EFFECT OF RHIZOBIA INOCULATION AND PHOSPHORUS FERTILIZER ON NODULATION AND YIELD OF SOYBEAN [*Glycine max* (L.) Merril] IN DEDZA, KASUNGU AND SALIMA DISTRICTS OF MALAWI

MASTER OF SCIENCE IN AGRONOMY

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UNIVERSITY OF MALAWI

BUNDA COLLEGE OF AGRICULTURE

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BSc. (Forestry), Malawi

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DECLARATION

I, Donald Siyeni, declare that this thesis is a result of my own original effort and work, and that to the best of my knowledge, the findings have never been presented to the University of Malawi or elsewhere for the award of any academic qualification. Where assistance was sought, it has been accordingly acknowledged.

Donald Siyeni

Signature_____________________________

Date__________________________________

CERTIFICATE OF APPROVAL

We, the undersigned, certify that this thesis is a result of the author's own work, and that to the best of our knowledge, it has not been submitted for any other academic qualification within the University of Malawi or elsewhere. The thesis is acceptable in form and content, and that satisfactory knowledge of the field covered by the thesis was demonstrated by the candidate through an oral examination held on 3rd February, 2016.

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DEDICATION

Regardless of our own upbringing, it’s up to us to willingly bend His loving hand and send forth strong arrows. The children of this world deserve nothing less_neither do you!!!

AMON LWENDA SIYENI; YOU DESERVE TO SEE YOUR DREAM!!!

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ABSTRACT

Malawi is experiencing a rise in soybean [*Glycine max* (L.) Merr.] production due to its widespread use in the food and feed industry. Productivity is, however, constrained by high nutrient depletion and land degradation. Unless efforts are taken in confronting the problems of nutrient depletion and land degradation, deteriorating productivity will seriously undermine the foundations of sustainable agricultural production. On-farm researcher-designed farmer managed trials were, therefore, conducted in Dedza (Linthipe EPA), Kasungu (Kaluluma EPA) and Salima (Chinguluwe EPA) districts of Malawi during 2012/2013 cropping season to evaluate the response of promiscuous and non-promiscuous soybean varieties to inoculation, and inorganic phosphorus fertilizer application. Firstly, two inoculated soybean varieties (Nasoko and Tikolore) were evaluated for their response to P fertilizer application (0 and 30 kg P_2O_5/ha) in Dedza, Salima and Kasungu districts. Secondly, P fertilizer was applied to determine its individual and interactive effect with *Rhizobia* inoculant on the nodulation, BNF and grain yield and yield components of promiscuous Tikolore soybean variety in Kasungu and Salima districts. The two experiments were laid out in a randomised complete block design (RCBD) with farmers as replicates. The modified nitrogen difference method was used to measure the amount of N_2 fixed and partial budget analysis was used to assess the economic feasibility of fertiliser and inoculum application to soybean. The combined effect of P fertilizer and *Rhizobia* inoculant significantly increased nodulation, BNF, yield and yield components of the soybean varieties with grain yield increase of 30% in the Tikolore promiscuous variety and 18% increase in Nasoko specific variety. Regression analysis showed some compelling effect of site parameters and yield components as likely predictors of soybean growth and development. Results suggest that P fertilizer application to soybean is needed in soils with available P below the critical level (<25ppm, Mehlich 3) and the *Rhizobia* inoculant application is also an important technology option. The synergy of *Rhizobia* inoculant and P fertilizer at 30 kg P_2O_5/ha contribute to increased yields, BNF and economic returns for smallholder farmers cultivating in responsive soils.

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LIST OF ABBREVIATIONS AND ACRONYMS

BNF	Biological nitrogen fixation
EPA	Extension Planning Area
CEC	Cation exchange capacity
OM	Organic matter
OC	Organic carbon
FAO	Food and Agricultural Organisation
IFPRI	International Food Policy Research Institute
IITA	International Institute for Tropical Agriculture
MGDS	Malawi Growth and Development Strategy
N_2	Nitrogen
P	Phosphorus
SSA	sub-Saharan Africa
UNDP	United Nation Development Programme
UNICEF	United Nations Children’s Fund

CHAPTER ONE

INTRODUCTION

1.1 Grain legumes and their importance in agriculture

The term "grain legumes" or "pulses" refer to leguminous plants producing dry edible seeds (Howieson*et al.*, 2000). Major grain legume species traditionally grown in the tropics include cowpea (*Vigna unguiculata* (L.) Walp.), black gram (*V. mungo* (L) Hepper), green gram (*V. radiata* (L.) Wilczek), common bean (*Phaseolusvulgaris* (L.)), lima beans (*P. lunatus*), pigeon pea (*Cajanus cajan* (L.) Millsp.), groundnut (*Arachis hypogaea L.*), bambara nut (*Vorandzeia subterranean L.*), chick pea (*Cicer arientum* L.) and soybean (*Glycine max* (L.) Merr.) (Raemaekers, 2001). Grain legumes are well known to contribute significantly towards reducing poverty, improving food security, improving nutrition and health, and sustaining the natural resource base (Rusike *et al.*, 2013). In Malawi, cowpea, groundnut, pigeon pea, common bean, soybean and bambara groundnut are grown across all the three regions for food security, improved nutrition, income and helping to maintain soil fertility (Abate *et al.*, 2012). The goal behind sustainable agriculture is to improve and sustain soil fertility and productivity with fewer external inputs (Peoples *et al.*, 1995). This is particularly crucial for the management of nitrogen (N); the nutrient that most limits plant growth in the majority of smallholder cropping systems in Malawi. Grain legumes have the inherent potential to supply this important soil nutrient in cereal based cropping systems through biological nitrogen fixation (BNF). The ability of legumes to fix atmospheric N_2 in symbiosis with *Rhizobia* strains makes them excellent colonizers of low-N environments (Graham and Vance, 2003). However, *Rhizobia* strains differ in their N_2 fixation efficiency and effectiveness. Likewise, grain legumes vary in their N contributions to cropping systems depending on the proportion of plant N removed in harvested seed and that from fixation (Salvagiotti *et al.*, 2008). The efficiency of the legumes to fix N biologically is affected by various factors such as soil moisture, temperature, available soil nutrients, biotic and abiotic stresses and the presence of efficient, competitive *Rhizobia* strains, cropping systems and field management practices (Thies *et al.,* 1995; Palmar and Young, 2000; Kiers *et al.,* 2003). Ojiem *et al.* (2007), Nyemba and Dakora (2010) and Mhango (2011) reported 22 to 124 kg/ha of total N fixed by groundnut under different cropping systems while Adu-Gyamfi *et al.* (2007) reported biological N_2 fixation of 20 to 118 kg/ha by pigeon pea. Common bean ranks low compared to most other legumes with reported N_2 fixation of less than 31 kg/ha/year (Hardarson *et al.*, 1993; Ojiem *et al.*, 2007). Efforts to optimize nodulation and BNF in grain legumes are critical challenges because of widespread increase in soil degradation in Africa.

Despite the numerous factors that compromise nodulation and nitrogen fixation, legumes generally assimilate 50 to 70% of their nitrogen from symbiotic nitrogen fixation (Vance, 1997). Relative to other grain legumes, soybeans vary in their ability to utilize nitrogen from N fixation in both irrigated and rain-fed production systems. On average, soybean N fixation under irrigation is typically around 175 kg N/ha/year, though amounts exceeding 300 kgNha/yearhave occasionally been recorded (Peoples *et al.,* 1995). Average N fixation under dryland conditions appears to be around 100 kg N/ha/year for soybean though up to 450 kg/ha/year of N in shoots being fixed in soybeans grown without supplementary water has been reported (Rennie *et al.,* 1988; Peoples and Crasswell, 1992).

In Malawi, production of tropical grain legumes is an activity mostly done by smallholder farmers (Edriss, 2003; Abate*et al.*, 2012). Despite previous investments in research and development having improved the productivity and quality performance of grain legumes, production challenges remain major setbacks to improved grain legumes productivity in smallholder farmers' fields in Malawi. The decrease in productivity of grain legumes is a consequence of several constraints that these smallholder farmers encounter. These constraints include use of low yielding varieties, unreliable rainfall, declining soil fertility, pests and diseases, inadequate support services and a clash in labour demand (Kumwenda and Madola, 2005). The general observation is that nutrient depletion of soil is a particular problem for small landholders in developing countries, where much grain legume production occurs, and many farmers cannot afford to use fertilizers. Sanchez (2002) suggests average annual nutrient depletion rates across 37 African countries of 22 kgN/ha, 2.5 kgP_2O_5/ha, and 15 kgK_2O/ha. Grain legumes are also susceptible to climate change and variability; both drought and heat can severely limit their productivity (Lipiec *et al.*, 2013). Unreliable markets have also hampered production of grain legumes in the country (Rusike *et al.*, 2013). Depending on the constraints, smallholder farmers end up choosing a particular grain legume for production looking at area specific requirements and advantages. For instance, the relay planting of common beans is a characteristic of the Blantyre/Shire Highlands of Southern Malawi, the Mwera Hills in Central Malawi, the Phoka Hills and Misuku Hills in Northern Malawi where off-season rains are utilized for a second crop of beans. The winter crop, grown in *dimbas,* under residual moisture, or irrigated conditions, is important mostly in lakeshore areas of Lake Malawi and in many riverine and flood plain areas (Mloza-Banda *et al.,* 2003). The groundnut crop is mainly produced in the Lilongwe-Kasungu plain, the Mchinji district in the central region of

Malawi and some areas in Salima as well as along the lakeshore owing to its wide adaptation to various environmental conditions (Ngulube *et al.,* 2001). The bulk of pigeon pea is confined to the southern region of Malawi as livestock damage to pigeon pea deters the adoption of pigeon pea production in the central and some part of northern Malawi. The long duration pigeon pea varieties take about 210-270 days to mature. As such, the crop is more prone to livestock damage in the central and some part of northern Malawi as farmers release their goats and cows to freely feed in the fields after harvesting maize.

In order to expand grain legume production to other areas and improve productivity, it is important to address some of the constraints and characterize the potential for a particular grain legume in a particular area based on concomitant management requirements as defined by the interplay of many factors, including climate, soil type and a range of socio-economic and biological factors (Mkandawire *et al.,* 1995). Research and development efforts in improving the production and productivity of the grain legumes in Malawi should focus on the adaptation of germplasm and means of production more precisely to specific environments and where development of appropriate technologies is based on a good understanding of constraints and opportunities of the grain legume growing ecosystems (Mloza-Banda *et al.,* 2003).

1.2 Importance of soybean

Malawi faces chronic food shortages leading to malnutrition, hunger and poverty. Malnutrition, particularly protein deficiency, is prevalent in many parts of Malawi as animal protein is too expensive for most populations (IFPRI, 2011). Despite efforts by government and development partners to improve Malawians' nutritional status, nutrition disorders continue to be widespread in the country. This poses a serious challenge to the attainment of the national growth and development goals as set out in the Malawi Growth and Development Strategy (MGDS) (FAO, 2010). Malnutrition contributes to approximately 55% of under-five child mortality and approximately 25% of Malawian children do not reach the age of 5 years and the United Nations Children's Fund (UNICEF) estimates that 22% of children younger than 5 years of age are underweight and 48% suffer from stunting (UNDP-Malawi, 2005). Bezner Kerr *et al.* (2008) reported that edible legumes which are an excellent source of dietary proteins and oils can play an important role in meeting food needs in present situation of food shortage and widespread malnutrition. In light of these circumstances, soybean has thus been variously described as a "miracle bean" or a "golden bean" because it is a relatively cheap and protein-rich grain (Sanginga *et al*., 1999). It contains 40% high quality protein, 20% edible vegetable oil and a good balance of amino acids (Fekadu *et al.,* 2009; Mahamood*et al.*, 2009) and has, therefore, tremendous potential to improve the nutritional status and welfare of the resource-poor families. Many leguminous crops provide some protein, but soybean is the only available crop that provides an inexpensive and high quality source of protein comparable to meat, poultry and eggs. Soybean protein has great potential as a major source of dietary protein. The oil produced from soybean is highly digestible and contains no cholesterol. Soybean cake, a by-product from the oil production, is used as a high-protein animal feed in many countries (FAO, 2008).

Soybean contributes to sustainable cropping systems by improving soil fertility through biological nitrogen fixation. It also provides useful crop residues for animal feed or left in the field to decompose, thereby increasing the organic matter content of the soil (Soko, 2000). Most African countries, including Malawi, can reduce expenditures on inorganic fertilizers through exploitation of atmospheric BNF (Giller, 2001). This is particularly important for resource poor farmers whose economics of inorganic fertilizer use is not attractive, a situation worsened by the escalating prices of fertilizers.

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

1.3 Benefits of inoculating soybean with *Rhizobia*

Rhizobia and legumes have specific requirements, and they must be properly matched. Soybean production requires good supply of N for high seed yield. However, like many other annual legumes, the crop has the ability to meet most of its N requirement through inoculation with *BradyRhizobia* (Kumaga and Ofori, 2004). Inoculation, defined as the process of adding effective bacteria to the host plant seed before planting with the purpose of ensuring that there is enough of the correct type of bacteria present in the soil for a successful establishment of legume-bacterial symbiosis (Benizri *et al.*, 2001), is a significant technology for the manipulation of *Rhizobia* for improving crop productivity and soil fertility through N_2-fixation (Keyser and Li, 1992). It is simply the process of applying suitable live *Rhizobia* to the soil where they can infect the roots and form effective nodules. It can lead to the establishment of large *Rhizobia* population in the rhizosphere and improved nodulation and N_2-fixation even under adverse soil conditions (Peoples *et al.*, 1995).

Inoculation of specific soybean varieties with *Rhizobia* is an important process to maximize BNF capacity in this crop. It has the potential of increasing dry matter yield, nitrogen yield, and residual N levels (Javaid andMahmood, 2010). This group of soybean varieties is different from promiscuous soybean varieties which nodulate with a wide range of *Rhizobia* strains. Promiscuous soybean varieties have the ability to perform well without inoculation with *Rhizobia* strain inoculants (Mpepereki *et al.*, 2000). Currently, Magoye and Tikolore are the only released promiscuous soybean varieties for cultivation by smallholder farmers in Malawi.

However, the performance of specific or promiscuous legumes is a function of legume species or variety in a particular environment. Yield responses to inoculation, even in fields with soil *Rhizobia* populations sufficient to infect the particular legume host, are common (but not universal) on-farm (Vessey, 2004). The high diversity in smallholder farming systems is often not accounted for in reporting yield differences in soybean and other grain legumes. Ignoring the heterogeneity within smallholder farming system has also been reason for the low success of inoculation technology interventions (Zingore *et al.*, 2007). The understanding of unique farm attributes could improve the development of appropriate inoculation and fertilization technologies for soybean in spatially heterogeneous farms in Malawi.

1.4 Importance of phosphorus supply to plant growth

No soil can sustain high yields if it is deficient in P (Rao *et al.*, 1999). Phosphorus is one of the essential macronutrients required by plants. As an essential plant nutrient, P is involved in a wide range of plant processes from permitting cell division to the development of a good root system to ensuring timely and uniform ripening of the crop. P is needed most by young, fast-growing tissues, and performs a number of functions related to growth, development, photosynthesis, and utilization of carbohydrates (Rao, 1996). P is a constituent of adenosine diphosphate (ADP) and adenosine triphosphate (ATP), two of the most important substances in life processes. ATP is a source of energy for physiological processes such as biological nitrogen fixation (Giller, 2001), photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement, root development, flowering, seed formation, fruiting and improvement of crop quality (Uchida, 2000; Sara *et al*., 2013). Because of the importance of P for plant growth and yield, many compound fertilizers (NPK) used to correct major deficiencies in soil contain P as a major element.

Optimal plant growth requires P in the range of 0.3–0.5% of dry matter during the vegetative growth stage. Dry matter P contents in excess of 1% may be toxic for most crops. However, many tropical food legumes are more sensitive to excess P, and toxicity may occur at much lower shoot P contents, for example, 0.3–0.4% in pigeonpea and 0.6–0.7% in black gram (Bell *et al.,* 1990). The partial productive efficiency of P for grain or seed is higher at early growth stages than at later stages, because P is needed for tillering or branching. If sufficient P is absorbed at early growth stages, it will be redistributed to other growing organs.

Plant roots acquire P as phosphate, primarily in the form of $H_2PO^-_4$, from the soil solution (Vance *et al.,* 2003). The concentration of $H_2PO^-_4$in the soil solution is often low (2 to 10 μM) (Raghothama, 1999) and, consequently, the supply of $H_2PO^-_4$to the root surface by diffusion is slow (Fitter and Hay, 2002). Therefore, P is one of the most unavailable and inaccessible macronutrients in the soil (Vance *et al.,* 2003) and frequently limits plant growth. For this reason, application of inorganic P fertilisers of low fertility soils enhance crop productivity. Site-specific P application to lower testing regions maybe a more profitable approach, although identifying lower P regions within fields is a challenge (Ferguson *et al.,* 2006). With increasing demand of agricultural production, phosphorus is receiving more attention as a non-renewable resource (Cordell *et al.,* 2009; Gilbert, 2009). In Africa, the rate of nutrient depletion is so high that even drastic measures, such as doubling the application of fertilizer or manure or halving erosion losses, would not be enough to offset nutrient deficits. Reports indicate that phosphorus loss is highest in Malawi, Burundi and Rwanda (Henao and Baanante, 1999). One unique characteristic of P is its low availability due to its slow diffusion and high fixation in acid and alkaline soils. As many African soils are old and highly-weathered, P fertilizers are required virtually everywhere for all crops. Legumes, in particular, tend to have a stronger requirement for P than cereals due to their less-branched and less fibrous root systems (Vanlauwe *et al.*, 2010). The higher protein content of grain legume seeds also requires greater amounts of photosynthate to be used in synthesizing the large amounts of protein (Sinclair and deWit, 1975), hence P availability is important in the supply of ATP to the crop.

The increase of whole plant growth and plant nitrogen concentration in response to increased soil P supply have been noted for several leguminous species including soybean (Israel, 1993). The application of P to soybean increases the amount of N derived from the atmosphere (Ndfa) by the soybean–rhizobium symbiotic system (Sanginga *et al.,* 1995). Phosphorus is especially important for growing nodules and nitrogen fixation processes in soybean as nodules have strong P sinks. Soybean can produce maximum seed yield with relatively low levels of available P (10 – 15 mg kg^{-1}) in the soil (Aune and Lal 1997; Ferguson *et al.*, 2006). Phosphorus application is not likely to increase seed yield at soil P concentrations of above 12 ppm P (Bray-1 test) because the available soil P is above the critical level for soybean growth and development.

1.5 Problem statement and justification

Low crop yields associated with predominantly nutrient-related soil constraints to crop production constitute an undoubted characteristic of subsistence cropping systems throughout Malawi. Research on crop nutrition has documented nitrogen and phosphorus as the most limiting nutrient elements for crop production (Kumwenda *et al.,* 1997). However, P has been reported to be more limiting than N in tropical legumes (Hedin *et al.,* 2003; Vitousek, 2004). Nodulation and N fixation for tropical legumes and survival of *Rhizobia* in soil are particularly affected under low P and acid soil conditions (Graham and Vance, 2003). Maximum benefits from N_2 fixation depend on soil P availability with 33% of the world's arable land limited in P (Kennedy and Cocking, 1997). Acid-weathered soils of the tropics and subtropics are particularly prone to P deficiency (Graham and Vance, 2003).

Soybean yields on smallholder farms in Malawi are still low averaging 800 kg/ha compared to the potential yield of 2000-3000 kg/ha (Kananji *et al.,* 2013). Efforts to improve soybean yields in smallholder farming systems remain a major challenge among research scientists. The high variability of productivity among smallholder farmers can be attributed to soil characteristics, quality of field management, input use,geophysical characteristics such as altitude and weather conditions, demographic and market situations (Bhatia *et al.,* 2006; Affholder*et al.,* 2013). For instance, *Rhizobia* growth, health and activity depend on the initial population of bacteria and the soil conditions that favour or hinder their development. Soil pH <5.6 or >8.0 creates a difficult environment for the bacteria to function efficiently (Hangria and Vargas, 2000). Nitrogen fixation is also sensitive to soil drying. Dry conditions can lead to excess sodium in the root zone, restricting water availability to the bacteria (Albrecht *et al.,* 1994). In some cases, the soybean plant may not initially need the bacteria due to excess residual nitrogen in the soil. In such cases, the soybean plant will not recognize the bacteria chemical reaction, and thus will not initiate nodular tissue formation (Zahran, 1999; Hungria *et al.,* 2005). Thus, in order for the inoculum *Rhizobia* to be useful to soybean, they must be able to survive, colonise, live saprophytically (outside the host) and compete with indigenous *Rhizobial* populations present in the soils (Jones *et al.,* 2007). However, the heterogeneity of farmers' fields create variation in response of soybean plants to phosphorus application and *Rhizobia* inoculation. Given the same treatments, some farmers observe exceptionally vigorous soybean plants with deep green leaf colour and prolific nodulation on plants growing in inoculated plots with *Rhizobia* and applied phosphorus which translates to increases in yield. In contrast, in other farmers' fields, there is no response of soybean to inorganic phosphorus fertilizer and inoculation.

In view of this, there is insufficient information on factors that contribute to variability in performance of soybean to inoculation and inorganic P fertilisers under smallholder farms. In response to the high diversity of smallholder farming systems in Malawi, identification of factors contributing to the enormous diversity in smallholder farms presents an opportunity to fine-tune recommendations to the farm type level that will probably improve adoption by farmers and make legume-based development projects more effective. The demand to provide context specific Integrated Soil Fertility Management (ISFM) recommendations to farmers is alarming.

