a**	2.67**c**	11**a**	3.01**a**	2.16**ab**	0.35**ac**
P_1I_o	42**a**	3.91**a**	11**a**	3.31**a**	2.43**a**	0.31**bc**
P_1I_1	42**a**	3.60**a**	10**a**	2.98**a**	2.11**b**	0.30**b**
P_2I_o	44**a**	3.06**bc**	10**a**	3.37**a**	2.45**a**	0.37**a**
P_2I_1	48**a**	3.11**b**	10**a**	2.92**a**	2.16**ab**	0.33**abc**
LSD ($p<0.05$)	9.5	0.43	1.42	0.38	0.31	0.04
CV (%)	3.274	17	16.7	14.9	17	14.1

❖ **Mean with the same letter are not significant. P_oI_o, = control, P_oI_1, = inoculation, P_1I_o = Triple super phosphate (TSP) without inoculation, P_1I_1, = Triple super phosphate (TSP) with inoculation, P_2I_o, = Morocco rock phosphate (MRP) without inoculation and P_2I_1 = Morocco rock phosphate (MRP) with inoculation respectively.**

4.5 Effect of genotype, P sources and rhizobia inoculation on nutrient concentration and uptake (Greenhouse Experiment).

The soybean genotypes did not show any significant ($p<0.05$) difference in the nitrogen and phosphorus concentration (%N and %P) in the shoot (Table 4.5). However, total N and P uptake in shoot by Jenguma genotype was higher than TGX1904-6F and TGX1955-4F. The TGX1904-6F and TGX1955-4F had higher N and P concentration in grain than Jenguma. The data also indicated that the concentration of N and P in the grain of the former genotypes were similar. Total N and P uptake was significantly ($p<0.05$) higher in TGX1955-4F and Jenguma than in Jenguma and TGX1904-6F. However, no significant ($p>0.05$) difference existed between the genotypes TGX1955-4F and Jenguma even though TGX1955-4F had the highest total N and P in grain.

The application of rhizobium inoculation had a more significant effect on the concentration of P and its uptake in both shoot and grain than the N content (Table 4.5). N concentration and its uptake in shoot in the inoculated and uninoculated treatments were not significant ($p>0.05$) different. However, rhizobium inoculation significantly influenced both N and P concentrations in the grain with the inoculated treatments had higher N concentration in the grains than the uninoculated treatment. There was no significant ($p>0.05$) effect of rhizobium inoculation on total N and P uptake in the grain even though inoculated treatment were higher than uninoculated treatments.

The application of P had no significant ($p<0.05$) effect on N concentration in the shoot (Table 4.5), however, the application significantly ($p<0.05$) enhanced shoot uptake. TSP amended treatments had higher N uptake than MPR. Similar trend was observed for the grains but the difference between TSP and MPR were not significantly ($p<0.05$) different.

The application of P significantly (p0.05) increased %P and P uptake in both shoot and grain. These increases were higher in TSP than MPR for the shoot but this was only for % P in grain. In the grain, total N and P were significantly higher in TSP and MPR than the control.

Table 4.5: Effect of genotype, rhizobia inoculation and P sources on nutrient concentration and uptake in the greenhouse.

	Concentration in Shoot		Shoot –Uptake		Concentration in Grain		Uptake of N and P in Grain	
Treatment	**% Nitrogen**	**% Phosphorus**	**N (mg/pot)**	**P (mg/pot)**	**% Nitrogen**	**% Phosphorus**	**N mg/pot**	**P mg/pot**
Genotype								
JENGUMA	1.88**a**	0.69**a**	90**a**	33**a**	3.93**b**	0.69**b**	77**a**	14**a**
TGX1904-6F	1.89**a**	0.68**a**	82**b**	30**b**	3.99**a**	0.72**a**	68**b**	12**b**
TGX1955-4F	1.90**a**	0.68**a**	78**b**	29**b**	4.00**a**	0.72**a**	80**a**	15**a**
LSD (p<0.05)	0.04	0.01	6	2.3	0.04	0.01	7.4	1.3
CV (%)	3.4	1.4	12.5	12.7	1.6	3	17	17
Rhizobium inoculation								
Uninoculation	1.88**a**	0.66**b**	81**a**	29**b**	3.86**b**	0.69**b**	77**a**	14**a**
Inoculation	1.90**a**	0.71**a**	85**a**	32**a**	4.03**a**	0.74**a**	74**a**	13**a**
LSD (p<0.05)	0.03	0.004	4.9	1.9	0.03	0.01	6	1.1
CV (%)	3.4	1.4	12.5	12.7	1.6	3	17	17
P sources								
Control	1.89**a**	0.53**c**	72**c**	20**c**	3.66**b**	0.57**c**	65**b**	10**b**
TSP	1.87**a**	0.77**a**	96**a**	40**a**	4.14**a**	0.80**a**	80**a**	15**a**
MRP	1.91**a**	0.75**b**	82**b**	32**b**	4.12**a**	0.77**b**	81**a**	15**a**
LSD (p<0.05)	0.04	0.01	6	2.3	0.04	0.01	7.4	1.3
CV (%)	3.4	1.4	12.5	12.7	1.6	3	17	17

Mean with the same letter are not statistically significant. No I = without rhizobia inoculation and Plus I = with rhizobia inoculation. TSP = Triple super phosphate and MRP = Morocco rock phosphate.

4.6 Interaction effect between P sources and Rhizobium inoculation on nutrient concentration and uptake (greenhouse Experiment)

The interaction between P sources and rhizobium inoculation did not significantly affect percent N in soybean shoot (Table 4.6). However, percent N in grain was significantly affected by the interaction. The N concentration was higher with TSP + I (P_1I_1) application but statistically similar to what was obtained with MPR + I (P_2I_1) and P alone (Table 4.6). Nonetheless, the interaction between rhizobium inoculation and P sources were significantly higher than the N concentration obtained from rhizobium inoculation alone (P_0I_1). The concentration of P in both shoot and grain was significantly higher with TSP + I and MPR + I than rhizobium inoculation alone (P_0I_1).

The uptake of N and P in shoot were also significantly influenced by the interaction between the P sources and rhizobium inoculation. There was no significant ($p>0.05$) difference between MPR (P_2I_0) alone, MPR + I (P_2I_2) and rhizobia inoculation (P_0I_1) alone in N uptake even though the uptake of N from MPR + I was higher than that of rhizobia inoculation alone.

N and P uptake from the P sources alone and also P sources plus inoculant were similar (Table 4.6). Total N and P contained in grain were significant ($p<0.05$) among the treatments. P_2I_0 had the highest total N uptake in the grain but not significantly ($p<0.05$) different from P_1I_0 and P2I2. Total P in grain was significantly influenced by the interaction of P sources and rhizobia inoculation. There was no significant difference between TSP + I (P_1I_1) and MPR + I (P_2I_1) in total P in grain but the two were statistically higher than inoculation alone treatment. Similarly there was no difference between TSP and MPR in total P but the two had more P than only inoculated seeds.

Table 4.6: Interaction effects between the P sources and Inoculation on N and P concentration and uptake (Greenhouse Experiment).

Treatment	Concentration in Shoot		Shoot -Uptake		Concentration in Grain		Total N & P in Grain	
	% Nitrogen	% Phosphorus	N (mg/pot)	P (mg/pot)	% Nitrogen	% Phosphorus	N (mg/pot)	P (mg/pot)
P_oI_o	1.87**a**	0.46**d**	65**c**	16**d**	3.50**d**	0.52**d**	60**d**	9**c**
P_oI_1	1.91**a**	0.60**c**	78**b**	25**c**	3.82**c**	0.63**c**	70**cd**	11**c**
P_1I_o	1.86**a**	0.77**a**	99**a**	41**a**	4.12**ab**	0.79**ab**	85**ab**	16**a**
P_1I_1	1.88**a**	0.78**a**	94**a**	39**a**	4.16**a**	0.80**a**	75**bc**	14**b**
P_2I_o	1.92**a**	0.75**b**	80**b**	31**b**	4.11**a**	0.76**b**	86**a**	16**a**
P_2I_1	1.90**a**	0.76**b**	83**b**	33**b**	4.15**ab**	0.78**b**	76**abc**	14**b**
LSD ($p<0.05$)	0.05	0.01	8.5	3.2	0.05	0.02	10.5	1.9
CV (%)	3.4	1.4	12.5	12.7	1.6	3	17	17

Mean with the same letter are not significant. P_oI_o, = control, P_oI_1, = inoculation, P_1I_o = Triple super phosphate (TSP) without inoculation, P_1I_1, = Triple super phosphate (TSP) with inoculation, P_2I_o, = Morocco rock phosphate (MRP) without inoculation and P_2I_1 = Morocco rock phosphate (MRP) with inoculation respectively.

PART B: Field Results

4.7 Effect of soybean genotype, P sources and rhizobia inoculation on growth, nodulation and grain yield in Field experiment.

The results of the effects of genotype, P sources and rhizobia inoculation on soybean growth, nodulation and grain yield in the field experiment are given in Table 4.7. Soybean genotype showed significant ($p<0.05$) differences in plant height, nodule dry weight and HI with TGX1955-4F having the tallest plants but not statistically ($p>0.05$) different from TGX 1904-6F. Soybean genotype also significantly ($p<0.05$) influenced nodule dry weight, grain yield and harvest index (Table 4.7). TGX1955-4F produced the highest number of nodules and a higher number of effective nodules than the other genotypes, though the differences were not significant ($p>0.05$). The highest nodule dry weight was recorded by TGX 1955-4F while the lowest was recorded by Jenguma, that of TGX1955-4F and TGX1904-6F were similar. Jenguma produced more pods (483) per ten plants than the new genotypes TGX 1955-4F (397) and TGX1904-6F (393) but these were different. Pod weight was heavier in Jenguma and TGX1955-4F than TGX1904-6F but the differences were not significant ($p>0.05$). The genotype TGX1955-4F produced the highest mean grain yield which was statistically ($p<0.05$) different from Jenguma and TGX1904-6F. The highest biomass yield was obtained in TGX1955-4F but this was not significantly ($p>0.05$) different from the other genotypes namely; Jenguma and TGX1904-6F. The Harvest Index of genotype TGX1955-4F was significantly ($p<0.05$) higher than that of Jenguma but not ($p>0.05$) different from TGX1904-6F.