Understanding the variability among farmers' fields and the underlying drivers behind the farm management options is an important step in designing policies tailored for improved soybean production. In most cases, data collection is limited to grain yield, which does not allow an explanation of the variation in technology performance between sites. Environmental factors like soil parameters, daily rainfall, radiation, temperature and management practices might affect the response of soybean to P fertiliser and *Rhizobia* inoculation. As such, there is need to understand the inoculation and phosphorus interaction system in soybean plants grown in soils of contrasting soil fertility and contrasting climatic conditions. The study presents an opportunity of understanding why inputs give a response in one place but fail in another place. There is also a need to identify types of soybean varieties which yield more but also respond favourably to the application of phosphorus fertilizer and *Rhizobia* inoculation in Malawi.

Assessment of impact of inoculants and P fertilizer on soybean production would generate additional information to enhance soybean scaling up strategies and opportunities among smallholder farmers. Such information provides an opportunity to better manage soybean production systems and increase grain yields.

1.6 Main objective

To determine the effect of *Rhizobia* inoculation and phosphorus fertilization on nodulation, biological nitrogen fixation and yield of promiscuous and specific soybean varieties under smallholder farms in Dedza, Salima and Kasungu Districts in Malawi.

1.6.1 Specific objectives

i. To study the effect ofinorganic P fertilization and *Rhizobia* inoculant on nodulation and biological nitrogen fixation of promiscuous and specific soybean varieties

ii. To study the effect ofinorganic P fertilization and *Rhizobia* inoculant on yield of promiscuous and specific soybean varieties

1.6.2 Research hypothesis

The study hypothesized that:

i. Application of inorganic P fertilizer and *Rhizobia* inoculant increase nodulation and biological nitrogen fixation of promiscuous and specific soybean varieties

ii. Application of inorganic P fertilizer and *Rhizobia* inoculant increase yield of promiscuous and specific soybean varieties

CHAPTER TWO

LITERATURE REVIEW

2.1 Role of biological nitrogen fixation in cropping systems

Nitrogen compounds comprise 40 to 50 per cent of the dry matter of protoplasm, the living substance of plant cells (Dreyfus *et al.,* 1987). For this reason, nitrogen is required in large quantities by growing plants and is indeed the key to soil fertility. The nutrient is needed by the plantas an integral part of all proteins, and is one of the main nutrients required for plant growth and photosynthesis which occurs at high rates when there is sufficient nitrogen. A plant receiving sufficient nitrogen will typically exhibit vigorous plant growth, leaves will also develop a dark green colour. Nitrogen represents about 72% of atmospheric gases but it is required by the plants in form of ammonium (NH_4^+) and nitrate (NO_3^-). As microorganisms decompose organic matter, ammonium is released in a process called mineralization for plant uptake. In addition to organic N, plants are also supplied with inorganic N fertilizer for plant growth and development when soil N is deficient.

Legumes have the potential to contribute to the soil N budget through biological N_2-fixation (BNF), a process which is becoming more important for not only as potential cheap alternative to mineral N fertilizers for providing N to crops but also in seeking more sustainable agricultural production (Boddey *et al.,* 1997; Giller *et al.,* 1997). Biological nitrogen fixation makes a significant contribution to N supply in cropping systems where legumes are grown in rotation or intercropped with cereals either as crops in their own right or as green manures. Evidence of N transfer from legume to cereal has been obtained in some intercropping and rotation studies through root excretion, N leached from leaves and leaf fall (Fujita *et al*., 1992; Stern, 1993; Ledgard and Giller, 1995; Yusuf *et al.,* 2009). For example, Eaglesham *et al.* (1981) showed that 24.9 % of N fixed by cowpea was transferred to maize. Up to 35% of N in maize grown after pigeon pea was shown by isotope dilution to be from nitrogen fixation and part of the fixed nitrogen was from below ground parts. Similarly, Mandimba (1995) revealed that the nitrogen contribution of groundnut to the growth of maizein intercropping systems is equivalent to the application of 96 kg of N/ha at a ratio of plant population densities of one maize plant to four groundnut plants. Osunde*et al.* (2004) found that without the addition of fertilizer the proportion of N derived from N_2-fixation was about 40 % in the intercropped soybean and 30 % in the sole crop.

For many farmers, BNF is, therefore, an essential, cost effective alternative or complementary solution to industrially manufactured N fertilizers for staple cereal crops (Carlsson and Huss-Danell, 2003). Legumes such as soybean that have been subject to intense breeding efforts are very efficient at translocating their N into the grain ranging from 50-150 kgN/ha (Matusso *et al.,* 2014), and even when the residues are returned to the soil there is generally a net removal of N from the field (Giller *et al.,* 1994). Soybean residues at harvest are lignified (10% lignin) with C/N ratios around 45:1 and these tend to immobilize N when they are added to the soil on short term and released for plant uptake in the long term (Toomsan *et al.*, 1995).

Specifically, soybean can fix 49-450 kgN/ha (Peoples and Crasswell, 1992); and net benefits ranging from 100 to 260kg N/ha (Maphumo, 2011). Positive net N balances of up to 136 kg/hafor several legume crops such as cowpea, pigeon pea, green gram and groundnuts following seed harvest havebeen shown by Peoples and Craswell (1992). However, if crop residues are removed from the field, the net N balances for soybean ranges from 28 to 104 kg/ha. Some promiscuous soybean varieties that produce large quantities of leafy biomass have a greater potential to add N to the soil, and are potentially more appropriate for cultivation by smallholder farmers than the recommended varieties grown on commercial farms in southern Africa requiring *Rhizobia* inoculant (Mpepereki *et al.,* 2000). While legumes can improve soil fertility through BNF, low soil fertility limits N fixation and the overall growth and yield of legumes grown on smallholder farms.

2.2 The role of phosphorus in biological nitrogen fixation

Phosphorus is one of the essential nutrients for legume growth and BNF (Giller and Cadisch, 1995; Whitbread *et al.,* 2004; Mhango *et al.,* 2008). Phosphorus deficiency can limit nodule number, leaf area, and biomass and grain development in legumes. Symbiotic nitrogen fixation has a high P demand because the process consumes large amounts of energy (Schulze *et al.,* 2006) and energy generating metabolism strongly depends upon the availability of P (Plaxton, 2004). Several reports have documented that nodules are a strong P sink and nodule P concentration normally exceeds that of roots and shoots (Sa and Israel, 1991; Drevon and Hartwig, 1997). Phosphorous affects root development and hence uptake of nutrients and water. Phosphorus, apart from its effect on the nodulation process and plant growth, has also been found to exert some direct effects on soil *Rhizobia* (Singleton *et al.,* 1992). Singh and Sale (2000) reported that P fertilization stimulates root growth, photosynthesis and increases hydraulic conductibility of roots.

Phosphorus fertilizer application to soybean is an important step in attaining high yield under low soil P (<10 mg kg^{-1}-Bray-1) (Aune and Lal 1997; Martin, 2005). Soybean plant requires an application of 20-30 kgP$_2$O$_5$/haduring the growing season to sustain a high crop yieldin low soil P. Soil phosphorus availability during plant seedling development is an important determinant of plant growth, N_2 fixation and grain formation of soybean (Vance, 2001). Low P availability in soils results in a decrease in shoot growth, affects the photosynthetic activity, and limits the transport of photosynthates to nodules (Jakobsen, 1985) with significant decline in N_2 fixation by the plant (Israel, 1987).

There are inconsistent reports on the response of soybean to P application on highly weathered soils. Chiezey *et al.* (1991, 1992) and Chiezey (2001) reported significant yield increase in soybean with P application on savanna soils. Similar reports were made elsewhere by other workers on soybean (Anzaku and Azanaku, 2002; Alpha *et al.,* 2006). However, Chiezey (1999) Erhabor *et al.* (1999) and Slaton *et al.* (1999) reported that grain yield in soybean was not significantly influenced by P application. Kumaga and Ofori, (2004) reported that under on-farm conditions, increased application of phosphorus had quite prominent effects on nodulation and other growth and yield parameters of promiscuous soybean variety (naturally-nodulating) but it was almost the reverse in the case of non-promiscuous soybean variety (requires specific bacteria to nodulate), where only seed yield was increased. P application at 30 kgP/ha coupled with inoculation with *BradyRhizobia* significantly favoured all the parameters studied, in the two varieties. The application of P in higher quantities under inoculated conditions proved beneficial only to non-promiscuous soybean variety and not the promiscuous soybean variety. A study conducted by Kamanga *et al.,* (2010) in Dowa district, central region of Malawi reported that P fertilizer increased the yield of soybean but no reasons to this positive response were revealed. The report indicate that grain yields of P fertilized legumes were higherthan yields of unfertilized treatments for soybean, pigeon pea, cowpea and groundnuts. Soybean showed response to P (20 kg/ha) with 0.5 t/ha extra grain yield than unfertilized plots. Fertilizer application increased biomass of these legumes. Soybean fertilized with P had 1.5 t/ha of biomass on top of the unfertilized treatment.Similar studies by Khonje (1994) reported that the population of *Bradyrhizobium* species and *Rhizobium* species in soils of Malawi are not uniform such that nodulation of promiscuous soybean will also depend on initial levels of indigenous populations of these nodule-forming bacteria. However, the study failed to ascertain why soybean variety surprisingly reduced nodulation after application of phosphate fertilizer at one of the sites in Malawi as compared to other sites used in the study which showed positive response to phosphate fertilizer application. In spite of these inconsistencies, the importance of P in soybean cultivation has been determined by many scientists (Vance, 2001; Mahamood *et al.*, 2009; Shahid *et al.*, 2009; Sharma *et al.*, 2011). Studies in Nigeria savanna showed that uninoculated soybean required 24-39 kgP/ha at low soil P levels below critical limits to produce higher yields (Pal *et al.*, 1989) and rhizobium inoculation increased the yield of promiscuous soybeans, particulary in soils having a low population of indigenous *BradyRhizobia* (Olufajo, 1990).

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

In Malawi, low yield of legumes grown by smallholder farmers may be strongly linked to minimal use of P fertilizer (Mwalwanda *et al.*, 2003) among other factors, and this was also identified in studies on the response of maize to legumes and N fertilizer in central Malawi (Robertson *et al.*, 2005). Studies have shown that there are benefits of P fertilization in legume cropping systems. Giller (2001) reported that the application of P fertilizer can overcome the deficiency in soils that do not strongly adsorb P. Given the high variability of soil fertility in smallholder farming systems, soil testing remains the most precise available tool to: 1) determine whether P deficiencies are the cause of low soybean yields, and 2) prescribe adequate P fertilization rates (Melgar *et al.*, 1995). Arable land tends to vary in soil fertility and this gives rise to varying responses to nutrient fertility management. The different crop species have varied responses to the different nutrient management interventions (Nyirenda, 1998); and thus it is worthwhile to test the response of different soybean varieties to nutrient management under on-farm conditions. Root hairs, root tips and the outermost layers of root cells are the most pathways of P entering the plants (Rotaru, 2010). Once P is inside the plant roots, phosphorus may be stored in the root or transported to the upper part of the plants (Singh and Sale, 2000). During various chemical reactions, P is integrated into organic compounds, including nucleic acids (DNA and RNA), phosphoproteins, phospholipids; sugar phosphate compounds like adenosine triphosphate (ATP) (Bashir *et al.*, 2011). Nitrogen is reduced to NH_3 under consumption of ATP and redox equivalents, and is associated with the formation of H_2 as a by-product. Thus, adding P fertilizer may reduce stress in the symbiotic relation between root bacteria and legume plant by providing this energy. The enzyme that catalyses the reaction is called nitrogenase and consists of the dinitrogenase reductase protein (Fe protein) and the dinitrogenase (MoFe protein) which actually catalyses the reduction of N_2.

2.3 The role of *Rhizobia* in biological nitrogen fixation

Rhizobia are symbiotic bacteria that facilitate formation of nodules on the roots of legume hosts, within which the bacteria fix atmospheric nitrogen into ammonia. Symbiotic nitrogen fixation is the main route for sustainable input of nitrogen into ecosystems. Nitrogen fixation in agriculture can be improved by inoculation of legume crops with suitable *Rhizobia*. Knowledge of the biodiversity of *Rhizobia* and of local populations is important for the design of successful inoculation strategies (Lindstrȍm *et al.,* 2010). A fully functional symbiosis requires successful completion of numerous steps, beginning with the exchange of recognition signals between the plant and bacteria. The signalling process is started by the plant that releases root exudates, including flavonoids and nutrients. *Rhizobia* are chemotactic toward them and respond to a characteristic plant flavonoid composition by initiating synthesis of specific lipochitooligosaccharide. These compounds, which are known as nod factors, trigger the early stages of nodule development, including root hair deformation, parenchyma cell division and nodule morphogenesis (Denarie *et al.,* 1992). *Rhizobia* are either indigenous to the soil or inoculated for a particular legume. Unfortunately, adaptation of indigenous *Rhizobia* populations to local environments, which is a big advantage for selection of inoculant strains, poses a challenge to productivity. Adaptability of indigenous *Rhizobia* to their environment results in high levels of saprophytic competence (Zengeni *et al.,* 2006). Sometimes indigenous *Rhizobia* may be found in greater numbers than those of the inoculated strains which are also limited in mobility. This challenge must be overcome by the inoculant strains, and it raises the standard of the inoculant required with regard to competitiveness with the native *Rhizobia* and nitrogen fixation (Tas *et al.,* 1995). *Rhizobia* may also be an indicator of soil properties as they are affected by soil acidity, temperature, moisture and other factors and, therefore, the diversity may be an indicator of the soil condition. Fields which receive consistent fertility management and legume cropping host higher *Rhizobia* numbers and diversity (Zengeni *et al.,* 2006).

2.3.1 *Rhizobia* diversity and specificity

In the plant family *Leguminosae*, the members with which *Rhizobia* form symbioses are very diverse, ranging from field grain annual legumes such as soybean to perennial trees such as *Sesbania sesban*. Whilst there may be cross-inoculation of strains compatible with any given legume (Abaidoo *et al.,* 2007), the wide diversity of *Rhizobia* requires more precise matching in symbioses. This specificity of an association enables maximization of nitrogen fixation. Currently, the taxonomy of *Rhizobia* is huge and the complete list of valid species of *Rhizobia* is constantly updated (Khan *et al*., 2010; Weir, 2012). However, based on growth habit, root nodule bacteria of leguminous plants are presently classified into two genera, *Rhizobium* and *Bradyrhizobium*. *Rhizobium* are fast-growing species and produce an acid growth reaction when cultured whilst *Bradyrhizobium* are slow-growing species and produce an alkaline growth reaction when cultured. *Rhizobium* includes three species, *Rhizobium meliloti, Rhizobium loti,* and *Rhizobium leguminosarum. Bradyrhizobium* has only one species, *Bradyrhizobium japonicum,* andincludes strains that are capable of nodulating soybeans**.** The soybean and cowpea *Rhizobia* are slow growers. The type strain for *Bradyrhizobium japonicum* (USDA 6^T)*, Bradyrhizobium japonicum* strain USDA 110, and the type strain for *Bradyrhizobium elkanii* (USDA 76^T) are bacteria forming a nitrogen-fixing symbiosis with soybean and are often used as reference strains in the characterization of newly cultured *Rhizobia* (van Berkum and Fuhrmann, 2009).

Subsequently, grain legumes can yield up to about 300 kgN/ha/year, whilst some tree legumes fix as much as 600 kgN/ha/year (Giller, 2001) with well-matched symbionts. For example, a wide diversity of soybean-nodulating *Rhizobia* has been found in Zimbabwean soils (Musiyiwa *et al.*, 2005). However, only three out of the 129 isolates obtained from one study had higher nitrogen fixation efficiency than the standard commercial strain MAR 1491 used in the country. This emphasizes the possibility of closer matching of strain and crop for improved symbiotic efficiency by careful strain selection. However, cross-inoculation strains represent a group of legumes which can form an effective symbiosis with a wide range of *Rhizobium species*. These have an advantage of not requiring *Rhizobia* inoculants; yet nodulation failures may still occur because of low numbers of *Rhizobia* in the soil or high levels of soil mineral nitrogen (Peoples *et al.*, 1995).

The occurrence of a wide diversity of strains increases the opportunity for a legume host to find a compatible rhizobium in any particular soil. Some smallholder farmers prefer using promiscuous varieties of soybean, as opposed to the higher yielding specific varieties because of the challenges they face in getting access to inoculants (Mpepereki *et al.*, 2000; Musiyiwa *et al.*, 2005). Many developing countries do not have inoculant factories and therefore indigenous *Rhizobia* become an important resource in their natural state. Native or indigenous *Rhizobia* are important in symbiotic BNF of legumes and play a significant role on growth and yield of most leguminous crops (Sinsiri and Homchan, 2002). However, symbiotic performance of indigenous *Rhizobia* strains depends on population size, survival and effectiveness in the soil

(Sanginga *et al.,* 2000; Abaidoo*et al.,* 2007). Further, differences in the amount of N fixed depends on the legume crop, length of growing period and the environmental conditions such as rainfall and temperature. Other studies which reported that nodulation and N_2 fixation require that host and microorganism are compatible, but also that the soil environment be appropriate for the exchange of signals that precedes infection (Leibovitch *et al*., 2001; Zhang *et al*., 2002; Hirsch *et al*., 2003). Soil environment is critical to the persistence of *Rhizobia* in the soil between hosts (Vlassak *et al*., 1997). Earlier reviews have also chronicled the influence of biotic and abiotic soil factors on *Rhizobium* ecology (Amarger, 2001; Sessitsch *et al*., 2002). For example it has been estimated that cowpea can fix up to 200 kgN/haunder on-farm field conditions, while common bean can fix about 125 kg N/ha, about half of the N fixed by cowpea (Giller, 2001). Little is known about *Rhizobia* variation in the farming systems of Malawi. Therefore, understanding soil variation in the cropping systems of Malawi would enhance exploitation of BNF through manipulation of the *Rhizobia*–legume symbioses that will enhance legume production by resource poor farmers.

2.4 Factors limiting biological nitrogen fixation

The BNF efficiency depends on: (i) soil fertility conditions and macro- and micronutrient supply; (ii) the interaction between environmental factors and the soybean plant, such as the efficiency of a soybean cultivar in fixing atmospheric nitrogen; (iii) climatic factors (temperature and photoperiod); and (iv) bacterial strain competitiveness, the amount and the quality of the inoculant, the care in the inoculation process and the absence of antagonistic agrochemicals on the seed (Campo and Hungria, 2004). A legume-*Rhizobium* symbiosis might perform well in a loamy soil but not in a sandy soil, in the sub-humid region but not in the Sahel, or under tillage but not in no-till plots (Vincent, 1990). Active N_2 fixation begins at V_2-V_3 growth stages and highest at R_5-R_6 growth stages. The process requires energy and microbes obtain this energy from photosythetically-derived carbohydrates; thus it is more energy efficient to take up available soil nutrients from organic matter, manure or fertilizer application and 36-74% of N uptake from N_2 fixation (Salvagotti *et al.,* 2008).

These factors affect the microsymbiont, the host-plant, or both. Various other factors may also influence nitrogen fixation in soybean, as described below.

2.4.1 Edaphic factors

Edaphic factors relate to the soil. Soil is the most important factor influencing the rate and amount of nitrogen fixation in soybean. The physical, chemical and biological characteristics of soil have a profound influence on BNF activity. Among the physical properties of soil, the type, texture and structure, having an effect on water holding capacity, groundwater table and so on, affect the nitrogen-fixing microbes and thereby the amount of nitrogen fixed. In general, loamy and clay soils favour better nitrogen fixation than sandy soils. This is attributed to the poor microbial activity and lesser water-holding capacity of the latter types of soil. On the other hand, soils with water pooling on the surface for unusually longer periods after rains or heavy irrigations or shallow ground water affect the aeration in the rhizosphere and consequently the microbes involved in nitrogen fixation (Puiatti and Sodek, 1999). Soils rich in available nitrogen tend to subdue the activity of nitrogen fixation (Bo *et al.,* 1997). In addition, soils inherently high in salts or acidity leading to either unusually high or low pH affect nitrogen fixation. Lack of organic matter in the soil is often a major factor, resulting in little or no microbial activity and rendering BNF less effective. Waluyo *et al.* (2004) reported that under acidic soil conditions, calcium and phosphorus were limiting factors for BNF.

Graham (1992) reported six main edaphic factors limiting BNF are: excessive soil moisture, soil acidity, P deficiency, excess mineral N, and deficiency of Ca, Mo, Co and B. Excessive moisture and waterlogging prevent the development of root hairs and sites of nodulation, and interfere with a normal diffusion of O_2 in the root system of plants (Zahran, 1999). Root nodules are rich in molybdenum, phosphorus, cobalt, iron, zinc, sulphur and nitrogen (Zahran, 1999). High concentration of these nutrients in the nodules is associated with high bacteroidal concentration of nucleotides, cobalamines and proteins, including Fe-, S- and Mo-proteins, and the presence of iron in legheamoglobin. Soilacidity and related problems of Ca deficiency and aluminium and manganese toxicity adversely affect nodulation, N_2 fixation and plant growth (Graham, 1992). Deficiencies or toxicities of these nutrients in the soil will affect legume-*Rhizobium* symbiosis. It is known, for example, that symbiotic plants need higher rates of P fertilization than nitrogen fed plants (Shenoy and Kalagudi, 2005), and when P requirements are not satisfied, nodule formation and functioning are adversely affected (Vincent, 1990). Phosphorus deficiency is commonplace in tropical Africa and reduces nodulation, N_2 fixation, plant growth and causes substantial economic losses (Giller, 2001; Sinclair and Vadez, 2002). Phosphorus requirement has previously been shown to vary among soybean genotypes (Gunawardena *et al.*, 1993) while the degree of nodulation has also been reported to depend on plant genotype and field site (Sanginga *et al.*, 2000). Identification of plant species adapted to low-P soils is a good strategy to overcome this soil constraint.

Mineral nutrients perform several functions. They participate in various metabolic processes in the plant such as proteins, nucleic acids, cell walls synthesis, maintenance of osmotic concentration of cell sap, electron transport systems, component of the chlorophyll molecule, enzymatic activity and act as major constituents of macromolecules, co-enzymes and nitrogen-fixing. This section review recognizes the role of some mineral nutrients in biological nitrogen fixation.

Nitrogen (N)

Inorganic N inhibits the *Rhizobium* infection process and also inhibits N_2 fixation (Zahran, 1999; Anne-Sophie *et al.,* 2002). The former problem probably results from impairment of the recognition mechanisms by nitrates, while the latter is probably due to diversion of photosynthates toward assimilation of nitrates. The inhibitory effects of mineral N on nodulation and N_2 fixation of soybean are clear at high concentrations (>5 mM), but far less at lower concentrations. Although there are a few reports on positive effects of low nitrate concentrations on N_2 fixation in legume species such as soybean (Gulden and Vessey, 1997), Olsson *et al.* (2005) showed that plants reduce carbon allocation to arbuscular mycorrhizae when grown in high compared to low nitrogen agar media. Application of large quantities of fertilizer N inhibits N_2 fixation, but low doses (<30 kgN/ha) of fertilizer N can stimulate early growth of legumes and increase their overall N_2 fixation.The nodulation process begins when the bacteria infect the root hairs of soybean during the early stages of the crop after planting. At this stage, nitrogen is needed to stimulate early growth of soybean before nodulation begins in soils with low residual N or low soil organic matter. Thereafter, soybean regulates this process - lack of available nitrogen triggers the nodulation process and N_2 fixation. The consesus is that some level of N stress is required for symbotic relationship to fully develop. In-season application of N is not recommneded for soybean as yield increase is rarely economic. The amount of this starter N must be defined in relation to available soil N (Graham, 1992).

Phosphorus (P)

Phosphorus is used in numerous molecular and biochemical plant processes, particularly in energy acquisition, storage and utilization (Epstein and Bloom, 2005). The deficiency of phosphorous supply and availability remains a severe limitation on nitrogen fixation and symbiotic interactions. This requirement might be higher than for root or shoot growth of the host plant. Nodules themselves are strong sinks for P and nodulation and N_2 fixation are strongly influenced by P availability (Singleton *et al.,* 1985; Saxena and Rewari, 1991). The phosphorus content per unit dry weight is usually considerably higher in the nodules than in the roots and shoots, particularly at low external phosphorus supply. Nitrogen fixing plants have an increased requirement for P over those receiving direct nitrogen fertilization, probability due to need for nodule development and signal transduction, and to P-lipids in the large number of bacterioids (Graham and Vance, 2000).

Potassium (K)

Potassium is not an integral constituent of any metabolite but serves to activate numerous enzymes, serves as a counter ion and is the major cationic inorganic cellular osmoticum (Epstein and Bloom, 2005). A qualitative requirement for K has been demonstrated for some *Rhizobia*. Vincent (Vincent, 1977) suggested that *R. trifolii* and *R. meliloti* show restricted growth when K is omitted from a defined medium and a linear response in cell yield up to 0.006 mM was obtained in batch culture.

Calcium (Ca)

Calcium is a macronutrient for plants, yet it is actively excluded from plant cytoplasm Calcium has several distinct functions within higher plants. Inhibition of nodulation is a major limiting factor in N_2 fixation of many legume species grown in acid mineral soils. Various factors are responsible for poor nodulation in acid mineral soils, high concentrations of protons and of monomeric aluminium (Alva *et al.,* 1991) and in particular, low calcium concentrations. Calcium deficiency decrease nitrogen fixation in nodules of soybean as it affects attachment of Rhizobia to root hairs (Smit *et al.,* 1992) and nodulation and nodule development (Alva *et al.*, 1991). Lastly, a calcium-spiking phenomenon is initiated in root-hair cells of legumes by nodulation actors and *Rhizobia* (Wais *et al.*, 2002).

Sulphur (S)

Sulphur is an essential element for growth and physiological functioning of plants. The sulphur containing amino acids cysteine and methionine play a significant role in the structure, conformation, and function of proteins and enzymes in vegetative plant tissue. Like molybdenum, sulphur is a constituent of the enzyme nitrogenase and important nutrient for the *Rhizobia* (Alves *et. al.,* 2003). Calcium deficiency in soybeans grown in nutrient solution led to optimal bacterial growth and attachment to the root hair surfaces (Kadreva and Ignatov, 1995). These effects were reversed with increasing Ca supply.

Boron (B)

Boron (B) is one of the eight essential micronutrients, also called trace elements, required for the normal growth of most plants. Yamagishi and Yamamoto (1994) reported strong alterations in N_2 fixation in soybean plants with a low B supply. Bolanos *et al.* (1996) made a study of the boron effect on Rhizobium-legume cell-surface interaction and nodule development in pea. In boron-deficient plants, the number of *Rhizobia* infecting the host cells and the number of infection threads were reduced and the infection threads developed morphological aberrations. The cell walls of root nodules of boron-deficient plants showing structural aberrations are reported to lack the covalently bound hydroxyproline/proline rich proteins (Bonilla *et al.,* 1997) which contribute to an O_2, barrier, preventing inactivation of nitrogenase and associated decrease in N_2 fixation.

Iron (Fe)

All plants require the micronutrient iron for optimum growth. However, legumes, which develop symbiotic relationships with nitrogen-fixing bacteria, have an increased demand for the micronutrient (Tang *et al.,* 1990). Both the plant and bacteria individually have an innate requirement, but it is also essential for the establishment, development, and function of the symbiosis (O'Hara, 2001). Nitrogen fixation is a process very dependent on iron since nitrogenase contents 18-36 atoms of iron in the Mo-protein and a single 4Fe-4S cluster in its complex, and a specific ferredoxin is the ultimate electron donor to nitrogenase (Fillat *et al.,* 1995).

The requirement for iron by legumes with an active symbiosis is large because many symbiotic proteins incorporate iron. Iron is required by the very numerous bacteroids for the synthesis of the nitrogen-fixing enzyme, nitrogenase, as well as cytochromes, ferredoxin, and hydrogenase (O'Hara, 2001; Dixon and Kahn, 2004; Peters and Szilagyi, 2006). This requirement for iron by the symbiosis is highlighted by the proportion of iron within the nodule compared to other plant organs. At nodule maturity soybean nodules have the highest iron concentration, approximately 44% of the iron within soybean plants is present in the nodule compared to 31% in leaves, 7% in seed, and 5% in roots (Burton *et al.,* 1998). At seed maturity, the seed has the highest iron concentration of all organs approximately 35% compared to 27% in the nodule, 23% in leaves, 9% in roots, and 3% in the stem (Burton *et al.,* 1998). A particular high iron requirement exists in legumes for the heme component of hemoglobin. Therefore, in legumes iron is required in a greater amount for nodule formation than for host plant growth (Tang *et al.,* 1990). Leghaemoglobin is an oxygen-binding protein. The single most abundant protein that the plant host makes in the nodule is leghaemoglobin, an iron protein. In the bacteria, nitrogenase and nitrogenase reductase contain FeS clusters and the former has the cofactor FeMoCo at the active site for N_2 reduction. Further, bacteroids have a very high respiratory demand, requiring abundant cytochromes and other electron donors, each with their own Fe centres (Delgado *et al.,* 1998). Nitrogenase is a metalloenzyme, which catalyses the conversion of atmospheric dinitrogen to ammonia. Iron is essential in the two components that make up nitrogenase. The iron protein is the smaller component, which is reduced and provides electrons to the molybdenum-iron protein, a larger, heterotetrameric component that contains the catalytic site (Dixon and Kahn, 2004). At the catalytic site, dinitrogen binds and is reduced (Peters and Szilagyi, 2006). Both the iron protein and the molybdenum-iron protein are sensitive to oxygen.

Other iron-containing proteins essential for the symbiosis include ferredoxin, a non-heme protein, involved in transferring electrons and reducing the iron component of nitrogenase (Dixon and Kahn, 2004), and cytochrome components of the bacterial respiratory electron transport chain, essential for providing the energy for nitrogen fixation (Delgado *et al.,* 1998). Although iron deficiency did not significantly affect shoot growth, it severely depressed nodule mass and particularly leghemoglobin content, number of bacteroids and nitrogenase activity, compared with those plants five days after a foliar spray of iron (Tang *et al.,* 1990).

Molybdenum (Mo)

Molybdenum is a micronutrient specifically for plants that form root nodules with nitrogen-fixing bacteria, though plants that do not form nodules also use trace amounts of it in a protein involved with nitrogen metabolism and uptake (Wiedenhoeft, 2006). Its relevance to N_2 fixation is clear, given that the Mo in 'FeMoCo' cofactor is at the heart of the nitrogen reduction process - at least for most nitrogenases. The Mo–Fe protein contains two atoms of molybdenum and has oxidation–reduction centres of two distinct types: two iron–molybdenum cofactors called FeMoCo and four Fe-S (4Fe-4S) centers. The Fe–Mo cofactor (FeMoCo) of nitrogenase constitutes the active site of the molybdenum-containing nitrogenase protein in N_2-fixing organisms (Allen *et al.,* 1999). Although at low supply, molybdenum is preferentially transported into the nodules (Brodrick and Giller, 1991), molybdenum deficiency-induced nitrogen deficiency in legumes relying on N_2 fixation is widespread, particularly in acid mineral soils of the humid and sub-humid tropics. There are reports that foliar applications of Mo to grain legumes in field conditions increase levels of N_2 fixation and nodule mass, resulting in higher overall N content and seed yield (Yanni, 1992; Vieira *et al.,* 1998). It is also reported that a *B. japonicum* strain deficient in molybdenum transport showed impaired nitrogen fixation activity when inoculated to soybean roots (Delgado *et al.*, 2006).

2.4.2 Crop factors

Among crop factors, the genetic constitution of the crop, its compatibility with nitrogen-fixing microbes, crop duration, different phenological stages and yield potential have all been found to affect the quantum of BNF (Nicolas *et al.,* 2006; Abaidoo *et al.*, 2007). Reports indicate the variability of varieties in influencing the nitrogen fixation activity of microbes (Farnia *et al.*, 2005). Furthermore, the duration of different phenological stages and total crop duration also have an important role (Shiraiwa *et al.,* 1994; Botha *et al.*, 1996). Since BNF starts only after the initial seedling growth stage, the time taken for initiating the vegetative phase will determine the time period available for BNF. Likewise, BNF tends to decline with the onset of podding and the grain-filling stage. Thus, the different phenological phases decide the total amount of nitrogen fixed. Finally, high-yielding varieties requiring rapid translocation of photosynthates as well as longer time periods tend to affect the rate and amount of nitrogen fixed by the crop (Pandey, 1996).

2.4.3 Climatic factors

One of the important challenges facing crop physiologists and agronomists is to understand and overcome the major abiotic stresses in agriculture which reduces crop productivity and yield (Habibpor *et al.,* 2011). Interest in crop response to environmental stresses has increased greatly in recent years because of severe losses that result from drought, heat and cold stress (Diab *et al.*, 2007). Leguminous plants in association with *Rhizobium* species have the potential to fix large amounts of atmospheric N which contributes to the soil N pool provided that the N fixation is not restricted by other environmental or microbial factors (Achakzai *et al.,* 2002). Rainfall, drought, salinity, acidity, low P and the presence of toxic ions hinders the establishment of symbiotic N fixation (Graham, 1992; Rajput *et al.,* 2001).

The two important climatic determinants affecting BNF are temperature and light. Extreme temperatures affect N_2 fixation adversely because N_2 fixation is an enzymatic process. However, there are differences between symbiotic systems in their ability to tolerate high (>35°C) and low (<25°C) temperatures (Brockwell *et al.,* 1991). The availability of light regulates photosynthesis, upon which BNF depends. This is demonstrated by diurnal variations in nitrogenase activity. Very few plants like cowpea can grow and fix N_2 under shade (Hungria and Vargas, 2000). Yield of a soybean crop is a function of light interception, dry matter production, and partition of dry matter into the plant's seed. Optimal crop growth rate is achieved when leaf area index is large enough to intercept 95% of the sun light (Board, 2000). Edward *et al.* (1985) observed a linear relationship between weeds and soybean and showed that the variation in soybean performance accounts for 86% due to shading by the weeds. It was predicated that 19 to 25% yield loss was observed due to 44-56% shading of the crop by the weeds.

Drought reduces the number of *Rhizobia* in soils, and inhibits nodulation and N_2 fixation (Napoles *et al.,* 2009). Prolonged drought will promote nodule decay (Benjamin and Nielsen, 2006). Reports indicate that drought severely inhibits nitrogenase activity (Streeter, 2003), N_2 fixation and nodulation (Pimratch *et al.*, 2008). As with other grain legumes, soybean is very sensitive to drought stress which leads to reduced yield and seed quality. Sadeghipou and Abbasi (2012) reported that water stress decreased number of pods per plant, number of seeds per pod, 100-seed weight and seed yield of soybean. Water stress increases the abortion of flowers and pods but also decreases fertilization values, photosynthates mobilization to seeds and seed filling period. The decrease in yield and yield components of soybean, due to water stress, has also been reported by other researchers (Mirakhori *et al.,* 2009; Masoumi *et al.,* 2011; Shafii *et al.,* 2011). In soybean, drought not only results in losses in CO_2 accumulation and leaf area development but also its symbiotic N_2 fixation is especially vulnerable to drought. With declining soil water content, soybean has decreased N_2 fixation rates in advance of declines of other physiological processes. This means a decrease in N availability to support cell and tissue development throughout the plant (Sinclair *et al.,* 2007). Decrease in N_2 fixation with soil drying causes yield reductions due to inadequate N for protein production which is the critical seed product (Sinclair *et al.,* 2007).

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

2.4.4 Biotic factors

Among biotic factors, the absence of the required *Rhizobia* species constitutes the major constraint in the nitrogen fixation process. The other limiting biotic factors could be: excessive defoliation of host plant; crop competition; weeds; insects and nematodes (Acker *et al.*, 1993; Wrather, 1998; Niblack *et al.*, 2006).

Defoliation decreases the photosynthetic ability of legumes. The soybean plant produces 95% of its total dry matter through photosynthesis (Taiz and Zeiger, 2002). Defoliation impairs N_2 fixation and can lead to nodule decay (Basavaraju and Nanjappa, 1996). Bayne *et al.*, (1984) reported that photosynthetic stress as a result of defoliation affected nodulation and nodule activity most severely in soybean.

Weeds are misfits and are one of the major limiting factors of soybean production throughout the world. Like other crop plants, weeds require light, moisture, nutrients, space and carbon for their growth and development (Moolani and Sachan, 1996). Losses caused by weeds and the cost of weed control are among the most expensive items in crop production. Losses caused by weeds exceed the losses from any other category of agricultural pests like insects, nematodes, rodents, etc. as weeds compete with crop plants for their nutrients, soil moisture, space and radiation and also sometimes serve as hosts for various pests and diseases (Everarts, 1992). Data available for weed infestation of soybean in tropical environments show that weeds reduce crop quality and yield by as much as 40 to 80% depending on the density and species of weed (Haygood *et al.,* 1980). They also indicate that soybeans in weed-infested fields are more liable to lodging than those in fields where weeds are adequately controlled because weeds remove some nutrients that would otherwise have been used by the plants, thus leaving the plants spindly, etiolated, and liable to lodging (Nnagju, 1980). Weed interference significantly reduced soybean leaf area index, total above ground soybean dry weight and crop growth rate (Acker *et al.*, 1993). This was in agreement with other studies that reported that weed competition reduces the leaf area index which ultimately influences the photosynthetic efficiency of the plant (Everarts, 1992; Basavaraju and Nanjappa, 1996; Singh *et al.,* 1999). In related studies, dry matter accumulation in soybean plants was inversely proportional to total weed dry matter (Kuruchania *et al.*, 1996; Billore *etal.*, 1999).

Insects and nematodes have also been reported to interfere with nodule formation, development, and functions (Vincent, 1990). Like many other agricultural field crops, soybean plants are exposed to attacks by a variety of pests. For example, soybean aphids (*Aphis glycines* Matsumura) feed on plant phloem; bean leaf beetles (*Cerotoma trifurcata* Forster) attack leaves and roots; and soybean cyst nematodes (*Heteroderaglycines* Ichinohe) feed on roots. In addition, soybean cyst nematode lives in the soil and is an endoparasitic obligate parasite infecting and reproducing in the roots of soybean and can cause serious yield losses (Wrather, 1998). Soybean aphid occurs aboveground (on leaves and stems) and feeds on the phloem while soybean cyst nematode occupies belowground parts (i.e., roots) of soybean and feeds intracellularly after degrading cell walls with enzymes (Niblack *et al.,* 2006).

2.4.5 Management factors

Various agronomic practices (i.e. time of sowing, depth of sowing, and cropping practices such as sole or intercropping, tillage operations, seed inoculation, irrigation method and frequency, use of plant protection chemicals, inter-cultivation practices and so on) have a profound influence on microbial activity, rhizosphere aeration and crop performance. These, in turn, influence the rate of nitrogen fixation. Seed inoculation with efficient strains of *Bradyrhizobium,* a starter dose of nitrogen through fertilizers, light irrigations to avoid waterlogging and avoiding the use of plant protection chemicals that harm the microbes, positively influence the BNF and lead to greater amounts of nitrogen fixation. On the other hand, untimely sowing, a poor or uneven plant stand, lack of seed inoculation, heavy doses of nitrogen fertilizers, result in shy nodulation and a lower amount of nitrogen fixation. In an experiment to study the impact of different residue management and tillage practices, no significant difference in soybean yield or nitrogen accumulation was observed, but BNF was higher in zero tillage as compared to conventional tillage (Alves *et al.,* 2002). Tillage stimulates mineralization of organic matter in the soil; this results in the availability of high levels of nitrate, which may depress nodulation and nitrogen fixation. It implies that no-tillage conditions are preferred to repeated tillage operations as far as BNF is concerned.

2.4.6 Inoculation of legumes

Inoculation of legume seeds with *Rhizobia* is perhaps the oldest agro-biotechnological innovation. Nitrogen fixation in agriculture can be improved by inoculation of legume crops with suitable *Rhizobia* in form of inoculants. Peat-based inoculants are applied to legume seed as a slurry just before sowing (Bashan and Carrillo, 1996). Inoculation of legume seed is an efficient and convenient way of introducing viable *Rhizobia* to the soil and subsequently to the rhizosphere of legumes (Deaker *et al.,* 2004). Knowledge of the biodiversity of *Rhizobia* and of local populations is important for the design of successful inoculation strategies. Inoculation of legumes with *Rhizobia* is a less expensive and more effective agronomic practice for supplying N to these crops, compared with the application of N fertilizers (Crews and Peoples, 2004). Selection of *Rhizobia* for inoculants that are well adapted to the edaphic and climatic conditions of the agricultural region is essential to maximize the BNF in legume systems. Despite the importance of inoculation of legumes with appropriate *Rhizobia*, poor nodulation and the lack of response to inoculation in field experiments has frequently been reported worldwide (Hardarson and Atkins, 2003). Lack of response to inoculation can be attributed to intrinsic characteristics of both the host plant and the bacteria, as well as the great sensitivity of the symbiosis to environmental stresses, such as high temperatures, soil dryness and low soil fertility (Graham *et al.,* 1994, Brockwell *et al.*, 1991). Success of inoculation is limited by several factors, including the presence of competing indigenous *Rhizobia*, which presents a ‘‘competition barrier’’ against nodulation by the inoculum strain (Thies *et al.*, 1991). When the *Rhizobia* in a soil are infective (capable of colonizing and nodulating a legume) but poorly effective, constitute a barrier to the successful exploitation of rhizobium inoculants. Introduced *Rhizobia* must, therefore, be more aggressive and competitive as nodulators than the native strains (Peoples *et al.*, 1995; Bogino *et al.*, 2006).

BradyRhizobia inoculants for soybean are very diverse, yet classification and characterization of strains have long been difficult. Genetic characterization methods permit more reliable identification and will improve our knowledge of local populations (Tas *et al.,* 1995). Seed inoculation increases yield and yield component of specific soybean varieties (Rajput *et al.*, 2001; Oad *et al.,* 2002). Inoculation ensures successful symbiosis by introducing effective *Rhizobia* strains into soils, in proximity of seeds, thus enhancing nitrogen fixation by soybean plants (Dobereiner *et al.,* 1995; Seneviratne *et al.,* 2000) but the success of inoculation was found to be highly variable (Peoples and Craswell, 1992). Formation of effective (functional) nodules in specific soybean varieties when inoculated with compatible *Rhizobia* leads to fixation of atmospheric nitrogen making nitrogenous fertilization of the soybean unnecessary (Alves *et al.,* 2003). Remarkable positive response of soybean to *Rhizobia* inoculation has been obtained in many tropical countries.In Brazil, for example, inoculation increased soybean grain yields by up to 750 kg/ha (Coutinho *et al.*, 1999). Previous studies with promiscuous soybean varieties, however, have shown considerable variability in the effectiveness and population of indigenous *BradyRhizobia* in a given location (Sanginga *et al.*, 1999; Fening and Danso, 2002).

Sanginga *et al.* (1995) also found a direct relationship between *BradyRhizobia* cell counts and promiscuous soybean response. Thus, promiscuous soybean may also need inoculation with exotic *BradyRhizobia* depending on effectiveness and population of indigenous *BradyRhizobia* in the locality (Okereke *et al.,* 2000), as well as the degree of promiscuity of variety (Sanginga *et al.,* 1999).

2.5 Bradyrhizobium strains as inoculants for soybean

2.5.1 *BradyRhizobia* as inoculants

High-yielding soybean plants require a lot of N and it is estimated that BNF can contribute 60 to 70% of the N requirement of the plant (Herridge *et al.,* 2008; Salvagiotti *et al.* (2008). Optimizing the process through *BradyRhizobia* inoculants is, therefore, key and contribute to N residual effect for subsequent crops. Reports, however, point to a lack of data to assess the real contribution of varying levels of below ground N and call for more research to elucidate whether optimized BNF systems can sustain optimal yields with minimal input of additional N to the subsequent crop. Despite the negative N balances for grain legumes grown in rotation or as intercrops (attributed to higher N mining from the soil than cereals), reported benefits of legumes to succeeding non-legume crops have been observed consistently by several authors (Wani *et al.,* 1994; Przednowek*et al.,* 2004; Getachew *et al.,* 2006; Malhi *et al.,* 2011). Grain legumes in rotations also improve organic matter, mineral N content, and N mineralization potential of soil compared with non-legume crops. Therefore, crop rotations that include grain legumes are recommended for improving sustainability of soil productivity and fertility/quality/health. However, annual grain legumes cannot be relied on for substitution of entire N removed by other crops. Improvement in cereal yield following monocropped legumes lie mainly in the 0.5 to 3 t ha^{-I} range, representing around 30 to 350% increase over yields in cereal-cereal cropping sequences (Peoples and Crasswell, 1992). Cereal plants require large amounts of mineral nutrients including N for their growth, development and grain production.