The application of rhizobium inoculation increased significantly ($p<0.05$) nodule number, nodule dry weight and effective nodule number per ten plants, and harvest index of soybean

as compared to the control (Table 4.7). Pod number and pod weight were higher with rhizobium inoculation than without inoculation treatments but the difference was however not significant ($p>0.05$). It was observed that inoculation did not affect plant height, biomass weight and grain yield either. A significant difference was observed with the biomass in which uninoculated treatments produced significantly higher biomass yield than the inoculated plants.

The application of TSP had significant ($p<0.05$) influence on plant height, pod number and pod weight compared with MPR and control (Table 4.7). Even though application of TSP produced higher nodule number, nodule dry weight and effective nodule number than either MPR or the control, the differences were not significant ($p>0.05$). The highest shoot biomass was obtained from TSP and the control and the least was from MPR. Pod number and pod weight were highly significant ($p<0.05$) with TSP over the MPR applied and the control. Neither the TSP nor MPR produced grain yield equal to the control treatment. Harvest index generally, was better with MRP and control than with TSP.

Table 4.7: Effect of genotype, rhizobia inoculation and P sources on growth, nodulation capacity and grain yield in soybean in the field (Field Experiment).

Treatment	Plant Height (cm) 10WAP	Nod. No/10 plants	Nod Dry wt/10 plants	Nod Effective No/10 plants	Pod No	Pod wt. (kg/ha)	Grain yield (kg/ha)	Biomass weight (kg/ha)	Harvest Index
Genotype									
JENGUMA	69**b**	24**a**	0.18**b**	12**a**	483**a**	1160**a**	694**b**	2407.99**a**	0.23**b**
TGX1904-6F	75**ab**	27**a**	0.20**ab**	14**a**	393**a**	1095**a**	754**b**	2405.90**a**	0.24**ab**
TGX1955-4F	82**a**	31**a**	0.23**a**	15**a**	397**a**	1160**a**	1033**a**	2573.96**a**	0.29**a**
LSD (p<0.05)	6.8	7.08	0.05	4.1	96	278.9	200.6	458.34	0.05
CV (%)	8.3	26.8	35.1	26.5	29.6	23.9	16.4	16.6	12.9
Rhizobium inoculation									
Unioculation	76**a**	22**b**	0.17**b**	12**b**	420**a**	1080**a**	852**a**	2684.95**a**	0.24**b**
Inoculation	74**a**	33**a**	0.23**a**	16**a**	429**a**	1197**a**	803**a**	2240.28**b**	0.26**a**
LSD (p<0.05)	3.01	3.56	0.03	1.78	60.8	131.7	65.4	197.42	0.02
CV (%)	8.3	26.8	35.1	26.5	29.6	23.9	16.4	16.6	12.9
P Sources									
Control	71**b**	27**a**	0.21**a**	13**a**	385**b**	1075**b**	933**a**	2557.99**a**	0.27**a**
TSP	85**a**	29**a**	0.22**a**	15**a**	471**a**	1265**a**	810**b**	2780.90**a**	0.23**b**
MRP	69**b**	27**a**	0.18**a**	14**a**	417**b**	1075**b**	739**b**	2049.96**b**	0.27**a**
LSD (p<0.05)	6.5	5.57	0.07	3.4	52.8	175.8	107	310.1	0.02
CV (%)	8.3	26.8	29.6	26.5	29.6	23.9	16.4	16.6	12.9

❖ **Mean with the same letter are not statistically significant. No I = without rhizobia inoculation and Plus I = with rhizobia inoculation. TSP = Triple super phosphate and MRP = Morocco rock phosphate.**

4.8 Interaction effects of P source and Rhizobium inoculation on plant growth, nodulation and yield (Field experiment).

The interaction effects of P sources and rhizobium inoculation on plant height, nodulation, and yield are given in Table 4.8. There was significant interaction between phosphorus source and rhizobia inoculation with respect to plant height, number of effective nodule, nodule number, nodule dry weight and harvest index grain yield and biomass yield (Table 4.8). Application of TSP and inoculation (P_1I_1) produced the tallest plant height while the control treatment (P_0I_0) produced the shortest plant height. The plant height obtained from MPR and rhizobium inoculation (P_2I_1) was lower than (P_1I_1) but similar to the control (P_0I_0). The combined application of TSP and rhizobium inoculation (P_2I_1) also resulted in the highest number of effective nodules (Table 4.8). Rhizobium inoculation alone (P_0I_1) resulted in the highest grain yield while the combined application of rhizobium inoculation with both TSP and MPR produced the lowest grain yield (Table 4.8). Application of TSP alone also produced the highest biomass while the application with rhizobium inoculation and MPR produced the lowest biomass.

The interaction between genotype and P sources, genotype and inoculation and among the three interaction that is genotype x P sources x inoculation were not significant statistically and are presented in Appendix II.

Table 4.8 Interaction effects of the P sources and Rhizobium inoculation on plant growth, nodulation capacity and yield components (Field experiment).

Treatment	Plant height (cm) 10WAP	Nod No./10 plants	Nod Dry wt./10 plants	Nod Effective No./10 plants	Pod No.	Pod wt. (kg/ha)	Grain Yield (kg/ha)	Biomass weight (kg/ha)	HI
P_oI_o	66**d**	21**b**	0.1b**a**	9**c**	383**a**	1050**a**	864**bc**	2407.64**bc**	0.26**ab**
P_oI_1	76**bc**	33**a**	0.27**a**	17**a**	386**a**	1100**a**	1001**a**	2708.33**b**	0.27**ab**
P_1I_o	94**a**	23**b**	0.21**ab**	14a**b**	477**a**	1180**a**	878**b**	3306.94**a**	0.21**c**
P_1I_1	77**b**	35**a**	0.22**ab**	16**ab**	466**a**	1350**a**	741**cd**	2254.86**c**	0.24**bc**
P_2I_o	67**d**	23**b**	0.15**b**	13**b**	400**a**	1010**a**	812**bc**	2340.28**bc**	0.26**ab**
P_2I_1	69**cd**	31**a**	0.21**ab**	15**ab**	435**a**	1140**a**	666**d**	1757.64**d**	0.28**a**
LSD (p>0.05)	7.34	6.88	0.08	3.95	88.9	231.7	130.3	382.36	0.03
CV (%)	8.3	26.8	35.1	26.5	29.6	23.9	16.4	16.6	12.9

❖ **Mean with the same letter are not significant. P_oI_o, = control, P_oI_1, = inoculation, P_1I_o = Triple super phosphate (TSP) without inoculation, P_1I_1, = Triple super phosphate (TSP) with inoculation, P_2I_o, = Morocco rock phosphate (MRP) without inoculation and P_2I_1 = Morocco rock phosphate (MRP) with inoculation respectively.**

The Table of the three interactions and their ANOVA Tables are provided in the appendices (Appendix1).

4.9 Influence of genotype, P sources and Rhizobia Inoculation on Nitrogen and Phosphorus accumulation (Field experiments).

Table 4.9 shows no significant variatoin ($p > 0.05$) among the genotypes in terms of nitrogen and phosphorus concentration in shoot. However, percent P (%P) in shoot was greater in TGX1955-4F than TGX1904-6F and Jeguma. The total N and P in shoot followed the trend TGX 1955-4F > Jenguma > TGX1904-6F. The concentration of N and P in grain were similar to the trend observed in P uptake in shoot. TGX1904-6F and TGX1955-4F had higher concentration of N and P than Jenguma but the variations were not significant ($p>0.05$). The genotype TGX1955-4F had higher total N uptake in grain in the field experiment and was significantly ($p<0.05$) higher than the other genotypes. Total P uptake was also significantly ($p<0.05$) higher in TGX1955-4F than in Jenguma and TGX1904-6F. There was no significant effect of rhizobium inoculation on total N and P uptake in grain.

Nitrogen concentration in both the shoot and grain was significant ($p<0.05$) with the application of rhizobium inoculant as shown in Table 4.9, while the application of rhizobium inoculation had no significant ($p>0.05$) effect on P concentration in both grain and shoot. Uptake N and P in the shoot was higher in the uninoculated treatment than in the inoculated treatment.

Addition of phosphorus (P sources) significantly ($p<0.05$) improved nutrient concentration of N & P and their uptake both in the shoot and grain over the control treatment (Table 4.9). The highest P concentration in shoot was observed in TSP while the least

concentration was found in the control. The trend was TPS > MPR> Control. Total uptake of P in shoot was different for the two P sources with TSP having the highest uptake; MPR was statistically similar to the control treatment (Table 4.9). Percent N in shoot and grain was significant and higher in MPR than in the TSP and control. P sources had no effect on total N uptake in the grain, however, the effect of P sources was significant ($p<0.05$) on total P uptake in the grain. Phosphorus uptake in grain from TSP was significantly higher than that of MPR and control.

Table 4.9: Effect of genotype, rhizobia inoculation and P sources on nutrient concentration and uptake in soybean (Field Experiment).

Treatment	Concentration in Shoot		Shoot -Uptake		Concentration in Grain		Total N and P in Grain	
	% Nitrogen	% Phosphorus	N (kg/ha)	P (kg/ha)	% Nitrogen	% Phosphorus	N (kg/ha)	P (kg/ha)
Genotype								
JENGUMA	2.93**a**	1.49**a**	70.51**a**	36.00**a**	3.93**a**	0.61**a**	27.23**b**	4.17**b**
TGX1904-6F	2.91**a**	1.49**a**	69.66**a**	36.35**a**	3.94**a**	0.62**a**	29.54**b**	4.60**b**
TGX1955-4F	2.90**a**	1.52**a**	74.23**a**	39.20**a**	3.97**a**	0.62**a**	41.01**a**	6.42**a**
LSD (p< 0.05)	0.05	0.09	13.5	6.3	0.05	0.01	7.87	1.26
CV (%)	2.8	7.4	16.7	16.7	2.4	3.7	17	16.2
Rhizobium inoculation								
Uninoculation	2.79**b**	1.50**a**	75.07**a**	41.24**a**	3.86**b**	0.61**a**	32.86**a**	5.23**a**
Inoculation	3.03**a**	1.50**a**	67.86**b**	33.13**b**	4.03**a**	0.62**a**	32.33**a**	4.90**b**
LSD (p<0.05)	0.04	0.054	5.8	3	0.05	0.01	2.68	0.4
CV (%)	2.8	7.4	16.7	16.7	2.4	3.7	17	16.2
P sources								
Control	2.90**b**	1.16**c**	74.30**a**	29.58**b**	3.67**c**	0.52**c**	34.47**a**	4.87**b**
TSP	2.90**b**	1.78**a**	80.31**a**	49.93**a**	4.05**b**	0.69**a**	32.79**a**	5.56**a**
MRP	2.94**a**	1.56**b**	59.79**b**	32.05**b**	4.13**a**	0.64**b**	30.51**a**	4.77**b**
LSD (p<0.05)	0.04	0.05	8.9	5.6	0.04	0.01	4.23	0.68
CV (%)	2.8	7.4	16.7	16.7	2.4	3.7	17	16.2

Mean with the same letter are not statistically significant. No I = without rhizobium inoculation and Plus I = with rhizobium inoculation. TSP = Triple super phosphate and MRP = Morocco rock phosphate.