The main *Rhizobia* symbiotic partners of soybean are the slow-growing *BradyRhizobia* and particularly the *Bradyrhizobium japonicum species* (Jordan, 1982), *Bradyrhizobium elkanii* (Kuykendall *et al.*, 1992) and *Bradyrhizobium liaoningense* (Xu *et al.*, 1995). Latter species is very close to *Bradyrhizobium japonicum* (van Berkum and Fuhrmann, 2000). In addition, the moderately fast-growing *Mesorhizobium tianshanense* (Chen *et al.*, 1995) and the fast-growing *Sinorhizobium (Ensifer) fredii* (Scholla and Elkan, 1984) and *Sinorhizobium xinjiangense* (Chen *et al.*, 1988) also nodulate soybean and can be as effective as *BradyRhizobia* in suitable ecological conditions (Albareda *et al.*, 2009). Possible synergistic effects promoting soybean plant growth by *BradyRhizobia* with other bacteria such as some strains of *Pseudomonas* (Chebotar *et al.*, 2001), *Bacillus thuringiensis* (Mishra *et al.*, 2009) and *Azospirillum brasilense* (Cassan *et al.*, 2009) have also been documented.

Commercial inoculant production is an important agricultural industry (Catroux *et al.*, 2001) and selected *BradyRhizobia* inoculant with superior symbiotic capacities are produced to improve production and grain yields. An inoculant or inoculation group is a culture of *Rhizobia species* that nodulate the same legume crop (O’Hara *et al.*, 2012). Different inoculation groups are nodulated by distinctly different *Rhizobia species.*The success of such introduced strains at establishing a symbiotic relationship and persisting in a field is dependent on local soil conditions and the presence of competing indigenous *Rhizobia* strains (Sadowsky, 2000; Botha *et al.*, 2004). This competition may comprise effective colonizers that are less effective nitrogen fixers. Commercial inoculant strains may evolve quickly in the soil (Farooq and Vessey, 2009). Over time, genetic exchange may dilute the beneficial capacities of introduced strains (Lo´pez-Garcı´a *et al.,* 2002) or introduced strains may disappear completely in the absence repeated inoculation (Obaton *et al.,* 2002), though it has also been reported that strains are highly adaptable to new environments (Andrade *et al.,* 2002; Alves *et al.,* 2003). There are even reports from Brazil of re-isolated Rhizobia that had become more competitive in their new environment while they maintained their nitrogen fixation ability (Alves *et al.,* 2003). When a legume is introduced into an area, there is the opportunity to co-introduce *Rhizobia* that are adapted to that environment. Knowledge of the local soil diversity is, therefore, indispensable to assess the potential benefits of an inoculation strategy (Catroux *et al.,* 2001; Obaton *et al.,* 2002; McInnes *et al.,* 2004; Musiyiwa *et al.,* 2005; Rickli-Binde *et al.,* 2009).

2.5.2 Specificity and effectiveness

A problem of great economic importance in microbial ecology concerns the efficiency of nitrogen-fixing bacteria to develop nodules on legumes such as groundnut, soybean, and bean. This is because the relationships between particular *Rhizobia* and particular legumes are very specific. Aim of inoculation is to provide sufficient numbers of viable (effective) *Rhizobia* to induce a rapid colonization of the rhizosphere of a particular legume (Catroux *et al.,* 2001). Only specific *Rhizobia* will nodulate and fix nitrogen with a particular legume host. This is why we have different inoculants.

There are roughly 1,300 leguminous plant species in the world. Of these, nearly 10% have been examined for nodulation, 87% of which nodulate. Thus, not all legumes are infected by *Rhizobia* (Thies *et al.,* 1991). A *Rhizobium* that nodulates cowpea may not nodulate *Leucaena* and vice versa (Fening and Danso, 2002). Leguminous species mutually liable to nodulation by a particular group of bacteria constitute a cross-inoculation group. Six cross-inoculation groups were defined in the early days of *Rhizobium* research in addition to the cowpea group (Lindstrӧm and Young, 2009). However, this classification scheme is undergoing modifications based on recent research findings.

Strains of *Rhizobia* must be successful in producing effective nodules in the cultivars of host species for which they are recommended (Vincent, 1990). One of the major problems for successful establishment and effective performance of newly introduced strains of *Rhizobia* into legume cropping systems is the competition from native established strains of the bacterium in the soil (Vessey and Chemining'wa, 2006). Not all symbioses fix N_2 with equal effectiveness. The free-nodulating promiscuous varieties of soybean can nodulate profusely and fix nitrogen depending on the effectiveness of the *Rhizobia* populations present (Kiers *et al*., 2003).

2.6 Synergistic effects of phosphorus and *Rhizobium* inoculation in soybean

Tahir *et al.* (2009) reported that application of *Rhizobium* inoculation and P fertilization significantly increased nodule numbers from 73 in un-inoculated control (with no P application) to 125 and 95 in *Rhizobium* inoculation and P treatments, respectively. Noticeable numbers of nodules in the control treatments reflected the presence of indigenous *Rhizobia species* capable of forming small sized nodules on lateral roots and most of them ineffective (white in colour). However, greater number of nodules due to inoculation suggested that there was better synergism between introduced *Rhizobia* and soybean. Larger response to inoculation and higher number of nodules per plant in comparison to un-inoculated treatments in a field that has no soybean cropping history was also reported by Revellin *et al.* (2000) and Abbasi *et al.* (2008).

Application of P alone enhanced nodulation by 30% while its combination with *Rhizobium* inoculation resulted in 47% more nodules than P alone. Similarly, Ashraf *et al.* (2002), Oad *et al.* (2002) and Abbasi *et al.* (2008) in Pakistan, reported increase in seed yield due to *Rhizobium* inoculation. Application of P alone increased pod plant^{-1}, seed and dry matter yield by 23, 18 and 46%, respectively, when compared with the control. This might be due to adequate supply of phosphorus which in turn increased the carboxylation efficiency and increased the ribulose-1-5-diphosphate carboxylase activity, resulting in increased photosynthetic rate, growth and yield (Jacob and Lawlor, 1992). Gentili and Huss-Danell, (2003) and Fatima *et al.* (2006) concluded that combined application of P and *Rhizobium* inoculation increased nitrogenease activity, growth, and grain yield as well as improved soil fertility for sustainable agriculture.

Higher P uptake due to *Rhizobium* inoculation is due to the ability of applied *Rhizobia* to solubilize precipitated P components thereby increased P uptake in plants as reported by Fatima *et al.* (2007). The higher P concentration in plant benefits the bacterial symbiont and the functioning of its nitrogenase activity, leading to increased nitrogen fixation. Two field experiments, which were established to determine the effectiveness of introduced *BradyRhizobia* on nodulation, growth and yield of a promiscuous and a non-promiscuous soybean variety under varying levels of phosphate fertilizer (0, 30 and 60 kg/ha) at the University of Ghana Farm, Legon, showed that *BradyRhizobia* inoculation had significant effects on nodulation, dry matter, total N and seed yield of both varieties, being more pronounced in the case of the promiscuous variety than the non-promiscuous variety. Under natural conditions, increased application of P had quite prominent effects on nodulation and other growth and yield components of promiscuous variety but it was almost the reverse in the case of the other non-promiscuous variety, where only seed yield was increased. Phosphorus application at

30 kg/ha coupled with inoculation with *BradyRhizobia* significantly improved all the parameters studied, in the two varieties (Kumaga and Ofori, 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Site description

The study was done on demonstration and dissemination on-farm trials of soybean in the Kasungu, Salima and Dedza districts of Malawi during the 2012/2013 growing season. These participatory demonstrations and dissemination on-farm trials were conducted within the framework of the N2Africa project. This is a large scale research project focused on putting N_2 fixation to work for smallholder farmers growing legume crops in Africa by increasing the average grain legume yields of groundnut, cowpea, soybean and common bean and increasing the amounts of atmospheric N_2 fixed by these crops. In Malawi, Dedza, Kasungu, and Salima are among the seven districts in which the project has been operating since the year 2010. These sites represent agro-ecological zones with different rainfall patterns, soil characteristics, temperatures and altitudes and located in central region of Malawi (Figure 3.1). All the sites experience unimodal rainfall pattern, with the planting season starting in November/December to April depending on the onset of effective planting rains (>30mm). The study was implemented in Linthipe Extension Planning Area (EPA) in Dedza district, Kaluluma EPA in Kasungu district and Chinguluwe EPA in Salima district.

Dedza district lies at the southern end of Malawi’s central region and combines agro-climatic and ecological features that are representative of conditions found in many parts of central and southern Malawi (Ellis *et al.*, 2002). The district is on grid reference 34^o 20' East and 14^o 10' South located about 85 km south of Lilongwe, the Capital City of Malawi and it covers an area of 3,624 km². Two distinct agro-ecological zones, Dedza hills and Dedza upland, exist in the district (Lorkeere and Venema, 1991). However, the current study was implemented in Linthipe EPA, part of mid-altitude zone (Dedza upland) about 1200m above sea level. It has a cool climate with annual rainfall of about 800 mm (David, 2003; Dedza District Assemby, 2012). The EPA covers an area of 1120 km^2 (26,050 ha) with an estimated population of more than 160,000 people (Lorkeere and Venema, 1991; David, 2003). The area has gently undulating plain with low rock hills commonly found and dominated by ferruginous and weakly ferruginous soils (Brown and Young, 1965).

Kasungu is characterized by wet and dry seasons, with temperatures ranging between 19 and 32^{o}C. It receives an average annual rainfall of about 763mm. The district is on grid reference 33^o 30' East and 13^o 03' South about 127 km north of Lilongwe (Kasungu District Assembly, 2009). The dominant soil types are the ferallitic soils (medium-textured sandy clay loam) (Brown and Young, 1965; Saka *et al.*, 2003). These are sandy loam soils that are well-drained. According to Kanyama-Phiri *et al.* (2007), Kaluluma EPA is an area with soils of pH 4.5 to 6.0, suggesting that the soils in question are acidic to moderately acidic. Njira *et al.* (2012) also reported acidic to moderately acidic soils in Mkanakhoti EPA in Kasungu district with organic matter content of 0.5-2.1% indicating that some soils have poor organic matter content while others have organic matter content above the critical values i.e. 1.5%.

In contrast, Salima district is located in the low altitude agro-ecological zone of Malawi bordering the western side of Lake Malawi with annual rainfall of 600 to 800 mm. The district is on grid reference 34^o 26' East and 13^o 47' South about 103 km east of Lilongwe. The area is characterized by warm tropical climate with mean temperature of 22^{0}C (Republic of Malawi, 2006; Seaman *et al.*, 2005). The district extends from the

Rift Valley lakeshore plain (altitude 200 to 500m) adjacent to Lake Malawi, to the central upland area in the west (altitude 500 to 1000m). The study was conducted in Chinguluwe EPA; which is part of central upland area of the district (Figure 3.1). Soils in Chinguluwe are ferruginous, low altitude type. Topsoil is dark or very dark reddish brown, varying considerably in texture, between sandy loam and sandy clay (Brown and Young, 1965). Lowole (1998) observed that the soils are slightly acidic within the rooting zone. The EPA has a research station within it and this strengthens the technical support that is required in crop and livestock production for the farmers in the area.

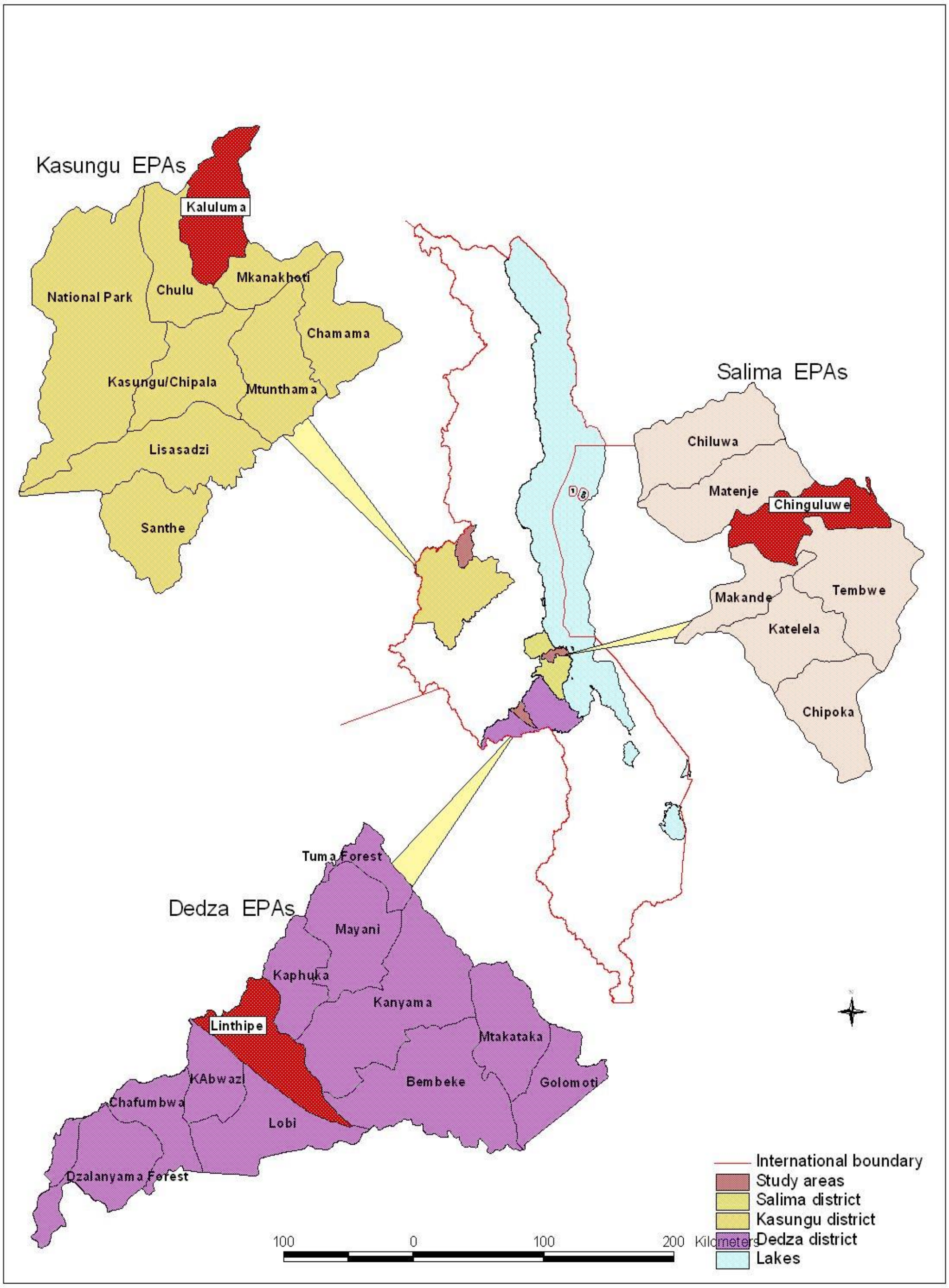

Figure 3.1 Map of Malawi with study sites indicated by Extension Planning Area (EPA) designation.

3.2 Selection of farmers hosting the on-farm trials

3.2.1 Choice of farmers

Thirty lead farmers' fields (10 in each district) distributed in the selected EPAs of the districts sites were randomly selected as replicates for the study. These farmers were part of those N2Africa project selected to implement their activities in the districts. In each district, five of the 10 farmers were selected to host a soybean variety demonstration and the remaining five were selected to host the soybean input demonstrations. Lead farmers were randomly selected after stratification was based on type of legume package the farmers received from N2Africa (groundnut, cowpea, soybean and common bean). Thereafter, lead farmers (both male and females) who received soybean seed package were included as part of the study. Lead farmers in N2Africa project represent a group of farmers with advanced farming and training skills, with some capable of facilitating communication with extension services (Ajeigbe *et al.*, 2010). The selected lead farmers were trained by N2Africa project in demonstration plot management and data recording.

3.2.1 Selection of farmers’ fields

At each site, the final selection of farmers’ fields was done after field inspection with the aim of choosing appropriate sites with minimal soil variation; within-farmer’s field soil fertility variability is a common feature within smallholder systems (Tittonell *et al.* 2005). Farmers’ fields with similar soil type, gentle slope, minimal shading and with no history of soybean cropping in the previous year were selected. There is probably high level of native *Rhizobia* for soybean in the fields with previous soybean cropping that might influence the results of the study (CIAT, 1988). Farmers’ knowledge about the variation in their fields was the key determinant in the location of the plots and any blocking scheme. Other factors considered in the selection of lead farmers were the willingness of the farmer to host the demonstrations and proximity of the field to main village paths for easy accessibility and viewing of the demonstrations plots by fellow farmers in the area and the in-coming visitors.

3.3 Treatments

Initially the study was planned to be conducted on 10 farmers' fields in each district where 5 farmers would host soybean input trial and 5 farmers would host soybean variety trial per district. However, due to other reasons, the study was successfully implemented on 5 farmers' fields in Dedza, 9 farmers' fields in Kasungu and 9 farmers' fields in Salima.

Experiment 1. Response of soybean varieties to applied P on biological nitrogen fixation, yield and yield components

5 lead farmers in each district implemented soybean varieties x P fertilizer trials that were established to test the response of two soybean varieties Nasoko and Tikolore to applied P fertilizer (triple superphosphate).

The experiment had the following treatments:

1. Tikolore variety without P fertilizer
2. Tikolore variety with applied P fertilizer (30 kgP_2O_5/ha)
3. Nasoko variety without P fertilizer
4. Nasoko variety with applied P fertilizer (30 kgP_2O_5/ha)

Note: All seeds in the four treatments were inoculated with *BradyRhizobia* soybean inoculant using recommended rate of 10g per kg of seed (Ajeigbe*et al*., 2010).

Experiment 2. Response of Tikolore variety to applied P fertilizer and soybean *BradyRhizobia* inoculant on biological nitrogen fixation, yield and yield components

The 4 lead farmers in Kasungu and Salima, respectively, implemented soybean on-farm trials that were established to test the response of Tikolore variety to applied P fertilizer (triple superphosphate) and soybean *BradyRhizobia* inoculant. The experiment had the following treatments:

1. Tikolore without both inoculant and P fertilizer
2. Tikolore with applied P fertilizer but without inoculant
3. Tikolore with applied inoculant but without P fertilizer
4. Tikolore with combined inoculant and P fertilizer

Nasoko soybean variety, released in 2003, is indeterminate, medium to late maturing. The variety is large seeded and has good lodging resistance. With good management, Nasoko produces high yields (3000 kg/ha) and is one of the recommended varieties in Malawi (Kananji *et al.*, 2013). It has a yellow seed coat and colourless helium, both desirable attributes for the manufacturing of soybean-based products, such as *Likuni phala* (soybean blend flour porridge) and soymilk. It grows to a height of 75 cm; matures in 123 days, and is adapted to a wide range of environments (Soko *et al.,* 2003).

Tikolore soybean variety was released in 2010 and is a self nodulating variety with grain and fodder yield potential of 3000 and 7000 kg/ha, respectively. It is an early maturing variety (102 days) with an average of 37 pods per plant and 100-seed weight of 14g. The variety is susceptible to rust disease, however, because of its early maturity it can escape rust infection and is able to produce high yields under such conditions (Mviha *et al.,* 2011).

Triple superphosphate (TSP) fertilizer was applied used as a source for P. This was applied in a furrow, 10 cm away from the row, 2 cm deep and covered after fertilizer application rate of 30 kg P_2O_5/ha.

A peat (Sphagnum moss) based inoculant was used. Inoculation procedure followed was described by Ajeigbe*et al*., (2010). The inoculated seeds were planted on the same day and prevented from being exposed to strong heat and sunshine when drying before planting. This was a deliberate measure of avoiding killing the *Rhizobia* (GoM/MoAFS, 2005).

3.4. Experimental design

Both soybean on-farm trials were laid out in a factorial arrangement in randomised complete block design (RCBD) with each lead farmer as a replicate for either soybean varieties x P fertilizer trial or inoculant x P fertilizer trial, depending on which on-farm trial hosted, respectively. Thus 5 farmers for soybean varieties x P fertilizer trials and 4 farmers for soybean inoculant x P fertilizer trials per district.

3.5 Plot dimensions and demo management

Four plots each 10 m x 10 m were prepared in each farmer's field intended for the trial treatments. According to recommendations by the GoM/MoAFS (2005), soybean was planted on ridges spaced at 75 cm apart; two rows of soybean were planted per ridge spaced at 30 cm apart and a spacing of 5 cm between planting stations and one seed per planting station.

The demonstrations were researcher-designed farmer-managed and therefore, all management activities on the demo plots were done by the farmers who hosted the demonstrations. Researchers provided P fertilizer, seed and field notebooks while participating farmers provided land and labour. Farmers and extension agents within the area assisted in making observations and help with data recording and were also involved in all the activities from planting to harvesting with the guidance of the researcher.

3.7 Data collection and analysis

3.7.1 Soil sampling and analysis

Baseline soil samples were collected with a soil auger from a soil depth of 0-30 cm before planting from 10 randomly selected points using the zigzag sampling pattern to collect a representative soil sample of the plot. The sub-samples were then mixed thoroughly and combined into a composite sample of approximately 1 kg per field. Soil samples were then air-dried at Chitedze Soil Laboratory, ground (to increase the surface area for chemical reactions) and sieved through 2 mm sieve and 0.25 kg of each sample was used for analyses.

The samples were analysed for pH (in H_20), soil texture (Bouyoucos), organic matter (Walkley-Black), nitrogen (Kjeldahl), cation exchange capacity (CEC) (extraction with ammonium acetate), K, Ca, and Mg content (atomic absorption spectrophotometry) and available phosphorus (Mehlich-3) using standard procedures as outlined by Anderson and Ingram (1993). Soil texture analysis was also done to determine the percentage of sand, silt and clay in the inorganic fraction of the soil based on Stoke’s law that governs the rate of sedimentation of particles suspended in water.

3.7.2 Daily rainfall

Rain gauges were mounted at every farmer's site to collect daily rainfall (Figure 3.2). The farmers were trained on how to record rainfall data. The rainfall data at the nearest agricultural office was accessed from the EPA office.

Figure 3.2 A rain gauge at one of the farmer's field

3.7. 3 Nodulation assessment

Nodulation may be assessed several times during early growth of the crop; nodule number, size and distribution can be indications of *Rhizobia* infectiveness and effectiveness. However, due to limited resources, a single observation was done at flowering growth stage. The assessment was done on the following parameters: vegetative growth (health, vigour and colour of plants), nodule number per plant and nodule colour. Healthy, vigorous and green plants are most likely to have fully effective symbioses and resultant nodule isolates may be at the upper end of the effective scale; but not always because localized soil environments, particularly with variations in soil nitrogen, may stimulate vigorous growth of the plant (Zaychuk, 2009). Such situations are only apparent when the plants are excavated and examined for the presence of active nitrogen-fixing nodules. Effective nodules generally have predominant pinkish or red internal colour, while ineffective nodules have green or white internal colour when dissected (Bala *et al.,* 2010; Tiwari, *et al.,* 2012). Using destructive sampling method, five randomly selected plants per plot were sampled at flowering stage when the number of nodules is at maximum and easier to see the nodules in the soybean crop (Zaychuk, 2009; Bala *et al.,* 2010). These nodules were collected when the soil was moist as dry conditions can cause the legume to shed its nodules or nodules to desiccate and rupture.

The randomly selected plants were carefully dug up and rinsed from the net plot (8 m x 8 m) of each plot with the help of spade by digging the soil core of 150 mm around the plant and 200 mm in depth. Where necessary, the plants were then placed into plastic bucket of water to loosen the adhering soil. After 15 to 20 minutes, the adhering soil was carefully removed to avoid losing some nodules. Thereafter, plants were separated into roots and shoots by cutting from the first node. Care was also taken not to damage to nodules. Roots were then placed in a cooler box to preserve the nodule colour and taken to microbiology laboratory at Chitedze Research Station where the roots were washed under running water with a screen underneath to catch the detached nodules. Nodules from roots were removed, counted, nodule colour assessed (15 nodules in each plot), weighed to obtain fresh weight and then oven-dried for 72 hours at 65-70°C (Jones, 2001) and weighed for nodule dry matter assessment. In addition to nodule number and nodule dry matter measurements, plant vegetative growth was also assessed. This is important to avoid relying only on nodule dry matter as it is hard to clean nodules completely from adhering soil and a single sand grain is often heavier than one nodule (Bala *et al.,* 2010).

Plant growth and vigour, nodule number and nodule colour were assessed using the procedure described by Zaychuk (2009).

Plant growth and vigour

- ✓ Plants predominantly green and vigorous ………………....5
- ✓ Plants predominantly green but relatively small………….4
- ✓ Plants slightly green and relatively small………………….3
- ✓ Plants slightly chlorotic (yellowing)……………………….2
- ✓ Plants very chlorotic……………………………………… 1

a. **Nodule number**

- ✓ Super-nodulated (>50 nodules)……………………………5
- ✓ Abundant (>20 nodules)…………………………………..4
- ✓ Moderate (11-20 nodules)……………………………….....3
- ✓ Few (5-10 nodules)…………………………………......2
- ✓ Root nodules absent (0-4)…………………………………1

b. **Nodule internal colour**

- ✓ Predominantly red in colour……………………………….5
- ✓ Some pink or reddish colour……………………………....4
- ✓ Some pink or greenish colour……………………………..3
- ✓ Some white or greenish colour……………………………2
- ✓ Predominantly white or greenish colour…………………..1

Overall nodulation assessment, which took into account plant growth and vigour, nodule numbers and nodule colour rating scores, was done using a scale of 1 to 15 to rate nodulation effectiveness (Table 3.1).

Table 3.1 Criteria for assessing nodulation (nodule number, nodule colour and vegetative growth) based on 1 to 15 rating system

Total scores	Assessment	Comments
10-15	Effective nodulation	Good N-fixation potential
7-9.9	Nodulation less effective	Reduced fixation potential. Needs probing
1-6	Generally unsatisfactory nodulation	Requires evaluation of growing conditions at the site

Source: Adapted from Zaychuk (2009); Bala *et al.* (2010)

3.7. 4 Plant biomass at flowering stage

At flowering stage, five soybean plants were randomly selected for nodulation assessment and the same plants were also used for determination of plant dry matter and for N and P analyses. Shoots and roots were immediately weighed to obtain fresh above ground dry matter (shoot weight) and below ground dry matter (root weight), respectively, in the field using a 0.01g precision electronic scale. All samples were then packed in paper bags and labelled, and then transported to Chitedze Microbiology Laboratory for determination of dry shoot and root matter. Shoot and root dry weight per plant were taken after oven drying the fresh plant samples for 48 hours at 70°C as described by Jones (2001).

3.7.5 Plant chemical analyses for N and P

In preparation for plant chemical analyses, plant shoots and roots were cut into small pieces and oven dried at 70°C for 48 hours. Dried plant samples were then milled and analysed for plant N and P using the methods described by Winkleman *et al.* (1985).

3.7.6 Plant biomass and grain yield; and calculation of harvest index

In order to get biomass yield, plants were uprooted by hand from the harvest area of each net plot (8 m x 8 m), sun dried and weighed to determine biomass yield. The senesced leaves were estimated per plot by systematically collecting them from the 2 furrows per net plot, sun dried and calculated the average weight for 1 furrow and then multiplied the weight of senesced leaves for 1 furrow by number of furrows per net plot; the computed figure was added to the biomass yield to obtain the total biomass yield. The biomass yields of each net plot were then threshed and winnowed.

After threshing and winnowing the harvested plants, the grain yield per plot was weighed and recorded. Grain yield was adjusted to standard storage moisture content of 13%.

Harvest index (%) was calculated using the following formula:

$$Harvest\ Index\ (\%) = Grain\ Yield\ \left(kgha^{-1}\right)\ /\ Biomass\ Yield\ \left(kgha^{-1}\right) \times 100$$

3.7.7 Determination of biological nitrogen fixation

Modified nitrogen difference method technique was used to quantify the nitrogen fixed by soybean. The method compares total N of the N_2fixing species with that of a neighbouring non N_2-fixing species, with the difference between the two values assumed to be due to N_2 fixation (Peoples *et al.,*1989;Unkovich*et al.,* 2008). In this method, the differences in post-harvest soil mineral N are also determined in the fixing and non-fixing plots and added to the differences in total N yields of the two crops. Underlying assumptions are that the N accumulated by the non N_2-fixing control is derived only from soil N, and its N content represents the amount of soil mineral N available for plant growth. The N_2-fixing plants use the same amount of soil mineral N as the non N_2fixing control. It also assumes that mineralization, leaching and denitrification are identical under each crop (Peoples *et al.,* 1989).

The method is expressed in the following equation:

$$BNF \quad \left[kgha^{-1}\right] = \left(shootN_{Leg} + rootN_{Leg}\right) \ \left[kgha^{-1}\right] - \left(shootN_{\mathrm{Re}\,f} + rootN_{\mathrm{Re}\,f}\right) \ \left[kgha^{-1}\right] + \left(SoilN_{Leg} - SoilN_{\mathrm{Re}\,f}\right) \ \left[kgha^{-1}\right]$$

Where:

$\left(shootN_{Leg} + rootN_{Leg}\right)$ = Nitrogen amount in soybean shoots and roots

$\left(shootN_{\mathrm{Re}\,f} + rootN_{\mathrm{Re}\,f}\right)$ = Nitrogen amount in shoots and roots of reference crop

$\left(SoilN_{Leg} - SoilN_{\mathrm{Re}\,f}\right)$ = Difference between soil nitrogen amount under soybean and soil nitrogen amount under reference crop

In line with the requirements of the method used, unfertilized maize was used as non-nitrogen fixing plant species; planted adjacent to the main soybean plots. At mid-podding stage (before leaf fall), five plant samples for each species were taken at random in each plot for determination of crop N. The plant samples were oven dried at 70°C for 48 hours and ground. Nitrogen content was determined using the Kjedahl's digestion method. Soil samples were taken at 0-15 cm depth from the respective plots of soybean and the control reference species for analysis of soil nitrogen; calculated on the basis of soil and plant N and adjusted to area basis by using the soil bulk density and soil-depth used to collect soil samples i.e. (10000 m^2 * 0.1 m x bulk density in g/cm^{-3}) x BNF measured.

3.7.8 Partial budget analysis

The economic effect of P fertilizer application and inoculant use on soybean was evaluated using a partial budget analysis after harvesting. Inoculant and P fertilizer costs were considered as the variable costs. On soybean P fertilizer x Inoculant trial with Tikolore variety, average grain yield obtained under different inputs (P fertilizer and inoculant) less the grain yield from the control (Tikolore without inputs) were used to obtain the yield gain due to input application. This yield gain less the yield required to cover input cost was then converted into monetary value and regarded as the net input use benefit (Tables 4.22 and 4.23) gained by the farmer per hectare after paying for their input cost. Official Malawi government farm gate price for soybean was taken as the market price to compute income in the experimentation year. Prices for inputs (inoculant and P) were those used during the purchase of the products. The exercise was done to evaluate if it warrants investing on fertilizer or inoculant use at small-scale farming level focusing on the returns obtained from different sites used in this study.

3.7.9 Data analysis

Data on yield and yield components of soybean was statistically subjected to linear mixed models analysis using GenStat statistical software 15th edition. Prior to subjecting data to statistical analysis (confirmatory analysis), an investigation of data pattern was carried out to ensure that there was no pattern in residual part of the data (data exploration). Square root transformation of data on nodule counts was done prior to analysis to improve homogeneity of variance of the data. Appropriateness of the transformation was evaluated by the examination of the pattern of the residuals plots. Significant differences were assessed at 5% level and means separation were using Fisher's protected least significant difference (LSD) procedure. Regression and correlation analyses were also performed to determine the association between measured variables. Criteria used to evaluate the strength of the associations were the coefficient of correlation (r), coefficient of determination (r^2) and P value. The statistical models used in analysing the data were as follows:

Statistical model for the soybean varietiesand P application trial

$$Y_{ijt} = \mu + \mathrm{b}_{\mathrm{i}} + d_j + s_t + bd_{ij} + bs_{it} + ds_{jt} + bds_{itj} + e_{itj}$$

Where:

Y_{itj} is response variable within i^{th} variety treatment, j^{th} P treatment and t^{th}site

µ is the overall mean

b_i is the fixed effect of ith variety treatment

d_j is the fixed effect of jth P treatment

s_t is the random effect of tth site

bd_{ij} is the interaction effect of variety x P

bs_{it} is the interaction effect of variety x site

ds_{jt} is the interaction effect of P x site

bds_{ijt} is the interaction effect of P x variety x site

e_{ijt} is the random error

Statistical model for the soybean inoculant and P application trial

$$Y_{it} = \mu + \mathrm{b}_{\mathrm{i}} + s_t + bs_{it} + e_{it}$$

Where:

Y_{it} is response variable within the i^{th} treatment and t^{th} site

µ is the overall mean

b_i is the fixed effect of ith treatment

s_t is the random effect of tth site

bs_{it} is the of interaction effect of treatment x site

e_{it} is the random error

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Soil characterisation

Table 4.2 shows results on initial soil chemical properties and texture. Inorganic P, total N, CEC and OM for Linthipe EPA in Dedza were above the critical levels for soybean growth and development. Soybean grows well in soils with more than 25 ppm available P, 0.1% N, 25 meq/100g CEC and 1.5% OM. Soil analysis results showed that most of the nutrients analysed were below the critical levels for soybean growth and development in Kaluluma EPA in Kasungu. Low nutrient levels in Kaluluma may be attributed to the higher percentage (72 to 90%) of sand in the most of the farms. Soil values for Chinguluwe EPA in Salima were high in OM, N, and CEC. However, P values were below the critical levels for soybean growth and development and therefore, necessitating the need to apply P to support soybean growth and development.

Soil reaction was moderately acidic (pH 5.7) in Kaluluma and Linthipe EPAs and slightly acidic (pH 6.2) for Chinguluwe EPA. Soybean grows well in soil of pH 6.0 or higher, but can also tolerate pH 4.3 to 4.8. Soil pH is an important factor for plant growth, as it affects nutrient availability, nutrient toxicity, and has a direct effect on the protoplasm of plant root cells (Alam *et al.*, 1999). It also affects the abundance and activity of soil organisms (from microorganisms to arthropods) responsible for transformations of nutrients (Nicol *et al.*, 2008).

Soil texture for Linthipe EPA range from sandy clay loam soils to sandy clay soils. Kaluluma EPA has majority of the soils with high percentage of sand between 72 to 90% giving loamy sand and sand textures. Chinguluwe EPA has soil texture ranging from sandy clay loam soils to sandy loam soils and (Table 4.2). Soybean tolerates a wide range of soil texture; but, the crop performs well in sandy loam soil.

Table 4.2 Soil characterisation of the sites in Linthipe EPA, Kaluluma EPA and Chinguluwe EPA

Soil variable	**Critical value**	**Linthipe EPA (n=5)**		**Kaluluma EPA (n=9)**		**Chinguluwe EPA (n=9)**	
		Mean	Std dev.	Mean	Std dev.	Mean	Std dev.
pH	5-7	5.7	0.23	5.7	0.4	6.2	0.4
CEC (meq/100g)	25	51.7	9.06	16.4	9.3	35.4	7.4
Total N (%)	0.1	0.15	0.04	0	0	0.1	0
P_Av.(ppm)	25	32.4	26.4	24.4	8.9	8.7	5.8
K ($cmol_c$/kg)	0.13-0.20	0.7	0.31	0.4	0.2	0.8	0.4
Ca ($cmol_c$/kg)	2.0	18.2	2.6	4.9	2.9	10.1	2.2
Mg ($cmol_c$/kg)	0.24	3.6	1.02	1.1	0.5	2.3	0.6
% OM	1.5	2.98	0.8	0.9	0.5	1.6	0.6
% Sand	-	60.4	11.3	83.8	6.2	73.6	4.8
% Clay	-	28.8	10.0	12.4	5.0	19.8	3.9
Textural class	SL	SCL	-	SL	-	SL	-

Std. Dev = standard deviation; Critical value = A value below which implies low amount; n = number of observations; SL = sandy loam soil; SCL = sandy clay loam soil

Soil analyses results indicate a wide variability in the soil parameters among the districts and within farms in the EPA (Appendices 1 to 5). This affected the performance of the soybean varieties in growth and development and therefore, discussed in the subsequent crop growth performance results; identified through morphological and physiological symptoms. However, soybean grain yield is the final product of numerous factors that affect crop growth and development during the growing season. Soils in Linthipe EPA were the only ones with the potential to successfully support soybean growth and development with minimal inorganic fertilizer application.

4.2 Effect of variety and phosphorus on nodulation, yield and yield components of promiscuous and specific soybean varieties in Dedza, Kasungu and Salima districts

Two inoculated soybean varieties were evaluated for their response to P fertilizer application in Dedza, Salima and Kasungu districts. These varieties were of two distinct categories: one specific variety (Nasoko) and the other one promiscuous variety (Tikolore). The results of the potential role(s) of P in *Rhizobia* inoculated soybean varieties are presented with respect to nodulation, BNF, economic benefits, yield and yield components is this section.

4.2.1 Effect of variety and phosphorus fertilizer on soybean nodulation

Unlike soybean varieties, application of P significantly ($p < 0.001$) increased soybean nodulation in all the study sites (Table 4.3). There were significant differences ($p < 0.001$) among the sites in nodulation scores. Results suggest that soil fertility status among the districts had much influence on soybean response to P fertilizer application and confirmatory analysis using regression models showed the compelling effect of Ca, Mg, K, OM, and pH to be significant with high correlation of the variables with CEC and N in accounting for variation in grain yield (Table 4.15). Linthipe EPA in Dedza had much better soils parameters to support soybean growth and development than the other two sites (Table 4.2).

A nodulation score of 10-15 signifies effective nodulation; 7-9.99 signifies less effective nodulation and 1-6 signifies unsatisfactory nodulation (Bala *et al.,* 2010). Nodulation assessment score comprised a combined analysis of vegetative growth, nodule numbers, nodule weights and nodule effectiveness and these parameters favoured the treatment with an application of P. Phosphorus requirement has previously been shown to vary among soybean genotypes with the degree of nodulation reported to depend on plant genotype and field site (Sanginga *et al.,* 2000). Several studies have also reported that soils deficient in P limit the extent of soybean nodulation (Israel, 1993; Ankomah *et al*., 1995). Results demonstrate that inoculation alone may not be the only solution to improved soybean nodulation. Kwari (2005) concluded that the poor soil-P status would have implications for the production of legumes, such as cowpea and soybean, which would require P for their root development, nodulation and N_2 fixation. Deficiencies of soil nutrients, especially P, may restrict the development of a population of free-living Rhizobia in the rhizosphere, limit the growth of the host plant, restrict nodulation itself, and cause an impaired nodule function (Danso, 1993; Wasike *et al.,* 2009).

4.2.2 Effect of variety and phosphorus fertilizer on number of nodules per plant

As evident from the results presented in Table 4.3, inorganic P fertiliser significantly increased nodule numbers in both Nasoko and Tikolore soybean varieties by 40-50% (P = 0.007). This suggests that P fertilizer application has a positive impact on the initiation and development of soybean root nodules. This is in agreement with reports from earlier workers who showed significant influence of P fertilizer application on soybean root and nodule development (Kumaga and Ofori, 2004; Aduloju *et al.,* 2009; Tahir *et al.,* 2009). Phosphorus plays a very important function in almost every plant process that involves energy transfer. High-energy phosphate, detained as a part of the chemical structures of adenosine diphosphate (ADP) and adenosine triphosphate (ATP), is the source of energy that drives the huge number of chemical reactions within the plant. During nodulation, P is an essential ingredient for *Rhizobium* bacteria to convert atmospheric N_2 into an ammonium (NH_4^+) form usable by plants. Inadequate P restricts root growth, the process of photosynthesis, translocation of sugars and other such functions which directly influence N fixation by legume plants (Fatima *et al.,* 2007).

However, site differences significantly affected the response of soybean varieties to nodule formation (Table 4.3) indicating that varying soil physical and chemical properties have an impact on nodule number production. Dedza produced 87 and 115% more nodules than Salima and Kasungu respectively.Even though inoculant was applied in some plots, yellowing and chlorosis due to nitrogen deficiency were evident in many of the soybean fields in Kasungu and few in Salima. Reduced nodulation leads to nitrogen deficiency symptoms in soybeans if residual nitrogen is not available (Keyser and Li, 1992). On the other hand, *Rhizobia* growth, health and activity depend on the initial population of bacteria and the soil conditions that favour or hinder their development (Keyser and Li, 1992). Therefore, in sites with insufficient nodulation, in-crop season assessment of nutrient deficiency is key; if the number and quality of the nodules is not sufficient, supplemental nutrient element can be applied before flowering even though it's a daunting task for smallholder farmers in Malawi.

The extent to which different management practices contribute to changes in symbiont effectiveness is another area of research. However, the study suggests (i) fertilizer use in poor fertile soils could be key; (ii) high quality Rhizobia inoculant adds beneficial strains to the soil to support soybean nodulation; (iii) using the best adapted soybean genotype with a fully compatible inoculant; and (iv) increasing the breadth of breeding programmes to include selection for effective symbioses under low soil fertility, poor management/agronomic practices and extreme environmental conditions could prove an effective tool for symbiont management to improve nodulation and overall N_2-fixation in legumes. If management/agronomic practices are driving symbioses towards increased or reduced nodulation, modifying the practices in Kasungu and Salima districts is key to successful nodulation in these areas.

Table 4.3 Main effects of phosphorus (P) fertilizer application and sites on soybean nodulation and nodule numbers per plant

P_rate(kg P_2O_5 ha^{-1})	**Nodulation score**	**Number of nodules/plant**
P_0	9	15
P_{30}	11	22
SED	0.007	0.08
P-value	<0.001	0.007
Site	Nodulation score	Number of nodules/plant
Dedza	12^a	28^a
Kasungu	8^b	13^b
Salima	10^c	15^b
SED	0.007	0.12
P-value	<0.001	<0.001

NB: SED is the average standard error of difference as estimated under generalised linear mixed model in GenStat; score 10-15 = Effective nodulation; 7-9.9 = Nodulation less effective; 1-6 = Unsatisfactory nodulation;P_0 = 0 kg P_2O_5 ha^{-1}; P_{30} = 30 kg P_2O_5 ha^{-1}

4.2.3 Effect of variety and phosphorus fertilizer on biological nitrogen fixation (BNF)

The results in Table 4.4 indicate that P fertilizer application to soybean varieties has a significant positive contribution to the quantities of biologically fixed nitrogen in the soybean farming system ($P < 0.001$). This is in agreement with the earlier presented results on nodulation assessment (N_2 fixing potential) of the soybean varieties which showed that P fertilizer application has a positive influence on nodulation.