4.10 Interaction effects of the P sources and Rhizobium inoculation on nutrient concentration and uptake (Field experiment).

Table 4.10 shows the results of the effect of the interaction between P sources and Rhizobium inoculation on the concentration and uptake of N and P. The concentration of N and P in shoot were significantly ($p<0.05$) influenced by the interaction of the P sources and rhizobium inoculation. The concentration of N in shoot was higher with the application of MPR + inoculant P_2I_1 than P_1I_1. MPR and inoculation varied significantly ($p<0.05$) from TSP and inoculation. The %P in shoot was higher with TSP application (P_1I_0) alone than with TSP + I (P_1I_1). However, phosphorus concentration in TSP + I (P_1I_1) was better than that of MPR + inoculant (P_2I_1) in both shoot and grain. The concentration and uptake was also significant ($p<0.05$) with MPR + I (P_2I_1) application over application of Rhizobium inoculation alone (Table 4.10).

Table 4.10: Interaction effects of the P sources and Rhizobium inoculation on nutrient (N&P) concentration and uptake (Field experiment)

	Concentration in Shoot		Shoot -Uptake		Concentration in Grain		Total N & P in Grain	
Treatment	**% Nitrogen**	**% Phosphorus**	**N (kg/ha)**	**P (kg/ha)**	**% Nitrogen**	**% Phosphorus**	**N (kg/ha)**	**P (kg/ha)**
P_oI_o	2.75**d**	1.05**e**	66.26**c**	25.15**d**	3.41**e**	0.52**d**	29.47**cd**	4.52**bc**
P_oI_1	3.05**a**	1.26**d**	82.33**b**	34.00**bc**	3.94**d**	0.52**d**	39.47**a**	5.22**ab**
P_1I_o	2.84**c**	1.87**a**	93.70**a**	61.98**a**	4.04**c**	0.67**b**	35.49**ab**	5.88**a**
P_1I_1	2.96**b**	1.68**b**	66.93**c**	37.88**b**	4.05**b**	0.71**a**	30.09**cd**	5.24**ab**
P_2I_o	2.79**cd**	1.57**c**	65.26**cd**	36.58**b**	4.14**a**	0.65**c**	33.62**b**	5.29**ab**
P_2I_1	3.09**a**	1.56**c**	54.33**d**	27.52**cd**	4.11**abc**	0.64**c**	27.41**d**	4.25**c**
LSD (p>0.05)	0.06	0.08	11.09	6.55	0.07	0.02	5.2	0.82
CV (%)	2.8	7.4	16.7	16.7	2.4	3.7	17	16.2

❖ **Mean with the same letter are not significant. P_oI_o, = control, P_oI_1, = inoculation, P_1I_o = Triple super phosphate (TSP) without inoculation, P_1I_1, = Triple super phosphate (TSP) with inoculation, P_2I_o, = Morocco rock phosphate (MRP) without inoculation and P_2I_1 = Morocco rock phosphate (MRP) with inoculation respectively.**

CHAPTER FIVE

5.0 DISCUSSION

This chapter focused on discussing the results obtained from this research work and made comparisons with references to available data from elsewhere. The discussion came out with conclusions and gave possible suggestions and recommendations at the end for future study on Morocco phosphate rock in the yield of soybean because it has higher solubility rate than that of Togo phosphate rock.

5.1 Soil Characterisation

The Tanchera soil series used in this experiment was moderately acidic with pH of 5.55 in water. This might be due to the nature of inherent parent material. Soil pH has been identified as one of the most important soil chemical properties which adversely affects nutrient bioavailability and microbial activity (Liu and Haulon, 2012). pH impedes rhizobium growth and root infection which leads to symbiotic failure. Colonization of rhizobia around the root zone of the legume may decrease by extreme soil pH and thereby reducing amount of nitrogen fixed. The low organic carbon content of 0.27% of the soil might be partly attributed to low organic matter and high temperature that accelerates decomposition of the soil's organic matter (Agboola and Aiyelari, 2000). The nitrogen level of the Tanchera series was 0.05 g/kg which is very low when compared to the overall ratings as defined by Landon (1984) as follows: very high (>10 g/kg), high (5-10 g/kg), medium (2-5 g/kg), low (1-2 g/kg), and very low (< 1 g/kg) of soil. The low level of available phosphorus of this soil might be as a result of the nature of parent materials that formed the soil and also iron pans (concretions) of these soils in the savannah zones of Ghana (Abekoe, 1989). The high sand and low silt and clay contents have clearly indicated

that the soils are exposed to varying temperature which can be as high as 42^{o}C and as low as 16^{o}C which accelerates decomposition of organic matter which is the binding factor. The textural characteristics indicate that the soil was generally loamy sand. The bulk density of this soil was high an indication of high gravel content of the soil. The cation exchange capacity (CEC) of the soil could be attributed to the fact that the soils are susceptible to the washing away of the basic elements from surface soil, which is of agricultural importance. It has been stated that, clay content of a soil accommodates more organic carbon, total N and P than sand and silt fractions (Jones *et al*., 2006) and these are contributors of the low soil fertility status of the northern savannah soils. This corresponds to the findings of (Buri *et al*., 2009) who reported that soil nutrient levels of the northern savannah zones are very low with respect to soil organic matter, P, N and also with high pH.

5.2 Nodulation potential of the three soybean genotypes in the Tanchera soil.

The initiation of nodule in the *legume-Rhizobium* symbiosis is brought about with a complex interaction between host roots, rhizobial strain, and the environment. The process of proliferation and attachment of rhizobia strain on root surfaces followed by infection thread formation in susceptible host root cells may be affected by many biotic and abiotic factors. However, a successful nodulation of legumes by rhizobium depends mostly on the presence of a specific and compatible strain in soil for a particular legume and this seems to be true for the three soybean genotypes used in this experiment.

The nodulation by the soybean genotypes in the uninoculated fresh soil indicates the presence of indigenous rhizobial population of the soil are compatible to the three genotypes with Jenguma producing higher nodulation than TGX1955-4F and TGX1904-

6F in the initial rhizobial population analysis. This could be because Jenguma have been cultivated in northern Ghana by smallholder farmers over a long period of time and has become compatible with the native rhizobium strains in the soil. In the residual (after harvest) rhizobia population analysis, again the uninoculated control showed nodulation with the various genotypes with TGX1955-4F producing the highest population with the application of MRP only followed by Jenguma then TGX1904-6F. The result obtained in residual analysis showed that there was a high effective competition between the commercial inoculant and the native rhizobial population in this soil causing their increase in plots where P alone was applied and a decrease in plots where P and commercial inoculant were applied. The findings agree with reports made by Graham, (1981); Singleton and Tavares, (1986); and Brockwell *et al*., (1995) that high population density of naturalized and indigenous rhizobia population of 1×10^2 rhizobia cells/g soil could prevent nodulation and dislocate applied inoculums. The soil used for these studies contained high indigenous rhizobia population number with the various soybean genotypes which is higher than the 1×10^2 rhizobia cells/g soil and therefore could have displaced applied inoculums in the field.

5.3 Response of soybean genotypes to growth, nodulation and grain yield.

The soybean genotypes showed high variability in growth, nodulation and yield components indicating their differences in the traits studied. The significant differences in plant height, in both experiments might primarily be due to the growth habit and differences in their ability to adapt to the soil, and other environmental conditions. Variations among genotypes in plant height were reported by El Naim and Jaberelder, (2010). In similar study, Talaka *et al.,* (2013), reported significant difference in growth of five different

soybean varieties strictly under rain-fed condition at 6 WAP (weeks after planting) but not at 8 WAP.

The results of the experiment showed no difference in nodulation by the soybean genotypes. However, the greatest nodule number was recorded by TGX1955-4F followed by TGX1904-6F and Jenguma respectively in the field experiment. Genotype effect on nodule dry weight was significant and this followed the trend observed in nodule number and effective nodule number. The genotype influence may be attributed to differences in their ability to withstand adverse weather condition in the region. According to Lie, (1974); and Lee *et al*., (1998), the environment has a profound impact on genotypes nodulation that can be confirmed by comparing the nodule number and mass developed by the genotype. This implies that whether the environmental condition favours or not, differences in nodule weight between genotypes will still occur. The results showed that the higher the nodule number the higher the dry weight which is in conformity with results of Oti and Agbim (2000) that a simple relationship ensues between nodule number and nodule dry weight and they are indices of nitrogen fixation. The high nodule number and effective nodules obtained in TGX1955-4F may suggests its potential to fix more atmospheric nitrogen in this soil. Effective nodulation is crucial for a functioning legume-rhizobium symbiosis and so plants most susceptible to infection and capable of producing high effective nodules have the utmost capacities to fix more atmospheric nitrogen.

Genotypic differences affected pod numbers and pod weight only in the greenhouse. The number of pods per plot which is also the main component of yield ultimately determines the potential productivity of soybean. Jenguma the local genotype produced the highest pod number in the greenhouse only. This agrees with the assertion of Graham (1992) that,

selection of genotypes is one of the essential factors for improving pod yield in soybean. The non-significant difference in pod number and pod weight among the genotypes in the field experiment might be due to the reduction in soil moisture during flowering and pod filling as a result of late planting. Masoumi *et al*., (2011) and Behtari and Abadiyyan, (2009), reported similar result that soybean yields reduce as result of water stress.

Significant grain yield difference was observed among the soybean genotypes. The result showed that TGX1955-4F in the field study produced the highest grain yield compared with the grain yield of TGX1904-6F and Jenguma which were similar. The genotype TGX1955-4F produced as high as 1033 kg/ha grain yield compared with 754 and 694 kg/ha obtained with TGX1904-6F and Jenguma respectively. Also TGX1955-4F and Jenguma produced statistically similar amount of grain yield in the greenhouse experiment. The significant differences in the performance of these soybean genotypes especially TGX1955-4F in grain yield in this study might be due to differences in genetic potentials of the different genotypes. This might also be due to fact that TGX1955-4F had optimum conditions favouring its growth and yield in this experiments. Other workers have reported significant yield variations among soybean genotypes (Alam *et al*. 2009, Malik *et al*., 2007; Rahman *et al*. 2011). The grain yield of Jenguma in this study is lower than what was observed by Aziz *et al*., 2014 in similar environment. This might be attributed to drought that set in during pod filling as explained earlier. According to Gardner *et al*., (1985) increasing available soil moisture during vegetative and reproductive growth increases yield and its components of soybean plants.

The results from the greenhouse experiment, revealed that the soybean genotype Jenguma which produced significantly highest biomass yield (4.77g/pot), might be due to its longer

growth period which supported higher biomass at maturity under controlled environment (greenhouse). In the field experiment, biomass production did not differ, although TGX1955-4F appears to have produced the highest biomass (2574kg/ha). Research has revealed that soybean straw (biomass) yield is dependent on the variety and the environment (Martinov, 2008). In addition, the differences in the root morphology of the different genotypes to take up nutrients and moisture from the soil might have contributed to this behavior of the soybean genotypes. The result conformed to the findings of Salisbury and Ross, (1992), who reported that shoot dry yield by varieties of the same crop under similar growth conditions is an indication of similar potential. Soybean has the potential to producie biomass (straw) and seed. Harvest index is an important determinant of seed yield in soybean production. It is the ratio between grain yield and biomass (grain yield/grain yield + straw). Harvest index varied significantly among the soybean genotypes in both experiments. The harvest index average of the three genotypes was 0.30 in the greenhouse experiment and 0.25 in the field experiment which showed that a total of 30% and 25% of the total yield was grain yield and the rest 70% and 75% was biomass yields respectively. The genotype TGX1955-4F had the highest grain yield (2.35 g/pot) in the greenhouse experiment and (1033 kg/ha) in the field, and also the highest biomass yield (2573.96 kg/ha) in the field experiment with the highest harvest index value of 0.33 and 0.29 in both experiments. Also the genotype Jenguma had the highest dry matter yield (straw yield) in the greenhouse experiment but its harvest index was lower than that of the TGX1955-4F.This response might also be due to the genetic background of the soybean genotypes.

5.4 Effect of P sources on plant growth, nodulation and grain yield.

The results showed a positive response of soybean to the application of TSP at 30 kg P/ha, which significantly influenced plant height. Plant grown with TSP at 30 kg/ha were taller than with MPR at 30 kg/ha and control in both the field and greenhouse experiments. This indicated that triple super phosphate (TSP) was readily soluble in water for plant use hence reflected in early plant growth and development as observed in both the field and the greenhouse experiments. The increase in plant growth in response to phosphorus supply had been reported by Kwari (2005); and Rebafka, *et al*. (2003). The observation also agrees with the findings of Bekere *et al*. (2012) who showed that phosphorus application of 60, 120 and 180 mg P/kg significantly increased soybean height under controlled environment. Similar observations were made by Tomar *et al*. (2004); and Rani (1999).

Application of P did not affect nodulation significantly in the field. Similar results have been reported by Jemo *et al*. (2010). Studies by Tagoe *et al*. (2008); and Waluyo *et al*. (2004) have shown that phosphorus helps to initiate nodule formation as well as the development and functioning of formed nodules. Absence of phosphorus in legume significantly affects the development of effective nodules and nodule leghaemoglobin content in the nodule as reported by Singleton *et al.,* (1984). This shows that phosphorus must be present in soil in adequate quantities to support nodulation and hence nitrogen fixation. According to Miller *et al*. (1982) the weight of nodule is a factor contributing to N_2 fixation activity while nodule number is important in relation to nodule weight. It may be possible that the quantity of P applied (30 kg P/ha) in this experiment was not adequate to allow sufficient nodulation.

Shoot biomass obtained with TSP application was significantly higher than that of MRP but similar to the control. However, in the greenhouse experiment, shoot biomass increased significantly with TSP over MPR and the latter over the control. Lamptey *et al*., (2014) reported of similar increases in shoot biomass of soybean with the application of TSP fertilizer. Asia *et al*. (2005) also reported of 20.7% increase in shoot biomass yield due to phosphorus application. Roughley *et al*. (1993) reported how shoot dry matter yield reduced drastically upon the omission of P from optimum nutrition of soybean. The poor influence of MPR on the growth and yield parameters of the soybean may be due to its low solubility as compared with TSP. Both the greenhouse and the field experiments produced similar results with MPR.

Pod number and pod weight were also significantly influenced by the application of phosphorus with TSP having higher number of pods and pod weight than MRP and the control in the field experiment. Similar results have been reported by Mohan (1997); Rani, (1999); CSIR-SARI Annual Report, (2013).

Grain yield was higher in the control treatment and statistically different from the P sources although TSP tended to increase pod number and pod weight in the field experiment. While in the greenhouse experiment, the results seem to be statistically similar among the P sources. This observation may be due to the fact that the P fertilizer treated plots convert the nutrient to vegetative growth at the expense of grain yield production. As observed by Blackman (1959a), that, during juvenile stages of plants, vigorous exponential vegetative growth occurs, leaf area index then increases which in turn leads to increasing rate of photosynthesis and hence dry matter yield.

Several studies have shown higher grain yield of soybean with application of phosphorus than without P (Sabir *et al*., (2001); Malik *et al*., (2006); SARI, (2012); Lamptey *et al*., (2014). It has been reported that soybean has high requirement for phosphorus and seed and yield quality could be improved by phosphorus fertilizer in soils with low phosphorus levels (Shahid *et al*., 2009). Harvest index was higher and significant with MPR and control over TSP in both experiments indicating that the higher the shoot dry matter yield the lower the HI and the vice versa. The higher shoot biomass yield produced by TSP treated plots and the poor filling of the pods led to its low HI in this experiment which is equally beneficial to the farmers in the region as crop residues are either used in feeding livestock or sold to livestock farmers for cash income.

5.5 ***Effect of rhizobium inoculation on plant growth, nodulation and yield.***

Rhizobium inoculation significantly improved nodule number, nodule dry weight and effective nodule number but reduced shoot biomass in the field. It however had no significant effects on plant height, pod number, pod weight and grain yield in the field experiment suggesting that inoculation has no influence on plant height under field conditions. This is contrary to the finding of Alam *et al*. (2015) who reported increase in biological yield (biomass) of soybean with rhizobium inoculated seeds. However, plant height was significantly affected by rhizobia inoculation in the greenhouse experiment which agrees with assertion made by Hernandez *et al*. (2003) that *Rhizobium* inoculation increased plant height.

Nodulation of soybean was significantly affected in inoculated plants than uninoculated treated plants. This is in line with the reports of Elkoca *et al*. (2010) who recorded a significant increase in nodulation by inoculating legume with native soil rhizobium in

single inoculation. The findings are also in line with observation made by Kumaga *et al*. (2002) which suggested that nodulation and N_2 fixation of promiscuous soybean may be increased by inoculation with effective *Bradyrhizobia*. Okereke *et al*. (2000) attributed the increase in nodulation to the competitive ability of *Bradyrhizobium* used.

Pod number and pod weight were not affected by rhizobium inoculation in the field, and this may be due to the extreme drought condition experienced during pod filling of soybean which occurred at the terminal part of the season. Schultz and Thelen (2008) also reported similar findings about potential negative yield response from inoculation under extreme drought condition occurring during pod filling stage. This result is in disagreement with reports of Malik *et al*. (2006) and Bhuiyan *et al*. (2008), who concluded significant increase of pod number of mung bean and soybean by *Bradyrhizobium.* Shahid *et al*. (2009) also reported that inoculated soybean produced more pods per plant than uninoculated plants.

Rhizobium inoculation did not have significant effect on grain yield in both experiments. Similar result was reported by Otieno *et al*. (2007) and Chemining' wa *et al*. (2004) about lack of significant increase on grain yield improvement by inoculation. The significant effect shown by rhizobia inoculation (nodumax) in the field experiment on HI may be primarily due to smaller amount of shoot biomass produced by the inoculated plants.

5.6 Interaction effects between the P sources & Inoculation on Plant growth, nodulation capacity and grain yield.

Results of both field and greenhouse experiment, showed significant interaction effect between P sources and rhizobium inoculation resulting in significant effects on most of the parameters measured (plant height, grain yield, biomass yield, nitrogen and phosphorus concentration in both shoot and grain, N and P uptake in both shoot and grain. The

significant effect of the interaction on plant height confirmed the observation made by Gunarto (2000) that increase in plant growth from seeds appears to be as a result of improved soil productivity due to bacterial activity and available nutrients. Rhizobium inoculation combination with P significantly increased biomass and grain yield. Similar results were obtained by Khan *et al*. (2000) and Amos *et al*. (2004), who reported that P application with rhizobium inoculation significantly increased pod number, grain yield and dry matter yield when compared with uninoculated treatments. Phosphorus plays an important role in physiological and developmental process in plants life. N and P concentration as well as their uptake in both shoot and grain were significantly influenced by phosphorus alone and its interaction with rhizobium (P x I). This may be due to the supply of P needed for rhizobium to fix more nitrogen for plant use resulting in the increase in growth yield and N uptake by root to the shoot. However the effect was not significant in the greenhouse experiment. The genotypes x P sources as well as the other interaction were found non-significant for all parameters in both experiments except % P in shoot and grain of the greenhouse experiment.

5.7 Nutrient concentration and uptake in the three soybean genotypes.

The importance of P in symbiotic nitrogen fixation in legumes has received considerable attention in modern farming systems. Results from our studies showed that, soybean genotypes response to the concentration and uptake of N and P in both shoot and grain was not significant statistically at ($p>0.005$) but there was a remarkable difference among them when compared. TGX1955- 4F was the most efficient in taking up P in the shoot from the fertilizer P sources applied followed by TGX1904-6F and Jenguma especially in the field (Table 4.9). The uptake of nutrient reflected significantly on the growth and yield

parameter of TGX1955-4F genotype. N and P concentrations in grain were also higher in TGX1955-4F. However, P concentration in shoot and N and P uptake was higher in Jenguma than TGX1955-4F and TGX1904-6F in the greenhouse experiment (Table 4.5). The results may be attributed to the root morphology of the genotypes used. The response may also be attributed to the inherent genetic composition of the new genotypes especially for TGX1955-4F and their specific affinity to the P sources applied. According to Zhao *et al*., (2004), plants with shallow root architecture had better spatial configuration in the higher-P cultivated soil surface layer and hence had higher P efficiency and yield. Also soybean varieties are known to differ in their ability to grow under low P conditions and the more efficient varieties may exhibit internal or external mechanisms that allow greater P uptake and grain yield (Neue, 1991).This finding agrees with the reports of Solomon *et al*., (2012) that varietal differences exist in soybean with respect to N concentration. The high performance of TGX1995-4F in terms of growth, nodulation and yield showed its high affinity for P in this soil.