Differences in the performance of the two varieties were also evident in this study. Tikolore promiscuous soybean variety had fixed significantly ($P = 0.033$) higher amount of N than Nasoko specific variety (Table 4.4). Keyser and Li (1992) reported that the average amount of soybean N derived from BNF is estimated at 75 kg N ha^{-1}. Chibwana (1996) reported that up to 50 kg N ha^{-1} can be fixed by soybean varieties in Malawi. As results indicate, however, the symbiosis is specific and depends upon soybean genotype and *Rhizobia* strains under various edaphic and climatic conditions (Abaidoo *et al.,* 2007; Chalk *et al.,* 2010). Further research is needed to correlate the number of native *Rhizobia* strains under different soils with the amount of BNF measured per particular site. Elsewhere, it is reported that the nitrogen requirement of a soybean crop is estimated at 350 kg N ha^{-1} (Abendroth *et al.,* 2006). With adequate supply of P, soybeans can fix up to 450 kg N ha^{-1} (Unkovich and Pate, 2000) making it possible for the crop to satisfy its nutritional requirements and leave some residual N for use by associated crops. Results of this study imply that a lot has to be done in order to meet this requirement in smallholder farming systems. Among other factors, the amount of fixed N which is ultimately used by soybean crop is a function of available N, with the plants utilizing available soil N prior to fixed N (Salvagiotti *et al.,* 2008).

Table 4.4 The effect of variety and P fertilizer on biological nitrogen fixation (kg N ha^{-1})

	P_rate (kg P_2O_5ha^{-1})		
Variety	**P_0**	**P_{30}**	**Mean**
Nasoko	21	32	27
Tikolore	28	32	30
Mean	25	32	
	Variety	P_rate	Variety* P_rate
SED	1.92	1.92	2.72
P-value	0.033	<0.001	0.06

NB: SED is the average standard error of difference as estimated under generalised linear mixed model in GenStat; P_0 = 0 kg P_2O_5 ha^{-1}; P_{30} = 30 kg P_2O_5 ha^{-1}

4.2.4 Variety and phosphorus effect on soybean shoot and root dry weight at 50% flowering stage

The study results showed significant effect of P fertilizer application to soybean and significant effect of using different soybean varieties on shoot dry weight at 50% flowering stage (Table 4.5). Phosphorus fertilizer application significantly influenced the growth of soybean varieties both above-ground and below-ground. Two soybean varieties also differed significantly ($p < 0.001$) in their response to P fertilization with Nasoko variety giving 32% higher shoot dry matter yield than the promiscuous soybean variety. The significant ($p < 0.05$) interaction effect of soybean variety and site suggests that shoot and root dry matter yield might depend on the variety you use in a particular site or agro-ecological zone (Tables 4.6 and 4.8). Higher values of root dry matter were obtained from Linthipe EPA; an area with observed and measured better physical and chemical soil properties, suggesting that edaphic factors largely affected the yield of root dry matter of soybean varieties. In particular calcium levels, an element required in high amount by legumes, Mg, OM and K were much higher in Linthipe, Dedza.

Regression analysis for these factors to grain yield was significant, suggesting that they contributed to the yield variation among the three sites.

Aside from the main effects of P fertilizer application and soybean varieties on shoot dry weight, there was significant interaction between inorganic P fertilizer application and the soybean varieties in root dry matter yield (Table 4.7); suggesting differences in P use efficiency between the two varieties. Results suggest that P fertilization played a very big role in the performance of the soybean varieties depending on the available soil nutrients. Results of the study showed positive correlations of root dry matter yield with CEC, N, Mg, Ca. OM and K (Table 4.15). Soil physical-chemical factors such as CEC, pH, P, N and micronutrient levels are also important in root development and growth of soybean (Brady, 2002; Mohammadi *et al*., 2012).

Results obtained in this study were in agreement with Mahamood *et al.* (2009), Mugendi *et al*. (2010) who reported significant differences between soybean varieties and observed significant positive responses to P application. Lukiwatid and Simanungkalit (2002) also stated that the presence of P in the soil with Rhizobium strains, individually or cumulative, effect on root development, nodule weight per plant and nitrogen fixation parameters. Higher values of root dry matter in the fertilized treatments suggest that the soybean varieties responded to P fertilizer application and influenced their root growth and development. Bekere *et al.* (2012) also observed that both inoculation and P fertilizer application significantly influenced root dry matter of soybean. Elsewhere, Tahir *et al*. (2009) reported that application of P alone significantly increased root length and root dry weight by 33 and 64% over the control. Kumaga and Ofori (2004) concluded that inoculation of promiscuous soybean varieties with effective *Bradyzhizobia* may be a more important strategy for increasing soybean yields than addition of P fertilizers. Results of this study indicate that application of P fertilizer for root growth and development and rhizobium inoculation for nodulation collectively enhance nodulation, plant growth and development.

Table 4.5 Shoot dry weight (g/plant) as affected by variety and phosphorus (P) fertilization at 50% flowering stage

	P_rate(kg P_2O_5 ha^{-1})		
Variety	**P_0**	**P_{30}**	**Mean**
Nasoko	11.56	14.75	13.16
Tikolore	9.14	10.77	9.96
Mean	10.35	12.76	
	Variety	P_rate	Variety* P_rate
SED	1.51	1.51	2.15
P-value	<0.001	<0.001	0.215

SED is the average standard error of difference as estimated under generalised linear mixed model in GenStat; P_0 = 0 kg P_2O_5 ha^{-1}; P_{30} = 30 kg P_2O_5 ha^{-1}

Table 4.6 Shoot dry weight (g/plant) as affected by variety and site at 50% flowering stage

		Site		
Variety	**Dedza**	**Kasungu**	**Salima**	**Mean**
Nasoko	15.21	13.11	11.15	13.16
Tikolore	9.45	10.29	10.13	9.96
Mean	12.33	11.70	10.64	
	Variety	Site	Variety*site	
SED	1.51	0.76	1.07	
P-value	<0.001	0.09	0.011	

SED is the average standard error of difference as estimated under generalised linear mixed model in GenStat

Table 4.7 Root dry weight (g/plant) of soybean as affected by variety and phosphorus (P) fertilization at 50% flowering stage

	Variety		
P_rate (kg P_2O_5 ha^{-1})	**Nasoko**	**Tikolore**	**Mean**
P_0	1.1	0.7	0.9
P_{30}	1.2	1.3	1.3
Mean	1.1	1.0	
	Variety	P_rate	Variety* P_rate
SED	0.08	0.08	0.115
P-value	0.137	<0.001	0.002

SED is the average standard error of difference as estimated under generalised linear mixed model in GenStat; P_0 = 0 kg P_2O_5 ha^{-1}; P_{30} = 30 kg P_2O_5 ha^{-1}

Table 4.8 Root dry weight (g/plant) of soybean as affected by different sites at 50% flowering stage

Site	**Dedza**	**Kasungu**	**Salima**
Mean	1.65	0.41	1.19
SED	0.1		
P-value	<0.001		

SED is the average standard error of difference as estimated under generalised linear mixed model in GenStat

4.2.5 Effect of variety and phosphorus fertilizer on soybean shoot nitrogen (N) and phosphorus (P) at 50% flowering stage

Phosphorus fertilization did not significantly ($p < 0.05$) affect shoot P content in both varieties. Significant differences were observed for shoot N content for the two varieties (Table 4.9). Results suggest that inoculation technology with the right Rhizobia could be beneficial for soybean N and P uptake, however, further research might be needed to evaluate the N and P use efficiency of soybean varieties with application of *Rhizobium* inoculant and P fertilizer in the soils of Malawi. Large amount of P applied as fertilizer enters into the immobile pools through precipitation reaction with highly reactive aluminium (Al^+) and iron (Fe^{3+}) in acidic soils, and calcium (Ca^{2+}) in calcareous or normal soils (Gyaneshwar *et al.*, 2002; Hao*et al.*, 2002). Other researchers reported significant positive responses of soybean varieties in terms of yield components (including shoot N and P content) to phosphorus fertilizer application only at higher levels of P i.e. > 60 $kgPha^{-1}$ (Islam*et al.*, 2004; Shahid *et al.*, 2009; Ali *et al.*, 2013; Jaidee *et al.*, 2013; Mahmoodi *et al.*, 2013). However, wide differences in P acquisition and use have been documented among and within many legumes including soybean (Alves *et al.*, 2003; Sanginga *et al.*, 2000). It is, therefore, pertinent to place more emphasis on P use efficiency other than increased application of P fertilizer. Significant ($p < 0.001$) differences were observed for shoot N and P content among the different sites (Table 4.10). Results showed that higher values for total N were obtained in trials from Salima (low-altitude zone) which had lowest values of shoot P content and lowest values for shoot N content were obtained in Dedza (mid-altitude) which had higher values for shoot P content. Differences in soil physical and chemical parameters among the agro-ecological sites tested could be the major influence in shoot P content in the soybean. Reports indicated that nutrient uptake by the plant is influenced by nutrient variability, water content, aeration and pore continuity conditions in the rhizosphere, which govern nutrient diffusion from soil solid phase to plant roots (Barber, 1995). The changes in the forms and distribution of soil P are related to soil texture and soil pH. Huffman *et al.* (1996) reported that soil texture had a greater effect on labile P transformations than the combined effects of crop residues, nutrient additions and residue placement. In Malawi, soil P is higher on farms that received relatively adequate P fertilization, indicating that there has been a build-up of P in the soils that received P fertilisation (Maida, 2013).

Table 4.9 Effect of soybean variety on shoot nitrogen (N) and phosphorus (P) at mid-flowering

Site	**Shoot P %**	**Shoot N%**
Nasoko	0.17	2.5
Tikolore	0.18	2.7
SED	0.01	0.13
P-value	0.272	0.04

SED is the average standard error of difference as estimated under generalised linear mixed model in GenStat

Table 4.10 Effect of site on shoot nitrogen (N) and phosphorus (P) at mid-flowering

Site	**Shoot P %**	**Shoot N%**
Dedza	0.24^{a}	2.2^{c}
Kasungu	0.19^{b}	2.6^{b}
Salima	0.10^{c}	2.9^{a}
SED	0.01	0.16
P-value	<0.001	<0.001

SED is the average standard error of difference as estimated under generalised linear mixed model in GenStat

4.2.6 Effect of variety and phosphorus fertilizer on soybean harvest index, grain and biomass yields

The interaction of soybean varieties and site was significant indicating that the genotypes perform differently in different sites (Table 4.11). Aside to genetic differences between the two soybean varieties, this could also be due to differences in environmental conditions of the tested sites. Nasoko gave highest grain yield in Dedza but no differences were observed in Salima and Kasungu. Lowest yields were observed in Kasungu for Tikolore soybean variety and no differences were in Salima and Dedza. Tikolore soybean variety out-yielded Nasoko variety in low-altitude zone whilst

Nasoko out-yielded Tikolore soybean variety in mid-altitude zone (Kasungu and Dedza). Early maturing varieties are suited in low-altitude agro-ecological zones which have fewer number of rainfall months than medium or high altitude agro-ecological zones. Similarly, Tikolore variety produced significantly higher biomass yield in Salima than the other two districts and Nasoko produced significantly higher biomass yield in Dedza than the other two districts (Table 4.13). Farmers in Salima could therefore be advised to adopt Tikolore soybean variety which is early maturing than Nasoko variety. Nasoko soybean variety could be recommended in Kasungu and Dedza districts.

Phosphorus application increased grain yield by 27% (Table 4.12) and increased soybean biomass by 24% (Table 4.14). Higher positive influences of fertilizer application were observed in fertilized soybean varieties over the unfertilized soybean varieties. Tahir *et al.*, (2009) reported that combination of *Rhizobium* inoculation and P fertilizer application resulted in 21% increase in grain yield. Fatima *et al.* (2006) observed similar findings and concluded that combined application of P with *Rhizobium* inoculation increased growth, yield and nitrogenase activity as well as improved soil fertility. Kumaga and Ofori (2004), however, reported that P fertilizer additions did not result in significant increases in shoot growth and seed yield against the inoculated soybean variety; significant differences were only observed on the un-inoculated variety. Similar results were measured in Dedza district where P fertilization did not significantly influence soybean grain and biomass yield in Nasoko specific soybean variety. This was a site with available soil P above critical level (P > 25ppm, Mehlich 3). Ferguson *et al.* (2006) also reported that P application is not likely to increase seed yield at soil P concentration above 12 ppm (Bray 1 method); except under low P status. Results from this study suggest that there is need to establish threshold values of P beyond which P fertilization has no effect in Malawi. Under low P levels, the net profits for soybean varieties under the best performing intervention (P + *Rhizobium* inoculant) have been observed for other sites in sub-Saharan Africa (SSA) under the N2Africa project (Woomer *et al.,* 2012). The significant response ($P < 0.001$) to P fertilizer application in Kasungu and Salima districts is noteworthy given the generally P deficient status of the experimental sites (Table 4.2). P fertilization in Kasungu increased grain yield by 56 and 34% in Salima district. Results suggest that P fertilizer application should be economical in sites with low soil P levels ($P < 25$ppm, mehlich-3). Phosphorus replenishment is particularly important in soils that have very low native P availability levels (Martin, 2005) even though identifying lower P regions within fields is problematic (Ferguson *et al.,* 2006).

Similar to results reported by Kamara *et al.* (2007), there were no significant differences ($P < 0.05$) in harvest index between the inoculated and the fertilized plus inoculated soybean varieties in both promiscuous and specific varieties. However, harvest index was significantly affected by site conditions ($P = 0.009$). Kasungu district had the lowest harvest index of 44% which was significantly different to 53% and 54% for Salima and Dedza respectively.

Table 4.11 Effect of variety and site on soybean grain yield (kg ha^{-1})

		Site	
Variety	**Dedza**	**Kasungu**	**Salima**
Nasoko	2422^{b}	1344^{a}	1589^{a}
Tikolore	1385^{b}	910^{a}	1755^{b}
	Variety	Site	Variety*Site
SED	126.4	154.8	201.4
P-value	<0.001	<0.001	<0.001

SED is the average standard error of difference as estimated under generalised linear mixed model in GenStat; Means in a row followed by the same letter are not different at p=0.05

Table 4.12 Effect of phosphorus (P) fertilizer application on soybean grain yield (kg ha^{-1})

P_rate (kg P_2O_5 ha^{-1})	**Grain yield (kg ha^{-1})**
P_0	**1378**
P_{30}	**1756**
SED	116.3
P-value	0.002

SED is the average standard error of difference as estimated under generalised linear mixed model in GenStat; P_0 = 0 kgP_2O_5ha^{-1}; P_{30} = 30 kgP_2O_5ha^{-1}

Table 4.13 Effect of variety and site on soybean biomass yield (kgha^{-1})

		Site	
Variety	**Dedza**	**Kasungu**	**Salima**
Nasoko	3962^{a}	2670^{b}	2917^{b}
Tikolore	2657^{b}	2092^{b}	3200^{a}
	Variety	Site	Variety*Site
SED	181.3	222	314
P-value	0.005	<0.001	0.004

SED is the average standard error of difference as estimated under generalised linear mixed model in GenStat; Means in a row followed by the same letter are not different at p=0.05

Table 4.14 Effect of phosphorus (P) fertilizer application on soybean biomass yield (kg ha^{-1})

P_rate (kg P_2O_5 ha^{-1})	**Biomass yield (kg ha^{-1})**
P_0	2609
P_{30}	3224
SED	181.3
P-value	0.001

SED is the average standard error of difference as estimated under generalised linear mixed model in GenStat; P_0 = 0 kgP_2O_5ha^{-1}; P_{30} = 30 kgP_2O_5ha^{-1}

4.2.7 Correlation and regression analyses for the studied parameters

Correlation analysis of 8 major soil components with soybean growth parameters showed varied significant correlations with grain yield, biomass, root dry matter yield, shoot N and shoot P. Available soil P showed significant correlation with only shoot P whilst most soil parameters showed significant correlation with root dry matter yield (Table 4.15). Soil pH had significant ($P < 0.001$) positive and negative correlation with only shoot N and shoot P respectively. As natural stress, pH has far-reaching effects on the growth and nutrient uptake by plants. Positive correlation of soil pH and total nitrogen concentration in the plant materials is mediated by the processes of ammonification and nitrification; ammonification increases soil pH and nitrification reduces the soil pH (Yan *et al.*, 1996). Decreases in rate of phosphate absorptions with increasing pH are also documented (Marschner, 1995; Tyler, 1999; Soti *et al.*, 2015).

Table 4.15 Correlation of soil parameters with various studied parameters in soybean varieties

	Grain yield	Biomass	Root dry matter yield	Shoot P	Shoot N
K	0.14	0.33**	0.28*	-0.35**	0.39**
Mg	0.21	0.17	0.72***	0.07	-0.16
N	0.41***	0.43***	0.73***	0.25	-0.28*
OM	0.41***	0.42***	0.74***	0.25	-0.28*
P	-0.05	0.05	0.10	0.55***	-0.14
pH	0.03	0.13	0.09	-0.59***	0.58***
Ca	0.33**	0.30*	0.67***	0.32*	-0.29*
CEC	0.27*	0.26*	0.73***	0.19	-0.11

*, ** and *** correlation is significant at <0.05, <0.01 and <0.001 levels of probability respectively

Correlation matrix of the studied parameters revealed significant positive association between grain yield and the following parameters: biomass yield (r^2 = 0.77), harvest index (r^2 = 0.46), nodulation assessment, shoot dry matter yield (r^2 = 0.25), root dry matter yield (r^2 = 0.26), Nitrogen (r^2 = 0.17), OM (r^2 = 0.17), Ca (r^2 = 0.11) and CEC (r^2 = 0.07). Significant ($P < 0.001$) positive correlation were also observed between nodulation and biomass yield, grain yield, nodule dry weight, nodules per plant and root dry weight (Table 4.16). Direct relationships of biomass yield, nodulation, shoot and root dry matter yield with grain yield or nodulation are strongly in support of Achakzai and Kayani (2002) and Bekere *et al.* (2012). Therefore, a successful breeding program can rely on these positive relationships in order to improve grain or biomass yield of soybean varieties. On the other hand, parameters with positive relationships with grain yield can be used for soybean yield prediction in some cases.

Table 4.16 Correlation matrix among the various studied parameters in Nasoko and Tikolore soybean varieties

	Biomass	Grain yield	Harvest index	Nodule dry wt	Nodules plant^{-1}	Root DM	Shoot N%	Shoot P%	Nodulation	Shoot DM
Biomass	-									
Grain yield	0.88***	-								
Harvest index	0.27*	0.68***	-							
Nodule dry wt	0.18	0.12	-0.02	-						
Nodules plant^{-1}	0.34**	0.30*	0.10	0.79***	-					
Root DM	0.51***	0.51***	0.31*	0.53***	0.56***	-				
Shoot N%	-0.07	-0.22	-0.31*	-0.02	-0.06	-0.24	-			
Shoot P%	0.02	0.07	0.04	0.17	0.35**	0.08	-0.27*	-		
Nodulation	0.47***	0.42***	0.16	0.60***	0.73***	0.67***	-0.12	0.26*	-	
Shoot DM	0.46***	0.50***	0.27*	0.17	0.27*	0.20	-0.10	0.24	0.24	-

*, ** and *** correlation is significant at <0.05, <0.01 and <0.001 levels of probability respectively; DM = dry matter; wt = weight

Correlation analysis of the soil parameters and yield components that correlated with grain yield showed presence of multicollinearity among the parameters if subjected to regression analysis (Table 4.17). Biomass yield correlated with each and every parameter and had the highest coefficient of determination (76.2) and lowest standard error (330) after running simple linear regressions of these parameters with grain yield. Due to multicollinearity of the studied parameters, stepwise regression analysis singled out biomass yield as strongest predictor of grain yield among the measured parameters as well as harvest index as second important predictor after biomass yield. Other predictors were highly collinear to biomass yield. Generallinear mixed model analysis of the factors used in the study showed compelling‘site’ and ‘variety’ interaction effect on soybean grain yield (Table 4.18).

Table 4.17 Correlation matrix among the parameters with significant correlation with grain yield

	Nodulation	Root dry weight	Nodule plant	OM	N	Harvest Index	Biomass yield	Dry matter yield	Ca	CEC
Nodulation	-									
Root dry weight	0.67***	-								
Nodule plant	0.73***	0.56***	-							
OM	0.57***	0.74***	0.55***	-						
N	0.56***	0.73***	0.56***	0.99***	-					
Harvest Index	0.16	0.3112	0.1037	0.17	0.16	-				
Biomass yield	0.47***	0.50***	0.34**	0.42***	0.43***	0.27*	-			
Dry matter yield	0.24***	0.20***	0.27*	0.14	0.15	0.27*	0.46***	-		
Ca	0.46***	0.67***	0.37**	0.85***	0.85***	0.20	0.30**	0.12	-	
CEC	0.51***	0.73***	0.56***	0.85***	0.85***	0.15	0.26*	0.10	0.90***	-

*, ** and *** correlation is significant at <0.05, <0.01 and <0.001 levels of probability respectively

Table 4.18 Summary results of the GLM model for explaining the variability in soybean grain yield in the on-farm trials. Significant effects are shown in bold.

Model	F statistic	*P*-Value	df
P_rate	10.57	**0.002**	1
Variety	13.98	**<0.001**	1
Site	15.68	**<0.001**	2
P_rate x Variety	0.39	0.535	1
P_rate x Site	0.92	0.406	2
Variety x Site	8.91	**<0.001**	2
P_rate x Variety x Site	0.01	0.985	2

4.3 Effect of *Rhizobia* inoculation and phosphorus fertilizer on nodulation, BNF, grain yield and yield components of promiscuous soybean variety in Kasungu and Salima districts

In this study, P fertilizer was applied to determine its individual and interactive effect with *Rhizobium* inoculant on the nodulation, BNF and grain yield and yield components of promiscuous soybean variety in Kasungu and Salima districts of Malawi. The trial had the following treatments: Tikolore without both inoculant and P; Tikolore with inoculant only; Tikolore with P fertilizer only; and Tikolore with inoculant and P fertilizer application. Results are presented with respect to nodulation, BNF, economic benefits, yield and yield components of the soybean variety.