5.8 Effect of P sources on N &P concentrations and their Uptake in shoot.

Mineral nutrient deficiencies are the major constraints limiting legume growth and yield (O'Hara *et al*., 1988). Results from these studies have confirmed the assertion O'Hara et al. The uptake of nutrient was highly influenced by the application of the P sources when compared to the control treatment in both experiments. This showed the importance of phosphorus in soybean production, and supports the conclusion of earlier workers who showed significant effect of phosphorus on growth, nodulation and yield of soybean (Okogun *et al*. (2005); Kamara *et al*. (2007); Lamptey *et al*. (2014). The accumulation of P in the soybean resulted in corresponding improvement in growth, shoot biomass and

grain yield (Table 4.5 & 4.9). The significantly high N concentration in both shoot and grain of plants that received Morocco rock phosphate in this soil may probably be due to its chemical composition causing the synergistic effect on the N concentration and even its uptake. Nutrient interactions occur when the applied affects the uptake, distribution or function of another nutrient. According to Robson and Pitman, (1983), nutrient interaction can be assessed by examining the relationship between nutrient supply and nutrient concentration in plants or the plant growth. Morphological features of root mainly root hairs are important for plant P uptake in low-P field by expanding the absorptive surface area and increasing the soil volume (Yan *et al*. 2004). This confirms the important role phosphorus availability plays in terms of P uptake (Marschner 1993). Similar results were obtained from the field experiment.

5.9 Effect of Rhizobia Inoculation on nutrient concentration and uptake of N & P.

In the case of the greenhouse experiment, the concentration of N in shoot was high but not significantly affected by rhizobium inoculation. However, rhizobium inoculation significantly influenced uptake of P and P concentration in shoot. Also N and P concentration in grain were significantly affected by rhizobium inoculation. This is in agreement with report made by Biswas *et al*., (2000) that, rhizobium inoculation may induce an increase in number of root hairs and thereby favouring nutrient uptake by exploration of a greater soil volume. Rhizobia inoculation significantly increased uptake of P. This is contrary to the observation made by Basu *et al*., (2008) that P concentration in soybean decreased by 0.1% the same trend as N and K with respect to P concentration when soybean was inoculated. Whereas N uptake was not affected even though it was higher than that of the uninoculated treatment.

The field experiment showed that Rhizobium inoculation increased N concentration in both shoot and grain. The accumulation of nitrogen in grain was enhanced by rhizobium inoculation alone implying a positive effect of nodulation on N concentration of the soybean. Similar findings was reported by Hussain (1995) who studied N uptake in lentil with Rhizobium inoculation alone or in combination with L-TRP (L-tryptophan) and reported an increase in nitrogen contents in grains. Also Qureshi *et al*. (2009) indicated an increase in N concentration and its uptake in *Albizia lebbeck.* Rhizobium inoculation had no significant effects on P concentration, and uptake of N and P in this soil. In this case, the P concentration and the uptake of N and P were higher in the uninoculated treatment.

5.10 Interaction effects between the P sources and Rhizobium inoculation on nutrient concentration and uptake in both experiments.

The combination of P application and rhizobium inoculation had significant effect on the concentration and uptake of N and P in both experiments. This agrees with conclusion made by Rodelas *et al*. (1999) that root development and water and mineral uptake by root increased with the application of mix *Rhizobium* strain and phosphorus on faba beans. However, the interaction was not effective on N concentration in the greenhouse experiment. In general the combination of P and the inoculation P x I had no positive effects on the three genotypes in terms of growth and nutrient uptake in both experiments.

5.11 Effect of genotype, P sources and Rhizobium inoculation on total nitrogen and phosphorus uptake in grain.

The results indicate that the main effect of soybean genotypes, and P sources was significant in relation to total N and P in grain. The highest total N and P was found in TGX 1955-4F genotype. This may be due to the fact that this particular genotype was able

to take up nutrient efficiently and transported it from other plant parts to the seed at the start of seed filling as confirmed by Greenwood *et al*., (1991). Genetic factors of the soybean genotypes may have contributed to the differences in their accumulation of N and P in their grains. The main effect of rhizobium inoculation was not significant on total N and P in grain. The inability of the rhizobium inoculant to significantly influence N and P in grain of the soybean may be due to poor uptake of nutrient as has occurred in the field experiment. For the greenhouse experiment, the nutrient were not transported to the grain during seed filling. Addition of phosphorus tended to increase total N and P in grain of the soybean plant. Total P significantly increased with TSP but statistically similar to MPR in the greenhouse experiment.

6.0 CONCLUSIONS

The primary purpose of this study was to evaluate the effect of two P sources and Rhizobium inoculation on the growth, nodulation, N & P uptake and the yield performance of three soybean genotypes (TGX1955-4F, TGX1904-6F and Jenguma) in Tachera soil series. Jenguma soybean variety (TGX 1448/2E) produced the highest nodule number than either TGX1955-4F or TGX1904-6F. Among the three genotypes, however, TGX1955-4F produced more nodules with the application of MRP only followed by Jenguma and TGX1904-6F.

Application of the P sources especially TSP increased growth, and N and P uptake of soybean in both experiments. Nodulation was heavily influenced by P availability. The interaction between P and rhizobium inoculation affected various parameters such as plant height, grain yield and biomass in both experiments.

The major conclusion from the study was that the two soybean genotypes (Jenguma and TGX1955-4F) that have thrived well in the low P soil have the potential of taking up their phosphorus from the readily available P source Triple superphosphate (TSP). The study further revealed that more P was taken up from TSP than from Morocco phosphate rock (MPR). These two genotypes TGX1955-4F and Jenguma had high mean performance in terms of yield, nutrient concentration and uptake but TGX1955-4F was the highest performing genotype. It is concluded from the study that the Tanchera soil has high potential for soybean production with significant grain and biomass yield.

6.1 RECOMMENDATIONS/ SUGGESTIONS

The study had shown that application of the P sources especially TSP increased growth, and N and P uptake of soybean in both experiments. Also genotype TGX1955-4F performed better in terms of growth, nodulation, nutrient uptake and yield than the other genotypes. Therefore the genotype TGX1955-4F can be considered important and be recommended to farmers in the northern Guinea savannah zone of Ghana for better growth and improve grain and biomass yield. The farmers should use the Triple superphosphate despite its cost. However, further studies could be undertaken on the suggestions below to improve on the nodulation and yield capacity as well as the contribution capacity of TGX1955-4F and MPR to biological nitrogen fixation in the three northern regions of Ghana.

SUGGESTIONS

1. Future work should increase the application rate of 30 kg P/ha to 35 or 40 kg P/ha of MPR and the experiment should be conducted in two farming seasons in the field under rain fed conditions to ascertain it effectiveness on these genotypes especially TGX1955-4F. Estimation of the amount of N fixed should be included as one of the specific objectives of the experiment in future.

2. Future research should also increase the rate of rhizobium inoculation (Nodumax), from 7g/kg seed to 8 or 9 g/kg seed in this soil if the same inoculum should be used.

3. Future work on this soil should also apply small amount N fertilizer may be in the form of urea as a starter for early growth and development in combination with P sources and inoculants.

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APPENDICES

Analysis of variance (ANOVA) Table for the various parameters.

Greenhouse Results

Variate: Plant Height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	125.53	62.76	3.92	0.026
Fert_Sources	2	778.53	389.26	24.33	<.001
Inoculant	1	80.22	80.22	5.01	0.029
Variety.Fert_Sources	4	155.14	38.78	2.42	0.059
Variety.Inoculant	2	41.19	20.60	1.29	0.284
Fert_Sources.Inoculant	2	61.03	30.51	1.91	0.158
Variety.Fert_Sources.Inoculant	4	22.81	5.70	0.36	0.839
Residual	54	864.00	16.00		
Total	71	2128.44			

Variate: Dry matter g/pot

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	2.9815	1.4907	5.37	0.007
Fert_Sources	2	19.7614	9.8807	35.62	<.001
Inoculant	1	0.0416	0.0416	0.15	0.700
Variety.Fert_Sources	4	1.5749	0.3937	1.42	0.240
Variety.Inoculant	2	0.0597	0.0299	0.11	0.898
Fert_Sources.Inoculant	2	1.5234	0.7617	2.75	0.073
Variety.Fert_Sources.Inoculant	4	0.6957	0.1739	0.63	0.645
Residual	54	14.9793	0.2774		
Total	71	41.6173			

Variate: Pod No.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	49.361	24.681	8.16	<.001
Fert_Sources	2	2.528	1.264	0.42	0.660
Inoculant	1	5.014	5.014	1.66	0.203
Variety.Fert_Sources	4	6.306	1.576	0.52	0.720
Variety.Inoculant	2	0.528	0.264	0.09	0.917
Fert_Sources.Inoculant	2	2.528	1.264	0.42	0.660
Variety.Fert_Sources.Inoculant	4	13.806	3.451	1.14	0.347
Residual	54	163.250	3.023		
Total	71	243.319			

Variate: Pod weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	2.1381	1.0690	4.94	0.011
Fert_Sources	2	0.0956	0.0478	0.22	0.803
Inoculant	1	1.5961	1.5961	7.37	0.009
Variety.Fert_Sources	4	0.8507	0.2127	0.98	0.425
Variety.Inoculant	2	0.4531	0.2265	1.05	0.358
Fert_Sources.Inoculant	2	0.3652	0.1826	0.84	0.436
Variety.Fert_Sources.Inoculant	4	0.9500	0.2375	1.10	0.367
Residual	54	11.6909	0.2165		
Total	71	18.1395			

Variate: Grain Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	1.6519	0.8260	5.81	0.005
Fert_Sources	2	0.6506	0.3253	2.29	0.111
Inoculant	1	0.4337	0.4337	3.05	0.086
Variety.Fert_Sources	4	0.2343	0.0586	0.41	0.799
Variety.Inoculant	2	0.9161	0.4580	3.22	0.048
Fert_Sources.Inoculant	2	0.8030	0.4015	2.82	0.068
Variety.Fert_Sources.Inoculant	4	0.2595	0.0649	0.46	0.767
Residual	54	7.6771	0.1422		
Total	71	12.6262			

Variate: Harvest index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	0.035897	0.017948	7.95	<.001
Fert_Sources	2	0.038004	0.019002	8.42	<.001
Inoculant	1	0.013633	0.013633	6.04	0.017
Variety.Fert_Sources	4	0.006608	0.001652	0.73	0.574
Variety.Inoculant	2	0.007392	0.003696	1.64	0.204
Fert_Sources.Inoculant	2	0.001710	0.000855	0.38	0.686
Variety.Fert_Sources.Inoculant	4	0.008168	0.002042	0.90	0.468
Residual	54	0.121859	0.002257		
Total	71	0.233270			

Variate: %Nitrogen in Grain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	0.080811	0.040406	9.51	<.001
Fert_Sources	2	3.633953	1.816976	427.48	<.001
Inoculant	1	0.310735	0.310735	73.11	<.001
Variety.Fert_Sources	4	0.030172	0.007543	1.77	0.147
Variety.Inoculant	2	0.010178	0.005089	1.20	0.310
Fert_Sources.Inoculant	2	0.320336	0.160168	37.68	<.001
Variety.Fert_Sources.Inoculant	4	0.006289	0.001572	0.37	0.829
Residual	54	0.229525	0.004250		
Total	71	4.621999			

Variate: %Phosphorus in Grain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	0.0177333	0.0088667	19.42	<.001
Fert_Sources	2	0.7063000	0.3531500	773.63	<.001
Inoculant	1	0.0338000	0.0338000	74.04	<.001
Variety.Fert_Sources	4	0.0010667	0.0002667	0.58	0.675
Variety.Inoculant	2	0.0000000	0.0000000	0.00	1.000
Fert_Sources.Inoculant	2	0.0381000	0.0190500	41.73	<.001
Variety.Fert Sources.Inoculant	4	0.0095500	0.0023875	5.23	0.001
Residual	54	0.0246500	0.0004565		
Total	71	0.8312000			

Variate: %N in Shoot

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	0.002711	0.001356	0.34	0.715
Fert_Sources	2	0.016878	0.008439	2.10	0.132
Inoculant	1	0.003472	0.003472	0.86	0.357
Variety.Fert_Sources	4	0.009289	0.002322	0.58	0.680
Variety.Inoculant	2	0.006978	0.003489	0.87	0.425
Fert_Sources.Inoculant	2	0.013878	0.006939	1.73	0.187
Variety.Fert Sources Inoculant	4	0.008422	0.002106	0.52	0.718
Residual	54	0.216900	0.004017		
Total	71	0.278528			

Variate: %P in Shoot

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	0.00194444	0.00097222	10.10	<.001
Fert_Sources	2	0.87631944	0.43815972	4550.12	<.001
Inoculant	1	0.05335556	0.05335556	554.08	<.001
Variety.Fert_Sources	4	0.00763889	0.00190972	19.83	<.001
Variety.Inoculant	2	0.00254444	0.00127222	13.21	<.001
Fert_Sources.Inoculant	2	0.06900278	0.03450139	358.28	<.001
Variety.Fert_Sources.Inoculant	4	0.00237222	0.00059306	6.16	<.001
Residual	54	0.00520000	0.00009630		
Total	71	1.01837778			

Variate: Total Nitrogen uptake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	1766.6	883.3	8.21	<.001
Fert_Sources	2	7274.1	3637.1	33.80	<.001
Inoculant	1	200.5	200.5	1.86	0.178
Variety.Fert_Sources	4	718.2	179.6	1.67	0.171
Variety.Inoculant	2	59.4	29.7	0.28	0.760
Fert_Sources.Inoculant	2	915.9	457.9	4.26	0.019
Variety.Fert_Sources.Inoculant	4	143.7	35.9	0.33	0.854
Residual	54	5810.9	107.6		
Total	71	16889.5			

Variate: Total Phosphorus uptake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	269.55	134.78	8.79	<.001
Fert_Sources	2	4626.88	2313.44	150.83	<.001
Inoculant	1	148.15	148.15	9.66	0.003
Variety.Fert_Sources	4	92.09	23.02	1.50	0.215
Variety.Inoculant	2	12.49	6.24	0.41	0.668
Fert_Sources.Inoculant	2	321.61	160.81	10.48	<.001
Variety.Fert_Sources.Inoculant	4	25.92	6.48	0.42	0.792
Residual	54	828.23	15.34		
Total	71	6324.93			

Variate: Total Nitrogen in Grain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	1787.5	893.7	5.47	0.007
Fert_Sources	2	3855.2	1927.6	11.80	<.001
Inoculant	1	197.7	197.7	1.21	0.276
Variety.Fert_Sources	4	204.8	51.2	0.31	0.868
Variety.Inoculant	2	1185.3	592.7	3.63	0.033
Fert_Sources.Inoculant	2	1600.1	800.1	4.90	0.011
Variety.Fert_Sources.Inoculant	4	296.1	74.0	0.45	0.770
Residual	54	8821.1	163.4		
Total	71	17947.8			

Variate: Total Phosphorus in Grain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	58.727	29.364	5.52	0.007
Fert_Sources	2	407.163	203.582	38.25	<.001
Inoculant	1	2.741	2.741	0.52	0.476
Variety.Fert_Sources	4	5.239	1.310	0.25	0.911
Variety.Inoculant	2	37.554	18.777	3.53	0.036
Fert_Sources.Inoculant	2	77.627	38.814	7.29	0.002
Variety.Fert_Sources.Inoculant	4	12.203	3.051	0.57	0.683
Residual	54	287.399	5.322		
Total	71	888.653			

Field Results

Variate: Plant Height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	1854.89	618.30	6.66	
Block.Variety stratum					
Variety	2	2076.77	1038.38	11.18	0.009
Residual	6	557.30	92.88	0.80	
Block.Variety.Fert_Sources stratum					
Fert_Sources	2	3743.06	1871.53	16.12	<.001
Variety.Fert_Sources	4	546.25	136.56	1.18	0.354
Residual	18	2089.20	116.07	3.01	
Block.Variety.Fert_Sources.Inoculant stratum					
Inoculant	1	70.01	70.01	1.81	0.189
Variety.Inoculant	2	14.67	7.33	0.19	0.828
Fert_Sources.Inoculant	2	2255.34	1127.67	29.21	<.001
Variety.Fert_Sources.Inoculant	4	187.24	46.81	1.21	0.329
Residual	27	1042.47	38.61		
Total	71	14437.20			

Variate: Mean_Nodule No.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	731.71	243.90	2.43	
Block.Variety stratum					
Variety	2	627.51	313.76	3.12	0.118
Residual	6	603.32	100.55	1.19	
Block.Variety.Fert_Sources stratum					
Fert_Sources	2	71.84	35.92	0.43	0.660
Variety.Fert_Sources	4	176.05	44.01	0.52	0.721
Residual	18	1519.59	84.42	1.55	
Block.Variety.Fert_Sources.Inoculant stratum					
Inoculant	1	1946.88	1946.88	35.81	<.001
Variety.Inoculant	2	3.21	1.61	0.03	0.971
Fert_Sources.Inoculant	2	66.15	33.08	0.61	0.551
Variety.Fert_Sources.Inoculant	4	58.17	14.54	0.27	0.896
Residual	27	1467.75	54.36		

Total	71	7272.18			

Variate: Nodule Dry Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.192737	0.064246	45.28	
Block.Variety stratum					
Variety	2	0.034891	0.017446	12.30	0.008
Residual	6	0.008513	0.001419	0.11	
Block.Variety.Fert_Sources stratum					
Fert_Sources	2	0.024512	0.012256	0.99	0.392
Variety.Fert_Sources	4	0.045487	0.011372	0.92	0.476
Residual	18	0.223394	0.012411	2.47	
Block.Variety.Fert_Sources.Inoculant stratum					
Inoculant	1	0.068081	0.068081	13.57	0.001
Variety.Inoculant	2	0.000108	0.000054	0.01	0.989
Fert_Sources.Inoculant	2	0.026039	0.013020	2.60	0.093
Variety.Fert_Sources.Inoculant	4	0.008175	0.002044	0.41	0.802
Residual	27	0.135431	0.005016		
Total	71	0.767368			

Variate: Nodule Effective No.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	57.86	19.29	0.58	
Block.Variety stratum					
Variety	2	106.53	53.26	1.60	0.277
Residual	6	199.41	33.23	1.05	
Block.Variety.Fert_Sources stratum					
Fert_Sources	2	44.13	22.07	0.70	0.511
Variety.Fert_Sources	4	48.66	12.16	0.38	0.817
Residual	18	569.64	31.65	2.34	
Block.Variety.Fert_Sources.Inoculant stratum					
Inoculant	1	256.89	256.89	19.01	<.001
Variety.Inoculant	2	3.32	1.66	0.12	0.885
Fert_Sources.Inoculant	2	140.04	70.02	5.18	0.012
Variety.Fert_Sources.Inoculant	4	16.09	4.02	0.30	0.877
Residual	27	364.95	13.52		
Total	71	1807.50			

Variate: Pod No.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	194524.	64841.	3.51	
Block.Variety stratum					
Variety	2	123518.	61759.	3.35	0.106
Residual	6	110717.	18453.	2.44	
Block.Variety.Fert_Sources stratum					
Fert_Sources	2	91514.	45757.	6.05	0.010
Variety.Fert_Sources	4	18748.	4687.	0.62	0.654
Residual	18	136171.	7565.	0.48	
Block.Variety.Fert_Sources.Inoculant stratum					
Inoculant	1	1431.	1431.	0.09	0.766
Variety.Inoculant	2	9304.	4652.	0.29	0.747
Fert_Sources.Inoculant	2	6854.	3427.	0.22	0.806
Variety.Fert_Sources.Inoculant	4	32305.	8076.	0.51	0.728
Residual	27	426827.	15808.		
Total	71	1151914.			