4.3.1 Effect of *Rhizobia* inoculation and phosphorus fertilizer on nodulation of Tikolore promiscuous soybean variety

Results showed that there were no significant differences ($P < 0.05$) in soybean nodulation among the treatments and between the sites tested. *Rhizobium* inoculation and P fertilizer application did not significantly influence soybean nodulation in promiscuous soybean variety compared to the control.

Figure 4.3. Farmer assessment of treatments at vegetative stage

4.3.2 Effect of *Rhizobia* inoculation and phosphorus fertilizer on number of nodules per plant for Tikolore promiscuous soybean variety

Application of *Rhizobium* inoculant and P fertilizer to promiscuous soybean variety significantly (P = 0.002) influenced the number of nodules per plant depending on site (Table 4.19). Fertilizer application produced higher number of nodules in Salima than in Kasungu and *Rhizobium* inoculation had more effect on number of nodules in Kasungu than in Salima; raising questions on the differences in *Rhizobia* populations and competiveness between the two districts? Fertilizer effect was much evident in Salima than in Kasungu district and combined application of P fertilizer and *Rhizobia* inoculant had highest positive influence on number of nodules produced in both districts. Response to P fertilizer application could also be attributed to the measured low P status of the soils which were below the critical level in the sites where the on-farm trials were mounted. Under low P status, P fertilizer application to soybean is important in attaining high yield (Martin, 2005). Kumaga and Ofori (2004) reported that the number of nodules and nodule dry weight appeared to have reached their maximum when P fertilizer was applied at 30 kg P_2O_5 ha^{-1} in a promiscuous soybean variety as compared to where only inoculant was applied and where no inputs were applied. Similar results have also been reported by other workers (Tsvetkova and Georgiev, 2003; Tahir *et al.,* 2009; Bekere *et al.,* 2012).

4.3.3 Effect of *Rhizobium* inoculation and phosphorus fertilizer on biological nitrogen fixation (BNF) for Tikolore promiscuous soybean variety

The study revealed that application of *Rhizobium* inoculant and P fertilizer significantly ($p < 0.001$) increased the amount of N_2 fixed by the promiscuous soybean variety (Table 4.19). Phosphorus fertilizer and *Rhizobium* inoculant application increased BNF by 66% and 16% respectively and a combined effect of *Rhizobium* inoculant and P fertilizer increased BNF by 100%. Several factors have been shown to influence the amount or proportion of N_2 fixed in legume crops, including inoculation with *Rhizobium* strains, soil P status, soil inorganic N status and plant cultivars (Keyser and Li, 1992). Results agree with Sanginga *et al.* (2000) who noted that promiscuous soybean were incapable of nodulating effectively with indigenous *Rhizobia* in all locations in the moist savannah zone of Nigeria. Khonje (1998) observed that nodulation of two self-nodulating cultivars, Magoye and Hernon 147, was ubiquitous but intensity varied with soil types in Malawi including Kaluluma EPA. In some areas, nodulation was very profuse with more than 40 nodules per plant while other sites registered a single nodule per plant or none at all. Observation plots including promiscuous lines from International Institute of Tropical Agriculture (IITA) also indicated varied response across sites, depending upon soil type. Similarly, Bala (2008) observed that it is not clear whether promiscuous soybean cultivars are effectively nodulated by indigenous *Rhizobia* populations in all soils and under all conditions. Sridhara *et al.* (1995), Woomer *et al.* (2012), Njira *et al.* (2012) reported that N_2 fixation in soybean ranges from 13.4 to 35.8 kg ha^{-1} per season in Malawi against the estimated

103 to 313 kg ha^{-1} per season in Australia (Peoples *et al.*, 1995). The variable extent of nitrogen fixation by soybean cultivars is probably due to differences in symbiotic effectiveness of *Rhizobia* strains and their compatibility. Selections of host cultivar-compatible inoculant have been recognized as an important method for increasing nodulation and nitrogen fixation in soybean (Solomon *et al.,* 2012). The results, however, symbolise the importance of Rhizobia inoculation and P fertilizer application to promiscuous soybean varieties in some areas of Malawi. It may be safer to rely on effective inoculant strains and P fertilizer application rather than breed for the ability to nodulate with indigenous *Rhizobia* strains of unknown potential. On the other hand, using promiscuity of soybean varieties as a selection criterion in breeding programs requires support with in-depth microbiological studies. For instance, soybean growing in a soil with a small number of effective *Rhizobia* (20–50 cells g^{-1} soil) will likely respond to inoculation (Thies *et al.,* 1991). Sometimes the presence of a large indigenous population of compatible *Rhizobia* does not necessarily preclude response to inoculation, provided the inoculant *Rhizobia* strains are competitive and highly effective (Giller, 2001; Osunde *et al.,* 2003). The high variability of soil fertility in Malawi calls for more studies aimed at establishing the degree of promiscuity of soybeans with indigenous *Rhizobia* to be further investigated in order to confirm these results.

Considering that the study was conducted in fields where nitrogen is a major limiting factor for plant growth, BNF is thus a key element in integrated soil management strategy in smallholder farming systems where cropping sequences are poorly planned and do not maximise on agro-ecological soil nutrient cycles. It is advocated in sub-Saharan Africa (SSA) that low cost and sustainable technical solutions compatible with the socio-economic conditions of smallholder farmers are needed to solve soil fertility problems (Chianu *et al.,* 2009). Development and adoption of technologies that optimise BNF under on-farm conditions represent the future towards sustainable agricultural productivity of crops in maize-legume cropping systems. Despite the combined and independent positive effects of the inputs used in the study, there is need for a further research on the social-economic implications of the inputs used in the study under the smallholder farming system. It is argued that soybean yields exceeding 1200 kg ha^{-1}, farmers are likely to make profits but at less than 700 kg ha^{-1} farmers may not be able to recoup the cost of production (Mutengi and Zingore, 2013). Growing need for inorganic fertilizers to enhance crop yields in Malawi and the rest of SSA (Morris *et al.,* 2007; World Bank, 2008) is hampered by the fact that the majority (about 60%) of African smallholder farmers are unable to afford the high prices of mineral fertilizers (African Fertilizer Summit, 2006). Soybean is estimated to fix 80% of its N needs (Hungria *et al.,* 2006; Smaling *et al.,* 2008); however, P requirement through mineral fertilizer still remains a challenge for soybean production since almost all of the P fertilizers used in Malawi are imported.

Table 4.19 Effect of *Rhizobium* inoculation and P fertilizer application on nodule numbers and biological nitrogen fixation of Tikolore promiscuous soybean variety in Kasungu and Salima districts

	Nodule numbers/plant			BNF (kg N ha^{-1})
Treatment	Kasungu	Salima		
T_0	17^a	8^b		20^B
T_I	19^a	9^b		27^A
T_F	14^b	26^a		27^A
T_{I+F}	24^b	25^a		29^A
	Treatment	Site	Treatment*Site	BNF
SED	0.12	0.06	0.25	0.84
P-value	0.003	0.283	0.002	<0.001

SED is the average standard error of difference as estimated under generalised linear mixed model in GenStat;T_0 = no inoculant & no P applied; T_I = Inoculant applied; T_F = P applied; T_{I+F} = Inoculant + P applied

Means in a row followed by same small case letter are not statistically different at p=0.05

Means in a column followed by same upper case letter are not statistically different at p=0.05

4.3.4 Effect of *Rhizobia* inoculation and phosphorus fertilizer on phosphorus and nitrogen concentration of promiscuous soybean variety at 50% flowering

Rhizobia inoculation and phosphorus fertilization of promiscuous soybean variety showed no significant effect on P and N concentration at 50% flowering (Table 4.20). However, significant effects of site were observed on the shoot P concentration in that Kasungu district had higher shoot P than Salima district (Table 4.2). Reports suggest that even if available P in sandier soils may appear high, reserve available P is actually low (Zhang *et al.,* 2004). Typically sandy soils are more vulnerable to changes in the pools while clay soils are more resistant (Zheng *et al.,* 2003; Zhang *et al.,* 2004). At rates below crop uptake, the labile soil P fractions can be depleted and transfers from less available pools are necessary to sustain plant development (Blake *et al.,* 2003; Zheng *et al.,* 2003; He *et al.*, 2004). Thus, there is need for a further research to determine the response of promiscuous soybean variety under different rates of P fertilizer application in the soils of Malawi. The fate of fertilizer P in soil during crop production has to be determined to evaluate the long-term economic value and sustainability of fertilizer practices (Zhang *et al.,* 2004). The total quantity of P and plant-available P often differ greatly in soils of the tropics, which typically range in weathering intensity and diverse soils (Guo *et al.,* 2000). Nutrient uptake by the plant is influenced by nutrient variability, water content, aeration and pore continuity conditions in the rhizosphere, which govern nutrient diffusion from soil solid phase to plant roots (Barber, 1995).

Critical levels in total plant tissue that separate deficiency and adequacy of N and P in soybean are 3.5 and 0.30%, respectively (Campbell, 2000). Thus, results indicate that all the values for plant N and P observed in the study were below the critical levels in total plant tissues and hence, growth and development of the soybean variety could not reach its maximum; implying that some of the growth parameters of the soybean crop were affected in the sites under study. Higher P concentration in plant benefits the bacterial symbiont and the functioning of its nitrogenase activity, leading to increased nitrogen fixation (Tahir *et al.,* 2009). Studies elsewhere have indicated highly significant uptake of P and N being pronounced in *Rhizobia* inoculated varieties or combined *Rhizobium* inoculation with P fertilization over the non-inoculated soybean varieties while in others, the results were inconsistent (Kumaga and Ofori, 2004; Kamara *et al.,* 2007; Fatima *et al.,* 2007; Bekere *et al.,* 2012).

Table 4.20 Effect of *Rhizobia* inoculant and phosphorus fertilizer application on P and N content in promiscuous soybean variety at 50% flowering in Kasungu and Salima

	Nitrogen (%)			**Phosphorus (%)**		
Trt	**Kasungu**	**Salima**	**Mean**	**Kasungu**	**Salima**	**Mean**
T_0	2.8	2.4	2.6	0.20	0.12	0.16
T_I	2.5	2.7	2.6	0.17	0.12	0.15
T_F	2.7	2.5	2.6	0.21	0.11	0.16
T_{I+F}	2.7	2.8	2.7	0.21	0.13	0.17
Mean	2.7	2.6		0.20	0.12	
	Trt	Site	Trt*Site	Trt	Site	Trt*Site
SED	0.38	0.27	0.53	0.02	0.01	0.03
P-value	0.97	0.75	0.81	0.62	<0.001	0.56

SED is the average standard error of difference as estimated under generalised linear mixed model in GenStat;T_0 = no inoculant & no P applied; T_I = Inoculant applied; T_F = P applied; T_{I+F} = Inoculant + P applied; Trt = Treatment

4.3.5 Effect of *Rhizobia* inoculation and phosphorus fertilizer on shoot and root dry matter yield of Tikolore promiscuous soybean variety

Individual effect of *Rhizobia* inoculation and P fertilization was not significant ($p < 0.05$) on shoot dry matter yield of promiscuous soybean variety. There was significant ($p = 0.02$) interaction of the treatments with the individual sites (Table 4.21). The results show that input application of either *Rhizobia* inoculant or P fertilizer to Tikolore soybean variety is required in Salima more than in Kasungu district for shoot dry matter yield. Kumaga and Ofori (2004) reported that P fertilizer addition did not significantly affect shoot dry matter production of promiscuous soybean variety, irrespective of inoculation status. Interactive effect with sites signifies the importance of site conditions for soybean growth and development so long as management practices are optimal among smallholder farmers in Malawi.

Significant ($p = 0.019$) interaction between treatments and sites also affected root dry matter yield of the soybean variety (Table 4.22). Input application was again important in Salima in influencing root dry matter yield than in Kasungu district with significant positive responses to P fertilizer application. The increase in the root dry weight emphasizes the role of P in soil in ensuring adequate root growth and development for the soybean crop in areas where P is deficient. Bekere *et al.* (2012) reported that both factors significantly ($p < 0.05$) influenced root dry matter of soybean variety at three P application rates (60, 120, 180 mg kg^{-1}) and rhizobium inoculation. Similar findings were also reported by Abbasi *et al.* (2008) and Jabbar and Saud (2012). The rate of root growth and the plasticity of root architecture, through either root growth or extension of root hairs, are clearly important for effective exploration of soil and interception of nutrients (Richardson and Barea, 2009). However, the importance of different root traits is dependent on the nutrient in question and other factors that include plant species and soil type. It is reported that inadequate P restricts root growth, the process of photosynthesis, translocation of sugars, and other such functions, which directly or indirectly influence nitrogen fixation by legume plants (Olivera *et al*., 2004).

Table 4.21 Effect of *Rhizobia* inoculant application and phosphorus fertilization on dry matter yield (g/plant) of promiscuous soybean variety

	Site	
Treatment	**Kasungu**	**Salima**
T_0	14.07^a	07.38^b
T_I	11.51^b	10.65^b
T_F	10.56^b	12.08^b
T_{I+F}	11.81^b	14.58^b
	Treatment*site	
SED	2.09	
P-value	0.02	

SED is the average standard error of difference as estimated under generalised linear mixed model in GenStat;T_0 = no inoculant & no P applied; T_I = Inoculant applied; T_F = P applied; T_{I+F} = Inoculant + P applied. Means in a row followed by same letter are not statistically different at p=0.05

Table 4.22 Effect of *Rhizobia* inoculant application and phosphorus fertilizer on root dry matter yield (g/plant) of promiscuous soybean variety

	Site	
Treatment	**Kasungu**	**Salima**
T_0	1.16^a	0.79^b
T_I	0.75^a	1.07^a
T_F	1.08^b	1.54^a
T_{I+F}	1.11^a	1.26^a
	Treatment*site	
SED	0.18	
P-value	0.02	

SED is the average standard error of difference as estimated under generalised linear mixed model in GenStat;T_0 = no inoculant & no P applied; T_I = Inoculant applied; T_F = P applied; T_{I+F} = Inoculant + P applied; Means in a row followed by same letter are not statistically different at p=0.05

4.3.6 Effect of *Rhizobia* inoculation and phosphorus fertilizer on harvest index, grain and biomass yield of Tikolore promiscuous soybean variety

The results showed significant effect of *Rhizobium* inoculation and P fertilization on grain (P = 0.04) and biomass (P = 0.05) yield of promiscuous soybean variety. Significant (P < 0.001) differences were also observed between the two sites (Table 4.23). Salima district produced 27 and 49% more grain and biomass yield respectively than Kasungu district. This could be attributed to better soils in Salima than in Kasungu; but also considering that the promiscuous soybean variety used is an early maturing variety (105 days) that might have suited well in the low-altitude zone. Most of the important soil parameters (CEC, N, and OM) were below the critical levels in Kasungu district. The higher root dry weight observed in Salima than Kasungu indicate that soybean roots exploited more nutrients for plant growth and development. This is confirmed by the high correlation of root dry matter and grain yield (Table 4.27). These are not stand alone findings; they mirror results of previous studies by Kamara *et al.* (2007), Mhango *et al.* (2008), Wasike *et al.* (2009), Kamanga*et al.* (2010) and Sharma *et al.* (2011). In particular, Kamanga *etal*. (2010) reported that grain yields of P fertilized legumes were higherthan yields of unfertilized treatments for all legumes tested including soybean in the area of Chisepo, Dowa District. Soybean showed response to P with 0.5 t ha^{-1} extra grain yield than unfertilized plots. At this site, legume biomass yields were significantly different with fertilized treatments giving higher biomass yield than unfertilized treatments for all legumes planted. Fertilized soybean had 1.5 t ha^{-1} of biomass higher than that of the unfertilized treatment. Nevertheless, the results of this study were in contrast with the findings of other workers who reported that yield components and grain yield in soybean were not significantly influenced by P application (Chiezey, 1999; Olofintoye, 2007). It has however, been observed that the soybean crop response to P is dependent on soil available P (Mallarino and Reuben, 2005). Below critical levels of available P in Chinguluwe EPA in Salima district justifies why there were significant responses to P fertilizer application in promiscuous soybean variety.

Like Mabapa *et al.* (2010) observation, *Rhizobium* inoculation and P fertilization did not also affect the harvest index for the promiscuous soybean variety used in this study. However, site differences significantly ($P < 0.001$) influenced the harvest index of

Tikolore soybean variety with Kasungu district having a higher harvest index than Salima district (Table 4.23).

Table 4.23 Main effects of *Rhizobia* inoculant and phosphorus fertilizer application and sites on grain yield, biomass and harvest index of Tikolore soybean variety

Treatment	**Grain yield (kgha^{-1})**	**Biomass (kgha^{-1})**	**Harvest index (%)**
T_0	1301^{b}	2666^{b}	51
T_I	1432^{ab}	3014^{ab}	49
T_F	1635^{a}	3365^{a}	49
T_{I+F}	1703^{a}	3477^{a}	49
SED	143.5	296.2	3
P-value	0.041	0.05	0.714
Site	**Grain yield (kgha^{-1})**	**Biomass (kgha^{-1})**	**Harvest index (%)**
Kasungu	1338	2511	53
Salima	1697	3750	45
SED	101.5	209.4	2
P-value	0.002	<0.001	<0.001

SED is the average standard error of difference as estimated under generalised linear mixed model in GenStat; T_0 = no inoculant & no P applied; T_I = Inoculant applied; T_F = P applied; T_{I+F} = Inoculant + P applied

4.3.7 Correlation and regression analysis for the studied parameters

Correlation analysis of soil parameters with some studied soybean growth parameters showed significant correlations. Most of the soil parameters showed positive and significant correlation with soybean biomass and negative but significant correlation with shoot P. Available soil P showed positive and significant ($P < 0.001$) correlation with shoot P (Table 4.24). Okogun *et al.* (2004) reported significant correlations for promiscuous soybean growth parameters with CEC, soil P, total N and pH.

Correlation analysis was also done for the soybean growth parameters. The analysis showed that soybean grain and biomass yields of promiscuous soybean variety significantly ($P < 0.01$) correlated positively with shoot and root dry matter yields, shoot N, and nodulation assessment (Table 4.26). Measure of association between grain and biomass yield showed a coefficient of determination of 86%. Nodulation assessment also showed positive and significant correlation with related parameters i.e. nodule numbers per plant, nodule dry weight, root dry weight and shoot N%.

Table 4.24 Correlation of soil parameters with some of the studied parameters in Tikolore soybean variety

	Grain yield	**Biomass**	**Shoot P**	**Shoot N**
K	0.62***	0.75***	-0.29	0.48*
Mg	0.32	0.51**	-0.46**	-0.06
N	0.10	0.27	-0.68***	-0.29
OM	0.13	0.30	-0.68***	-0.27
P	-0.02	-0.23	0.68***	0.37*
pH	0.32	0.50**	-0.13	0.15
Ca	0.26	0.45**	-0.52**	-0.06
CEC	0.26	0.39*	-0.61***	-0.21

Grain yield correlated significantly ($p < 0.05$) with biomass yield, dry matter yield, root dry weight, nodule numbers per plant, shoot N, nodulation and K. Correlation analysis among these parameters showed presence of multicollinearity if subjected to multiple regression analysis (Table 4.25). Therefore, when these parameters were subjected to stepwise regression analysis, biomass yield was chosen as strongest single predictor of the 7 potential predictors of grain yield in the soybean variety. However, dry matter yield was also statistically important predictor of soybean grain yield in the study. Simple linear regression showed that biomass yield accounted for 85.9% variation in grain yield with a standard error of 181 (lowest value among the 7 predictors).

Table 4.25 Correlation matrix among the parameters with significant correlation with grain yield

	Biomass	Dry matter per plant	K	Nodules per plant	Root dry weight	Shoot N	Nodulation
Biomass	-						
Dry matter per plant	0.47**	-					
K	0.75***	0.23	-				
Nodules per plant	0.34*	0.59***	-0.02	-			
Root dry weight	0.55**	0.51**	0.41**	0.37*	-		
Shoot N	0.61***	0.60***	0.48**	0.25	0.43**	-	
Nodulation	0.56***	0.64***	0.29	0.65***	0.45**	0.57***	-

*, ** and *** correlation is significant at <0.05, <0.01 and <0.001 levels of probability respectively

Table 4.26 Correlation among the various studied parameters on the effect of *Rhizobia* inoculation and phosphorus application on Tikolore soybean variety

	Nodule dry wt	Nodules plant^{-1}	Root DM	Shoot N%	Shoot P%	Nodulation	Harvest index	Grain yield	Shoot DM	Biomass
Nodule dry wt	-									
Nodules plant^{-1}	0.54**	-								
Root DM	0.38*	0.37*	-							
Shoot N%	0.15	0.25	0.43*	-						
Shoot P%	-0.34	-0.14	-0.08	0.10	-					
Nodulation	0.61***	0.65***	0.45**	0.57***	-0.19	-				
Harvest index	0.07	0.00	0.13	0.10	0.41*	0.05	-			
Grain yield	0.25	0.41*	0.64***	0.67***	-0.23	0.66***	-0.05	-		
Shoot DM	0.44*	0.59***	0.52**	0.60***	-0.01	0.64***	0.29	0.64***	-	
Biomass	0.21	0.34*	0.55***	0.61***	-0.36*	0.56***	-0.40*	0.93***	0.47**	-

*, ** and *** correlation is significant at <0.05, <0.01 and <0.001 levels of probability respectively; DM = dry matter; wt = weight

4.3.8 Partial budget analysis for use of *Rhizobia* inoculant and phosphorus fertilizer in Tikolore promiscuous soybean variety

Economic constraints and opportunities for improving soybean production systems in Malawi must be understood as the basis for developing interventions. This study presents the partial budget analysis for the economic analysis of the on-farm soybean trials conducted with use of inorganic P fertilizer and *Rhizobia* inoculants. While there are other types of budgeting (e.g. whole farm and enterprise budgeting), the partial budgeting procedure is the most useful in farming system research (Worman *et al.*, 1990). It is a method of organising experimental data and information about the cost and benefits from some change in the technologies being used on the farm. Partial budgets do not calculate the total income and expenses for each of the alternative plan but list only those items of income and expense that change. They measure changes in income and returns to limited-resources, provide a limited assessment of risk and, through sensitivity analysis, suggest a range of prices or costs at which a technology becomes profitable.