Variate: Pod weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	7371.7	2457.2	3.27	
Block.Variety stratum					
Variety	2	326.0	163.0	0.22	0.811
Residual	6	4512.0	752.0	1.86	
Block.Variety.Fert_Sources stratum					
Fert_Sources	2	2785.5	1392.7	3.44	0.054
Variety.Fert_Sources	4	1844.1	461.0	1.14	0.370
Residual	18	7291.7	405.1	1.13	
Block.Variety.Fert_Sources.Inoculant stratum					
Inoculant	1	1181.5	1181.5	3.30	0.080
Variety.Inoculant	2	140.8	70.4	0.20	0.823
Fert_Sources.Inoculant	2	216.0	108.0	0.30	0.742
Variety.Fert_Sources.Inoculant	4	2029.3	507.3	1.42	0.255
Residual	27	9661.5	357.8		
Total	71	37360.1			

Variate: Grain Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	953314.	317771.	3.94	
Block.Variety stratum					
Variety	2	1577897.	788949.	9.78	0.013
Residual	6	484034.	80672.	2.58	
Block.Variety.Fert_Sources stratum					
Fert_Sources	2	460835.	230418.	7.36	0.005
Variety.Fert_Sources	4	84788.	21197.	0.68	0.617
Residual	18	563854.	31325.	1.71	
Block.Variety.Fert_Sources.Inoculant stratum					
Inoculant	1	43253.	43253.	2.36	0.136
Variety.Inoculant	2	36172.	18086.	0.99	0.385
Fert_Sources.Inoculant	2	309793.	154896.	8.47	0.001
Variety.Fert_Sources.Inoculant	4	55804.	13951.	0.76	0.559
Residual	27	493945.	18294.		
Total	71	5063688.			

Variate: Biomass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	1461361.	487120.	1.16	
Block.Variety stratum					
Variety	2	446350.	223175.	0.53	0.614
Residual	6	2526258.	421043.	1.61	
Block.Variety.Fert_Sources stratum					
Fert_Sources	2	6756350.	3378175.	12.92	<.001
Variety.Fert_Sources	4	262133.	65533.	0.25	0.905
Residual	18	4705845.	261436.	1.57	
Block.Variety.Fert_Sources.Inoculant stratum					
Inoculant	1	3559260.	3559260.	21.36	<.001
Variety.Inoculant	2	161443.	80721.	0.48	0.621
Fert_Sources.Inoculant	2	5661327.	2830664.	16.99	<.001
Variety.Fert_Sources.Inoculant	4	459958.	114989.	0.69	0.605
Residual	27	4499297.	166641.		
Total	71	30499582.			

Variate: Harvest index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.022058	0.007353	1.75	
Block.Variety stratum					
Variety	2	0.047815	0.023908	5.69	0.041
Residual	6	0.025210	0.004202	2.74	
Block.Variety.Fert_Sources stratum					
Fert_Sources	2	0.024881	0.012440	8.10	0.003
Variety.Fert_Sources	4	0.002538	0.000634	0.41	0.797
Residual	18	0.027652	0.001536	1.44	
Block.Variety.Fert_Sources.Inoculant stratum					
Inoculant	1	0.006850	0.006850	6.44	0.017
Variety.Inoculant	2	0.000939	0.000469	0.44	0.648
Fert_Sources.Inoculant	2	0.002528	0.001264	1.19	0.320
Variety.Fert_Sources.Inoculant	4	0.001282	0.000320	0.30	0.874
Residual	27	0.028706	0.001063		
Total	71	0.190457			

Variate: %N in Shoot

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.014156	0.004719	1.06	
Block.Variety stratum					
Variety	2	0.016878	0.008439	1.90	0.230
Residual	6	0.026678	0.004446	1.20	
Block.Variety.Fert_Sources stratum					
Fert_Sources	2	0.026678	0.013339	3.59	0.049
Variety.Fert_Sources	4	0.030489	0.007622	2.05	0.130
Residual	18	0.066967	0.003720	0.57	
Block.Variety.Fert_Sources.Inoculant stratum					
Inoculant	1	1.046422	1.046422	160.17	<.001
Variety.Inoculant	2	0.016878	0.008439	1.29	0.291
Fert_Sources.Inoculant	2	0.114878	0.057439	8.79	0.001
Variety.Fert_Sources.Inoculant	4	0.017422	0.004356	0.67	0.621
Residual	27	0.176400	0.006533		
Total	71	1.553844			

Variate: %Phosphorus in Shoot

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.01536	0.00512	0.30	
Block.Variety stratum					
Variety	2	0.02132	0.01066	0.63	0.564
Residual	6	0.10148	0.01691	2.41	
Block.Variety.Fert_Sources stratum					
Fert_Sources	2	4.77992	2.38996	339.85	<.001
Variety.Fert_Sources	4	0.00910	0.00227	0.32	0.859
Residual	18	0.12658	0.00703	0.57	
Block.Variety.Fert_Sources.Inoculant stratum					
Inoculant	1	0.00080	0.00080	0.06	0.802
Variety.Inoculant	2	0.06691	0.03345	2.69	0.086
Fert_Sources.Inoculant	2	0.49297	0.24649	19.83	<.001
Variety.Fert_Sources.Inoculant	4	0.17039	0.04260	3.43	0.022
Residual	27	0.33562	0.01243		
Total	71	6.12046			

Variate: Total Nitrogen uptake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	1273.2	424.4	1.16	
Block.Variety stratum					
Variety	2	284.3	142.1	0.39	0.695
Residual	6	2202.4	367.1	1.69	
Block.Variety.Fert_Sources stratum					
Fert_Sources	2	5341.4	2670.7	12.28	<.001
Variety.Fert_Sources	4	328.0	82.0	0.38	0.822
Residual	18	3915.0	217.5	1.52	
Block.Variety.Fert_Sources.Inoculant stratum					
Inoculant	1	936.4	936.4	6.55	0.016
Variety.Inoculant	2	68.0	34.0	0.24	0.790
Fert_Sources.Inoculant	2	5630.8	2815.4	19.70	<.001
Variety.Fert_Sources.Inoculant	4	422.8	105.7	0.74	0.573
Residual	27	3857.9	142.9		
Total	71	24260.1			

Variate: Total Phosphorus uptake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	497.27	165.76	2.06	
Block.Variety stratum					
Variety	2	148.14	74.07	0.92	0.448
Residual	6	482.04	80.34	0.94	
Block.Variety.Fert_Sources stratum					
Fert_Sources	2	5919.87	2959.93	34.51	<.001
Variety.Fert_Sources	4	80.84	20.21	0.24	0.915
Residual	18	1544.08	85.78	2.22	
Block.Variety.Fert_Sources.Inoculant stratum					
Inoculant	1	1183.57	1183.57	30.63	<.001
Variety.Inoculant	2	126.75	63.38	1.64	0.213
Fert_Sources.Inoculant	2	3264.99	1632.50	42.25	<.001
Variety.Fert_Sources.Inoculant	4	143.42	35.85	0.93	0.462
Residual	27	1043.19	38.64		
Total	71	14434.17			

Variate: Nitrogen concentration in Grain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.008194	0.002731	0.66	
Block.Variety stratum					
Variety	2	0.021519	0.010760	2.59	0.154
Residual	6	0.024881	0.004147	0.92	
Block.Variety.Fert_Sources stratum					
Fert_Sources	2	2.800078	1.400039	310.83	<.001
Variety.Fert_Sources	4	0.047514	0.011878	2.64	0.068
Residual	18	0.081075	0.004504	0.50	
Block.Variety.Fert_Sources.Inoculant stratum					
Inoculant	1	0.523606	0.523606	57.67	<.001
Variety.Inoculant	2	0.022669	0.011335	1.25	0.303
Fert_Sources.Inoculant	2	1.135478	0.567739	62.53	<.001
Variety.Fert_Sources.Inoculant	4	0.021297	0.005324	0.59	0.675
Residual	27	0.245150	0.009080		
Total	71	4.93146			

Variate: %P_concentration in Grain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.0006931	0.0002310	0.62	
Block.Variety stratum					
Variety	2	0.0032250	0.0016125	4.29	0.070
Residual	6	0.0022528	0.0003755	1.52	
Block.Variety.Fert_Sources stratum					
Fert_Sources	2	0.3573583	0.1786792	724.10	<.001
Variety.Fert_Sources	4	0.0004667	0.0001167	0.47	0.755
Residual	18	0.0044417	0.0002468	0.47	
Block.Variety.Fert_Sources.Inoculant stratum					
Inoculant	1	0.0008681	0.0008681	1.66	0.209
Variety.Inoculant	2	0.0009528	0.0004764	0.91	0.415
Fert_Sources.Inoculant	2	0.0087028	0.0043514	8.31	0.002
Variety.Fert_Sources.Inoculant	4	0.0061889	0.0015472	2.95	0.038
Residual	27	0.0141375	0.0005236		
Total	71	0.3992875			

Variate: Total Nitrogen in Grain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	1544.14	514.71	4.15	
Block.wplot stratum					
Variety	2	2615.54	1307.77	10.54	0.011
Residual	6	744.51	124.09	2.55	
Block.wplot.splot stratum					
Fert_Sources	2	189.31	94.66	1.95	0.172
Variety.Fert_Sources	4	126.03	31.51	0.65	0.636
Residual	18	875.93	48.66	1.59	
Block.wplot.splot.Ssplot stratum					
Inoculant	1	5.11	5.11	0.17	0.686
Variety.Inoculant	2	66.02	33.01	1.08	0.354
Fert_Sources.Inoculant	2	1001.01	500.50	16.35	<.001
Variety.Fert_Sources.Inoculant	4	96.64	24.16	0.79	0.542
Residual	27	826.76	30.62		
Total	71	8090.99			

Variate: Total Phosphorus in Grain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	39.1234	13.0411	4.11	
Block.wplot stratum					
Variety	2	68.2831	34.1416	10.77	0.010
Residual	6	19.0273	3.1712	2.51	
Block.wplot.splot stratum					
Fert_Sources	2	8.7986	4.3993	3.49	0.053
Variety.Fert_Sources	4	5.3208	1.3302	1.05	0.408
Residual	18	22.7213	1.2623	1.87	
Block.wplot.splot.Ssplot stratum					
Inoculant	1	1.9076	1.9076	2.82	0.105
Variety.Inoculant	2	1.9876	0.9938	1.47	0.248
Fert_Sources.Inoculant	2	10.0181	5.0090	7.41	0.003
Variety.Fert_Sources.Inoculant	4	1.9720	0.4930	0.73	0.580
Residual	27	18.2590	0.6763		
Total	71	197.4188			

Interaction effects between the P sources & Inoculation on Plant growth, and yield component of the three soybean genotypes (Greenhouse Results).

Genotype	Treatment	Plant Height (cm/pot) 10WAP	Biomass (g/pot) at harvest	Pod No/pot at harvest	Pod wt. (g/pot)	Grain yield (g/pot)	Harvest Index
JENGUMA	P_oI_o	34	4.01	13	3.56	2.03	0.30
	P_oI_1	35	4.67	12	3.33	2.51	0.31
	P_1I_o	45	5.38	12	3.26	2.34	0.27
	P_1I_1	49	5.15	11	3.09	2.29	0.28
	P_2I_o	42	4.57	11	3.20	2.35	0.30
	P_2I_1	40	4.86	11	3.26	2.33	0.29
		40.83	**4.77**	**11.67**	**3.28**	**2.31**	**0.29**
TGX1904-6F	P_oI_o	38	3.56	10	2.70	1.85	0.31
	P_oI_1	44	3.93	11	2.75	1.85	0.29
	P_1I_o	45	5.41	12	3.25	2.40	0.27
	P_1I_1	51	5.1	9	2.68	1.65	0.21
	P_2I_o	42	4.18	11	3.45	2.39	0.33
	P_2I_1	43	3.95	10	2.47	1.92	0.29
		43.83	**4.36**	**10.5**	**2.88**	**2.01**	**0.28**
TGX1955-4F	P_oI_o	39	2.94	10	3.11	2.17	0.39
	P_oI_1	41	3.64	9	2.96	2.12	0.33
	P_1I_o	44	5.15	10	3.41	2.54	0.30
	P_1I_1	45	4.72	10	3.20	2.40	0.30
	P_2I_o	43	3.83	10	3.49	2.62	0.37
	P_2I_1	43	4.35	9	3.00	2.23	0.30
		42.5	**4.11**	**9.67**	**3.2**	**2.35**	**0.33**
LSD ($p< 0.05$)		5.67	0.77	2.47	0.66	0.54	0.06
CV (%)		9.5	12.4	16.7	14.9	17	14.8

Mean with the same letter are not significant. P_oI_o, = control, P_oI_1, = Inoculation only, P_1I_o = Triple super phosphate (TSP) without inoculation, P_1I_1, = Triple super phosphate (TSP) with inoculation, P_2I_o, = Morocco rock phosphate (MRP) without inoculation and P_2I_1 = Morocco rock phosphate (MRP) with inoculation respectively.

Interaction effects between the Genotypes, P sources & Rhizobium Inoculation on nutrient concentration and uptake in the three soybean genotypes (Greenhouse Results).

Genotype	Treatment	N & P concentration in Shoot		N & P uptake (Shoot)		N & P concentration in Grain		Total N & P in Grain	
		% Nitrogen	% Phosphorus	N (mg/pot)	P (mg/pot)	% Nitrogen	% Phosphorus	N (mg/pot)	P (mg/pot)
JENGUMA	P_oI_o	1.87	0.48	75**g**	19	3.4	0.52	58	9
	P_oI_1	1.89	0.64	88	30	3.75	0.58	80	12
	P_1I_o	1.85	0.77	99	41	4.1	0.76	82	15
	P_1I_1	1.85	0.78	95	40	4.13	0.8	80	16
	P_2I_o	1.93	0.75	88	34	4.05	0.74	81	15
	P_2I_1	1.91	0.76	93	37	4.14	0.76	82	15
		1.88	**0.7**	**89.7**	**33.5**	**3.93**	**0.69**	**77.17**	**13.67**
TGX1904-6F	P_oI_o	1.87	0.43	67	15	3.56	0.52	56	8
	P_oI_1	1.91	0.6	75	23	3.85	0.64	60	10
	P_1I_o	1.84	0.76	99	41	4.14	0.81	84	17
	P_1I_1	1.91	0.79	97	40	4.16	0.81	58	11
	P_2I_o	1.89	0.75	79	31	4.15	0.77	84	16
	P_2I_1	1.91	0.76	75	30	4.13	0.78	67	13
		1.89	**0.68**	**82**	**30**	**4**	**0.72**	**68.17**	**12.5**
TGX1955-4F	P_oI_o	1.87	0.47	55	14	3.54	0.53	65	10
	P_oI_1	1.93	0.57	70	21	3.86	0.66	69	12
	P_1I_o	1.89	0.77	97	39	4.13	0.81	89	18
	P_1I_1	1.89	0.79	89	37	4.18	0.8	85	16
	P_2I_o	1.94	0.76	74	29	4.13	0.78	92	17
	P_2I_1	1.87	0.75	81	33	4.18	0.79	79	15
		1.9	**0.69**	**77.7**	**28.8**	**4**	**0.73**	**79.83**	**14.67**
LSD (p< 0.05)		0.09	0.01	14.7	5.6	0.09	0.03	18.1	3.3
CV (%)		3.4	1.4	12.5	12.7	1.6	3	17	17

Interaction effects between Genotypes, P sources & Inoculation on Plant growth, nodulation capacity and grain yield (Field Results).

Genotype	Treatment	Plant height (cm) 10WAP	Nod No./10 plants	Nod Dry wt./10 plants	Nod Effective No./10 plants	Pod No.	Pod wt. (kg/ha)	Grain Yield (kg/ha)	Biomass weight (kg/ha)	HI
JENGUMA	P_oI_o	56	18	0.15	8	473	1201	718	2310	0.24
	P_oI_1	64	28	0.24	15	404	1022	841	2625.0	0.24
	P_1I_o	88	20	0.21	12	543	1230	696	3179.2	0.18
	P_1I_1	71	34	0.2	14	480	1230	598	2168.6	0.21
	P_2I_o	64	18	0.08	10	452	960	654	2202.1	0.23
	P_2I_1	69	26	0.17	14	547	1230	654	1962.5	0.25
Mean		**68**	**24**	**0.18**	**12**	**483.2**	**1160.5**	**693.5**	**2407.99**	**0.23**
TGX1904-6F	P_oI_o	65	20	0.13	9	332	960	831	2295.8	0.26
	P_oI_1	80	33	0.27	17	407	1140	971	2775.0	0.26
	P_1I_o	97	22	0.17	12	414	1050	787	3420.8	0.19
	P_1I_1	75	31	0.19	15	451	1231	637	2183.3	0.23
	P_2I_o	66	26	0.21	14	373	1050	797	2289.6	0.26
	P_2I_1	63	33	0.24	16	382	1140	502	1470.8	0.26
Mean		**74**	**27**	**0.2**	**14**	**393.2**	**920.17**	**754.17**	**2405.9**	**0.24**
TGX1955-4F	P_oI_o	76	24	0.2	9	345	990	1044	2616.7	0.28
	P_oI_1	84	38	0.3	18	349	1140	1191	2725.0	0.30
	P_1I_o	96	29	0.24	17	474	1261	1152	3320.8	0.26
	P_1I_1	84	39	0.28	18	466	1590	988	2412.5	0.29
	P_2I_o	75	25	0.15	14	374	1022	985	2529.2	0.28
	P_2I_1	76	33	0.2	15	37	960	84	1839.6	0.31
Mean		**81**	**31**	**0.23**	**15**	**397.3**	**1160.5**	**1033.5**	**2573.96**	**0.29**
LSD ($p<0.05$)		12.26	12.1	0.12	6.8	165.7	431.3	263.4	701.84	0.06
CV (%)		8.3	23.6	35.1	26.5	29.6	23.9	16.4	16.6	12.9

❖ **Mean with the same letter are not significant. P_oI_o, = control, P_oI_1, = inoculation, P_1I_o = Triple super phosphate (TSP) without inoculation, P_1I_1, = Triple super phosphate (TSP) with inoculation, P_2I_o, = Morocco rock phosphate (MRP) without inoculation and P_2I_1 = Morocco rock phosphate (MRP) with inoculation respectively.**

Interaction effects between the Genotypes, P sources & Rhizobium Inoculation on nutrient concentration and uptake (Field Results).

Genotype	Treatment	N & P Concentration in Shoot		N & P Uptake (Shoot)		N & P Concentration in Grain		Total N & P in Grain (Field)	
		% Nitrogen	% Phosphorus	N (kg/ha)	P (kg/ha)	% Nitrogen	% Phosphorus	N (kg/ha)	P (kg/ha)
JENGUMA	P_oI_o	2.77	0.98	63.71	22.47	3.45	0.49**a**	24.75	3.5
	P_oI_1	3.05	1.28	79.83	33.3	3.95	0.53	33.24	4.4
	P_1I_o	2.87	1.82	91.38	57.94	3.99	0.67	27.79	4.26
	P_1I_1	2.94	1.74	63.76	37.46	4.05	0.69	24.06	4.09
	P_2I_o	2.87	1.64	63.32	35.88	4.12	0.65	26.96	4.65
	P_2I_1	3.12	1.47	61.08	28.95	4.06	0.63	26.56	4.13
Mean		**2.94**	**1.49**	**70.5**	**36**	**3.94**	**0.61**	**27.23**	**4.17**
TGX1904-6F	P_oI_o	2.77	1.08	63.66	24.66	3.37	0.53	28.02	4.38
	P_oI_1	3.08	1.25	85.51	34.48	3.86	0.52	37.51	5.03
	P_1I_o	2.8	1.94	95.78	66.38	4.1	0.67	33.06	5.27
	P_1I_1	2.98	1.56	64.91	34.15	4.02	0.71	20.82	3.18
	P_2I_o	2.77	1.55	63.36	35.65	4.15	0.66	32.21	5.28
	P_2I_1	3.05	1.54	44.7	22.79	4.14	0.63	25.61	4.48
Mean		**2.91**	**1.49**	**69.65**	**36.35**	**3.94**	**0.62**	**29.54**	**4.6**
TGX1955-4F	P_oI_o	2.73	1.09	71.42	28.34	3.42	0.55	35.64	5.67
	P_oI_1	3.01	1.27	81.66	34.2	4	0.52	47.67	6.24
	P_1I_o	2.73	1.86	93.93	61.62	4.03	0.67	40.83	6.34
	P_1I_1	3.12	1.74	72.1	42.02	4.1	0.72	34.86	5.48
	P_2I_o	2.84	1.51	69 09	38.22	4.15	0.64	46.47	7.7
	P_2I_1	2.98	1.68	57 20	30.83	4.14	0.65	40.6	7.1
Mean		**2.9**	**1.53**	**74.23**	**39.21**	**3.97**	**0.63**	**41.01**	**6.42**
LSD ($p<0.05$)		0.1	0.15	20.5	11.17	0.12	0.03	10.453	1.645
CV (%)		2.8	7.4	16.7	16.7	2.4	3.7	17	16.2