The study under review aim at estimating the change that will occur in farm profit or loss by use of *Rhizobia* inoculant and P fertilizer in soybean production systems. Tables 4.27 and 4.28 shows results on the partial budget for Tikolore soybean variety grown without *Rhizobia* inoculant nor P fertilizer application; grown with applied *Rhizobia* inoculant only; grown with P fertilizer application only; and grown with a combine application of *Rhizobia* inoculant and P fertilizer in form of TSP in Kasungu and Salima districts respectively. The majority of smallholder farmers in Malawi and in areas under study neither do they use *Rhizobia* inoculant nor inorganic fertilizer application to soybean production. *Rhizobia* inoculant and P fertilizer application to Tikolore promiscuous soybean variety had a positive net change in income as compared to the control treatment in Salima district. A combined application of inoculant and P fertilizer had a higher net income than single application of either *Rhizobia* inoculant or P fertilizer in the district (Table 4.28). However, the scenario was different in Kasungu district. Phosphorus fertilizer application alone showed a positive net benefit than *Rhizobia* inoculant or the combined application of P fertilizer and *Rhizobia* inoculant (Table 4.27). The results place more emphasis on the need to apply either P fertilizer or *Rhizobia* inoculant or a combined application of the two factors in order to increase the grain yield of Tikolore despite the promiscuous nature of the soybean variety in Salima district. As for Kasungu, we need to intensify the management practices that will improve the soil fertility status of the area as well as selection of adapted soybean genotypes in order to realise the benefit of *Rhizobia* inoculant and P fertilizer application. Either way, the economic analysis results of the study demonstrates an important step in unpacking economic viability of soybean production systems in order to identify opportunities and constraints that can be used as input information to devise improvement strategies that intensify soybean production in Malawi. The positive benefits of P fertilizer observed in the study and elsewhere strengthen the idea that P is important on the activation of metabolic processes necessary for vegetative growth resulting in high dry matter accumulation and therefore giving rise to high grain yield (Mugendi *et al.,* 2010; Hemalatha *et al.,* 2013). Besides economic benefits, Ogoke *et al.* (2001) studied the role of P in enhancing soybean residue contribution to soil fertility. They reported that litter residue increased by 42 to 46% with P application as compared to no phosphorus treatment. Further work of Ogoke *et al.* (2003) demonstrated that application of P fertilizer was justified and necessary in soils where P levels were below critical levels.

Table 4.27 Partial budget analysis for production of Tikolore promiscuous soybean variety in Kasungu districtas of April, 2014

	Unit	**Treatment**			
		T_0	**T_I**	**T_F**	**T_{I+F}**
Area	Hectare	1	1	1	1
Average grain yield	kg ha^{-1}	1250	1246	1480	1375
Average gain yield	kg	0	-4	230	125
Market price	MK kg^{-1}	290	290	290	290
Gross income	MK	362,500.00	361,340.00	429,200.00	398,750.00
TSP required	kg ha^{-1}	0	0	100	100
TSP cost	MK (100 $kg)^{-1}$	0	0	35,200.00	35,200.00
Fertilizer transport cost	MK	0	0	2,000.00	2,000.00
Fertilizer application cost	MK ha^{-1}	0	0	6,000.00	6,000.00
Post-harvest costs	MK	0	-48	2,760.00	1,500.00
Inoculant cost	MK	0	1,876.00	0	1,876.00
Total added cost	MK	0	1,828.00	45,960.00	46,576.00
Net income	MK	362,500.00	359,512.00	383,240.00	352,174.00
Net benefit	MK	0	-2,988.00	20,740.00	-10,326.00

T_0 = no inoculant & no P applied; T_I = Inoculant applied; T_F = P applied; T_{I+F} = Inoculant + P applied; Post-harvest cost (MK600/50kg bag) = winnowing, handling, grading and packaging costs; Fertilizer transport cost = maximum amount from main trading centre (7km radius)

Table 4.28 Partial budget analysis for production of Tikolore promiscuous soybean variety in Salima districtas of April, 2014

	Unit	**Treatments**			
		T_0	**T_I**	**T_F**	**T_{I+F}**
Area	Hectare	1	1	1	1
Average grain yield	kg ha^{-1}	1352	1617	1789	2031
Average gain yield	kg	0	265	437	679
Market price	MK kg^{-1}	290	290	290	290
Gross income	MK	392,080.00	468,930.00	518,810.00	588,990.00
TSP required	kg ha^{-1}	0	0	100	100
TSP cost	MK $(100\ kg)^{-1}$	0	0	35,200.00	35,200.00
Fertilizer transport cost	MK	0	0	2,000.00	2,000.00
Fertilizer application cost	MK ha^{-1}	0	0	6,000.00	6,000.00
Post-harvest costs	MK	0	3180	5,244.00	8,148.00
Inoculant cost	MK	0	1,876.00	0	1,876.00
Total added cost	MK	0	5,056.00	48,444.00	53,224.00
Net income	MK	392,080.00	463,874.00	470,366.00	535,766.00
Net benefit	MK	0	71,794.00	78,286.00	143,686.00

T_0 = no inoculant & no P applied; T_I = Inoculant applied; T_F = P applied; T_{I+F} = Inoculant + P applied; Post-harvest cost (MK600/50kg bag) = winnowing, handling, grading and packaging costs; Fertilizer transport cost = maximum amount from main trading centre (7km radius)

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Results from the two studies have shown that P fertilizer application is effective in improving growth, BNF, yield and yield componentsof soybean varieties in low P soils (<25ppm, Mehlich 3). Studies also revealed that inoculation alone may not be adequate enough to improve soybean grain yield under low P soils. Synergetic effect of *Rhizobia* inoculation and P fertilizer application on grain yield and N fixation was more pronounced than the application of *Rhizobia* inoculant or P fertilizer alone. Despite the promiscuous status of Tikolore soybean variety, the variety may also need inoculation or P fertilizer application or both for higher productivity. Soil physical and chemical properties influenced the response of soybean varieties to applied *Rhizobia* inoculant and P fertilizer. Thus, there exists interaction between soil fertility and soybean response to *Rhizobia* inoculation and P fertilizer application. Selection and development of efficient soybean varieties that are responsive to inoculant *Rhizobia* strain(s) form the basis increased soybean yields and BNF. Farmers should put extra efforts on farming practices that stimulate symbiotic effectiveness and improved soil productivity including (i) *Rhizobia* inoculation that adds beneficial strains to the soil (ii) crop rotation with well fertilized cereal crop to get the benefit of the residual N and P (iii) appropriate soil conservation practices and recycling of crop residues (iv) adequate amount of organic and inorganic fertilizer use. These attempts will be met with greater success through a consideration of other factors like crop protection, selection of right varieties, proper plant spacing, seed rate, timely planting and weed control.It is important to develop and disseminate crop and site-specific nutrient management technologies for sustainable crop management practices among smallholder farmers in Malawi.

5.2 Recommendations

Based on the results of both studies, the following recommendations are made:

i. Despite the promiscuous nature of Tikolore soybean variety, the variety responds to *Rhizobia* inoculation and, therefore, inoculation of *Rhizobia* can boost nodulation, BNF and growth performance of the variety.

ii. Combined application of *Rhizobia* inoculation and P fertilizer has more yield benefits than single application in both soybean varieties

iii. Further studies are needed to evaluate the response of soybean to different P fertilizer application rates in Malawi and other P containing fertilizers.

iv. Increasing the breadth of breeding programmes to include selection for effective symbioses under both high- and low P fertilization regimes could prove an effective tool for symbiotic efficiency in soybean.

v. Further research is needed to establish the effectiveness of promiscuous soybean varieties in locations of variable population of indigenous *Rhizobia*. Knowledge of the biodiversity of *Rhizobia* and local populations is important for the design of successful inoculation strategies.

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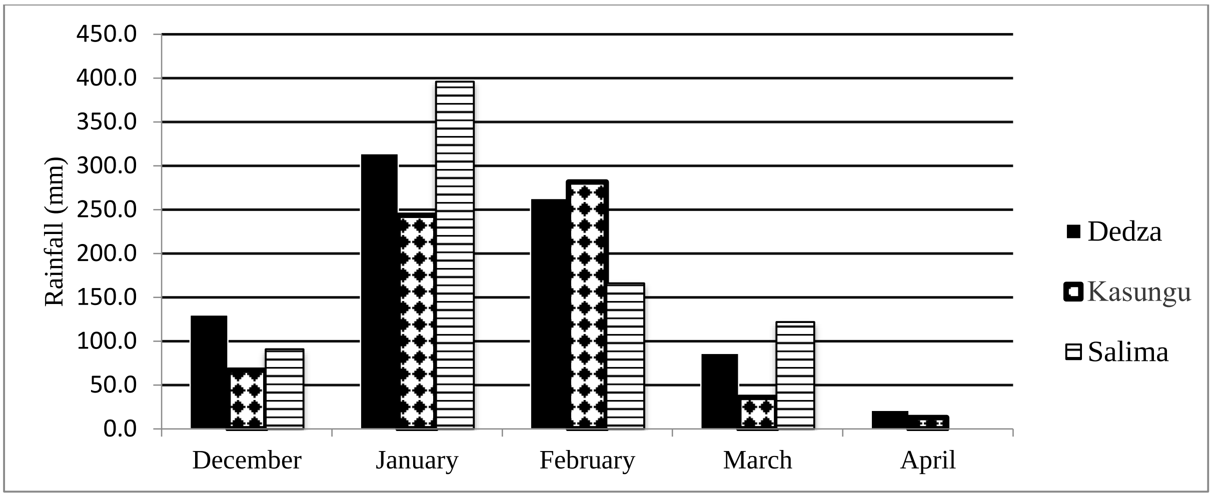
APPENDICES

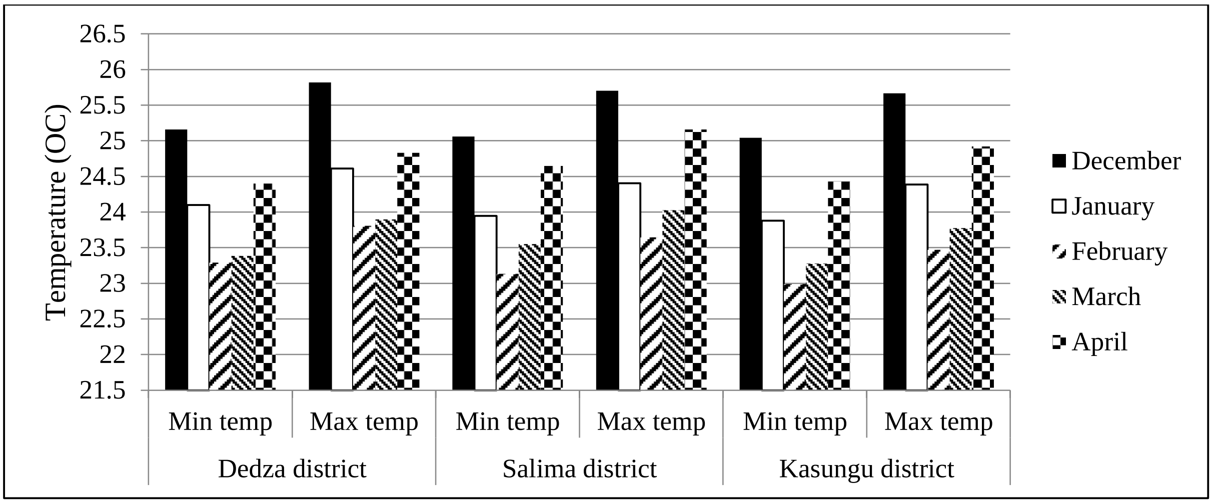
7.1 Rainfall, temperature and relative humidity

The study sites received different amounts of rainfall. There were, however, minimal variations in amount and distribution of rainfall within farmers' sites in an EPA. Relative to the historical rainfall amounts and distribution on the tested sites, there was an improvement in rainfall distribution and amounts in the 2012/2013 cropping season for crop growth and development. The total amount of rainfall was enough to support soybean growth and development; rainfall of 500 to 900 mm is required for better yields and better seed quality, depending on growth conditions.All the sites received much of the rainfall in January and February (Appendix 1). There were no significant dry spells to affect the vegetative, flowering and grain filling stages of soybean crop growth during 2012/2013 cropping season in all the sites. However, planting was delayed in Chinguluwe EPA in Salima district because of an initial 10-12 days dry spell that occurred towards end December of 2012 to early January, 2013.

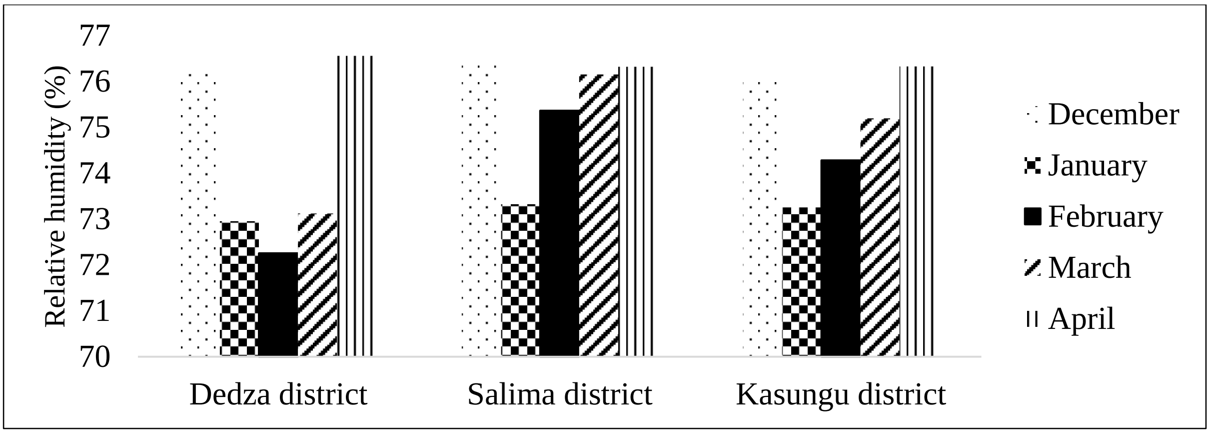
During the cropping season under review, the daily temperature had ranged from 22^oC to 27^oC at all the three sites and relative humidity (RH) of 68-83%; which is generally very moist (Appendices 2 and 3 respectively). Yields of soybean are adversely affected as temperatures rise above 30 °C, while temperatures below 13 °C for long periods during flowering stage inhibit flower and seed formation (Lindsey and Thomson, 2012). As such, the daily temperatures in all the research sites were within the optimal range for soybean production.



Appendix 1. Mean monthly rainfall (mm) for study sites, 2012/2013 cropping season



Appendix 2. Mean maximum and minimum temperature (^{O}C) for study sites, 2012/2013 cropping season



Appendix 3. Mean relative humidity (%) for study sites, 2012/2013 cropping season

Appendix 4. Baseline soil properties in the 0 - 20 cm depth range for the study sites under soybean varieties and P fertilizer application in Linthipe EPA, Dedza district

Soil property	Critical value	Farmer 1	Farmer 2	Farmer 3	Farmer 4	Farmer 5	Mean	Std. Dev
pH (H_20)	5-7	6.1	5.6	5.7	5.8	5.5	5.7	0.23
CEC (meq/100g)	25	49.4	51.4	38.3	57.1	62.4	51.7	9.06
Total N (%)	0.1	0.17	0.15	0.08	0.17	0.17	0.15	0.04
Mehlich-3 P (ppm)	25	40.1	31.8	83.8	38.5	174.4	73.7	59.9
K (cmol/kg)	0.13-0.20	1.2	0.4	0.5	0.57	0.6	0.7	0.31
Ca (cmol/kg)	2.0	18.5	21.2	16.1	20.3	15.1	18.2	2.6
Mg(cmol/kg)	0.24	4.3	4.8	2.1	3.4	3.6	3.6	1.02
OM (%)	1.5	3.45	3.05	1.58	3.45	3.35	2.98	0.8
Sand (%)	-	54	60	78	62	48	60.4	11.3
Clay (%)	-	36	28	14	26	40	28.8	10.0
Textural class	SL	SC	SCL	SL	SCL	SC	-	-

Std. Dev = standard deviation; Critical value = A value below which implies low amount; SL= sandy loam soil; SC = sand clay soil; SCL = sandy clay soil

Appendix 5. Baseline soil properties in the 0 - 20 cm depth range for the study sites under soybean varieties and P fertilizer application in Chinguluwe EPA, Salima district

Soil property	Critical value	Farmer 1	Farmer 2	Farmer 3	Farmer 4	Farmer 5	Mean	Std. Dev
pH (H_20)	5-7	6.3	6.5	6.6	6.1	6.7	6.4	0.2
CEC (meq/100g)	25	43.6	32.7	28.5	33.3	35.2	34.7	5.7
Total N (%)	0.1	0.06	0.13	0.03	0.09	0.06	0.07	0.04
Mehlich-3 P (ppm)	25	11.1	21.5	33.3	7.1	10.5	16.7	10.7
K (cmol/kg)	0.13-0.20	0.36	1.3	0.7	0.5	1.4	0.9	0.5
Ca (cmol/kg)	2.0	12.3	10.8	6.5	9.0	10.4	9.8	2.2
Mg(cmol/kg)	0.24	3.7	2.3	2.1	1.9	2.4	2.5	0.7
OM (%)	1.5	1.23	2.51	0.69	1.77	1.18	1.48	0.69
Sand (%)	-	70	82	76	74	70	74	5
Clay (%)	-	24	14	16	18	24	19	5
Textural class	SL	SCL	SL	SL	SL	SCL	-	-

Std. Dev = standard deviation; Critical value = A value below which implies low amount; SL= sandy loam soil; LS = loamy sand soil; SCL = sandy clay soil

Appendix 6. Baseline soil properties in the 0 - 20 cm depth range for the study sites under *Rhizobia* inoculation and P fertilizer application in Chinguluwe EPA, Salima district

Soil property	Critical value	Farmer 1	Farmer 2	Farmer 3	Farmer 4	Mean	Std. Dev
pH (H_20)	5-7	6.4	6.1	5.8	5.6	6.0	0.4
CEC (meq/100g)	25	39.8	33.3	24.1	48.1	36.3	10.2
Total N (%)	0.1	0.10	0.07	0.08	0.13	0.09	0.03
Mehlich-3 P (ppm)	25	47.5	16.1	21.7	9.6	23.7	16.6
K (cmol/kg)	0.13-0.20	1.5	0.6	0.8	0.4	0.8	0.4
Ca (cmol/kg)	2.0	13.5	8.4	8.5	11.4	10.5	2.5
Mg(cmol/kg)	0.24	2.6	2.0	1.9	2.2	2.2	0.3
OM (%)	1.5	2.07	1.48	1.63	2.16	1.84	0.33
Sand (%)	-	70	80	70	70	72.5	5
Clay (%)	-	22	16	20	24	20.5	3.4
Textural class	SL	SCL	SL	SCL	SCL	-	-

Std. Dev = standard deviation; Critical value = A value below which implies low amount; SL= sandy loam soil; SCL = sandy clay soil

Appendix 7. Baseline soil properties in the 0 - 20 cm depth range for the study sites under soybean varieties and P fertilizer application in Kaluluma EPA, Kasungu district

Soil property	Critical value	Farmer 1	Farmer 2	Farmer 3	Farmer 4	Farmer 5	Mean	Std. Dev
pH (H_20)	5-7	5.8	5.9	5.3	6.1	5.5	5.7	0.3
CEC (meq/100g)	25	20.0	39.1	18.5	13.0	11.0	20.3	11.1
Total N (%)	0.1	0.05	0.09	0.04	0.04	0.03	0.05	0.02
Mehlich-3 P (ppm)	25	20.1	38.7	45.0	53.3	70.1	45.4	18.4
K (cmol/kg)	0.13-0.20	0.5	0.8	0.4	0.5	0.3	0.5	0.2
Ca (cmol/kg)	2.0	4.5	11.8	3.4	4.6	3.2	5.5	3.6
Mg(cmol/kg)	0.24	1.5	2.1	0.7	0.8	0.9	1.2	0.6
OM (%)	1.5	1.03	1.72	0.74	0.79	0.64	0.98	0.44
Sand (%)	-	78	72	82	90	86	81.6	7.0
Clay (%)	-	16	22	14	8	12	14.4	5.2
Textural class	Sandy loam	SL	SCL	SL	LS	SL	-	-

Std. Dev = standard deviation; Critical value = A value below which implies low amount; SL= sandy loam soil; LS = loamy sand soil; SCL = sandy clay soil

Appendix 8. Baseline soil properties in the 0 - 20 cm depth range for the study sites under *Rhizobia* inoculation and P fertilizer application in Kaluluma EPA, Kasungu district

Soil property	Critical value	Farmer 1	Farmer 2	Farmer 3	Farmer 4	Mean	Std. Dev
pH (H_20)	5-7	6.1	5.0	5.8	6.0	5.7	0.5
CEC (meq/100g)	25	12.5	14.3	7.9	11.2	11.5	2.7
Total N (%)	0.1	0.05	0.04	0.00	0.05	0.04	0.02
Mehlich-3 P (ppm)	25	75.5	85.0	62.7	48.1	67.8	16.0
K (cmol/kg)	0.13-0.20	0.3	0.3	0.4	0.2	0.3	0.08
Ca (cmol/kg)	2.0	4.1	2.0	3.8	6.8	4.2	1.98
Mg(cmol/kg)	0.24	1.0	0.5	1.3	1.2	1.0	0.4
OM (%)	1.5	1.03	0.74	0.00	1.03	0.7	0.5
Sand (%)	-	88	80	88	90	86.5	4.4
Clay (%)	-	8	16	8	8	10	4
Textural class	SL	LS	SL	LS	LS	-	-

Std. Dev = standard deviation; Critical value = A value below which implies low amount; SL= sandy loam soil; LS = loamy sand soil